

Journal of Oil Palm Research

Vol. 34 (4) • December 2022

REVIEW ARTICLE

- Treated Oil Palm Frond and Its Utilisation as an Improved Feedstuff for Ruminants - An Overview
- The Synthesis of *sn*-2 Palmitate as Human Milk Fat Substitute from Palm Oil Fractions by Enzymatic Interesterification - A Review



Treated Oil Palm
Frond (OPF)



Infant Milk
Formula

eISSN 2811-4701



9772811470006



JOURNAL OF OIL PALM RESEARCH (formerly known as ELAEIS)

JOURNAL OF OIL PALM RESEARCH, an international refereed journal, carries full-length original research papers, short communications and scientific review papers on various aspects of oil palm and palm oil and other palms. JOURNAL OF OIL PALM RESEARCH is published four times per year, *i.e.* March, June, September and December.

© Malaysian Palm Oil Board, 2022

All rights reserved. No part of this publication may be reproduced in any form or by any means without the written permission of the Malaysian Palm Oil Board.

Impact Factor:

1.594

data from 2021 *Journal Citation Report*® Science Edition
– A Clarivate Analytics product.

For more information on advertisement for the JOURNAL OF OIL PALM RESEARCH, please write to:

Editor-in-Chief
Journal of Oil Palm Research
Malaysian Palm Oil Board
6 Persiaran Institusi, Bandar Baru Bangi
43000 Kajang, Selangor, Malaysia

Tel: 603-8769 4400

E-mail: pub@mpob.gov.my

Website: jopr.mpob.gov.my

DISCLAIMER

Views of writers expressed in this publication are not necessarily endorsed by or represent the views of the Malaysian Palm Oil Board.



Published by the Malaysian Palm Oil Board

JOURNAL OF OIL PALM RESEARCH

Vol. 34 (4) December 2022

C O N T E N T S

REVIEW ARTICLES

- 591 Treated Oil Palm Frond and its Utilisation as an Improved Feedstuff for Ruminants – An Overview
Mookiah Saminathan; Wan Nooraida Wan Mohamed; 'Abidah Md Noh; Nur Atikah Ibrahim; Muhammad Amirul Fuat; Suriya Kumari Ramiah; Eric Lim Teik Chung and Noor Lida Habi Mat Dian
- 608 The Synthesis of *sn*-2 Palmitate as Human Milk Fat Substitute from Palm Oil Fractions by Enzymatic Interesterification – A Review
Hasrul Abdi Hasibuan

RESEARCH ARTICLES

- 622 *Strategus aloeus* (Coleoptera: Scarabaeidae) Damage and Its Relationship with Rainfall and Hybrid Oil Palm Age in Colombia
Luis Guillermo Montes-Bazurto; Alex Enrique Bustillo-Parde and Anuar Morales Rodriguez
- 629 Cell Wall-glycolipids Profiling of Oil Palm Roots during *Ganoderma boninense* Infection Using Gas Chromatography-mass Spectrometry
Alexander, A; Abdullah, S; Dayou, J and Chong, K P
- 643 Oil Palm SSR Resource Interface (OPSRI) – Web-based Bioinformatic Analysis Pipeline for SSR Mining
Rozana Rosli; Mohd Amin Ab Halim; Ting Ngoot-Chin; Noorhariza Mohd Zaki; Jayanthi Nagappan; Rajinder Singh; Leslie Low Eng-Ti and Zeti-Azura Mohamed-Hussein
- 657 Field Evaluation of Oil Palm Genetic Materials for Partial Resistance in *Ganoderma* Hotspots of Tropical Peat Soil
Mohd Din, A; Marhalil M; Mohd Mustakim, M; Norziha, A; Kushairi, and Rajanaidu, N
- 668 Mechanical Design Analysis and Improvement of Aluminium Alloy CANTAS Sickle Blade Using Finite Element Method Simulation
Ahmad Syazwan Ramli; Mohd Azwan Mohd Bakri; Mohd Rizal Ahmad and Mohd Ikmal Hafizi Azaman
- 678 Characterisation of Palm Pyroligneous Acid and Its Effectiveness as Antifungal Agent for Oil Palm Trunk
Fazliana Abdul Hamid; Nahrul Hayawin Zainal; Nur Eliyanti Ali Othman; Fatiha Ismail; Noorshamsiana Abdul Wahab and Astimar Abd Aziz
- 686 Emulsifying Properties and Antioxidant Activity of Phospholipids Fractions from Palm Pressed Fibre
Kgs Ahmadi; Teti Estiasih and Ahmad Diaul Khuluq
- 699 Properties of Solid Polyurethanes Made from Palm Olein-based Polyols Prepared with Various Types of Nucleophiles
Tuan Noor Maznee Tuan Ismail; Nor Azowa Ibrahim; Kosheela Devi Poo Palam; Hoong Seng Soi; Mohd Azmil Mohd Noor; Yeong Shoot Kian; Emilia Abd. Malek; Ibrahim Sendijarevic and Vahid Sendijarevic
- 710 Method for the Determination of Total Chloride Content in Edible Oils
Abdul Niefazal Abdul Hammid; Azmil Haizam Ahmad Tarmizi; Muhamad Roddy Ramli; Ainie Kuntom and Lee Hock Chin
- 721 Effects of Palm Oil-based Surfactant Used in Pesticide Formulation on Terrestrial Plants
Asma Liyana Shaari; Noorazah Zolkarnain; Ismail Ab. Raman and Razmah Ghazali
- 731 Association of Fatty Acids and Polar Compound Fractions with Acrylamide Formation during Intermittent Frying
Su Lee Kuek; Azmil Haizam Ahmad Tarmizi; Raznim Arni Abd Razak; Selamat Jinap; Saparin Norliza and Maimunah Sanny
- 741 Assessment of Fatty Acid and Vitamin E Profiles in Edible Oils in Malaysia
Radhika Loganathan and Teng Kim-Tiu
- 752 Properties of Bread Made with Palm-based Fluid Shortening
Nur Haqim Ismail; Norazura Aila Mohd Hassim; Saw Mei Huey; Noor Lida Habi Mat Dian and Sivaruby Kanagaratnam

SHORT COMMUNICATION

- 762 Optimisation of Ethylene Treatment Conditions for Oil Palm Fruit Detachment Using Response Surface Methodology
Mohd Ibnur Syawal Zakaria; Arutchelvam Balakrishnan; Bee Aik Tan; Jaime Yoke Sum Low; Shwu Fun Kua; Chin Ming Lim and David Ross Appleton

Cover picture: 1. Treated oil palm frond (OPF), a biomass residue from oil palm plantations.
2. *Sn*-2 palmitate incorporated in infant formulas to mimic human milk's lipid profile.

EDITORIAL BOARD

(1 January 2022 – 31 December 2023)

Datuk Dr. Ahmad Parveez Ghulam Kadir
Malaysia (Editor-in-Chief)

Prof. Dr. Douglas G Hayes
USA

Dr. Carl Traeholt
Malaysia

Prof. Fredolin Tangang
Malaysia

Prof. Dr. Matthias Finkbenier
Germany

Prof. Dr. Norzulani Khalid
Malaysia

Dr. Julie Flood
United Kingdom

Prof. Dr. Ir. Azmi Yahya
Malaysia

Prof. Dr. Dirk Prufer
Germany

PUBLICATION COMMITTEE

CHAIRPERSON COMMITTEE MEMBERS

Datuk Dr. Ahmad Parveez Ghulam Kadir

Dr. Zainab Idris

Dr. Ramle Moslim

Rosidah Radzian

Dr. Astimar Abdul Aziz

SECRETARY

Dr. Mohamad Arif Abd Manaf

Anita Taib

Dr. Idris Abu Seman

Dr. Aki @ Zaki Aman

Dr. Zafarizal Aldrin Azizul Hasan

Ruba'ah Masri

Johari Minal

Mohd Saufi Awang

Iptisam Abdul Wahab

TREATED OIL PALM FROND AND ITS UTILISATION AS AN IMPROVED FEEDSTUFF FOR RUMINANTS - AN OVERVIEW

MOOKIAH SAMINATHAN^{1*}; WAN NOORAIDA WAN MOHAMED¹; ABIDAH MD NOH¹;
 NUR ATIKAH IBRAHIM¹; MUHAMMAD AMIRUL FUAT¹; SURIYA KUMARI RAMIAH²;
 ERIC LIM TEIK CHUNG³ and NOOR LIDA HABI MAT DIAN¹

ABSTRACT

Oil palm frond (OPF) is a biomass residue from oil palm plantations that have received increasing interest for its promise as roughage resources to overcome limitations of ruminant feeding. However, the use of OPF for livestock productivity is limited by its high lignocellulosic content and low nutritional value. Various physical, chemical, biological and physico-chemical treatments have been used to improve the nutritional value of OPF. This review was conducted to study the treatment methods of OPF as a value-added feedstuff and emphasise the effects of treated OPF on in vitro ruminal fermentation and in vivo ruminant digestibility and growth performance. The biological treatment produced favourable outcomes in improving the nutritional value of OPF and was more cost-effective, safe, and environmental-friendly compared to chemical and physical treatments. In vitro rumen fermentation studies on the potential use of treated OPF as feedstuffs for ruminants have been widely reported. Several in vivo studies have found that ruminants performed satisfactorily when fed diets with treated OPF at appropriate ratios. Nevertheless, chemically treated OPF has been successfully used in both beef and dairy cattle feeding trials as it is a good source of protein and has positive impacts on ruminal fermentation and animal production.

Keywords: feed, *in vitro* rumen fermentation, oil palm frond, ruminants, treatment.

Received: 12 May 2021; **Accepted:** 28 July 2021; **Published online:** 14 October 2021.

INTRODUCTION

Profitable and sustainable livestock productivity in tropical regions is often limited by the high cost of conventional feed resources and competition from human nutrition. Hence, bioconversion of

agro-industrial waste or by-products has become an alternative for the economic and sustainable production of feed for both ruminants and non-ruminants. The oil palm (*Elaeis guineensis* Jacq.) industry in Malaysia produces large quantities of biomass residues annually that can potentially be used as indigenous feed ingredients for livestock feeding, such as oil palm frond (OPF), oil palm trunk (OPT), palm press fibre (PPF), empty fruit bunches (EFBs), palm kernel cake (PKC), palm oil mill effluent (POME) and decanter cake (Soh *et al.*, 2018). Oil palm biomass residues have become increasingly explored and exploited as promising alternatives to conventional feed for their ability to overcome the problem of feed resource waste, promote the sustainability of oil palm cultivation, and does not harm the environment (Kushairi *et al.*, 2019). Current trends in feed science and

¹ Malaysian Palm Oil Board,
6 Persiaran Institusi, Bandar Baru Bangi,
43000 Kajang, Selangor, Malaysia.

² Institute of Tropical Agriculture and Food Security,
Universiti Putra Malaysia,
43400 Serdang, Selangor, Malaysia.

³ Department of Animal Science,
Faculty of Agriculture, Universiti Putra Malaysia,
43400 Serdang, Selangor, Malaysia.

* Corresponding author e-mail: saminathan@mpob.gov.my

technology in animal nutrition may provide appropriate means for effectively utilising locally available crop residues in appreciable quantities to maximise the benefits of feed in animal systems.

OPF consists of leaves and petioles of oil palms and is a major cellulosic solid crop residue utilised as a fibre-forage complete feed for ruminants (Kum and Zahari, 2011). OPF is generally obtained during pruning operations in plantations. The availability of OPF in 2020 was estimated to be approximately 49.0 million tonnes (dry weight basis) and is likely to increase in the future (Agensi Inovasi Malaysia, 2013). OPF can be utilised as a ruminant feed either as chopped green or fermented silage in combination with concentrate as a complete feed or total mixed rations (TMRs). Incorporating OPF into the ruminant diet might provide an effective way of overcoming the typical limitations of pastures for sustaining animal production in palm oil-producing countries, including Malaysia. Balanced and economical feeding of fibrous crop residues is fundamental for optimising the voluntary intake of feed, enhancing its utilisation, and improving animal husbandry (Beigh *et al.*, 2017).

The widespread use of OPF as ruminant feed suggests that it is important for meeting the net energy needs and nutritional requirements of animals. However, the inherent limitations of OPF as a feed include its complex biological structure, high lignocellulosic content, low nutritional value, and low energy content, which often fail to meet the nutritional requirements of ruminants (Rahman *et al.*, 2011). Furthermore, the abundance of lignin (~199 g kg⁻¹ dry matter, DM) in OPF reduces the accessibility and use of structural carbohydrates for rumen microbial fermentation (Ishida and Abu Hassan, 1997). In such circumstances, feeding ruminants with low-quality OPF can result in rumen dysfunction, nutritional inadequacy and poor growth performance. Hence, efforts are being made to develop effective treatment methods for OPF to enhance its nutritional quality as feedstuff needed for new fodders feeding strategies and prevent the scarcity of balanced feeds (Rusli *et al.*, 2019). The improvement of the nutritional value of OPF is one of the latest developments in oil palm waste management for exploiting the potential of locally available feedstuffs and optimising its utilisation as an animal feed resource (Wu *et al.*, 2020).

Many studies on the conversion of OPF to valuable feedstuffs via biological, chemical and physical methods have been conducted to elucidate how animals could utilise this lower-grade feedstuff more effectively. An abundance of information on the nutritional value, biochemical properties, treatment methods and feeding response of treated OPF as a ruminant feedstuff are also available. Thus, this article is aimed to investigate the ability

of various treatments in maximising the nutritional value of OPF as an improved feedstuff for ruminants through *in vitro* and *in vivo* studies. Specifically, the effects of different treatment methods of OPF to maximise digestibility coefficients, rumen fermentation, ruminant production performance, milk production and compositional traits of both cattle and goats will also be reviewed. Improving the feed value of OPF is becoming increasingly important as it permits the increased use of oil palm crop residues in ruminant diets while minimising feed costs and maximising feed production.

CHEMICAL COMPOSITION OF OPF

Much research has been conducted on the potential utility of OPF as an animal feed ingredient, including comprehensive proximate chemical studies. The chemical composition of OPF can be determined by the feed analysis method based on the neutral detergent system (Ishida and Abu Hassan, 1997). Nevertheless, the chemical properties of OPF biomass may vary depending on plant nutrient uptake, which is influenced by several factors, such as the age of OPF collected, geographical conditions, types of fertilisers applied, and soil chemistry (Ooi *et al.*, 2017). Table 1 shows the typical proximate chemical composition and metabolisable energy (ME) of OPF adopted from Ishida and Abu Hassan (1992), Hong *et al.* (2012) and Mathius (2008). The nutritional analysis showed that OPF is composed of 22% soluble carbohydrates and 70% fibre on a dry matter (DM) basis. Therefore, OPF has a low

TABLE 1. PROXIMATE CHEMICAL COMPOSITION AND METABOLISABLE ENERGY (ME) OF OIL PALM FROND (OPF)

Parameters	Composition (%)
Dry matter (DM)	31.10-48.78
Percentage in DM	
Crude protein (CP)	4.21-5.33
Ether extract (EE)	2.00-3.14
Crude fibre (CF)	48.25-50.20
Crude ash	4.48-4.75
Nitrogen free extract (NFE)	36.45-38.30
Neutral detergent fibre (NDF)	69.50-78.05
Acid detergent fibre (ADF)	50.90-56.93
Hemicellulose	11.91-21.12
Cellulose	27.94-39.63
Lignin	16.94-20.50
ME (MJ kg ⁻¹ DM)	4.90-5.60

Note: Adopted from Ishida and Abu Hassan (1992), Mathius (2008) and Hong *et al.* (2012).

ME value, ranging between 4.9 MJ kg⁻¹ and 5.6 MJ kg⁻¹ DM. Naturally, OPF has quantities of nutrients (% DM) similar to those in typical pastures used for grazing. The high levels of crude fibre (CF, 48.25%-50.20% of DM) make OPF a very promising source of feed for ruminants. Indeed, OPF consists of non-edible lignocellulosic plant materials (lignin, cellulose and hemicellulose), of which the fibrous elements of the cell wall make OPF less digestible for ruminants (Zakaria *et al.*, 2014).

In addition, OPF contains a minimum amount of plant secondary metabolites compounds (polyphenol compounds) such as tannins, flavonoids, saponins and catechins (Aiman-Zakaria *et al.*, 2017). Among these, tannins (both condensed and hydrolysable tannins) which represent the most abundant group of polyphenol compounds in OPF (30.9 g kg⁻¹), have been reported to help avoid biohydrogenation of fatty acids, improve rumen metabolism and mitigate methane production in ruminants (Jenkins *et al.*, 2008; Patra and Saxena, 2011). The smallest amount of amino acids, organic acids, minerals and vitamins are also present in the OPF (Ishida and Abu Hassan, 1997). OPF also contains a reasonable number of inorganic elements such as potassium (7.0 g kg⁻¹) and calcium (5.3 g kg⁻¹) which are essential to animal nutrition, metabolism, and growth performance (Ishida and Abu Hassan, 1997).

USE OF OPF AS A RUMINANT FEEDSTUFF

Research had shown that OPF could be used successfully as a viable ruminant forage feed. Moreover, fresh OPF has been extensively processed as pelleted feed for trade and export. Toxicity problems and adverse health effects have not been reported for OPF ruminant feeding (Ishida and Abu Hassan, 1992), and the product seems readily accepted and palatable for goat, sheep, dairy goat, beef cattle, and dairy cattle (Ooi *et al.*, 2017). Thus, OPF has been used as a source of roughage to replace rice straw and other low-quality roughage commonly used in ruminant feeding without detrimental effects on livestock production.

The use of OPF as a feedstuff for small ruminants and its effect on fibre digestion and nutrient intake has been widely discussed (Dahlan, 2000; Dahlan *et al.*, 1993; Ebrahimi *et al.*, 2014; Rajion *et al.*, 2001). Dahlan *et al.* (1993) found that the typical metabolisable energy intake (MEI) of goats fed a diet of pure OPF was 311.3 ME kg⁻¹ body weight^{0.75} (BW^{0.75}), which is within the range (230.5 to 580.8 ME kg⁻¹ BW^{0.75}) of the optimal energy maintenance requirements for goats. Besides meeting the energy requirements for small ruminants, several of the beneficial effects of using OPF have been achieved through its use as a feed. For example, Meng *et al.*

(2018) suggested that sheep diets composed of 50% OPF have beneficial effects on blood plasma lipid parameters and fatty acid (FA) profiles in rumen fluid and muscle tissues.

Moreover, OPF could be used as a replacement for native grasses when the availability of forage or fodder for beef and dairy cow feed becomes limiting. The recommended amount of OPF included in TMRs (on a DM basis) is 30% for dairy cattle and 50% for beef cattle. The nutritional needs of dairy cattle after calving are closely related to the live weight of animals and to the level of milk production, while feed intake is associated with the composite of rough fibre in feed. Advantages of OPF feeding include solving roughage/feed shortages, improving milk quality in dairy farms, and reducing feeding, operational and management costs (Dahlan *et al.*, 1993).

LIMITATIONS OF OPF AS A RUMINANT FEEDSTUFF

The effective application of OPF as whole feedstuff for animal production is limited by its large quantities of cellulolytic materials, poor nutrient digestibility coefficients, low ME values, resistance to enzymatic hydrolysis, low palatability and possession of some anti-nutritional factors such as high lignin content, non-starch polysaccharides, tannin and phenolic compounds (Febrina *et al.*, 2014). OPF contains substantial quantities of lignin, which is a large complex structure that adheres to the matrix of hemicellulose and cellulose of the cell wall to form crystalline structures called microfibrils or lignocellulose biopolymers. Plant lignin confers rigidity and impermeability to the cell wall, facilitating resistance to microbial attack and oxidative stress (Rizal *et al.*, 2018). The large biomass of lignocelluloses and low ligninolytic activity of rumen microbes reduce the digestibility of fibre in the rumen and restrict the efficient use of lignocelluloses as feedstuff in ruminants.

Moreover, OPF contains low levels of protein (4.0%-7.5%) unless processed (Ooi *et al.*, 2017). The insufficient protein content in OPF could lower the digestibility coefficient and decrease the absorption of nutrients needed to support the optimal productivity of ruminants. Furthermore, unprocessed OPF, which contains >50% moisture, tends to deteriorate and develop mould, reducing its palatability and decreasing the DM nutrient intake, weight gain, and milk production of ruminants (Dahlan, 2000). In addition, mouldy OPF may have an undesirable taste and texture, a low nutritional value, and may become heavily contaminated with fungal mycotoxins. Likewise, oil palm leaves are rich in secondary metabolite substances, such as tannins and phenolic compounds, which might be

detrimental to ruminant fermentation, microbial populations, the DM digestibility of feed, growth performance, and milk production (Ebrahimi *et al.*, 2015; Jaffri *et al.*, 2011). Saminathan *et al.* (2016; 2019) revealed that the secondary metabolites and bioactive compounds from plants, particularly hydrolysable and condensed tannins at a concentration of 20-30 g kg⁻¹ could be harmful to the fibrolytic anaerobic rumen bacteria such as *Fibrobacter succinogenes* and *Ruminococcus* spp., and anaerobic fungi species such as monocentric *Neocallimastix* spp., and *Piromyces* spp., in the rumen.

TREATMENT OF OPF

The structural arrangement of OPF elements and their chemical composition plays a significant role in dictating its utility as a feedstuff. Treatment of OPF to create value-added feedstuffs is an important step for enhancing the nutritional value of this material for ruminant feeding. Possible treatment is needed to alter the fibrous structure of OPF at both the macro- and microscopic scales to facilitate more efficient and rapid digestion and increased accessibility for the rumen microbiota (Hermiati *et al.*, 2013; Rizal *et al.*, 2018; Rusli *et al.*, 2019). OPF can be treated by biological, physical and chemical means, or their combinations as shown in Figure 1, whereby each treatment method has advantages and disadvantages (Table 2). OPF treatments mainly involve delignifying processes that increase the

cellulose content and effectively improve the availability of other nutrition for ruminants in a cost-effective and sustainable way (Galbe and Zacchi, 2012; Rizal *et al.*, 2018).

PHYSICAL AND PHYSICO-CHEMICAL TREATMENT METHODS

Several common physical treatment methods have been developed to enhance the value of OPF as a feed, which include pelleting, grinding, chopping and steaming. Grinding, pelleting and steaming process increased the digestibility of OPF hence, improved the feed intake and growth performance of ruminants (Wan Zahari *et al.*, 2003). Physico-chemical treatment comprises both physical and chemical interactions in the treatment of OPF. All the physico-chemical treatments of fibrous substances are carried out at extremely high pressure and temperature in a closed system (Rabemanolontsoa and Saka, 2016). Steam explosion, ammonia (NH₃) fibre explosion and fibre cracking technology (FCT) are among the most recently used methods to partially hydrolyse the glycosidic bonds of hemicellulose, decrease cellulose crystallinity, disrupt chemical bonding and increase the surface area of OPF lignocellulosic substances (Jayanegara *et al.*, 2019). The large surface area of OPF could increase the attachment of rumen microbes and the accessibility of enzymes, thereby increasing the biodegradability of plant cell walls and fibre fermentation. Wu *et al.* (2020)

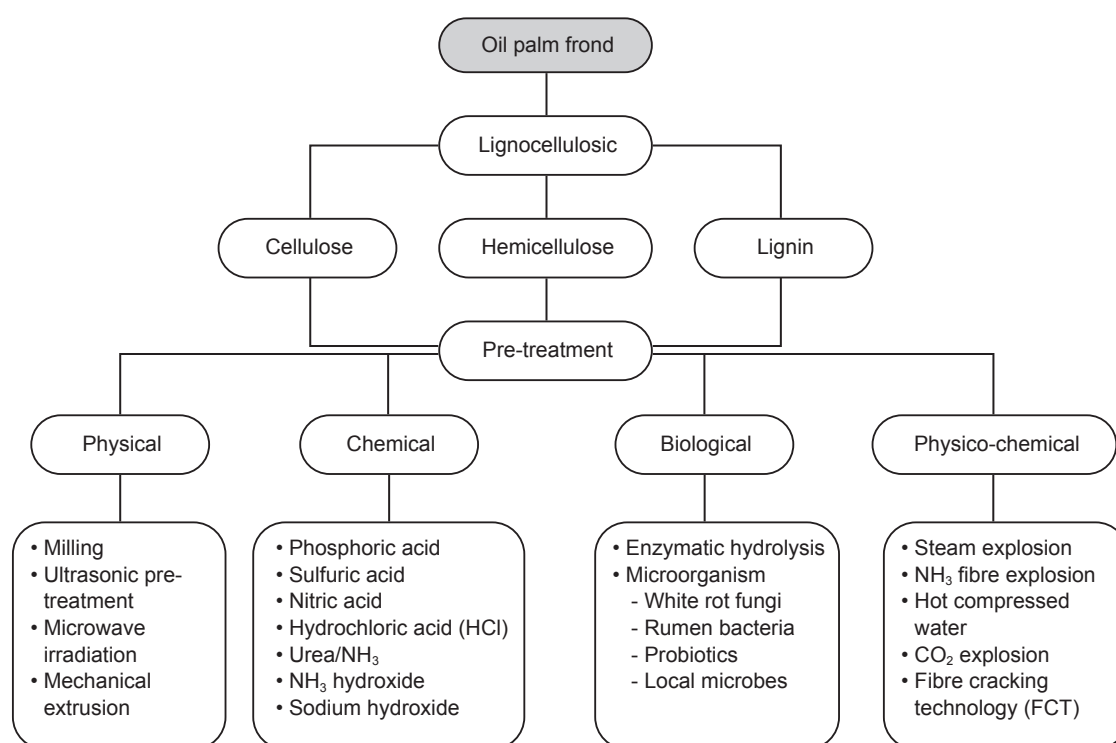


Figure 1. Different types of oil palm frond (OPF) pre-treatment methods.

TABLE 2. ADVANTAGES AND DISADVANTAGES OF PHYSICAL, PHYSICO-CHEMICAL, CHEMICAL AND BIOLOGICAL TREATMENT METHODS OF OIL PALM FROND (OPF)

Treatment category	Treatment methods	Advantages	Disadvantages	References
Physical	Pelleting/ grinding/ chopping/steaming	Short process duration; efficiently reduces the substrate size; increases the surface area of biomass	High cost for equipment and machinery setup; required additional treatment as it does not remove lignin	Galbe and Zacchi (2012)
Physico-chemical	Steam explosion/ fibre cracking technology (FCT)/ hot water	No chemicals used; adequate for large-scale application; environment-friendly; low energy input	Risk of condensation and precipitation of soluble lignin components; not effective in removing lignin; incomplete destruction of the lignin-carbohydrate matrix	Wan Zahari <i>et al.</i> (2003) Rabemanolontsoa and Saka (2016)
Chemical	Acid/alkali/ionic liquids/urea	Short process duration; selectively attacks the lignin; possible to fully hydrolyse the entire lignin	Toxic to enzymes and fermentation; not economically feasible; less effective if the lignin content is high in biomass; usually requires high temperature to dissolve lignin	Mosier <i>et al.</i> (2005) Kristiani <i>et al.</i> (2013) Noparat <i>et al.</i> (2015)
Biological	Fungi/bacteria/ ligninolytic enzymes/fibrolitic enzymes	Selectively degrades lignin; environment-friendly; requires mild operating conditions	Slow process and therefore it needs long process duration; requires large space; enzyme cost is very high; some carbohydrate fraction is consumed by the microorganism	Hassim <i>et al.</i> (2012) Yunilas <i>et al.</i> (2014) Barlianti <i>et al.</i> (2015)

showed that steam explosion could effectively improve the ME value of OPF and decrease the hemicellulose content of OPF from 22.81% to 18.80%. For instance, steam explosion treatment is an important method for aiding the release of OPF cellulose implanted between hemicelluloses and lignin and is more applicable to rumen fermentation, hydrolysis and densification processes. Moreover, higher yields of OPF hemicelluloses containing various monosaccharide subunits can be obtained from steam explosion technology relative to other treatment methods (Garrote *et al.*, 1999). Harahap *et al.* (2018) reported that the acid detergent fibre (ADF) and neutral detergent fibre (NDF) content of OPF decreased after the FCT treatment process, thereby increasing the hydrolysis of complex carbohydrates. In addition, microwave irradiation could enhance the rupture of the rigid structure of lignin biomass and eventually increase the susceptibility of lignocellulosic substances (Binod *et al.*, 2012; Zhang and Zhao, 2010).

CHEMICAL TREATMENT METHOD

Chemical treatment with diluted acid or alkaline solutions is one of the most common methods that has been used to treat fresh or dried OPF. Chemical treatment requires the maintenance of low temperature and pressure compared to other treatment strategies (Mosier *et al.*, 2005). Acid

treatment involves hydrolysis of hemicellulose components of OPF and the release of cellulose components for conversion into fermentable carbohydrates by rumen microbes. Application of diluted acid is among the major concerns requiring consideration in the chemical treatment of lignocellulosic substances to avoid overexposure of OPF to harsh conditions. To date, diluted acids that have been used for OPF treatment include phosphoric acid (H_3PO_4), sulphuric acid (H_2SO_4), nitric acid (HNO_3), and hydrochloric acid (HCl) (Awalludin *et al.*, 2015; Kristiani *et al.*, 2013; Noparat *et al.*, 2015). Kristiani *et al.* (2013) discovered that treatment of OPF with diluted acid could alter the surface structure and concurrently increase specific surface area, thereby reducing the crystallinity of cellulose matter in OPF. However, the utilisation of acid is less applicable for OPF treatment because of the formation of inhibitors that might inhibit the enzymatic activity of microbes during rumen fermentation. Likewise, high operational and maintenance costs are major limitations of this method that preclude the application of this technique on a commercial scale.

Diluted alkaline hydrolysis is perhaps the most common method used to treat OPF. Alkaline treatment might trigger the swelling of lignocellulosic materials of plants and the disintegration of hemicellulose and lignin. Inclusion of non-protein nitrogen (NPN) resources, such as NH_3 or urea N and ammonium hydroxide

(NH₄OH), is the method typically applied for poor-quality OPF given the ease with which they can be managed at low costs. Supplementation of OPF with NPN such as urea has been shown to improve the nutritional value and nutrient digestibility of OPF and, in turn, the ease with which the energy and protein requirements of ruminants can be met. Urea can break down plant lignocellulose materials and enhance the nitrogen (N) level available for microbial protein synthesis in the rumen (Gunun *et al.*, 2016). However, several factors require consideration for the urea treatment to be successful, including the concentration of urea, moisture content and duration of pre-treatment (Rusli *et al.*, 2019). Treating OPF silage with urea could prevent mould growth under aerobic conditions and concomitantly increase the level of organic acids (acetic and butyric acids) that animals might absorb as a major energy source. Generally, alkaline treatments are preferable to acid treatments, given that alkaline solutions delignify the lignin of OPF more effectively than acids. Barlianti *et al.* (2015) discovered that alkaline treatment improved the availability of carbohydrates up to 6 g L⁻¹ after pre-treatment of OPF with 1% of NaOH.

BIOLOGICAL TREATMENT METHOD

In recent decades, the biological treatment method, which is known for providing an environmental-friendly approach, has received considerable attention to enhance the nutritional properties and ruminal feeding value of OPF. Biological treatment of biomass generally involves two key methods: enzymatic and microorganism treatments. These biological methods are becoming famous, particularly in developing countries, due to the recent developments in fermentation technology to produce large quantities of fungi or their enzymes to meet the requirements, and the costs of these materials are expected to decline in the future (Rizal *et al.*, 2018).

The typical microorganisms used for OPF treatment include white fungi, rumen bacteria, and local microbes (Azmi *et al.*, 2019; Hassim *et al.*, 2012; Rahman *et al.*, 2011; Yunilas *et al.*, 2014). The number of bacterial strains which show lignin-degrading ability are quite limited. However, there are several fibre-degrading bacteria species (*Actinomyces*, *Nocardia*, *Streptomyces*, and *Eubacteria*) which are identified by their role in the hydrolysis of fibre content in biomass to some extent (Azmi *et al.*, 2019). Similar to chemical treatments, the microorganism's treatment method applied on OPF results in the breakdown of lignocellulosic fibres in OPF and thus, enhances the *in vitro* degradability of cellulose by up to 31% in the rumen (Rahman *et al.*, 2011). However, bacterial strains to treat OPF are no longer popular due to their slower reaction response, compared to that obtained with fungi. White-rot fungi (WRF) are extensively used in OPF treatment to efficiently break down plant lignin and cell walls. Indigenous probiotics derived from palm oil waste, which include *Bacillus* sp. YLB1, *Saccharomyces* sp. YLY3 and *Trichoderma* sp. YLF8, all have the capability of biodegrading OPF fibres and increasing crude protein (CP) content through the microbial fermentation process (Yunilas *et al.*, 2014). The proximate chemical analysis of OPF treated with WRF, rumen microorganisms and probiotics is shown in Table 3.

Numerous studies have shown that fungal degradation of OPF lignin via biological delignification using WRF could improve the chemical composition of OPF and may be of significant value to ruminant feedstuff and feeding (Chanjula *et al.*, 2015; 2018; Hamchara *et al.*, 2018; Rahman *et al.*, 2011). The effectiveness of WRF enzyme activity might be influenced by the type of fungal species or strain used. WRF, effective wood-decaying basidiomycetes in nature, actively secrete lignin-modifying enzymes (LMEs), specifically laccase (Lac), manganese peroxidase (MnP) and lignin peroxidase (LiP), which have high ligninolytic activity (Namoolnoy *et al.*, 2011). Among the edible

TABLE 3. PROXIMATE ANALYSIS OF TREATED OIL PALM FROND (OPF) WITH WHITE ROT FUNGI, RUMEN MICROORGANISMS AND PROBIOTICS

Type of microorganism	DM (%)	CP (%)	EE (%)	CF (%)	Ash (%)	ME (MJ kg ⁻¹)	References
<i>Lactobacillus plantarum</i> MTD1	ND	4.78	2.85	38.50	7.53	ND	Ebrahimi <i>et al.</i> (2015)
<i>Prolinas</i> (Pineapple skin probiotic)	83.57	6.58	2.18	32.70	6.99	ND	Mardalena <i>et al.</i> (2019)
<i>Produren</i> (a durian probiotic)	92.34	15.34	2.21	33.31	5.92	ND	Mardalena and Musnandar (2019)
Fungal strain (<i>Lentinus sajor-caju</i> spp.)	50.83	7.24	1.87	36.49	7.40	ND	Chanjula <i>et al.</i> (2017)
<i>Lentinus sajor-caju</i> spp.	39.47	6.18	1.82	28.38	8.29	4.33	Hamchara <i>et al.</i> (2018)

Note: CF - crude fibre; CP - crude protein; DM - dry matter; EE - ether extract; ME - metabolisable energy; ND - not determined.

WRF, *Schizophyllum* sp. and *Lentinus sajor-caju* are more active in breaking down lignocellulosic materials than other WRF species. *Lentinus sajor-caju* can only consume the lignin and hemicellulose of OPF, leaving the cellulose in perfect condition so that it can contribute to improving the supply of energy for ruminants. Additionally, fungi-treated OPF is an excellent source of amino acids, peptides and small proteins. Previous research has also shown that the mechanism of DM loss in OPF and the fermentation profile triggered by WRF are correlated to the selective disappearance of lignin (Hassim *et al.*, 2012). On the other hand, Azmi *et al.* (2019) isolated specific fungi from rotten OPF (non-WRF species); namely, *Fusarium solani* and *T. harzianum* strains, and demonstrated the optimum activity of the enzyme as a biological treatment agent for improving the use of OPF as a ruminant feedstuff.

Fibrolytic enzymes that catalyse hydrolysis play a vital role in animal feeding by enhancing the digestibility of feed and the conversion of feedstuff into added-value end-products (Sujani and Seresinhe, 2015). Enhancement of the nutritional value of OPF through the treatment of exogenous fibrolytic enzymes has been the subject of much research. Hemicellulose and cellulose can be digested enzymatically into glucose and other metabolisable sugar components, such as hexose and pentose, which can be absorbed by the bloodstream of animals as energy sources. The mixture of enzymes exhibiting fibrolytic activities (*e.g.*, hemicelluloses, cellulases, mannanases and xylanase) can be potentially used to enhance the degradability of low-quality fibrous substrates in OPF (Hassim *et al.*, 2012). OPF treated with Hemicell[®] containing mostly β -D-mannanase that degrade mannan might increase the rate of rumen fermentation due to the ease of access that several ruminal cellulolytic bacteria species have to nonstarch polysaccharides (NSPs) from OPF (Wahyuni, 2012). On the other hand, the inclusion of tannase in OPF feed can hydrolyse anti-nutritional phenolic substrates in the OPF, which might decrease feed intake and adversely affect rumen DM digestion (Abd El Tawab *et al.*, 2015; Curiel *et al.*, 2009). Abd El Tawab *et al.* (2015) reported that the supplementation of tannase as a feed additive for lactating goats could reduce the hydrolysable and condensed tannin contents in plant-based feed and enhance milk production and the rumen fermentation profile. The inclusion of fibre-degrading enzymes into fermented OPF with WRF should be more effective and exhibit synergistic effects relative to the outcome of OPF inoculation with WRF alone. However, Hassim *et al.* (2012) showed that the inclusion of both OPF treated with WRF and fibrolytic enzymes did not increase the *in vitro* digestibility of OPF, suggesting that no synergism occurs between treated OPF and enzymes. This finding indicates that there is a need

to further optimise enzyme combinations depending on the type of plant carbohydrates in the substrate.

A summary of different treatments (physical, physico-chemical, chemical, and biological treatment) effects on chemical composition and physical and biological properties of OPF is shown in Table 4.

EFFECTS OF TREATED OPF ON *in vitro* RUMEN FERMENTATION CHARACTERISTICS

A novel approach known as the *in vitro* rumen fermentation technique, and specifically the *in vitro* gas production technique, could provide an effective method for assessing the nutritional value of ruminant feeds. The chemical or proximate analysis of feedstuffs only suggests the nutritional value and composition but does not provide information about the actual ruminal fermentable and digestible values of feedstuff. Several studies of *in vitro* gas production techniques and rumen fermentation of treated OPF have been performed to predict its digestibility and potential use as a quality feedstuff for ruminants (Boonthep *et al.*, 2011; Hassim *et al.*, 2012; Mulianda *et al.*, 2018; Tafsir *et al.*, 2018).

Many beneficial effects of incorporating treated OPF feedstuffs into complete feeds during *in vitro* rumen fermentation have been documented. For example, Boonthep *et al.* (2011) showed that pre-digestion of OPF by *Aspergillus* spp. BCC274 could significantly increase *in vitro* gas production and OPF digestibility. Several studies have found that OPF when biologically treated with WRF, or an enzyme extract of WRF, shows promise for enhancing *in vitro* DM degradability (IVDMD) and *in vitro* organic matter degradability (IVOMD) by breaking down the lignocellulosic biomass in OPF fibres (Febrina *et al.*, 2016; Rahman *et al.*, 2011; Rusli *et al.*, 2019). WRF are the most effective microbes for delignification due to their production of fibrolytic enzymes and/or ligninolytic extracellular oxidative enzymes, which can improve the digestibility of DM of poor-quality forages, such as OPF. Nevertheless, the solid-state fermentation period, fungal strain, substrate, and culture conditions are key factors determining the digestibility coefficient of pre-digested OPF. Digestibility coefficient is a benchmark for early determination of the availability of the total quantity of nutrients in feedstuff consumed by livestock. The high digestibility coefficient of DM in treated OPF based on *in vitro* analysis reflects the significant contribution of specific nutrients to ruminants.

Rahman *et al.* (2011) carried out an *in vitro* study by screening 10 different WRF for their ability to convert plant lignin to carbohydrates and their effects on the *in vitro* ruminal degradability of OPF. Among the WRF tested, OPF inoculated with *L. edodes* and *P. brevispora* for nine weeks and

TABLE 4. DIFFERENT TREATMENT EFFECTS ON CHEMICAL COMPOSITION AND PHYSICAL AND BIOLOGICAL PROPERTIES OF OIL PALM FROND (OPF)

Treatment type	Treatment methods	Chemical composition and physical and biological properties	References
Physical	Pelleting, grinding and chopping	↓ particle size; ↑ OPF surface area; ↑ digestibility and feed intake	Wan Zahari <i>et al.</i> (2003)
Physico-chemical	Steam explosion	↓ NDF and ADF; improve energy value; ↓ hemicellulose content of OPF from 22.8% to 18.8%	Wu <i>et al.</i> (2020)
	Fibre cracking technology (FCT)	Disrupt chemical bonding; ↓ NDF and ADF by 29.8% and 14.2%, respectively	Harahap <i>et al.</i> (2018)
Chemical	Urea	Break down OPF lignocellulose materials; ↑ N availability; ↑ the level of an organic acid; ↑ CP content (10%-25%)	Wu <i>et al.</i> (2020)
	Diluted acid	↑ specific surface area of OPF from 2.23 m ² g ⁻¹ to 5.57 m ² g ⁻¹ ; ↓ crystallinity of cellulose molecule	Kristiani <i>et al.</i> (2013)
	Alkali treatment	Delignify the lignin of OPF, ↓ lignin content from 30.9% to 19.2%; lower cellulose crystallinity index; improve the availability of carbohydrates up to 6 g L ⁻¹	Barlianti <i>et al.</i> (2015)
Biological	White-rot fungi (WRF) (<i>Lentinus sajor-caju</i>)	Efficiently break down OPF lignin and cell walls; optimum enzyme activity; ↑ <i>in vitro</i> rumen degradability of carbohydrates by up to 31%	Rahman <i>et al.</i> (2011)
	White-rot fungi (WRF) (<i>Schizophyllum</i> spp. and <i>Lentinus sajor-caju</i>)	Actively secrete lignin-modifying enzymes; excellent source of amino acids	Namoolnoy <i>et al.</i> (2011)
	Non-white-rot fungi (<i>Fusarium solani</i> and <i>T. harzianum</i>)	↓ OPF lignin from 30% to 15%; highest laccase and manganese peroxidase (MnP) activities (0.89 U g ⁻¹ and 0.79 U g ⁻¹ of DW, respectively) Produces high lignolytic enzymes	Azmi <i>et al.</i> (2019)
	Probiotic (<i>Bacillus</i> spp. and <i>Saccharomyces</i> spp.)	Improve biodegradation of OPF fibres; ↑ CP content	Yunilas <i>et al.</i> (2014)
	Fibrolitic enzymes	Degradability of low-quality fibrous substrate in OPF; β-D-mannanase degrade mannan and ↑ rate of fermentation	Hassim <i>et al.</i> (2012)
	Tannase enzymes	Degrade non-nutritional component (secondary metabolite compounds) in OPF	Abd El Tawab <i>et al.</i> (2015)

Note: ADF - acid detergent fibre; CP - crude protein; DW - dry weight; NDF - neutral detergent fibre; OPF - oil palm frond; ↑ - increase; ↓ - decrease.

C. subvermispora for three weeks showed higher degrees of lignin loss, which were positively correlated with an increase in *in vitro* gas production and volatile fatty acid (VFA) concentrations. Suggested mechanisms for improving OPF nutrient content include the degradation of lignin to lignocellulose and the partial degradation of biological cellulose, which improved the accessibility of the substrate to rumen microorganisms. Furthermore, a decrease in fibre fractions in treated biomass via WRF treatment could increase the *in vitro* digestibility of bovine rumen fluid and the quantity of product palatable to cattle (Okano *et al.*, 2006). In another experiment, Hassim *et al.* (2012) concluded that the inclusion of *L. edodes* and *C. subvermispora* inoculum in OPF could improve the apparent rumen degradable carbohydrate (ARDC) and/or *in vitro* gas production rates; however, additional exogenous enzyme supplementation did not further improve

ARDC activity. Inclusion of the ideal combination of minerals (150 ppm Ca and 2000 ppm Mn) during OPF fermentation by *P. chrysosporium* significantly enhanced ($p < 0.05$) the *in vitro* digestibility of DM, organic matter, NDF, and cellulose and consequently increased the concentration of VFA (Febrina *et al.*, 2016). The addition of minerals capable of increasing the quantity of fungal mycelium and the production of ligninolytic enzymes can promote the cleavage of bonds within lignocellulose and lignohemicellulose. Moreover, the lignin degradation activity of fungi is influenced by MnP, a non-specific extracellular enzyme system, and the stability and activity of MnP are dependent on the concentration of Mn²⁺ (Pazarlioglu *et al.*, 2005).

The application of local microorganisms in OPF bio-fermentation and their effects on ruminant digestion efficiency and feed fermentability has been evaluated *in vitro* to measure the nutritional quality

of livestock feed. A stable microbial ecological condition within the rumen is one of the factors that can increase the consumption rate, thereby giving the ruminants more nutrients and energy. Likewise, Tafsir *et al.* (2018) showed that fermenting OPF with local microorganisms (*A. niger* and *S. cerevisiae*) and isolates of buffalo rumen bacteria improved the digestibility of DM and organic matter *in vitro*. Mulianda *et al.* (2018) found that application of local microorganisms at the level of 0.6% with seven days of fermentation tended to increase the quality of OPF, which resulted in an increase in the DM digestibility, total VFAs and $\text{NH}_3\text{-N}$ of rumen fluid *in vitro*. Indigenous microbes which are able to secrete multiple enzymes play a crucial role in feedstuff digestion and are also capable of converting the inorganic forms of N compounds into organic N (true protein), which can enhance CP availability in treated OPF-based feed. Fermentation of OPF by local microbes can also increase its digestibility and palatability and improve the vitamin and mineral contents in feedstuffs.

Ramin *et al.* (2008) found that *in vitro* fermentation of OPF with *C. kwangyangense*, a cellulolytic bacterium isolated from termite gut, increased the total concentration of VFAs in rumen fluids compared with that resulting from other termite bacterial strains. Endo- β -1,4-glucanase produced by termite bacteria plays a fundamental role in cellulose digestion and can concurrently enhance rumen microbial activity and ruminal metabolites (König, 2006). A selected group of probiotic strains that has a high potential to degrade lignin also appear to be essential in cellulose degradation by significantly increasing the degradability of DM *in vitro*, regardless of whether the rumen fluid was adapted to OPF. Mardalena and Musnandar (2019) found that *in vitro* fermentation of OPF fermented with 7.5% produren (a durian probiotic) enhanced DM digestibility and increased the total production of VFAs in bovine rumen fluid. Selected rumen microorganisms can actively secrete several enzymes, specifically amylase, cellulase and catalase, during fermentation that have the ability to degrade OPF into small molecules so that it can be absorbed by microbes for cell growth and concomitantly increase the concentration of by-products, such as VFAs, available for utilisation by animals as an energy source. In the microbial fermentation process, active decomposition of the substrate occurs by the action of appropriate enzymes on indigestible materials, such as hemicelluloses and lignin, converting them into simpler carbohydrate molecules.

Several *in vitro* studies using exogenous fibrolytic enzyme additives in the pre-digestion of OPF have been conducted to improve the digestibility of complex fibres (Beauchemin *et al.*, 2003; Rode *et al.*, 1999), but the results were often inconsistent. The

inconsistency in the findings of these studies stems from a variety of factors, including diet composition, type of enzyme preparation, enzyme quantity, enzyme stability, and the method of application. Enzyme additives constantly offer alternative ways for enhancing ruminant growth performance. These additives are primarily attributed to observe the improvements in ruminal fibre digestion and digestible energy intake (DEI). A study carried out by Boonthep *et al.* (2011) revealed that TMRs containing treated OPF (60% of DM) with 2 g kg⁻¹ DM fibrolytic enzymes reduced gas production without having negative effects on nutrient digestibility, suggesting that the environmental aspects (specifically, methane mitigation) of feeding ruminant animals could be improved. However, the soluble gas fraction slightly increased while the gas production rate slightly decreased in response to increases in the quantity of enzyme. The moderate application of exogenous enzyme in animal feedstuff might produce favourable, minor changes in feed surface structure either following or prior to ingestion; however, excessive enzyme use can contribute to extreme enzyme attachment to ingested feed, limiting the ability of microbial enzymes to attach to the ingested feed and thereby restricting its digestion (Nsereko *et al.*, 2002).

The increases in total gas production and the potentially fermentable soluble fraction *in vitro* with OPF treated with exogenous enzyme were probably caused by the rise in the rate of rumen fermentation and the efficient digestion of feeds in the rumen. Aside from OPF, Khatib and Tawab (2018) have found that the application of date palm frond feed in combination with fibrolytic enzymes (inclusion rate of 4 g kg⁻¹ DM) was beneficial and resulted in improvements in ruminal digestibility and fermentation. Rusli *et al.* (2019) used enzyme extract of *Ganoderma lucidum* and *Lentinula edodes* to measure the correlation between enzyme-specific activity and *in vitro* rumen fermentation of OPF. Biological treatment with enzyme extract of *G. lucidum* and *L. edodes* showed a similar pattern, wherein the structural carbohydrates in OPF were degraded, and the CP content increased. However, between these two WRF, *G. lucidum* seemed to be more promising for improving rumen degradability *in vitro*.

Synergistic effects of different treatments of biomass should be considered to achieve balanced and optimal fibre digestion and fermentation in ruminants. Hassim *et al.* (2012) suggested that combination of chemically (e.g., NH_3) treated fibrous matter (e.g., forage or roughage) and fibrolytic enzymes could significantly enhance the degradability of ruminal fibres in OPF *in vitro* compared with treatment by enzymes alone. The application of cellulase alone or in combination with lactic acid bacteria (*Lactobacillus plantarum* MTD1) improved the *in vitro*

fermentation profile, changed the FA properties, and enhanced the nutritional value of pre-digested OPF silage (Ebrahimi *et al.*, 2014). Biological treatment of OPF with enzyme extract of *G. lucidum* combined with physical treatment improved the nutritional values of OPF by decreasing the lignin content and, consequently, improving ruminal digestibility and increasing total gas production, VFAs and ARDCs (Rahman *et al.*, 2011). Harahap *et al.* (2018) reported that OPF treated with the physico-chemical method using FCT combined with *Indigofera* sp. and urea in the rumen-simulating, semi-continuous culture system (Rusitec) could facilitate the breakdown of lignocellulose bonds and the removal of lignin from OPF which is recognised as a valuable feedstuff. The effects of treated OPF on *in vitro* gas production, rumen fermentation profiles and digestibility are summarised in Table 5. Treatment of OPF, which uses various techniques including NH_3 fibre explosion, chemical treatment, biological treatments and steam explosion serve a similar function to alter the structure of cellulose biomass to make cellulose more accessible and susceptible to ruminal microbial to enzymatic degradation in its amorphous form (Rabemanolontsoa and Saka, 2016). However, most research mainly focused on biologically treated OPF in *in vitro* fermentation and digestibility studies in view of practical use of this treatment by considering significant cost effectiveness, safety, improvement of DM recovery, aerobic stability, and animal performance (Azmi *et al.*, 2019).

EFFECTS OF FEEDING TREATED OPF ON RUMINANTS

Several studies have assessed the effects of feeding treated OPF on different ruminant livestock species under various conditions, which are described below.

Effects of Feeding Treated OPF to Small Ruminants

The feeding of small ruminants with treated OPF appears to be the most promising feeding strategy for improving their productivity in palm oil producing countries. The nutritional value of OPF for small ruminants can be enhanced by chemical and/or biological treatment, resulting in improved fibre DM biodegradability and bioavailability of energy and protein. The inclusion of treated OPF in small ruminant feed rations at the optimal level could promote feed digestibility, growth performance, meat quality and milk yield. Several studies have demonstrated the beneficial effects of feeding treated OPF on the growth performance of small ruminants, as described in Table 6. Hamchara *et al.* (2018) found that feeding OPF treated with various levels of *L. sajor-caju* to 16 month-old crossbred male goats promoted digestibility and N retention, indicating that the application of fungus-treated OPF provides an alternative forage feedstuff constituting up to 100% OPF. This result is consistent with the findings of Febrina *et al.* (2017), who showed that the best

TABLE 5. EFFECTS OF TREATED OIL PALM FROND (OPF) ON *in vitro* GAS PRODUCTION, RUMEN FERMENTATION AND DIGESTIBILITY

Treatment methods	Inclusion level in OPF/ condition	Interpretation	References
White-rot fungi (WRF) (<i>L. edodes</i> and <i>P. brevispora</i>)	3 or 9 weeks of fermentation at 30°C	↑ IVGP; ↑ VFA; ↑ ARDC	Rahman <i>et al.</i> (2011)
Enzyme extract of <i>G. lucidum</i> + physical treatment	8 mL enzyme extract/ incubated for five days at 40°C	↓ lignin content; ↑ IVGP; ↑ VFA; ↑ ARDC	Rusli <i>et al.</i> (2019)
<i>Lactobacillus plantarum</i> + cellulose	1×10 ⁶ colonies forming units (CFU) g ⁻¹	↑ IVDMD; ↑ VFA; ↓ fat content	Ebrahimi <i>et al.</i> (2014)
Fibrolitic enzymes isolated from <i>Aspergillus</i> spp.	Levels of enzyme (0, 2, 4 and 6 g kg ⁻¹ of DM)	↓ IVGP; without having adverse effects on nutrient digestibility	Boonthep <i>et al.</i> (2011)
Local microorganisms	60 g kg ⁻¹ /7 days of fermentation	↑ IVDMD; ↑ VFA; ↑ N NH_3 of rumen fluid <i>in vitro</i>	Mulianda <i>et al.</i> (2018)
Cellulolytic bacterium from termite gut (<i>C. kwangyangense</i>)	231×10 ⁻⁶ CFU mL ⁻¹ , OD: 0.5	↑ IVVFA; ↑ IVDMD; ↑ IVOMD; improve NDF and cellulose content in rumen fluids	Ramin <i>et al.</i> (2008)
Produren (a durian probiotic)	7.5 g% DM/1 week fermentation at room temperature	↑ IVDMD; ↑ VFA	Mardalena and Musnandar (2019)

Note: ARDC - apparent rumen degradable carbohydrate; DM - dry matter; IVDMD - *in vitro* dry matter digestibility; IVGP - *in vitro* gas production; IVOMD - *in vitro* organic matter digestibility; NH_3 - ammonia; OD - optical density; VFA - volatile fatty acids; ↑ - increase; ↓ - decrease.

TABLE 6. EFFECTS OF TREATED OIL PALM FROND (OPF) ON RUMINANT PERFORMANCE, RUMEN FERMENTATION PROFILES, NUTRIENT DEGRADABILITY AND MILK PRODUCTION

Ruminant type	Treatment methods of OPF	Animals/optimal inclusion level in the diet	Effects	References
Small ruminant	Biological pre-treated OPF	Male cross-bred Boer goats / 20% DM basis	↑ UFA in <i>longissimus dorsimuscle</i> muscle (40.01%) compared to untreated OPF diet	Rusli <i>et al.</i> (2021)
	White-rot fungi (<i>Lentinus sajor-caju</i>)	Crossbred male goats (Thai Native × Anglo Nubian) / 30% DM basis	↑ nitrogen (N)-retention (63.37% based on g / d / animal); Improved microbial-N supply; DMI and nutrient intake – no changes compared to untreated OPF diet	Hamchara <i>et al.</i> (2018)
	<i>Phanerochaete chrysosporium</i> + minerals (S, P and Mg)	Male goats / 40% DM basis	↑ average daily gain (g/head / day) of 60.0% and feed efficiency of 29.25% compared to control (40% napier grass based diet); ↓ feed conversion (29.27%) compared to control (40% napier grass)	Febrina <i>et al.</i> (2017)
	OPF fermented with microorganisms	Katjang goats / 20% DM basis	↑ daily gain (g/head) of 27.01% compared to untreated OPF; ↑ feed efficiency by 51.93% compared to untreated OPF diet	Astuti <i>et al.</i> (2017)
	Fungal-treated OPF	Crossbred male goats (Thai Native × Anglo Nubian) / 30% DM basis	Growth performance, nutrient digestibility, feed intake, and rumen fermentation characteristics – no changes compared to untreated OPF diet	Chanjula <i>et al.</i> (2018)
	Steam-treated OPF supplemented with urea	Dairy goats (Saanen) / 89.2%-91.66% DM basis	↑ digestibility of nutrients; Improved ruminal fermentation, microbial yield, and N balance in goats fed with steam-treated OPF supplemented with 3% urea	Paengkoum <i>et al.</i> (2006)
Large ruminant	Pelleted OPF (physical treatment)	Male goats / 50% OPF pellet	↑ apparent DM digestibility (13%) and apparent CP digestibility (58%) compared to untreated OPF diet	Dahlan (2000)
	Fermented OPF with <i>prolinas</i> (pineapple skin probiotic)	Lactating Friesian Holstein Crossbred (PFH) dairy cattle / 22% DM basis	↓ feed consumption (11.87%) compared to control (diet containing forage); ↓ milk production (litre / day) of 17.33% compared to control treatment	Mardalena <i>et al.</i> (2019)
	NH ₃ treated OPF	Bali bulls / 50% DM basis	↑ carcass weight (CW), rib-eye muscle area (REMA), and meat chemical compared to a diet without NH ₃ -treated OPF	Muthalib and Afdal (2018)
	Sodium hydroxide (NaOH)-treated OPF	Kedah-Kelantan cross calves / 40% DM basis	↑ DM digestibility and DMI compared to untreated and OPF silage	Kawamoto <i>et al.</i> (2001)
	Urea treated OPF	Sahiwal-Friesian lactating dairy cows / 30% DM basis	↓ DMI (kg / day) of 21.98% compared to untreated OPF diet; 4% FCM: ME intake ratio not different between the untreated OPF and 30% urea-treated OPF silage; Yield of 4% FCM (kg / day) - no changes compared to untreated OPF diet	Ishida and Abu Hassan (1997)
	Urea treated OPF	Kedah- Kelantan bulls / 3% urea treated OPF	↑ TDN (7.5%) compared to 0% urea treated OPF; Improved DMI	Ishida and Abu Hassan (1992)

Note: CP - crude protein; DM - dry matter; FCM - fat-corrected milk; ME - metabolisable energy; NH₃ - ammonia; TDN - total digestible nutrients; UFA - unsaturated fatty acid; ↑ - increase; ↓ - decrease.

diet for goats, as indicated by body weight gain and low feed conversion, was OPF fermented with *Phanerochaete chrysosporium* plus minerals (S, P and Mg). The inclusion of minerals could support the growth of *P. chrysosporium* and enhance the activity of fungal ligninolytic enzymes. However, Chanjula *et al.* (2017) observed no significant difference in feed intake, performance, carcass traits, or meat quality in finishing crossbred male goats (Thai Native × Anglo Nubian) fed TMRs containing 30% OPF treated with *L. sajor-caju*.

Astuti *et al.* (2017) demonstrated that a diet containing at least 20% fermented OPF enhanced the N balance, feed efficiency, and growth response in Katjang goats. Likewise, Chanjula *et al.* (2018) suggested that feeding 30% fungal-treated OPF (FTOPF) in TMRs to crossbred (Thai Native × Anglo Nubian) male goats resulted in no undesired harmful effects on nutrient digestibility, feed intake, and rumen fermentation characteristics, thereby suggesting that FTOPF can be used as an alternative feed source for small ruminants. Wahyuni (2012) reported that feeding goats TMRs containing OPF silage supplemented with enzymes at 2 g kg⁻¹ DM could increase the supply of slowly fermentable carbohydrate fractions and help maintain their body weights. When lambs were given steam-treated OPF supplemented with up to 16 g urea kg⁻¹ OPF, the DM intake (DMI), OM intake, and DM digestibility then showed significant linear ($p < 0.001$) increases (Bengaly *et al.*, 2010). Paengkoum *et al.* (2006) found that feeding high energy levels (16.20-16.24 MJ kg⁻¹) of steam-treated OPF could improve the utilisation of urea (optimal level of 3%) supplementation in Saanen goat feedstuff, which revealed positive responses of animal performance in conjunction with improved ruminal fermentation, microbial yield, and N balance. Ebrahimi *et al.* (2015) reported that goats fed with 50% OPF silage with feed pellet showed promise in modulating the rumen environment so that the bio-hydrogenation of fatty acids could be decreased by rumen microbes. In ruminants, modification of FA profiles is mainly needed to regulate the antimicrobial activity of potential free fatty acids and to control microbial bio-hydrogenation to improve the nutritional value of animal feed (Jenkins *et al.*, 2008). The supplementation of OPF ensiled with lactic acid bacteria, and cellulose enzyme increases the n-3 polyunsaturated fatty acids in the plasma and subcutaneous fat in sheep, which in turn have less negative effects on consumers' health (Ebrahimi *et al.*, 2015).

Effects of Feeding Treated OPF on Beef Cattle and Dairy Cows

Treated OPF roughage for use in TMRs could provide an effective way to improve nutrient

utilisation to enhance the growth response and reduce the costs of feeding large ruminants (e.g., cattle and buffaloes). Previous studies have reported numerous positive effects of treated OPF supplementation on some characteristics (performance, digestion, and microbial populations) of beef and dairy cattle as described in Table 6 (Ishida and Abu Hassan, 1992; 1997; Kawamoto *et al.*, 2001; Mardalena *et al.*, 2019; Mhudmhan *et al.*, 2012; Muthalib and Afdal, 2018). In addition, several *in vivo* studies have reported that the incorporation of treated OPF with different feed additives improves the nutrient degradability of large ruminants (Kawamoto *et al.*, 2001; Muthalib and Afdal, 2018). However, there are very few studies which have reported the effects of feeding biologically treated OPF to ruminants, including the use of fungal and exogenous fibrolytic enzyme treated OPF on *in vivo* digestibility in cattle (Mardalena *et al.*, 2019). Indeed, treatment of OPF with NPN such as anhydrous NH₃, urea, or other NH₃-releasing compounds has been widely investigated as value-added ruminant feedstuffs for cattle and dairy cows. In addition, urea-treated OPF has been found to be effective in improving *in vivo* DM and CP degradability, and reducing the amount of supplemental N, and enhancing acceptability and voluntary intake of the treated OPF by ruminants (Ishida and Abu Hassan, 1992; 1997; Muthalib and Afdal, 2018).

The feeding of mixed rations consisting of up to 30% OPF treated with 3% urea with PKC-based concentrates to beef bulls (Australian Commercial Cross bulls - 6 bulls/treatment) did not adversely affect the growth performance or the quality of lean meat. However, body weight gain, feed intake, and carcass fat decreased as the quantity of OPF treated with 3% urea in the diet increased (Ishida and Abu Hassan, 1997). In another experiment, Ishida and Abu Hassan (1992) carried out digestion trials using Kedah-Kelantan bulls (the indigenous breed of cattle in Malaysia), to evaluate the nutritional value of OPF silage and the effects of different levels of urea inclusion on the nutritional value of OPF silage. The intake and digestibility data indicated that the nutritional value of OPF silage was high with the inclusion of 3% urea. However, the OPF digestibility in the 6% urea treatment had decreased by nutrient losses caused by prolonged bacterial activity. The addition of urea in OPF and the optimum inclusion level could increase the CP level and formation of NH₃ in rumen, which stimulates urea-splitting bacterial growth and the subsequent production of larger quantities of VFAs through ruminal fermentation. These findings suggest that treated OPF silage forage can be used as a potential feedstuff to meet the nutritional needs of beef cows.

A feeding trial conducted by Muthalib and Afdal (2018) demonstrated that OPF treated with 50% NH₃ tended to increase carcass weight, rib-eye muscle

area (REMA) and the meat chemical content of Bali bulls. Mhudmhan *et al.* (2012) observed that OPF silage mixed with 2%, 4% or 6% molasses positively impacted the nutritional utilisation and rumen fermentation profile in Thai native male cattle when they were fed a concentrate at 0.5% of mature cattle BW. Kawamoto *et al.* (2001) reported that sodium hydroxide (NaOH)-treated OPF had a higher DM digestibility and digestible DMI in cattle compared with that of OPF pellets and silage, although it had low palatability. It was suggested that the low fermentation quality might reduce the palatability of NaOH treated OPF silage based on animal feeding trials. The inclusion of nutritious alkali-treated OPF in the concentrate or roughage component of the complete feed has proved to be beneficial for promoting growth performance and improving digestibility, N-balance, and rumen fermentation of beef cattle. However, since NaOH is caustic and dangerous, a safer procedure for treatment and a more cost-effective method should be developed.

Feeding of treated OPF to dairy animals has variable effects on milk composition. OPF silage (solid-state fermentation of OPF without additives) made of fresh OPF could be fed to lactating dairy cows as a roughage source without adverse effects on animal condition or milk flavour. The optimum level of inclusion of OPF silage in the diet is approximately 30% (on a DM basis). Ishida and Hassan (1997) found that Sahiwal-Friesian lactating dairy cows fed 30% OPF (on a DM basis) silage as roughage with concentrate consisting of corn- and soybean-based feed produced the highest yields of milk and did not appear to have any adverse effects in terms of condition or milk flavour relative to those fed a diet of 50% OPF silage or 50% fodder. The results obtained indicated that OPF silage could be fed to lactating dairy cows for milk production. Similarly, Mardalena *et al.* (2019) found that corn straw substitution with fermented OPF with *prolinas* (pineapple skin probiotic) in lactating Friesian Holstein Crossbred (PFH) dairy cattle feed could decrease feed consumption but not adversely affecting feed efficiency and 4% fat corrected milk (FCM), milk composition and production of dairy cattle. Ishida and Abu Hassan (1992) suggested that feeding dairy cattle with OPF silage treated with optimum urea level could increase the level of protein content in milk and alter milk composition.

CONCLUSION

OPF is identified as the major biomass source used as feedstuffs for ruminants in the tropics as a substitute for tropical grasses. Its utilisation as a livestock feed is limited due to poor nutritive values with low ME and high lignocellulosic content. Therefore, OPF treatment is a crucial step for the

conversion of OPF into value-added feedstuffs. Extensive investigations have been carried out on the effects of different treatment methods on OPF, nutritional value, and chemical compositions. Analysis of various treatment methods brings us to the conclusion that each technique has shown promising results in relation to breakdown and altering the structure of lignocellulose in OPF to make lignocellulosic substrates readily accessible to enzymatic hydrolysis by rumen microbes, thereby increasing *in vitro* and *in vivo* rumen apparent DM and organic matter degradability and improving ruminant growth efficiency. Although there have already been several laboratory studies (*in vitro* and *in sacco* experiments) and on-farm trials, most research works have been carried out separately. Thus, there is still a limited systematic literature review on OPF treatment from laboratory to production. Moreover, the molecular approaches evaluating the effects of treated OPF as value-added feedstuffs in ruminant in associated expression patterns of growth performance and digestibility efficacy-related genes are still lacking. Therefore, further study is needed to determine how treated OPF may affect ruminant metabolism activities by regulating the transcription and translation of their DNA.

Although different biological, chemical, and physical treatments of OPF have been used to improve digestibility and feed intake of ruminant animals, the practical use of these treatments can be restricted by significant cost-effectiveness, energy-effectivity, environmental sustainability, and safety. Therefore, the question related to sustainable agriculture arises what strategies can be technically and socio-economically suitable and acceptable to farmers under local conditions. Biological treatment (*i.e.*, fungi and enzymes treatments) is considered a favourable option due to its practicality, environmental-friendly and safer approach for enhancing the nutritional value of OPF as animal feed. Thus, the biological treatment method could offer an optimisation approach in contributing to sustainable agriculture, particularly in ruminant production using OPF waste from oil palm plantations. Furthermore, ligninolytic enzymes combined with non-protein N sources such as urea or NH_3 could be an alternative way to shorten the silage incubation period or the delignification cycle. In conclusion, to date, the fundamental factor contributing to the lack of technology in producing a large number of fungi or enzymes is the difficulty in controlling the optimum environmental conditions that lead to problems in biological treatments procedure in commercial or small-scale farms. In this respect, future research should focus on optimisation of biological and economic effects of different treatments and development in alternative enzyme production technology for an effective OPF treatment.

ACKNOWLEDGEMENT

The authors would like to thank the Director-General of MPOB for permission to publish this article.

REFERENCES

- Abd El Tawab, A M; Matloup, O H; Kholif, A M; Abo El-Nor, S A H; Murad, H A; El-Sayed, H M and Khorshed, M M (2015). Influence of addition of tannase enzyme to reducing tannins effects in lactating goats diets. *Int. J. Dairy Sci.*, 10: 24-35.
- Agensi Inovasi Malaysia (2013). National biomass strategy 2020: New wealth creation for Malaysia's biomass industry. <https://www.cmtevents.com/MediaLibrary/BStgy2013RptAIM.pdf>, accessed on 25 November 2021.
- Aiman-Zakaria, A; Yong-Meng, G; Ali-Rajion, M; Jafari, S; Faseleh-Jahromi, M; Shokriyazdan, P and Ebrahimi, M (2017). The influence of plant polyphenols from oil palm (*Elaeis guineensis* Jacq.) leaf extract on fermentation characteristics, biohydrogenation of C18 PUFA, and microbial populations in rumen of goats: *In vitro* study. *Acta Agric. Scand. A-Anim. Sci.*, 67: 76-84.
- Astuti, T; Santoso, U and Amir, Y (2017). Nutritional value of fermented palm oil fronds as a basis for complete feed for ruminants. *Pak. J. Nutr.*, 16: 96-100.
- Awalludin, M F; Sulaiman, O; Hashim, R and Nadhari, W N A W (2015). An overview of the oil palm industry in Malaysia and its waste utilisation through thermochemical conversion, specifically via liquefaction. *Renew. Sust. Energ. Rev.*, 50: 1469-1484.
- Azmi, M A; Yusof, M T; Zunita, Z and Hassim, H A (2019). Enhancing the utilization of oil palm fronds as livestock feed using biological pre-treatment method. *IOP Conf. Ser.: Earth Environ. Sci. Vol. 230, No. 1*. p. 012077.
- Barlianti, V; Dahnum, D; Hendarsyah, H and Abimanyu, H (2015). Effect of alkaline pretreatment on properties of lignocellulosic oil palm waste. *Procedia Chem.*, 16: 195-201.
- Beauchemin, K A; Colombatto, D; Morgavi, D P and Yang, W Z (2003). Use of exogenous fibrolytic enzymes to improve feed utilization by ruminants. *J. Anim. Sci.*, 81: E37-E47.
- Beigh, Y A; Ganai, A M and Ahmad, H A (2017). Prospects of complete feed system in ruminant feeding: A review. *Vet. World.*, 10: 424-437.
- Bengaly, K; Liang, J B; Jelani, Z A; Ho, Y W and Ong, H K (2010). Utilisation of steam-processed oil palm (*Elaeis guineensis*) frond by ruminants in Malaysia: Investigations for nitrogen supplementation. *Afr. J. Agric. Res.*, 5: 2131-2136.
- Binod, P; Satyanagalakshmi, K; Sindhu, R; Janu, K U; Sukumaran, R K and Pandey, A (2012). Short duration microwave assisted pretreatment enhances the enzymatic saccharification and fermentable sugar yield from sugarcane bagasse. *Renew. Energy*, 37: 109-116.
- Boonthep, K; Ngampongsai, W; Wattanachant, C; Visessanguan, W and Boonpayung, S (2011). Effects of enzyme levels in total mixed ration containing oil palm frond silage on kinetics of gas production. In SAADC 2011 strategies and challenges for sustainable animal agriculture-crop systems, Volume III: full papers. *Proc. of the 3rd International Conference on Sustainable Animal Agriculture for Developing Countries*. Nakhon Ratchasima, Thailand. p. 563-567.
- Chanjula, P; Petcharat, V and Cherdthong, A (2017). Effects of fungal (*Lentinus sajor-caju*) treated oil palm frond on performance and carcass characteristics in finishing goats. *Asian-Australas. J. Anim. Sci.*, 30: 811-818.
- Chanjula, P; Petcharat, V and Cherdthong, A (2018). Rumen characteristics and feed utilization in goats fed with biologically treated oil palm fronds as roughage in a total mixed ration. *S. Afr. J. Anim. Sci.*, 48: 1049-1056.
- Chanjula, P; Petcharat, V and Promkot, C (2015). Nutritive value of oil palm fronds treated with white rot fungi. *Proc. of the 5th International Conference on Sustainable Animal Agriculture for Developing Countries*. Dusit Thani Pattaya Hotel, Thailand. p. 135-138.
- Curiel, J A; Rodríguez, H; Acebrón, I; Mancheño, J M; De Las Rivas, B and Muñoz, R (2009). Production and physicochemical properties of recombinant *Lactobacillus plantarum* tannase. *J. Agric. Food Chem.*, 57: 6224-6230.
- Dahlan, I (2000). Oil palm frond: A feed for herbivores. *Asian-Aus. J. Anim. Sci.*, 13: 300-303.
- Dahlan, I; Yamada, Y and Mahyuddin, M D (1993). Botanical composition and models of metabolizable energy availability from undergrowth in oil palm plantations for ruminant production. *Agroforest. Syst.*, 24: 233-246.
- Ebrahimi, M; Rajion, M A; Goh, Y M; Farjam, A S; Sazili, A Q and Schonewille, J T (2014). The effects

- of adding lactic acid bacteria and cellulase in oil palm (*Elaeis guineensis* Jacq.) frond silages on fermentation quality, chemical composition and *in vitro* digestibility. *Ital. J. Anim. Sci.*, 13: 557-562.
- Ebrahimi, M; Rajion, M A; Meng, G Y; Shokryzadan, P; Sazili, A Q and Jahromi, M F (2015). Feeding oil palm (*Elaeis guineensis* Jacq.) fronds alters rumen protozoal population and ruminal fermentation pattern in goats. *Ital. J. Anim. Sci.*, 14: 3877.
- Febrina, D; Jamarun, N and Zain, M (2014). Biological delignification by *Phanerochaete chrysosporium* with addition of mineral Mn and its effect on nutrient content of oil palm frond. *Proc. of the 16th AAAP Animal Science Congress Vol. II*. Yogyakarta, Indonesia. p. 1723-1726.
- Febrina, D; Jamarun, N; Zain, M and Khasrad (2016). Effects of calcium (Ca) and manganese (Mn) supplementation during oil palm frond fermentation by *Phanerochaete chrysosporium* on *in vitro* digestibility and rumen fluid characteristics. *Pak. J. Nutr.*, 15: 352-358.
- Febrina, D; Jamarun, N; Zain, M and Khasrad (2017). Effects of using different levels of oil palm fronds (FOPFS) fermented with *Phanerochaete chrysosporium* plus minerals (P, S and Mg) instead of napier grass on nutrient consumption and the growth performance of goats. *Pak. J. Nutr.*, 16: 612-617.
- Galbe, M and Zacchi, G (2012). Pretreatment: The key to efficient utilization of lignocellulosic materials. *Biomass Bioenergy*, 46: 70-78.
- Garrote, G; Domínguez, H and Parajó, J C (1999). Hydrothermal processing of lignocellulosic materials. *Holz als Roh- und Werkstoff*, 57: 191-202.
- Gunun, N; Wanapat, M; Gunun, P; Cherdthong, A; Khejornsart, P and Kang, S (2016). Effect of treating sugarcane bagasse with urea and calcium hydroxide on feed intake, digestibility, and rumen fermentation in beef cattle. *Trop. Anim. Health Prod.*, 48: 1123-1128.
- Hamchara, P; Chanjula, P; Cherdthong, A and Wanapat, M (2018). Digestibility, ruminal fermentation and nitrogen balance with various feeding levels of oil palm fronds treated with *Lentinus sajor-caju* in goats. *Asian-Australas. J. Anim. Sci.*, 31: 1619-1626.
- Harahap, R P; Jayanegara, A; Nahrowi and Fakhri, S (2018). Evaluation of oil palm fronds using fiber cracking technology combined with *Indigofera* sp. in ruminant ration by Rusitec. *AIP Conf. Proc. 2021*. AIP Publishing LLC. p. 050008.
- Hassim, H A; Lourenço, M; Goh, Y M; Baars, J J P and Fievez, V (2012). Rumen degradation of oil palm fronds is improved through pre-digestion with white rot fungi but not through supplementation with yeast or enzymes. *Can. J. Anim. Sci.*, 92: 79-87.
- Hermiati, E; Anita, S; Risanto, L; Styarini, D; Sudiyani, Y; Hanafi, A and Abimanyu, H (2013). Biological pretreatment of oil palm frond fibre using white-rot fungi for enzymatic saccharification. *Makara J. Technol.*, 17: 39-43.
- Hong, L S; Ibrahim, D and Omar, I C (2012). Oil palm frond for the production of bioethanol. *Int. J. Biochem. Biotechnol.*, 1: 7-11.
- Ishida, M and Abu Hassan, O (1992). Effect of urea treatment level on nutritive value of oil palm fronds silage in Kedah-Kelantan bulls. *Proc. of the 6th AAAP Animal Science Congress. Vol. III*. Kasetsart University, Bangkok, Thailand. p. 68.
- Ishida, M and Abu Hassan, O (1997). Utilization of oil palm frond as cattle feed. *Jpn. Agric. Res. Q.*, 31: 41-47.
- Jaffri, J M; Mohamed, S; Ahmad, I N; Mustapha, N M; Manap, Y A and Rohimi, N (2011). Effects of catechin-rich oil palm leaf extract on normal and hypertensive rats' kidney and liver. *Food Chem.*, 128: 433-441.
- Jayanegara, A; Ardhisty, N F; Dewi, S P; Antonius; Ridwan, R; Laconi, E B; Nahrowi and Ridla, M (2019). Enhancing nutritional quality of oil palm empty fruit bunch for animal feed by using fibre cracking technology. *Adv. Anim. Vet. Sci.*, 7: 157-163.
- Jenkins, T C; Wallace, R J; Moate, P J and Mosley, E E (2008). Board-invited review: Recent advances in biohydrogenation of unsaturated fatty acids within the rumen microbial ecosystem. *J. Anim. Sci.*, 86: 397-412.
- Kawamoto, H; Mohamed, W Z; Mohd Shukur, N I; Mohd Ali, M S; Ismail, Y and Oshio, S (2001). Palatability, digestibility and voluntary intake of processed oil palm fronds in cattle. *Jpn. Agric. Res. Q.*, 35: 195-200.
- Khattab, M S A and Tawab, A M A E (2018). *In vitro* evaluation of palm fronds as feedstuff on ruminal digestibility and gas production. *Acta Sci.-Anim. Sci.*, 40: e39586.
- König, H (2006). *Bacillus* species in the intestine of termites and other soil invertebrates. *J. Appl. Microbiol.*, 101: 620-627.

- Kristiani, A; Abimanyu, H; Setiawan, A H and Aulia, F (2013). Effect of pretreatment process by using diluted acid to characteristic of oil palm's frond. *Energy Procedia*, 32: 183-189.
- Kum, W H and Zahari, M W (2011). Utilisation of oil palm by-products as ruminant feed in Malaysia. *J. Oil Palm Res.*, 23: 1029-1035.
- Kushairi, A; Ong-Abdullah, M; Nambiappan, B; Hishamuddin, E; Bidin, M N I Z; Ghazali, R; Subramaniam, V; Sundram, S and Parveez, G K A (2019). Oil palm economic performance in Malaysia and R&D progress in 2018. *J. Oil Palm Res.*, 31: 165-194.
- Mardalena and Musnandar, E (2019). *In vitro* evaluation of oil palm fronds fermented with produren: A durian probiotic. *Pak. J. Nutr.*, 18: 186-196.
- Mardalena, M; Syarif, S and Zubaidah, Z (2019). The effect oil palm fronds fermented with *prolinas* to milk production of dairy cattle. *IOP Conf. Ser.: Earth Environ. Sci.* Purwokerto, Indonesia. p. 012016.
- Mathius, I W (2008). Development of beef cattle based palm oil industry. *Develop. Agr. Innov.* 1, 3: 206-224.
- Meng, G Y; Rajion, M A; Jafari, S and Ebrahimi, M (2018). Oil palm frond supplementation can change fatty acid composition of rumen fluid, muscle tissue and blood cholesterol level in crossbred male sheep. *Thai J. Vet. Med.*, 48: 137-146.
- Mhudmhan, S; Wattanachant, C; Ngampongsai, W and Kuprasert, S (2012). Effects of oil palm frond silage mixed with different levels of molasses on intake and nutrient utilization in native cattle. *Khon Kaen Agr. J.*, 40: 79-92.
- Mosier, N; Wyman, C; Dale, B; Elander, R; Lee, Y Y; Holtzapple, M and Ladisch, M (2005). Features of promising technologies for pretreatment of lignocellulosic biomass. *Bioresour. Technol.*, 96: 673-686.
- Mulianda, R; Tafsir, M; Hanafi, N D and Yulinas (2018). Utilizing local microorganisms with different dosages and duration of fermentation towards digestibility and rumen activity of oil palm frond *in vitro*. *E3S Web Conf.*, 52. p. 00036.
- Muthalib, R A and Afdal, M (2018). Effect of oil palm frond treated with ammonia on some carcass traits and meat chemical composition of Bali bulls-Indonesia. *Iraqi J. Vet. Sci.*, 32: 57-60.
- Namoolnoy, P; Phoolphundh, S and Wongwicharn A (2011). Biodegradation of lignin in oil palm fronds by white rot fungi. *Kasetsart J. (Nat. Sci.)*, 45: 254-259.
- Noparat, P; Prasertsan, P; O-Thong, S and Pan, X (2015). Dilute acid pretreatment of oil palm trunk biomass at high temperature for enzymatic hydrolysis. *Energy Procedia*, 79: 924-929.
- Nsereko, V L; Beauchemin, K A; Morgavi, D P; Rode, L M; Furtado, A F; McAllister, T A; Iwaasa, A D; Yang, W Z and Wang, Y (2002). Effect of a fibrolytic enzyme preparation from *Trichoderma longibrachiatum* on the rumen microbial population of dairy cows. *Can. J. Microbiol.*, 48: 14-20.
- Okano, K; Iida, Y; Samsuri, M; Prasetya, B; Usagawa, T and Watanabe, T (2006). Comparison of *in vitro* digestibility and chemical composition among sugarcane bagasses treated by four white-rot fungi. *Anim. Sci. J.*, 77: 308-313.
- Ooi, Z X; Teoh, Y P; Kunasundari, B and Shuit, S H (2017). Oil palm frond as a sustainable and promising biomass source in Malaysia: A review. *Environ. Prog. Sustainable Energy*, 36: 1864-1874.
- Paengkoum, P; Liang, J B; Jalan, Z A and Basery, M (2006). Utilization of steam-treated oil palm fronds in growing Saanen goats: II. Supplementation with energy and urea. *Asian-Australas. J. Anim. Sci.*, 19: 1623-1631.
- Patra, A K and Saxena, J (2011). Exploitation of dietary tannins to improve rumen metabolism and ruminant nutrition. *J. Sci. Food Agric.*, 91: 24-37.
- Pazarlioglu, N K; Urek, R O and Ergun, F (2005). Biodecolourization of Direct Blue 15 by immobilized *Phanerochaete chrysosporium*. *Process Biochem.*, 40: 1923-1929.
- Rabemanolontsoa, H and Saka, S (2016). Various pretreatments of lignocellulosics. *Bioresour. Technol.*, 199: 83-91.
- Rahman, M M; Lourenço, M; Hassim, H A; Baars, J J P; Sonnenberg, A S M; Cone, J W; De Boever, J and Fievez, V (2011). Improving ruminal degradability of oil palm fronds using white rot fungi. *Anim. Feed Sci. Technol.*, 169: 157-166.
- Rajion, M A; Goh, Y M; Dahlan, I and Abdullah, A S (2001). Dietary manipulation and increase in plasma unsaturated fatty acids in sheep. *Asian-Australas. J. Anim. Sci.*, 14: 1073-1077.
- Ramin, M; Alimon, A R; Panandam, J M; Sijam, K; Javanmard, A and Abdullah, N (2008). Digestion of rice straw and oil palm fronds by microflora from

- rumen and termite bacteria, *in vitro*. *Pak. J. Biol. Sci.*, 11: 583-588.
- Rizal, N F A A; Ibrahim, M F; Zakaria, M R; Abd-Aziz S; Yee P L and Hassan, M A (2018). Pre-treatment of oil palm biomass for fermentable sugars production. *Molecules*, 23: 1381.
- Rode, L M; Yang, W Z and Beauchemin, K A (1999). Fibrolytic enzyme supplements for dairy cows in early lactation. *J. Dairy Sci.*, 82: 2121-2126.
- Rusli, N D; Azmi, M A; Mat, K; Hasnita, C H; Wan-Zahari, M; Azhar, K; Zamri-Saad, M and Hassim, H A (2019). The effect of physical and biological pre-treatments of oil palm fronds on *in vitro* ruminal degradability. *Pertanika J. Trop. Agric. Sci.*, 42: 791-805.
- Rusli, N D; Mat, K; Hasnita, C H; Rahman, M M; Yusof, M T; Zamri-Saad, M and Hassim, H A (2021). Fatty acid profile of meat goats fed pre-treated oil palm frond. *IOP Conf. Ser.: Earth Environ. Sci.*, 756: 012018.
- Saminathan, M; Ramiah, S K; Gan, H M; Abdullah, N; Wong, C M V L; Ho, Y W and Idrus, Z (2019). *In vitro* study on the effects of condensed tannins of different molecular weights on bovine rumen fungal population and diversity. *Ital. J. Anim. Sci.*, 18: 1451-1462.
- Saminathan, M; Sieo, C C; Gan, H M, Ravi, S; Venkatachalam, K; Abdullah, N; Wong, C M V L and Ho, Y W (2016). Modulatory effects of condensed tannin fractions of different molecular weights from a *Leucaena leucocephala* hybrid on the bovine rumen bacterial community *in vitro*. *J. Sci. Food Agric.*, 96: 4565-4574.
- Soh, K L; Mohd, A K and Nurul, B (2018). Optimisation of process conditions for ethanol production from enzymatically saccharified empty fruit bunch using response surface methodology (RSM). *J. Oil Palm Res.*, 30: 642-654.
- Sujani, S and Seresinhe, R T (2015). Exogenous enzymes in ruminant nutrition: A review. *Asian J. Anim. Sci.*, 9: 85-99.
- Tafsin, M; Khairani, Y and Hanafi, N D (2018). *In vitro* digestibility of oil palm frond treated by local microorganism (MOL). *IOP Conf. Ser.: Earth Environ. Sci.*, 122 012134.
- Wahyuni, R D (2012). *Effect of enzyme supplementation in total mixed ration containing oil palm frond silage on productive performance of goat*. Ph.D thesis, Prince of Songkla University.
- Wan Zahari, M; Abu Hassan, O; Wong, H K and Liang, J B (2003). Utilization of oil palm frond-based diets for beef and dairy production in Malaysia. *Asian-Australas. J. Anim Sci.*, 16: 625-634.
- Wu, H; Zhou, Z; Yang, Y and Meng, Q (2020). Effect of steam explosion of oil palm frond and empty fruit bunch on nutrient composition and ruminal fermentation characteristics. *Trop. Anim. Health Prod.*, 52: 1223-1228.
- Yunilas, Y; Warly, L; Marlida, Y and Riyanto, I (2014). Quality improvement of oil palm waste-based feed product through indigenous microbial fermentation to reach sustainable agriculture. *Int. J. Adv. Sci. Eng. Inf. Technol.*, 4: 282-285.
- Zakaria, M R; Fujimoto, S; Hirata, S and Hassan, M A (2014). Ball milling pretreatment of oil palm biomass for enhancing enzymatic hydrolysis. *Appl. Biochem. Biotechnol.*, 173: 1778-1789.
- Zhang, Z and Zhao, Z K (2010). Microwave-assisted conversion of lignocellulosic biomass into furans in ionic liquid. *Bioresour. Technol.*, 101: 1111-1114.

THE SYNTHESIS OF *sn*-2 PALMITATE AS HUMAN MILK FAT SUBSTITUTE FROM PALM OIL FRACTIONS BY ENZYMATIC INTERESTERIFICATION – A REVIEW

HASRUL ABDI HASIBUAN^{1*}

ABSTRACT

Human milk fat substitute (HMFS) is structured lipids with characteristics similar to human milk fat used in infant formulas. HMFS is designed to contain 60%-70% palmitic acid at the *sn*-2 position and unsaturated fatty acids at the *sn*-1,3 positions in the triacylglycerol structure. HMFS is synthesised by enzymatic interesterification of vegetable oils or animal fats. Generally, the type of HMFS synthesised is *sn*-2 palmitate, with the main triacylglycerol being 1,3-dioleoyl-2-palmitoylglycerol (OPO). Palm oil fractions are used as raw material to synthesise *sn*-2 palmitate because it contains high palmitic acid. This article reviews the synthesis of *sn*-2 palmitate based on palm oil fractions via enzymatic interesterification. It gives a detailed description of the potential of palm oil fractions as substrates, lipases as biocatalysts, methods and reactors synthesis, and fractionation to increase the triacylglycerol of OPO. The information presented in this review can be used to develop future strategies for the synthesis of palm-based HMFS.

Keywords: enzymatic interesterification, human milk fat substitute, lipase, palm oil, *sn*-2 palmitate.

Received: 22 December 2021; **Accepted:** 13 June 2022; **Published online:** 5 August 2022.

INTRODUCTION

Human milk fat contains 32.0%-52.0% saturated fatty acid, 30.0%-50.0% monounsaturated fatty acid, and 2.5%-13.8% polyunsaturated fatty acid (Monaco *et al.*, 2016). The primary fatty acids contained in human milk fat are 30.0%-35.0% oleic acid, 20.0%-30.0% palmitic acid, 7.0%-14.0% linoleic acid, and 5.7%-8.0% stearic acid. Besides these fatty acids, the human milk fat also contains long-chain polyunsaturated fatty acids (LCPUFA) and they include docosahexaenoic acid, eicosapentaenoic acid, and arachidonic acid contained in with a concentration of less than 1.0% (Ferreira-Dias and Tecelão, 2014; Wei *et al.*, 2019). In human milk fat, about 60.0%-70.0% of palmitic acid is distributed

at the *sn*-2 position and unsaturated fatty acids (oleic acid, linoleic acid, docosahexaenoic acid, eicosapentaenoic acid, and arachidonic acid) at the *sn*-1,3 positions (He *et al.*, 2017; Wei *et al.* 2019).

Human milk fat substitutes (HMFSSs) are structured lipids with the distribution of fatty acids similar to human milk fat (Sahin *et al.*, 2005a; 2005b), commonly used as fat in infant formulas. HMFS has a similar function as human milk fat, increasing the permeation of calcium and fat, making the stool softer, and reducing obstipation (Zou *et al.*, 2017). HMFSSs are classified into four types, which include *sn*-2 palmitate (β -palmitate), LCPUFA, medium-chain fatty acid (MCFA), and milk fat globule membrane supplements. *Sn*-2 palmitate is the most common type of HMFS synthesised and it contains 1,3-dioleoyl-2-palmitoylglycerol (OPO), one of the primary triacylglycerols in human milk fat (Wei *et al.*, 2019).

In general, *sn*-2 palmitate is synthesised using oils and fats containing palmitic acid at the *sn*-2 position including, tripalmitin (Ilyasoglu *et al.*, 2011;

¹ Indonesian Oil Palm Research Institute, Brigjen Katamso 51, 20158 Medan, Indonesia.

* Corresponding author e-mail: hasibuan_abdi@yahoo.com

2013; Liu *et al.*, 2015; 2017; Tecelão *et al.*, 2010; Wang *et al.*, 2016; Yüksel and Yeşilçubuk, 2012; Zheng *et al.*, 2017), lard (Qin *et al.*, 2014; Wang *et al.*, 2010; Yang *et al.*, 2003; Zhang *et al.*, 2016), catfish oil (Zou *et al.*, 2016a; 2016b), oil from *Nannochloropsis oculata* (He *et al.*, 2017), butterfat oil (Rønne *et al.*, 2005) and palm oil fractions (Ghosh *et al.*, 2016; Karabulut *et al.*, 2007; Nagachinta and Akoh, 2013). Also, palmitic acid (Robles *et al.*, 2011; Turan *et al.*, 2013) and ethyl palmitate (Turan *et al.*, 2013) are used as palmitic acyl donors to enhance palmitic acid at the *sn*-2 position of oil containing high unsaturated fatty acids.

Palm oil fractions are the best substrates for HMFS synthesis among the vegetable oils synthesis (Mat Dian *et al.*, 2017). However, palm oil fractions contain high palmitic acid at the *sn*-1,3 positions (Lasekan *et al.*, 2017). The presence of palm oil fractions in infant formula would contribute to the physiological function of the body such as reduction of intestinal permeation of fat, palmitic acid, calcium and lower bone mass (Chen *et al.*, 2019; Koo *et al.*, 2006). Thus, palm oil fractions need to be modified so that more palmitic acid will be at the *sn*-2 position, hence resembling human milk fat. This article aims to scientifically update the usage of palm oil fractions for *sn*-2 palmitate synthesis as HMFS. This article will cover the potential of palm oil fractions as substrates, lipases as biocatalysts, reactors and methods synthesis, and fractionation to increase

OPO content in HMFS. Prospects of palm-based *sn*-2 palmitate synthesis were also discussed.

PALM OIL FRACTIONS AS SUBSTRATES FOR *sn*-2 PALMITATE SYNTHESIS

Palm oil fractions can be used as a substrate for *sn*-2 palmitate synthesis because of their high content of palmitic acid and oleic acid (Table 1) and can act as acyl donors for both acids. Apart from palm oil fractions, palm kernel oil is also utilised as a source of MCFA (especially lauric acid) for the HMFS synthesis (Hasibuan and Ijah, 2016; Karabulut *et al.*, 2007; Zou *et al.*, 2011; 2012b).

The palm oil fractions contain tripalmitin, a palmitic acid-containing triacylglycerol at the *sn*-2 position. Palm stearin is a solid palm fraction that contains high palmitic acid and tripalmitin compared to palm oil and palm olein (Table 2). Hence palm stearin is often used as a substrate for *sn*-2 palmitate synthesis (Wei *et al.*, 2019). In the palm oil industry, palm stearin can be fractionated through dry fractionation to produce soft palm stearin and hard palm stearin (Hasibuan and Siahaan, 2013). Hard palm stearin has palmitic acid higher than palm stearin and soft palm stearin. Hard palm stearin contains the main triacylglycerol, namely tripalmitin of 51.0% (Ibrahim *et al.*, 2006). Although palm stearin is rich in tripalmitin, it also contains

TABLE 1. FATTY ACIDS OF PALM OIL FRACTIONS

Fatty acids (%)	Palm oil*	Palm olein*	Palm stearin*	Soft palm stearin**	Hard palm stearin**	Palm kernel oil*
C6:0	ND	ND	ND	ND	ND	ND-0.8
C8:0	ND	ND	ND	ND	ND	2.4-6.2
C10:0	ND	ND	ND	ND	ND	2.6-5.0
C12:0	ND-0.5	0.1-0.5	0.1-0.5	0.01-0.25	0.14-0.32	45.0-55.0
C14:0	0.5-2.0	0.5-1.5	1.0-2.0	0.97-1.21	1.06-1.36	14.0-18.0
C16:0	39.3-47.5	38.0-43.5	48.0-74.0	50.94-54.84	76.36-81.04	6.5-10.0
C16:1	ND-0.6	ND-0.6	ND-0.2	ND-0.76	ND-0.09	ND-0.2
C17:0	ND-0.2	ND-0.2	ND-0.2	ND	ND	ND
C17:1	ND	ND-0.1	ND-0.1	ND	ND	ND
C18:0	3.5-6.0	3.5-5.0	3.9-6.0	4.44-5.46	3.61-4.87	1.0-3.0
C18:1	36.0-44.0	39.8-46.0	15.5-36.0	30.82-34.06	11.62-13.66	12.0-19.0
C18:2	9.0-12.0	10.0-13.5	3.0-10.0	6.91-8.71	1.95-3.33	1.0-3.5
C18:3	ND-0.5	ND-0.6	ND-0.5	ND-0.21	ND-0.09	ND-0.2
C20:0	ND-1.0	ND-0.6	ND-1.0	ND-0.18	ND-0.12	ND-0.2
C20:1	ND-0.4	ND-0.4	ND-0.4	ND-0.06	ND-0.09	ND-0.2
C20:2	ND	ND	ND	ND	ND	ND
C22:0	ND-0.2	ND-0.2	ND-0.2	ND	ND	ND
Iodine value (IV)	50-56	≥56	≤48	39.90-43.14	14.77-19.33	14.1-21.0

Note: ND - not detected.

Source: *Codex Alimentarius (2001), **Hasibuan and Siahaan (2013).

triacylglycerol with oleic acid at the *sn*-2 position (Table 2). When triacylglycerol containing oleic acid at the *sn*-2 position is acidolysed with oleic acid using a specific lipase of *sn*-1,3 will produce triolein (Wang *et al.*, 2020), which is not a *sn*-2 palmitate product. Table 3 shows that palm stearin has palmitic acid content at the *sn*-2 position ranging from 23.0%-70.1%. Thus, the palmitic acid content at the *sn*-2 position in palm stearin needs to be increased to produce a good substrate for HMFS synthesis (Hasibuan *et al.*, 2021b).

Technologies for improving the positioning of palmitic acid at the *sn*-2 position of palm stearin are solvent fractionation (Ghosh *et al.*, 2016; Lee *et al.*, 2010; Wang *et al.*, 2019; Zou *et al.*, 2012a), enzymatic interesterification (Jiménez *et al.*, 2010a; 2010b) or chemical interesterification (Zou *et al.*, 2011; Zou

et al., 2012b). Palm stearin is fractionated using acetone as a solvent to produce a tripalmitin-rich triacylglycerol (92.0%) (Lee *et al.*, 2010) and palmitic acid (88.57%-92.30%) (Ghosh *et al.*, 2016; Wang *et al.*, 2019; Zou *et al.*, 2012a). In general, the palm stearin solvent fractionation process condition is carried out at an acetone ratio of 5-9 and a fractionation temperature of 20°C-40°C for 3-24 hr.

Enzymatic interesterification between palm stearin (60.0% palmitic acid and 23.0% palmitic acid at the *sn*-2 position) with palmitic acid using Novozyme 435 produces a product with 68.0%-75.0% palmitic acid at the *sn*-2 position (Jiménez *et al.*, 2010a; 2010b). Meanwhile, chemical interesterification of palm stearin (41.7% palmitic acid at the *sn*-2 position) resulted in a product with 58.0% palmitic acid at the *sn*-2 position (Zou *et al.*,

TABLE 2. TRIACYLGLYCEROLS COMPOSITION OF PALM OIL FRACTIONS

Triacylglycerols (%)	Palm oil*	Palm olein*	Palm stearin*	Soft palm stearin**	Hard palm stearin**	Palm kernel oil*
CCLa						6.8
CLaLa						9.9
LaLaLa						21.2
LaLaM						17.0
LaLaO						5.3
LaMM						8.8
PLL				1.0	ND	
MMM	0.4	0.6	0.2	ND	ND	
LaLaP						1.2
LaMO						4.6
MPL	2.4	3.7	1.0	0.1	ND	
LaMP						4.6
LaOO						3.8
LaPO						4.3
LaPP+MMO						
OOL	0.7	0.8	0.1	0.8	ND	
MMP	1.8	2.6	0.8	ND	ND	0.7
MOO						2.0
POL	10.1	15.8	5.3	6.9	0.9	
PPL	9.8	11.2	7.8	8.1	3.8	0.6
MPP	0.6	ND	2.3	ND	ND	
OOO	4.1	5.6	1.8	4.1	4.5	1.4
POO	24.2	36.3	12.0	18.0	3.0	1.9
PPO	31.1	17.1	29.8	32.0	26.4	1.1
PPP	5.9	0.1	29.2	20.6	51.0	0.1
SOO	2.3	3.6	0.8	ND	ND	0.4
PSO	5.1	2.5	3.8	4.9	2.9	0.4
PPS	0.9	ND	5.2	3.5	7.4	
SSO	0.5	ND	ND	ND	ND	

Note: ND - not detected; L - lauric acid; M-myristic acid; O - oleic acid; P - palmitic acid; S - stearic acid.

Source: *Tan and Man (2002); ** Ibrahim *et al.* (2006).

2012b). In other studies, Zou *et al.* (2011) reported chemical interesterification of palm stearin (56.8% palmitic acid at the *sn*-2 position) and manufactured a product with 69.8% palmitic acid at the *sn*-2 position.

Palm oil fractions can also produce palmitic acid and oleic acid as palmitic and oleic acyl donors for the *sn*-2 palmitate synthesis. Both are manufactured by hydrolysis of palm oil fractions, then separated from other fatty acids (Esteban *et al.*, 2011; Jimenez *et al.*, 2010a). Besides palmitic acid, ethyl palmitate can also be used as a palmitic acyl donor (Pina-Rodriguez and Akoh, 2009; Turan *et al.*, 2013), produced through the esterification of palmitic acid with ethanol. Likewise, ethyl oleate is an oleic acyl donor (Lee *et al.*, 2010). Palmitic acyl donors are used as a substrate for improving palmitic acid at the *sn*-2 position of oil and fat. Meanwhile, acyl oleic donors are used to increase *sn*-1,3 oleic acid of oils and fats that contain high tripalmitin.

TABLE 3. FATTY ACIDS AT THE *sn*-2 POSITION OF PALM STEARIN

Fatty acids (%)	Total	<i>sn</i> -2
C12:0	ND - 0.9	ND - 0.3
C14:0	1.3 - 1.7	ND - 1.0
C16:0	68.8 - 70.1	23.0 - 70.1
C18:0	4.8 - 5.2	0.7 - 2.9
C18:1	18.7 - 29.0	30.9 - 65.2
C18:2	3.9 - 7.5	8.3 - 12.6
C18:3	ND - 0.3	ND - 0.1

Source: Jimenez *et al.* (2010a, 2010b); Zou *et al.* (2011; 2012b).

LIPASE FOR PALM-BASED *sn*-2 PALMITATE SYNTHESIS: TYPES AND SOURCES

Lipase (*triacylglycerol hydrolase*, EC 3.1.1.3) is a biocatalyst that naturally acts on carboxylate ester bonds to catalyse the hydrolysis of triacylglycerol (Araújo *et al.*, 2016). This substrate is insoluble in water, and the reaction usually occurs at the organic-water interface, where lipase works best (Adlercreutz, 2013). In non-aqueous media, lipase catalyses esterification, acidolysis, alcoholysis, and interesterification (Araújo *et al.*, 2016; Rodrigues and Fernandez-Lafuente, 2010; Speranza and Macedo, 2012). Lipase has serine-histidine-aspartate catalytic active sites, which is responsible for its catalytic activity (Fernandez-Lafuente, 2010; Ortiz *et al.*, 2019; Rodrigues and Fernandez-Lafuente, 2010). Lipase shows variable stability against the extreme pH conditions, the appearance of organic solvents, and ionic liquids (Kapoor and Gupta, 2012).

Lipase is an excellent biocatalyst for synthesising structured lipids, and triacylglycerol with fatty acids

at a specific position (Iwasaki and Yamane, 2000). HMFS is a structured lipid produced using various types of enzymes, substrates, and acyl donors (Soumanou *et al.*, 2013). The lipases commonly used for HMFS synthesis are Novozyme 435, Lipozyme RM IM, and Lipozyme TL IM (Table 4).

Lipozyme TL IM is obtained from *Thermomyces lanuginose* and is immobilised using silica. Lipozyme TLIM can maintain activity at 55°C-60°C (Fernandez-Lafuente, 2010) and shows positional specificity at the *sn*-1,3 (Soumanou *et al.*, 2013). Lipozyme RM IM is derived from *Rhizomucor miehei* and is immobilised using Duolite ES 562. Lipozyme RM IM is highly specific in the choice of substrate, stereospecific, regioselective, active and stable (Rodrigues and Fernandes-Lafuente, 2010; Zou *et al.*, 2014). Lipozyme RM IM also shows positional specificity at the *sn*-1,3 (Soumanou *et al.*, 2013). Novozyme 435 is generated from *Candida antarctica* lipase B and is immobilised using acrylic resin. Novozyme 435 is one of the most stable commercial lipases commonly used for various reactions (Ortiz *et al.*, 2019). Novozyme 435 can be used at 60°C-70°C (Soumanou *et al.*, 2013). When the substrate is triacylglycerol, Novozyme 435 does not show positional specificity (Jiménez *et al.*, 2010a; 2010b; Soumanou *et al.*, 2013).

METHODS FOR PALM-BASED *sn*-2 PALMITATE SYNTHESIS

Intesterification is an accepted oil and fat modification technique by redistributing the fatty acid groups between and within the triacylglycerol. After the interesterification of the substrate, the product has a distinct chemical composition and improved physical characteristics (Pacheco *et al.*, 2015). Enzymatic interesterification can be carried out non-specifically and specifically (Silva *et al.*, 2012). Non-specific enzymatic interesterification is a random process similar to chemical interesterification. Meanwhile, specific enzymatic interesterification is an acyl exchange process to a particular position, mainly at the *sn*-1,3 position using regioselective lipase (Gibon *et al.*, 2009).

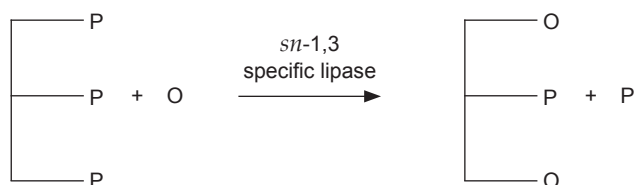
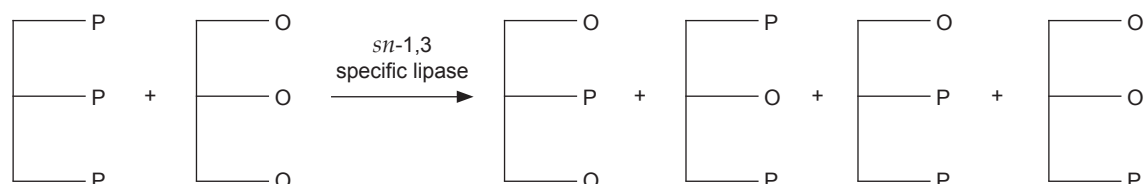
In general, *sn*-2 palmitate synthesis (especially OPO) is fabricated by a one-step reaction including transesterification or acidolysis and two-step reactions such as alcoholysis and esterification or two-step acidolysis (Hasibuan *et al.*, 2021) (Figure 1). Apart from oleic acid (monounsaturated fatty acid, MUFA), fatty acids that are incorporated at the *sn*-1,3 position of *sn*-2 palmitate are fatty acids of MCFA (Karouw *et al.*, 2012), LCPUFA (Ghosh *et al.*, 2016; Nagachinta and Akoh, 2012; 2013), MUFA and LCPUFA (Wang *et al.*, 2019; Zou *et al.*, 2012) or MCFA, MUFA and LCPUFA (Hasibuan and Ijah, 2016; Karabulut *et al.*, 2007; Zou *et al.*, 2011; 2012b). Process conditions for palm-based *sn*-2 palmitate

TABLE 4. CONDITION REACTION FOR THE SYNTHESIS OF PALM-BASED HMFS

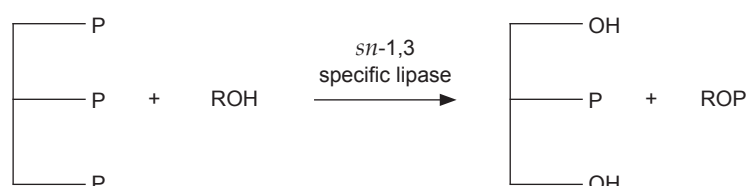
Type of reaction	Lipase	Substrate	Solvent system	Mode of operation	Lipase loading (%)	Substrate ratio (mol)	Temperature (°C)	Time (hr)	Characteristics of product	Reference
Transesterification	Lipozyme TL IM	Palm oil, palm kernel oil, olive oil, sunflower oil, and marine oil	Solvent-free system	Batch	10	4.0:3.5:1.0:1.5:0.2	60	6	23.00% palmitic acid and distributed at <i>sn</i> -2 41.5%	Karabulut <i>et al.</i> (2007)
Transesterification	Lipozyme TL IM	Palm stearin fractionates and ethyl oleate	Solvent-free system	Batch	10	1:5.5	50	3	31.43% OPO, 80.60% palmitic acid at the <i>sn</i> -2 position, and 64.90% <i>sn</i> -1,3 oleic acid	Lee <i>et al.</i> (2010)
Transesterification	Lipozyme TL IM	Palm stearin fractionates and PUFA-rich fish oil	Solvent-free system	Batch	10	2:1	60	12	75.98% palmitic acid at the <i>sn</i> -2 position, 0.27% arachidonic acid, 3.43% eicosapentaenoic acid, 4.25% docosahexaenoic acid, and melting point of 42°C	Ghosh <i>et al.</i> (2016)
Transesterification	Novozyme 435	Palm stearin, palm kernel oil, soybean oil, olive oil, and tuna fish oil	Solvent-free system	Batch	10	2.9:3.4:1.5:2.0:0.2	60	4	Fatty acids composition resembles human milk fat, melting point of 28°C	Hasibuan and Ijah (2016)
Acidolysis	Lipozyme RM IM	Palm stearin and a mixture of fatty acid from rapeseed oil, sunflower oil, palm kernel oil, stearic acid, and myristic acid	Solvent-free system	Batch	10.7	1:14.6	57	3.4	29.70% palmitic acid and 62.80% palmitic acid at the <i>sn</i> -2 position	Zou <i>et al.</i> (2011)
Acidolysis	Novozym 435	Palm olein, docosahexaenoic acid, and arachidonic acid	Hexane	Batch	10	1:18	60	24	Docosahexaenoic acid +arachidonic acid incorporated in triacylglycerol 25.25% (w/w) and docosahexaenoic acid +arachidonic acid incorporated at <i>sn</i> -2 17.20% (w/w)	Nagachinta and Akoh (2012)
Acidolysis	Novozym 435	Palm olein and a mixture of fatty acid (23.23% docosahexaenoic acid, 31.42% gamma-linoleic acid, and 15.12% palmitic acid)	Hexane	Batch	10	1:2	60	22.7	35.11% palmitic acid at the <i>sn</i> -2 position, 3.75% docosahexaenoic acid, and 5.03% gamma-linoleic acid	Nagachinta and Akoh (2013)

TABLE 4. CONDITION REACTION FOR THE SYNTHESIS OF PALM-BASED HMFS (continued)

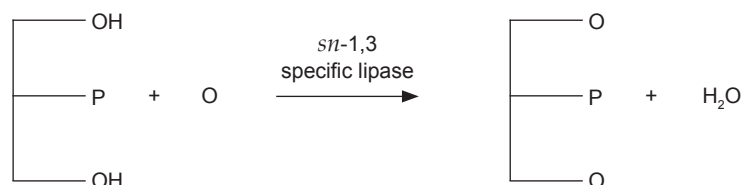
Type of reaction	Lipase	Substrate	Solvent system	Mode of operation	Lipase loading (%)	Substrate ratio (mol)	Temperature (°C)	Time (hr)	Characteristics of product	Reference
Acidolysis	Lipozyme RM IM	Palm stearin and fatty acid from rapeseed oil	Solvent-free system	Batch	8	1:10	60	4	39.60% palmitic acid and 70.50% distributed at <i>sn</i> -2	Zou <i>et al.</i> (2012a)
Acidolysis	Lipozyme RM IM	Palm stearin and a mixture of stearic acid, myristic acid, fatty acid from rapeseed oil, sunflower oil, and palm kernel oil	Solvent-free system	Packed bed reactor		1:9.5	58	2.7	28.80% palmitic acid and 53.20% distributed at <i>sn</i> -2	Zou <i>et al.</i> (2012b)
Acidolysis	Lipozyme RM IM	Palm stearin fractionates and fungal oil from <i>Mortierella alpina</i> ALK (1:0.3) and oleic acid	Solvent-free system	Batch	8	1:6	60	6	Oleic acid incorporated 53.50% in triacylglycerol, fatty acid at <i>sn</i> -2: 68.70% palmitic acid, 9.80% arachidonic acid, and 7.9% oleic acid	Wang <i>et al.</i> (2019)
Acidolysis	Lipozyme RM IM immobilised on macroporous acrylic resin	Palm stearin fractionates, oleic acid and linoleic acid	Solvent-free system	Batch	8	1:8.4	60	4	OPO and OPL 69.26%, 87.75% palmitic acid at the <i>sn</i> -2 position	Wang <i>et al.</i> (2020)
Alcoholysis and esterification	Lipozyme RM IM	2-monoacylglycerol from alcoholysis of palm stearin, then esterified with lauric fatty acid methyl ester from coconut oil	Hexane	Batch	10	1:3	50	12	MCFA 43.86% with lauric acid 39.37%, 24.18% palmitic acid at the <i>sn</i> -2 position	Karouw <i>et al.</i> (2012)
Two-step acidolysis	Acidolysis 1: Novozyme 435 Acidolysis II: Lipase DF (Rhizopus oryzae)	Acidolysis 1: Palm stearin and palmitic acid Acidolysis II: Palmitic acid-rich triacylglycerol from palm stearin and oleic acid	Hexane	Batch stirred tank reactor	Acidolysis I: 9.6 g lipase × h/g triacylglycerol Acidolysis II: 0.4 g lipase × h/g triacylglycerol	Acidolysis I: 1:3 Acidolysis II: 1:6	37	Acidolysis I: 24 Acidolysis II: 1	67.20% <i>sn</i> -1,3 oleic acid and 67.80% palmitic acid at the <i>sn</i> -2 position	Esteban <i>et al.</i> (2011)

Acidolysis**Transesterification****Alcoholysis and Esterification**

First-step: Alcoholysis



Second-step: Alcoholysis



Note: P - palmitic, O - oleic, ROH - alcohol, ROP - palmitic acid alkyl ester.

Figure 1. Mechanism of reaction for sn-2 palmitate synthesis using sn-1,3 specific lipase (especially OPO).

synthesis are presented in Table 4. Sn-2 palmitate synthesis can be performed in a solvent or solvent-free system. However, the solvent-free system is advisable for HMFS synthesis in terms of food safety and low production costs (Ferreira-Dias *et al.*, 2019).

Transesterification. Transesterification is a reaction between 1) triacylglycerol and triacylglycerol or 2) triacylglycerol and esterified fatty acids (Hassim *et al.*, 2018; Wei *et al.*, 2019). In the first type of reaction, Karabulut *et al.* (2007) conducted the interesterification of a mixture of palm oil, palm kernel oil, olive oil, sunflower oil, and marine oil using Lipozyme TL IM. The product contained 23.0% palmitic acid and 41.5% palmitic acid at the sn-2 position. Ghosh *et al.* (2016) reported transesterification between palm stearin fractionated with PUFA-rich fish oil with Lipozyme TL IM produced HMFS with 75.98% palmitic acid

at the sn-2 position, 0.27% arachidonic acid, 3.43% eicosapentaenoic acid, and 4.25% docosahexaenoic acid. In the synthesis using the second reaction, Lee *et al.* (2010) reported transesterification of palm stearin fractions and ethyl oleate using Lipozyme TL IM. The product contained 31.43% OPO, 80.6% palmitic acid at the sn-2 position, and 64.9% oleic acid at the sn-1,3 position.

Acidolysis. Acidolysis is a reaction of triacylglycerol and fatty acid (Hassim *et al.*, 2018; Wei *et al.*, 2019). Acidolysis between triacylglycerol contains high palmitic acid at the sn-2 position from palm stearin with MUFA, MCFA, and LCPUFA using Lipozyme RM IM has been reported by Zou *et al.* (2012a; 2012b) and Wang *et al.* (2019). Meanwhile, Nagachinta and Akoh (2012) used Novozyme 435 for acidolysis of palm olein with docosahexaenoic acid, and arachidonic acid to produce triacylglycerol with 25.25% (w/w) docosahexaenoic acid+arachidonic

acid incorporation and 17.20% (w/w) docosahexaenoic acid+arachidonic acid at the *sn*-2 position. Nagachinta and Akoh (2013) also reported Novozyme 435 for acidolysis between palm olein with palmitic acid, docosahexaenoic acid, and gamma linoleic acid to produce triacylglycerol containing palmitic acid at the *sn*-2 position, docosahexaenoic acid, and gamma linoleic acid 35.11%, 3.75% and 5.03%, respectively.

Two-step process. The two-step process can be carried out by alcoholysis of triacylglycerol using a specific lipase *sn*-1,3 to produce *sn*-2 monoacylglycerol and then *sn*-2 monoacylglycerol esterified with fatty acid (Wei *et al.*, 2019) or esterified fatty acid (Karouw *et al.*, 2012). This method produces high yield and purity (Soumanou *et al.*, 2013). The two-step process can also be conducted using two-step acidolysis, as reported by Esteban *et al.* (2011). First, palm stearin is acidolysed with palmitic acid using Novozyme 435 to produce triacylglycerol containing high palmitic acid at the *sn*-2 position. Second, triacylglycerol is acidolysed with oleic acid using lipase DF from *Rhizopus oryzae*. The product had 67.80% palmitic acid at the *sn*-2 position and 57.20% distributed at the *sn*-2 position.

REACTORS FOR PALM-BASED *sn*-2 PALMITATE SYNTHESIS

The challenge in structured lipid synthesis by enzymatic interesterification is that production costs are relatively high, so it is necessary to use continuous processes and cost-effective catalysts (Bourlieu *et al.*, 2009; Jala and Kumar, 2018). The selection of reactors is essential to fabricate high product yields. A batch and continuous reactor are used in the enzymatic interesterification for *sn*-2 palmitate. The batch reactor is usually suitable for operation at a laboratory scale, whereas the continuous system is very appropriate for an industrial scale. The optimal reaction conditions for palm-based *sn*-2 palmitate synthesis in a batch reactor are enzyme load of 8.0%-10.0% (w/w of the total substrate), temperature 40°C-60°C, and reaction time 3-24 hr.

Continuous enzymatic interesterification is an economical technology for large-scale production because of its minimal costs, ease of operation, and being able to control the fatty acid distribution due to the selectivity and regiospecific of lipases (Silva *et al.*, 2012). The continuous system commonly used in enzymatic interesterification is a packed bed reactor (Soumanou *et al.*, 2013; Zou *et al.*, 2012b). The advantages of a packed bed reactor over batch reactors are due to relatively high enzyme stability, ease of operation on large scales, high reaction rates, and mass transfer, thereby reducing the occurrence of acyl migration (Sen *et al.*, 2016; Zou *et al.*, 2012b).

A packed bed reactor is best applied continuously on an industrial scale to minimise the labour and costs of the processes (Nielsen *et al.*, 2006). Zou *et al.* (2012b) used a packed bed reactor in acidolysis between interesterified palm stearin with a mixture of stearic acid, myristic acid and fatty acids from rapeseed oil, sunflower oil, and palm kernel oil. The optimum conditions obtained using the response surface methodology approach were a substrate ratio of 9.5 mol/mol with a residence time of 2.7 hr at 58°C. The final product contained 28.8% palmitic acid and 53.2% palmitic acid at the *sn*-2 position.

ENHANCING OF *sn*-2 PALMITATE-RICH HMFS THROUGH FRACTIONATION

Karabulut *et al.* (2007) reported on the enzymatic interesterification of a mixture of palm oil, palm kernel oil, olive oil, sunflower oil, and marine oil was not optimum enough to produce a product with high palmitic acid at the *sn*-2 position. Fractionation can be applied in HMFS products obtained through enzymatic interesterification to increase triacylglycerol containing high palmitic acid at the *sn*-2 position (Hasibuan *et al.*, 2021c).

Fractionation of the acidolysis product between butterfat and a mixture of fatty acids from rapeseed oil and soybean oil using acetone as solvent at a ratio of 2.5, temperature of 0°C for 3 hr was reported by Sørensen *et al.* (2010). The product contained 56.12% palmitic acid at the *sn*-2 position. This value was higher than the product obtained from butterfat's acidolysis fractionated first (47.26%). In another study, Lee *et al.* (2015) reported the OPO content enhanced from 25.2% to 53.3% after fractionation at 22°C for 12 hr of the interesterified palm oil and camellia oil.

FUTURE OUTLOOK: CHALLENGES AND OPPORTUNITIES IN *sn*-2 PALMITATE SYNTHESIS

Betapol is commercial *sn*-2 palmitate developed by Lodders Croklaan in 1995 through acidolysis of the tripalmitin-rich palm stearin with oleic acid from high oleic sunflower oil using Lipozyme RM IM as a biocatalyst (Wei *et al.*, 2020). Palm oil fractions will continue to be developed as a substrate for *sn*-2 palmitate production because of the following advantages; high palmitic acid content, abundant availability, and low price (Hasibuan, 2021a). In addition, palm oil is one vegetable oil that does not contain cholesterol (Gesteiro *et al.*, 2019). Palm stearin as a substrate in the *sn*-2 palmitate synthesis is very interesting. However, the melting point of palm stearin is relatively high, so in the enzymatic process, it is necessary to add organic solvents (such

as hexane) or be carried out at a sufficiently high reaction temperature (Jimenez *et al.*, 2010a; 2010b).

The addition of solvents can lead to an increase in production costs and potential toxicity. In addition, the use of solvents is not recommended in terms of food safety (Ferreira-Dias and Tecelão, 2014). Thus, the production of HMFS in solvent-free systems is preferred in terms of food safety, cost, environmental friendly and ease of product purification (Ferreira-Dias and Tecelão, 2014; Tecelão *et al.*, 2019). However, the reaction in a solvent-free system needs to be carried out at a high temperature (Hasibuan, 2021a), which affects the lipase stability. For this reason, the reaction in a solvent-free system can use immobilised enzymes, which have higher stability than the original free suspended enzymes. Enzyme immobilisation can prevent denaturation and leakage of enzymes so that the number of batches or the duration of synthesis can be increased (Adlercreutz, 2013).

Several studies reported that the use of commercial immobilised lipases such as lipases of Novozyme 435, Lipozyme TL IM, and Lipozyme RM IM in the synthesis of HMFS show good activity and stability (Hasibuan, 2021a). Jimenez *et al.* (2010a) reported that lipase *Alcaligenes* sp. immobilised on diatomaceous earth remained stable for at least 11 times using acidolysis between palm stearin and palmitic acid at a mole ratio of 1:3, reaction temperature of 65°C and reaction time of 24 hr. In addition, Esteban *et al.* (2011) reported that the lipase *Rhizopus oryzae* immobilised on Accurel MP1000 remained stable for at least ten times usage in acidolysis between palm stearin high in palmitic acid at the *sn*-2 position and oleic acid at a mole ratio of 1:6, 50°C for 19 hr. Exploring new biocatalysts with high catalytic activity and operational stability through isolation and genetic engineering is of interest for future research (Wei *et al.*, 2020). Efficient and stable biocatalysts will reduce operating costs (Tecelão *et al.*, 2019).

In contrast to lard, catfish oil, and butterfat oil, palm stearin has high *sn*-1,3 palmitic acid, requiring a high ratio of fatty acyl donors. The use of a high acyl donor ratio is unattractive because of the difficulty of the separation process (Zou *et al.*, 2016b), so the cost for separation after processing is high (Zhang *et al.*, 2016). Thus, it is necessary to develop specific lipases to synthesise palm-based HMFS using palm stearin with low acyl donor ratios. Faustino *et al.* (2016) reported that the acidolysis between tripalmitin and fatty acids from camelina oil at a low fatty acid mole ratio of 1:1.2 using lipase *Rhizopus oryzae* immobilised on Lewatit VPOC 1600 at a reaction temperature of 65°C could use up tripalmitin at 62.7% w/w.

Specialised treatments such as crystallisation fractionation are important to enrich palmitic acid at the *sn*-2 position. Acyl donors (MUFA, MCFA,

and PUFA) will continue to be explored to produce palm-based HMFS resembling human milk fat (Hasibuan *et al.*, 2021c). In addition, palm-based HMFS formulation for infant formula needs to be developed according to the baby's needs (age and condition) and regulations related to infant formula. Commercial formula is divided into three stages depending on the age of the baby, namely infant formula (0-6 months, stage 1), follow-up formula (6-12 months, stage 2), growth formula (12-36 months, stage 3). The rules of the formulas of the various stages may differ (Wei *et al.* (2019).

The relevant regulations enacted by several authorities to regulate infant formula include the Codex Alimentarius Commission (CAC), the US Food and Drug Administration (FDA), the European Commission (EC), and the National Health Commission of the People's Republic of China (NHC). CAC, EC and NHC require that α -linolenic acid is required to be more than 50 mg/100 kcal, the amount of lauric acid and myristic acid should not exceed 20.0% of the total fatty acids, *trans*-fatty acids should be less than 3.0% of the total fatty acids, erucic acid should be less than 1.0% of total fatty acids and eicosapentaenoic acid levels should be no more than docosahexaenoic acid levels. Docosahexaenoic acid is recommended as the essential constituent (4.8-12 mg/100 kJ). The EC and NHC recommend that docosahexaenoic acid do not exceed 2.0% and 0.5% of total fatty acids, respectively, and arachidonic acid levels should not exceed 1.0%. The EC recommended that the ω -3 and ω -6 LCPUFAs be less than 1.0% and 2.0% of the total fatty acids, while the CAC allowed the addition of LCPUFAs, respectively. CAC requires no commercial hydrogenated fats and oils to be used as raw materials. The EC also states that sesame seed oil and cottonseed oil are not allowed in infant formula because of potential allergens (Wei *et al.*, 2019).

CONCLUSION

Primarily, palm stearin is used as a substrate for *sn*-2 palmitate synthesis for HMFS. Triacylglycerol rich in palmitic acid at the *sn*-2 position of palm stearin needs to be increased to be an excellent substrate for the production of HMFS with palmitic acids at the *sn*-2 position >60%. The selection of enzyme types and loading, substrate types, synthesis methods, and reactor configuration is essential to improve the efficiency of HMFS synthesis. Generally, the optimal reaction conditions for palm-based *sn*-2 palmitate synthesis in a batch reactor are enzyme load of 8%-10% (w/w of the total substrate), temperature of 40°C-60°C, and reaction time of 3-24 hr. The OPO content in palm-based HMFS produced by enzymatic interesterification can be increased using fractionation. The challenge in HMFS synthesis is

the high production cost. In addition, the resulting product must resemble HMF, hence the complete set of acyl donors from fatty acids such as MUFA, MCFA and PUFA must be present in HMF. The reduction in the production cost of HMFS can be accomplished through the exploration of acyl donors and novel lipase enzymes with high catalytic activity and stability at a low cost.

ACKNOWLEDGEMENT

The author wishes to thank the Indonesian Oil Palm Research Institute.

REFERENCES

- Adlercreutz, P (2013). Immobilisation and application of lipases in organic media. *Chem. Soc. Rev.*, 42(13): 6406-6436. DOI: 10.1039/c3cs35446f.
- Araújo, M E M B; Campos, P R B; Alberto, T G; Contesini, F J and Carvalho, P D O (2016). Synthesis of structured triacylglycerols enriched in *n*-3 fatty acids by immobilized microbial lipase. *Braz. J. Microbiol.*, 47(4): 1006-1013. DOI: 10.1016/j.bjm.2016.07.003.
- Bourlieu, C; Bouhallab, S and Lopez, C (2009). Biocatalyzed modifications of milk lipids: Applications and potentialities. *Trends Food Sci. Technol.*, 20(10): 458-469. DOI: 10.1016/j.tifs.2009.05.005.
- Chen, Y; Zhang, X; Li, D; Yi, H; Xu, T; Li, S and Zhang, L (2019). Fatty acid and triacylglycerol comparison of infant formulas on the Chinese market. *Int. Dairy J.*, 95: 35-43. DOI: 10/1016/j.idairyj.2019.02.017.
- Codex Alimentarius (2001). *Codex Standard for Named Vegetable Oils*. CX-STAN 210-1999. Vol. 8-2001. https://www.fao.org/fao-who-codexalimentarius/sh-proxy/ar/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252Fstandards%252FCXS%2B210-1999%252FCXS_210e.pdf, assessed on 20 July 2021.
- Esteban, L; Jiménez, M J; Hita, E; González, P A; Martín, L and Robles, A (2011). Production of structured triacylglycerols rich in palmitic acid at *sn*-2 position and oleic acid at *sn*-1,3 positions as human milk fat substitutes by enzymatic acidolysis. *Biochem Eng. J.*, 54: 62-69. DOI: 10.106/j.bej.2011.01.009.
- Faustino, A R; Osório, N M; Tecelão, C; Canet, A; Valero, F and Ferreira-Dias, S (2016). Camelina oil as a source of polyunsaturated fatty acids for the production of human milk fat substitutes catalyzed by a heterologous *Rhizopus oryzae* lipase. *Eur. J. Lipid Sci. Technol.*, 118: 532-544. DOI: 10.1002/ejlt.201500003.
- Fernandez-Lafuente, R (2010). Lipase from *Thermomyces lanuginosus*: Uses and prospects as an industrial biocatalyst. *J. Mol. Catal. B Enzym.*, 62: 197-212. DOI: 10.1016/j.molcatb.2009.11.010.
- Ferreira-Dias, S and Tecelão, C (2014). Human milk fat substitutes: Advances and constraints of enzyme-catalyzed production. *Lipid Technol.*, 26(8): 183-185. DOI: 10.1002/lite.201400043.
- Ferreira-Dias, S; Osório, N M; Rodrigues, J and Tecelão, C (2019). Structured lipids for foods. *Encyclopedia of Food Chemistry* (Melton, L; Shahidi, F and Varelis, P eds.). p. 357-369. DOI: 10.1016/B978-0-08-100596-5.21766-6.
- Gesteiro, E; Guijarro, L; Sánchez-Muniz, F J; Vidal-Carou, M C; Troncoso, A; Venanci, L; Jimeno, V; Quilez, J; Anadón, A and González-Gross, M (2019). Palm oil on the edge. *Nutrients*, 11 (2008): 1-36. DOI: 10.3390/nu11092008.
- Ghosh, M; Sengupta, A; Bhattacharya, D K and Ghosh, M (2016). Preparation of human milk fat analogue by enzymatic interesterification reaction using palm stearin and fish oil. *J. Food Sci. Technol.*, 53(4): 2017-2024. DOI: 10.1007/s13197-016-2180-5.
- Gibon, V; Ayala, J V; Dijckmans, P; Maes, J and Greyt, W D (2009). Future prospects for palm oil refining and modifications. *OCL*, 16(4): 193-200. DOI: 10.1051/ocl.2009.0268.
- Hasibuan, H A and Siahaan, D (2013). Characteristic of CPO, palm kernel oil and its fractions. *Pocket Book Series 30*. Indonesian Oil Palm Research Institute (IOPRI) Indonesia.
- Hasibuan, H A and Ijah (2016). Synthesis and microencapsulation of palm oil based human milk fat substitute. *J. Technology and Food Industry*, 27(2): 105-114. DOI: 10.6066/jtip.2016.27.2.105 (in Indonesia).
- Hasibuan, H A (2021a). Synthesis of lipids rich in 1,3-dioleoyl-2- palmitoyl-glycerol (OPO) from hard palm stearin. Dissertation. IPB University Bogor Indonesia.
- Hasibuan, H A; Sitanggang, A B; Andarwulan, N and Hariyadi, P (2021b). Solvent fractionation of hard palm stearin to increase the concentration of tripalmitoylglycerol and dipalmitoyl-stearoylglycerol as substrates for human milk fat substitute

synthesis. *Int. J. Food Sci. Technol.*, 56: 4549-4558. DOI: 10.1111/ijfs.15206.

Hasibuan, H A; Sitanggang, A B; Andarwulan, N and Hariyadi, P (2021c). Enzymatic synthesis of human milk fat substitute - A review on technological approaches. *Food Technol. Biotechnol.*, 59(4): 475-495. DOI: 10.17113/ftb.59.04.21.7205.

Hassim, N A M; Ismail, N H and Dian, N L H M (2018). Enzymatic interesterification of palm fractions for the production for the production of cocoa butter alternatives. *J. Oil Palm Res.*, 30(4): 537-547. DOI: 10.21894/jopr.2018.0038.

He, Y; Qiu, C; Guo, Z; Huang, J; Wang, M and Chen, B (2017). Production of new human milk fat substitutes by enzymatic acidolysis of microalgae oils from *Nannochloropsis oculata* and *Isochrysis galbana*. *Bioresour. Technol.*, 238: 129-138. DOI: 10.1016/j.biortech.2017.04.041.

Ibrahim, N H; Idris, N A; Abdullah, A; Said, M; Dian, N L H M and Man, Y C (2006). Polymorphism of anhydrous milk fat as affected by the addition of palm stearin. *J. Oil Palm Res.*, Special Issue-April 2006: 58-64.

Ilyasoglu, H; Gultekin-Ozguven, M and Ozcelik, B (2011). Production of human milk fat substitute with medium-chain fatty acids by lipase-catalyzed acidolysis: Optimization by response surface methodology. *LWT - Food Sci. Technol.*, 44: 999-1004. DOI: 10.1016/j.lwt.2010.11.027.

Ilyasoglu, H (2013). Production of human milk fat analogue containing α -linolenic acid by solvent-free enzymatic interesterification. *LWT- Food Sci. Technol.*, 54: 179-185. DOI: 10.1016/j.lwt.2013.05.036.

Iwasaki, Y and Yamane, T (2000). Enzymatic synthesis of structured lipids. *J. Mol. Catal. B: Enzym.*, 10: 129-140.

Jala, R C R and Kumar, C G (2018). Designer and functional food lipids in dietary regimes: Current trends and future prospects. *Alternative and Replacement Foods* (Holban, A M and Grumezescu, A M eds.). Elsevier Inc. Massachusetts, USA. p. 283-316.

Jiménez, M J; Esteban, L; Robles, A; Hita, E; González, P A; Muñío, M M and Molina, E (2010a). Production of triacylglycerols rich in palmitic acid at position 2 as intermediates for the synthesis of human milk fat substitutes by enzymatic acidolysis. *Process Biochem.*, 45: 407-414. DOI: 10.1016/j.probio.2009.10.018.

Jiménez, M J; Esteban, L; Robles, A; Hita, E; González, P A; Muñío, M M and Molina, E (2010b). Production of triacylglycerols rich in palmitic acid at *sn*-2 position by lipase-catalyzed acidolysis. *Biochem. Eng. J.*, 51: 172-179. DOI: 10.1016/j.bej.2010.06.015.

Kapoor, M and Gupta, M N (2012). Lipase promiscuity and its biochemical applications. *Process Biochem.*, 47: 555-569. DOI: 10.1016/j.procbio.2012.01.011.

Karabulut, I; Turan, S; Vural, H and Kayahan, M (2007). Human milk fat substitute produced by enzymatic interesterification of vegetable oil blend. *Food Technol. Biotechnol.*, 45(4): 434-438.

Karouw, S; Suparmo, P; Hastuti and Utami, T (2012). High lauric human milk fat analog prepared from palm stearin and coconut oil by enzymatic interesterification. *African J. Food Sci.*, 6(17): 441-448. DOI: 10.5897/AJFS12.090.

Koo, W W K; Hockman, E M and Dow, M (2006). Palm olein in the fat blend in infant formulas: Effect on the intestinal absorption of calcium and fat, and bone mineralization. *J. Am. Coll. Nutr.*, 25(2): 117-122.

Lasekan, J B; Hustead, D S; Masor, M and Murray, R (2017). Impact of palm olein in infant formulas on stool consistency and frequency: A meta-analysis of randomized clinical trials. *Food Nutr. Res.*, 61(1): 1-11. DOI: 10.1080/16546628.2017.1330104.

Lee, J H; Son, J M; Akoh, C C; Kim, M R and Lee, K T (2010). Optimized synthesis of 1,3-dioleoyl-2-palmitoylglycerol-rich triacylglycerol via interesterification catalyzed by a lipase from *Thermomyces lanuginosus*. *New Biotechnol.*, 27(1): 38-45. DOI: 10.1016/j.nbt.2009.10.006.

Lee, N K; Oh, S W; Kwon, D Y and Yoon, S H (2015). Production of 1,3-dioleoyl-2-palmitoyl glycerol as a human milk fat substitute using enzymatic interesterification of natural fats and oils. *Food Sci. Biotechnol.*, 24(2): 433-437. DOI: 10.1007/s10068-015-0057-4.

Liu, X; Wang, X; Pang, N; Zhu, W; Zhao, X; Wang, F; Wu, F and Wang, J (2015). APA-style human milk fat analogue from silkworm pupae oil: Enzymatic production and improving storage stability using alkyl caffeates. *Sci. Rep.*, 5(17909): 1-15. DOI: 10.1038/srep17909.

Liu, S; Dong, X; Wei, F; Wang, X; Lv, X; Wu, L; Quek, S Y and Chen, H (2017). Lipase catalyzed synthesis of ABA-type structured lipid from single cell oil and

- tripalmitin. *J. Food Process. Preserv.*, 41: e12843. DOI: 10.1111/jfpp.12843.
- Mat Dian, N L H; Hamid, R A; Kanagaratnam, S; Isa, W R A; Hassim, N A M; Ismail, N H; Omar, Z and Sahri, M M (2017). Palm oil and palm kernel oil: Versatile ingredients for food applications. *J. Oil Palm Res.*, 29(4): 487-511. DOI: 10.21894/jopr.2017.00014.
- Monaco, M H; Kim, J and Donovan, S M (2016). Human milk: Composition and nutritional value. *Encyclopedia of Food and Health* (Caballero, B; Finglas, P M and Toldrá, F eds.). Elsevier Inc. Massachusetts, USA. p. 357-362. DOI: 10.1016/B978-0-12-384947-2.00413-X.
- Nagachinta, S and Akoh, C C (2012). Enrichment of palm olein with long chain polyunsaturated fatty acids by enzymatic acidolysis. *LWT- Food Sci. Technol.*, 46: 29-35. DOI: 10.1016/j.lwt.2011.10.027.
- Nagachinta, S and Akoh, C C (2013). Production and characterization of DHA and GLA-enriched structured lipid from palm olein for infant formula use. *J. Am. Oil Chem. Soc.*, 90(8): 1141-1149. DOI: 10.1007/s11746-013-2255-7.
- Nielsen, N S; Yang, T; Xu, X and Jacobsen, C (2006). Production and oxidative stability of a human milk fat substitute produce from lard by enzyme technology in a pilot packed-bed reactor. *Food Chem.*, 94: 53-60. DOI: 10.1016/j.foodchem.2004.10.049.
- Ortiz, C; Ferreira, M L; Barbosa, O; Santos, J C S; Rodrigues, R C; Berenguer-Murcia, A; Briand, L E and Fernandez-Lafuente, R (2019). Nozovyme 435: the “perfect” lipase immobilized biocatalyst? *Catal. Sci. Technol.*, 9: 2380-2420. DOI: 10.1039/c9cy00415g.
- Pacheco, C; Crapeste, G H and Carrín, M E (2015). Study of acyl migration during enzymatic interesterification of liquid and fully hydrogenated soybean oil. *J. Mol. Catal. B: Enzym.*, 122: 117-124. DOI: 10.1016/j.molcatb.2015.08.023.
- Pina-Rodriguez, A M and Akoh, C C (2009). Enrichment of amaranth oil with ethyl palmitate at the *sn*-2 position by chemical and enzymatic synthesis. *J. Agric. Food Chem.*, 57: 4657-4662. DOI: 10.1021/jf900242g.
- Qin, X L; Zhong, J F; Wang, Y H; Lan, D M and Wang, F H (2014). 1,3-dioleoyl-2-palmitoylglycerol-rich human milk fat substitutes: Production, purification, characterization and modeling of the formulation. *Eur. J. Lipid Sci. Technol.*, 116: 282-290. DOI: 10.1002/ejlt.201300343.
- Robles, A; Jiménez, M J; Esteban, L; González, P A; Martín, L; Rodríguez, A and Molina, E (2011). Enzymatic production of human milk fat substitute containing palmitic and docosahexanoic acids at *sn*-2 position and oleic acid at *sn*-1,3 positions. *LWT-Food Sci. and Technol.*, 44: 1986-1992. DOI: 10.1016/j.lwt.2011.05.022.
- Rodrigues, R C and Fernandez-Lafuente, R (2010). Lipase from *Rhizomucor miehei* as a biocatalyst in fats and oils modification. *J. Mol. Catal. B: Enzym.*, 66: 15-32. DOI: 10.1016/j.molcatb.2010.03.008.
- Rønne, T H; Yang, T; Mu, H; Jacobsen, C and Xu, X (2005). Enzymatic interesterification of butterfat with rapeseed oil in a continuous packed bed reactor. *J. Agric. Food Chem.*, 53: 5617-5624.
- Sahin, N; Akoh, C C and Karaali, A (2005a). Enzymatic production of human milk fat substitutes containing γ -linolenic acid: Optimization of reactions by response surface methodology. *J. Am. Oil Chem. Soc.*, 82(8): 549-557.
- Sahin, N; Akoh, C C and Karaali, A (2005b). Lipase-catalyzed acidolysis of tripalmitin with hazelnut oil fatty acids and stearic acid to produce human milk fat substitutes. *J. Agric. Food Chem.*, 53: 5779-5783.
- Sen, P; Nath, A and Bhattacharjee, C (2016). Packed-bed bioreactor and its application in dairy, food, and beverage industry. *Current Developments in Biotechnology and Bioengineering: Bioprocesses, Bioreactors and Controls* (Larroche, C; Sanroman, M; Du, G and Pandey, A eds.). Elsevier Inc. Massachusetts, USA. p. 235-277. DOI: 10.1016/B978-0-444-63663-8.00009-4.
- Silva, R C; Soares, F A S D M; Hazzan, M; Capacla, I R; Gonçalves, M I A and Gioielli, L A (2012). Continuous enzymatic interesterification of lard and soybean oil blend: Effects of different flow rates on physical properties and acyl migration. *J. Mol. Catal. B: Enzym.*, 76: 23-28. DOI: 10.1016/j.molcatb.2011.11.021.
- Soumanou, M M; Pérignon, M and Villeneuve, P (2013). Lipase-catalyzed interesterification reactions for human milk fat substitutes production: A review. *Eur. J. Lipid Sci. Technol.*, 115: 270-285. DOI: 10.1002/ejlt.201200084.
- Sørensen, A D M; Xu, X; Zhang, L; Kristensen, J B and Jacobsem, C (2010). Human milk fat substitute from butterfat: Production by enzymatic interesterification and evaluation of oxidative stability. *J. Am. Oil Chem. Soc.*, 87: 185-194.

- Speranza, P and Macedo, G A (2012). Lipase mediated production of specific lipids with improved biological and physical properties. *Process Biochem.*, 47: 1699-1706. DOI: 10.1016/j.prcbio.2012.07.006.
- Tan, C P and Che Man, Y B (2002). Differential scanning calorimetric analysis of palm oil, palm oil based products and coconut oil: Effects of scanning rate variation. *Food Chem.*, 76: 89-102.
- Tecelão, C; Silva, J; Dubreucq, E; Ribeiro, M H and Ferreira-Dias, S (2010). Production of human milk fat substitutes enriched in omega-3 polyunsaturated fatty acids using immobilized commercial lipases and *Candida parapsilosis* lipase/acyltransferase. *J. Mol. Catal. B: Enzym.*, 65: 122-127. DOI: 10.1016/j.molcatb.2010.01.026.
- Tecelão, C; Perrier, V; Dubreucq, E and Ferreira-Dias, S (2019). Production of human milk fat substitutes by interesterification of tripalmitin with ethyl oleate catalyzed by *Candida parapsilosis* lipase/acyltransferase. *J. Am. Oil Chem. Soc.*, 96: 777-787. DOI: 10.1002/aocs.12250.
- Turan, D; Yeşilçubuk, N S and Akoh, C C (2013). Enrichment of *sn*-2 position of hazelnut oil with palmitic acid: optimization by response surface methodology. *LWT- Food Sci. Technol.*, 50: 766-772. DOI: 10.1016/j.lwt.2012.07.009.
- Wang, Y H; Qin, X L; Zhu, Q S; Zhou, R; Yang, B and Li, L (2010). Lipase-catalyzed acidolysis of lard for the production of human milk fat substitute. *Eur. Food Res. Technol.*, 230: 769-777. DOI: 10.1007/s00217-010-1218-6.
- Wang, J; Liu, X; Wang, X D; Dong, T; Zhao, X Y; Zhu, D; Mei, Y Y and Wu, G H (2016). Selective synthesis of human milk fat-style structured triacylglycerides from micro algal oil in a microfluidic reactor packed with immobilized lipase. *Bioresour. Technol.*, 220: 132-141. DOI: 10.1016/j.biortech.2016.08.023.
- Wang, X; Zou, S; Miu, Z; Jin, Q and Wang, X (2019). Enzymatic preparation of structured triacylglycerols with arachidonic and palmitic acids at the *sn*-2 position for infant formula use. *Food Chem.*, 283: 331-337. DOI: 10.1016/j.foodchem.2019.01.057.
- Wang, X; Jiang, C; Xu, W; Miu, Z; Jin, Q and Wang, X (2020). Enzymatic synthesis of structured triacylglycerols rich in 1,3-dioleoyl-2-palmitoylglycerol and 1-oleoyl-2-palmitoyl-3-linoleoylglycerol in a solvent free system. *LWT- Food Sci. Technol.*, 118: 108798. DOI: 10.1016/j.lwt.2019.108798.
- Wei, W; Jin, Q and Wang, X (2019). Human milk fat substitutes: Past achievements and current trends. *Prog. Lipid Res.*, 74: 69-86. DOI: 10.1016/j.plipres.2019.02.001.
- Wei, W; Sun, C; Wang, X; Jin, Q; Xu, X; Akoh, C C and Wang, X (2020). Lipase-catalyzed synthesis of *sn*-2 palmitate: A review. *Eng.*, 6: 406-14. DOI: 10.1016/j.eng.2020.02.008.
- Yang, T; Xu, X; He, C and Li, L (2003). Lipase-catalyzed modification of lard to produce human milk fat substitutes. *Food Chem.*, 80: 473-481.
- Yüksel, A and Yeşilçubuk, N S (2012). Enzymatic production of human milk fat analogues containing stearidonic acid and optimization of reactions by response surface methodology. *LWT- Food Sci. Technol.*, 46: 210-216. DOI: 10.1016/j.lwt.2011.10.004.
- Zhang, X; Nie, K; Zheng, Y; Wang, F; Dieng, L and Tan, T (2016). Lipase *Candida* sp. 99-125 coupled with β -cyclodextrin as additive synthesized the human milk fat substitutes. *J. Mol. Catal. B: Enzym.*, 125: 1-5. DOI: 10.1016/j.molcatb.2015.12.009.
- Zheng, M; Wang, S; Xiang, X; Shi, J; Huang, J; Deng, Q; Huang, F and Xiao, J (2017). Facile preparation of magnetic carbon nanotubes-immobilized lipase for highly efficient of 1,3-dioleoyl-2-palmitoylglycerol-rich human milk fat substitutes. *Food Chem.*, 228: 476-483. DOI: 10.1016/j.foodchem.2017.01.129.
- Zou, X Q; Huang, J H; Jin, Q Z; Liu, Y F; Song, Z H and Wang, X G (2011). Lipase-catalyzed preparation of human milk fat substitutes from palm stearin in a solvent-free system. *J. Agric. Food Chem.*, 59: 6055-6063. DOI: 10.1021/jf200619v.
- Zou, X Q; Huang, J H; Jin, Q Z; Liu, Y F; Tao, G J; Cheong, L Z and Wang, X G (2012a). Preparation of human milk fat substitutes from palm stearin with arachidonic and docosahexaenoic acid: Combination of enzymatic and physical methods. *J. Agric. Food Chem.*, 60: 9415-9423. DOI: 10.1021/jf3017354.
- Zou, X Q; Huang, J; Jin, Q; Liu, Y; Song, Z and Wang, X G (2012b). Lipase-catalyzed synthesis of human milk fat substitutes from palm stearin in a continuous packed bed reactor. *J. Am. Oil Chem. Soc.*, 89: 1463-1472. DOI: 10.1007/s11746-012-2046-6.
- Zou, X G; Hu, J N; Zhao, M L; Zhu, X M; Li, H Y; Liu, X R; Liu, R and Deng, Z Y (2014). Lipozyme RM IM-catalyzed acidolysis of *Cinnamomum*

camphora seed oil with oleic acid to produce human milk fat substitutes enriched in medium-chain fatty acids. *J. Agric. Food Chem.*, 62(43): 10594-10603. DOI: 10.1021/jf503691p.

Zou, X; Jin, Q; Guo, Z; Huang, J; Xu, X and Wang, X (2016a). Preparation of 1,3-dioleoyl-2-palmitoylglycerol-rich structured lipids from basa catfish oil: Combination of fractionation and enzymatic acidolysis. *Eur. J. Lipid Sci. Technol.*, 118: 708-715. DOI: 10.1002/ejlt.201500226.

Zou, X; Jin, Q; Guo, Z; Xu, X and Wang, X (2016b). Preparation of human milk fat substitutes from basa catfish oil: Combination of enzymatic acidolysis and modeled blending. *Eur. J. Lipid Sci. Technol.*, 118: 1702-1711. DOI: 10.1002/ejlt.201500591.

Zou, X; Ali, A H; Abed, S M and Guo, Z (2017). Current knowledge of lipids in human milk and recent innovations in infant formulas. *Curr. Opin. Food Sci.*, 16: 28-39. DOI: 10.1016/j.cofs.2017.06.010.

Strategus aloeus (COLEOPTERA: SCARABAEIDAE) DAMAGE AND ITS RELATIONSHIP WITH RAINFALL AND HYBRID OIL PALM AGE IN COLOMBIA

LUIS GUILLERMO MONTES-BAZURTO^{1*}; ALEX ENRIQUE BUSTILLO-PARDEY¹
 and ANUAR MORALES RODRIGUEZ¹

ABSTRACT

The adults of *Strategus aloeus* (L.) feed on the bulb and cause damage to young oil palm that can lead to lesions, and ultimately, to rotting. The aim of this study was to determine the *S. aloeus* damage in young hybrid oil palms, to propose an alternative method for its monitoring and control. Initially, destructive samplings of the palms were made at 8, 10, 12 and 15 days after insect attack. Additionally, weekly insect attack monitoring and rainfall register were made. About 44.4% of the oil palms were damaged after eight days of the adult's infestation. During the first year of the crop's establishment, there was a positive correlation between the number of galleries and rainfall. During the second and third year, there was a negative correlation between the number of galleries and oil palm age ($p=-0.93$; $p<0.0001$; $n=105$). Our study found that the damage caused by *S. aloeus* in hybrid cultivars can be observed eight days after the adult's arrival. The monitoring and control of *S. aloeus* in hybrid cultivars should be performed on a weekly basis from establishment to the first three and a half years of the crop.

Keywords: *Elaeis oleifera* x *Elaeis guineensis*, gallery, monitoring, rhinoceros beetle, rot.

Received: 26 May 2021; **Accepted:** 22 November 2021; **Published online:** 31 January 2022.

INTRODUCTION

The rhinoceros beetle *Strategus aloeus* (L.) (Coleoptera: Scarabaeidae) (Figure 1) is one of the most important pest in South America during the first few years of the establishment of oil palm crops (Ahumada *et al.*, 1995; Aldana-De La Torre *et al.*, 2017; Bedford, 1980; Chinchilla, 2003a). *Strategus aloeus* is a species with the largest geographic distribution within the genus *Strategus*, extending from the south of the United States to Brazil and Bolivia (Genty *et al.*, 1978; Kim and Brou, 2018; Ratcliffe, 1976). In Colombia, it has been reported

in replanting areas or in new crop establishments across all oil palm growing areas (Aldana-De La Torre *et al.*, 2010; Calvache and Gomez, 1991). In addition to oil palm crops, it also infests coconut (*Cocos nucifera* L.) (Arecales: Arecaceae) plantations (Sanchez and Ortiz, 1997).

Strategus aloeus adult male causes damage by building a gallery that extends close to the palm bulb (Aldana-De La Torre *et al.*, 2017; Bustillo-Pardey *et al.*, 2016; Chinchilla, 2003b; Pallares *et al.*, 2000) (Figure 2). Once the gallery is built, the male emits an aggregation pheromone, comprising a mixture of butanone, pentanone, and sec-butyl acetate (Rochat *et al.*, 2000) that attracts the female. Both the female and male feed on the petiole bases, bulb, and stem of the oil palm, causing a lesion (Aldana-De La Torre *et al.*, 2010; Aldana *et al.*, 2000; Chinchilla, 2003a).

In oil palm [*Elaeis guineensis* Jacq. (Arecales: Arecaceae)], the damage can reach the meristem

¹ Colombian Oil Palm Research Centre, Cenipalma, Palmar La Vizcaina Experimental Field, KM 132 Vía Puerto Araujo-La Lizama, Barrancabermeja, Santander, Colombia.

* Corresponding author e-mail: lgmontesb@unal.edu.co



Figure 1. Adult male of *Strategus aloeus*.

eight days after the arrival of the male, leading to the death of the young palm (of less than one year old). If this does not occur, the lesion can be the entry point for microorganisms that cause stem or basal rots. One of the factors that influence the increase in gallery numbers in young palms is the start of the rainy season (Ahumada *et al.*, 1995; Aldana-De La Torre *et al.*, 2010). Similarly, the replanting system and the organic matter disposal in the field seem to influence the population abundance of *S. aloeus*. Replanting methods that produce stacking of palm into the plots tend to favour an increase in beetle populations (Ávila *et al.*, 2014; Bustillo-Pardey *et al.*, 2016) because the *S. aloeus* larvae are saprophagous (Aldana-De La Torre *et al.*, 2010; Rivera-Gasperín and Escobar-Hernández, 2020).

The natural enemies of *S. aloeus* include the fungus *Metarhizium anisopliae* (Metchnikoff) Sorokin (Ascomycota: Hypocreales), which affects the larvae and adults and can be a promising alternative for pest control of this species (Bustillo-Pardey, 2014;

Bustillo-Pardey *et al.*, 2016; Valencia *et al.*, 2011), *Nudivirus oryctes* (Wang *et al.*, 2007); previously called *Baculovirus oryctes* Huger virus that is pathogenic to *S. aloeus* adults (Lomer, 1987), and entomopathogenic nematodes that affect the larvae of *S. aloeus* (Gómez and Sáenz, 2015). In addition, *Phileurus* sp. (Coleoptera: Scarabaeidae) a predator of the larvae and pupae of *S. aloeus* can be found in the same organic matter in which *S. aloeus* reproduces (Aldana-De La Torre *et al.*, 2010).

The management of *S. aloeus* in *E. guineensis* consists of eliminating the breeding sites (Bustillo-Pardey *et al.*, 2016; Ulloa *et al.*, 2010), monitoring and controlling the galleries in young palms on a weekly basis. Insecticides are commonly sprayed in each gallery to control the adult *S. aloeus*, and the sprayed gallery is subsequently covered with soil (Aldana-De La Torre *et al.*, 2010; Aldana *et al.*, 2000; Bustillo-Pardey *et al.*, 2016).

Currently, Latin American countries are affected by bud rot disease and there have been an increase in oil palm replanting with O×G hybrid cultivars (*E. oleifera* × *E. guineensis*). Currently, there is a lack of knowledge regarding the damage caused by *S. aloeus* and its behaviour on these cultivars. Therefore, this study was designed to determine the damage caused by *S. aloeus* and to propose an alternative method for its monitoring and control in young hybrid cultivars.

MATERIALS AND METHODS

Area of Study

The study was conducted in plots of Coarí × La Mé hybrid oil palm cultivar that were infested by *S. aloeus*, at the Palmas Monterrey plantation,

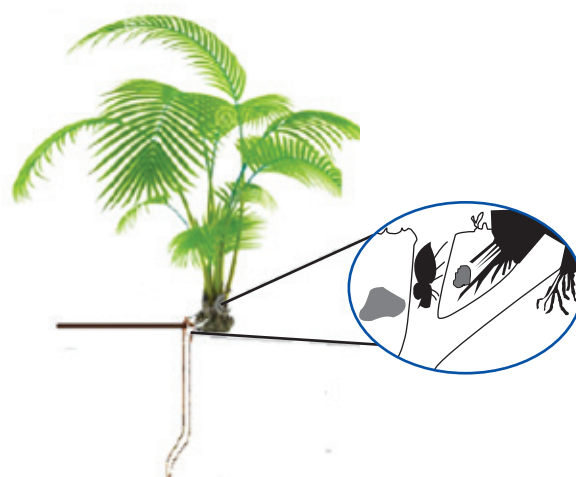


Figure 2. Galleries made by *Strategus aloeus* to palms during the first few years of crop establishment. Left image shows a real image of the galleries entrance beside of oil palm. Right image shows a graphical image of the gallery into the soil beside of oil palm.

in Puerto Wilches, Santander, Colombia, (7° 16' 14" North; 73° 50' 21" West) with the following climatic characteristics: Mean temperature of 29.5°C $\pm$ 5.9°C, 86.3% $\pm$ 19.8% relative humidity, 2152 mm of rainfall per year, and an elevation of 94 m above sea level.

Description of the Damage Caused by *S. aloeus*

Initially, 45 palms at 12 months of age were planted in an area that had a natural infestation of *S. aloeus*. The high infestation in the selected area of the plantation was due to the eradication method used when preparing the plot for replanting, which consisted of knocking down and stacking the eradicated palms. The eradication was performed three years prior to the start of this study.

After the establishment of the palms, daily monitoring was performed to document the arrival of adult *S. aloeus*. Once a gallery was found, the palm was marked and the evaluation of the treatments to the resulting damage was scheduled at 8, 10, 12 and 15 days after the arrival of the adults to the palm. There were nine replicates for each treatment and the experimental unit was the palm naturally infested by *S. aloeus*.

The evaluation was destructive, as it was necessary to remove the palm from its site to examine the entire bulb. The damage was documented following examination of the bulb; determination whether the damage in the petiole base or in the bulb, and the diameter of the lesion was measured. The information was analysed by determining the proportion of damage in the young oil palms (%), the mean diameter of the lesion and its confidence intervals (CI) with $\alpha=0.05$.

Strategus aloeus Infestation and Its Correlation with Rainfall and Palm Age

Two commercial plots of the O $\times$ G hybrid palm cultivar, Coarí $\times$ La Mé, were selected to study the infestation by *S. aloeus* at different times from the establishment of the crop. In the first plot (P1), 250 palms (2.2 ha) were monitored weekly during the first year of cultivation from the establishment in the plantation, and the number of galleries per palm was recorded for one year. Once a gallery was found, it was sprayed with chlorpyrifos (Lorsban, Dow AgroSciences. Bogotá, Colombia) at the rate of 2.4 g of active ingredient/gallery, and the gallery was subsequently covered with soil. To spray the insecticide, the boom of a hand-operated 20-L backpack sprayer (Royal Condor, Bogota, Colombia) was introduced into 5 cm of the gallery.

Simultaneously, in the second plot (P2), the infestation by *S. aloeus* was monitored during the second and third year from the establishment of the palm in 432 palms (3.8 ha) that were planted

18 months prior to the start of this study. In this plot, weekly control treatments were conducted for two years, recording the number of *S. aloeus* galleries, spraying them with chlorpyrifos using the same spraying protocols used in P1 at the rate of 2.4 g of active ingredient/gallery, and then covering them with soil. Simultaneously, the weekly rainfall was recorded for each plot.

The analysis of the presence of *S. aloeus* galleries, age of the palm (in weeks), and rainfall in the two study plots was performed using Spearman's correlations. Data analysis was performed separately for the two study plots (P1 and P2) because of the experimental design and the availability of similar plots with different ages in the same conditions of replanting management. A second analysis was made together to analyse the complete time period of evaluation (three years and seven months).

RESULTS AND DISCUSSION

Description of the Damage in Coarí $\times$ La Mé Hybrid Palms

After eight days of the infestation of adult *S. aloeus* to the palms, 44.4% of the palms were injured at the petiole bases, while all palms were damaged after 15 days of the adult's arrival (Table 1). Damage to the meristem was not observed in any of the evaluated palms (Figure 3). There were no differences in the diameter of the lesion caused by the adult *S. aloeus* between the days after their arrival to the palms (Figure 4).

After reviewing the information on damage caused by *S. aloeus* in oil palms in previous studies compared to this study, it was found that the damage caused by the adult *S. aloeus* in hybrid oil palms (Coarí $\times$ La Mé) and *E. guineensis* oil palms is similar in both cultivars. However, according to this study, the severity of the damage is lower in the hybrid palms as there were no death of palms occurred after 15 days of *S. aloeus* adults' infestation on the young palms. In *E. guineensis* palms, it has been reported that the damage to the meristem at

TABLE 1. PROPORTION OF DAMAGE IN YOUNG OIL PALMS CAUSED BY *Strategus aloeus* IN COARÍ $\times$ LA MÉ HYBRID OIL PALM CULTIVAR, RECORDED AFTER THE ARRIVAL OF THE ADULTS TO THE PALM IN FIELD CONDITIONS

Days after arrival of the adult	n	Proportion of palms with damage (%)
8	9	44.4
10	9	55.6
12	9	77.8
15	9	100.0

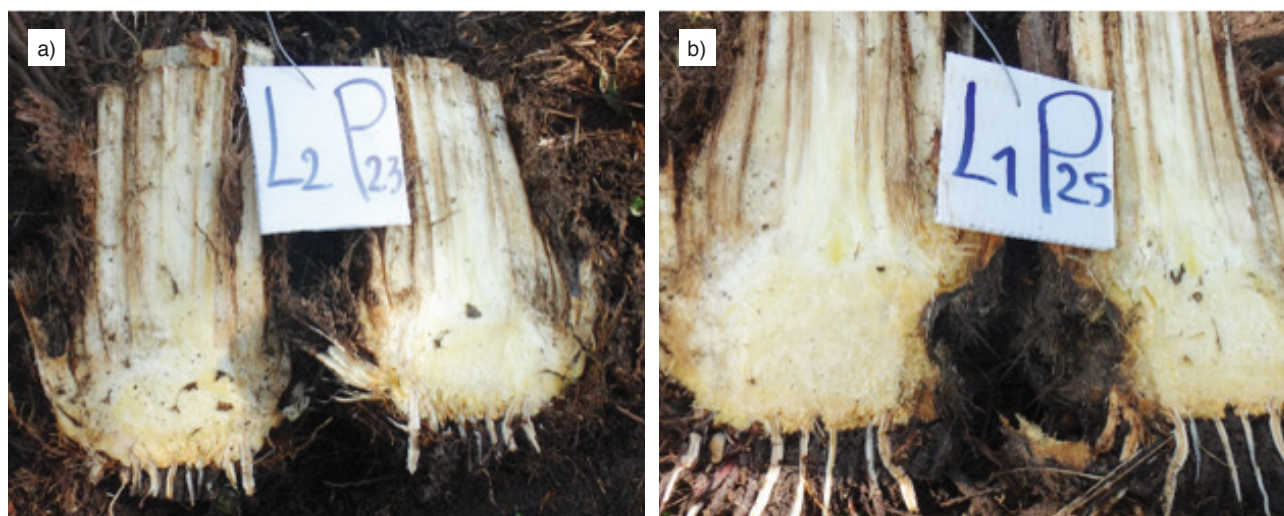


Figure 3. Bulbs of 12-month-old Coarí × La Mé hybrid palm planted in a plot infested with *Strategus aloeus*. a) Bulb with a vertical cut without damage. b) Bulb with a vertical cut with damage without affecting the meristem.

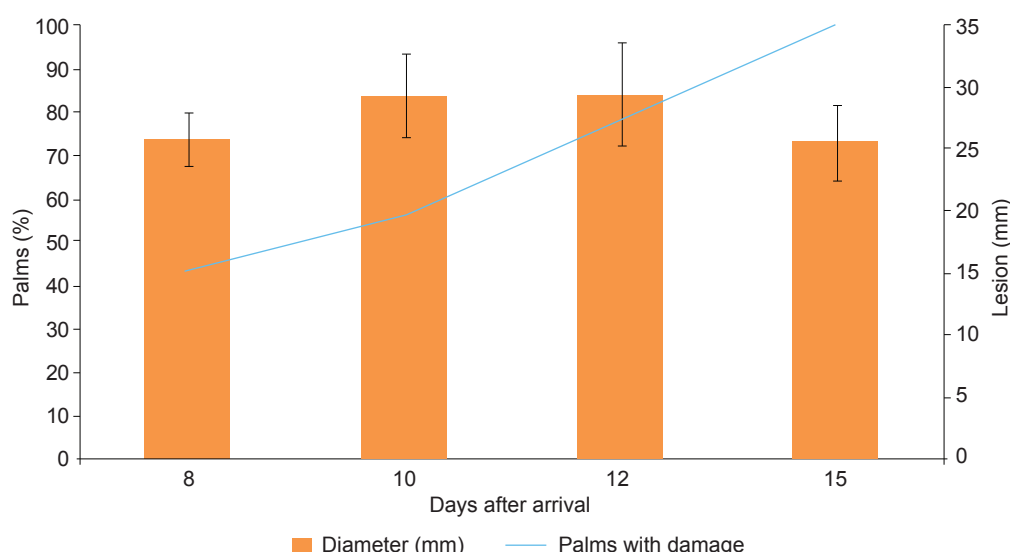


Figure 4. Mean diameter of the lesion and its standard deviation (SD) caused by *Strategus aloeus* in the petiole base of the Coarí × La Mé hybrid oil palm cultivar and the young oil palm proportion with damage; recorded between 8 and 15 days after the arrival of the adults to the palm at field conditions.

eight days after the arrival of the adult can lead to the death of the young palm (Aldana-De La Torre *et al.*, 2010; Genty *et al.*, 1978).

Furthermore, the lesions caused by *S. aloeus* in palms under the age of three years can be the entry point for pathogens that cause palm stem rots (Ahumada *et al.*, 1995; Aldana *et al.*, 2000; Bustillo-Pardey *et al.*, 2016). Therefore, it is necessary to control the galleries on a weekly basis in *E. guineensis* and hybrid cultivars (Morales Ipuz *et al.*, 2017). The difference in damage between both cultivars can be associated with the robustness of the tissues of the hybrid cultivars, by having less damage severity. However, it is necessary to conduct further studies to corroborate or rule out this hypothesis.

***Strategus aloeus* Infestation and its Correlation with Rainfall and Palm Age**

The first gallery built by *S. aloeus* was observed 22 days after the establishment of the palm in the P1. During the first year of establishment, there was a positive correlation between the number of galleries and rainfall ($\rho=0.44$; $p=0.0042$; $n=40$) (Figure 5). There was no correlation between the number of galleries and the age of the hybrid cultivar palm during the first year of the establishment of the crop ($\rho=0.18$; $p=0.2640$; $n=40$).

Based on the weekly controls conducted in the P2, there was no correlation between the number of galleries made by *S. aloeus* and the weekly rainfall ($\rho=0.05$; $p=0.5956$; $n=105$). However, there

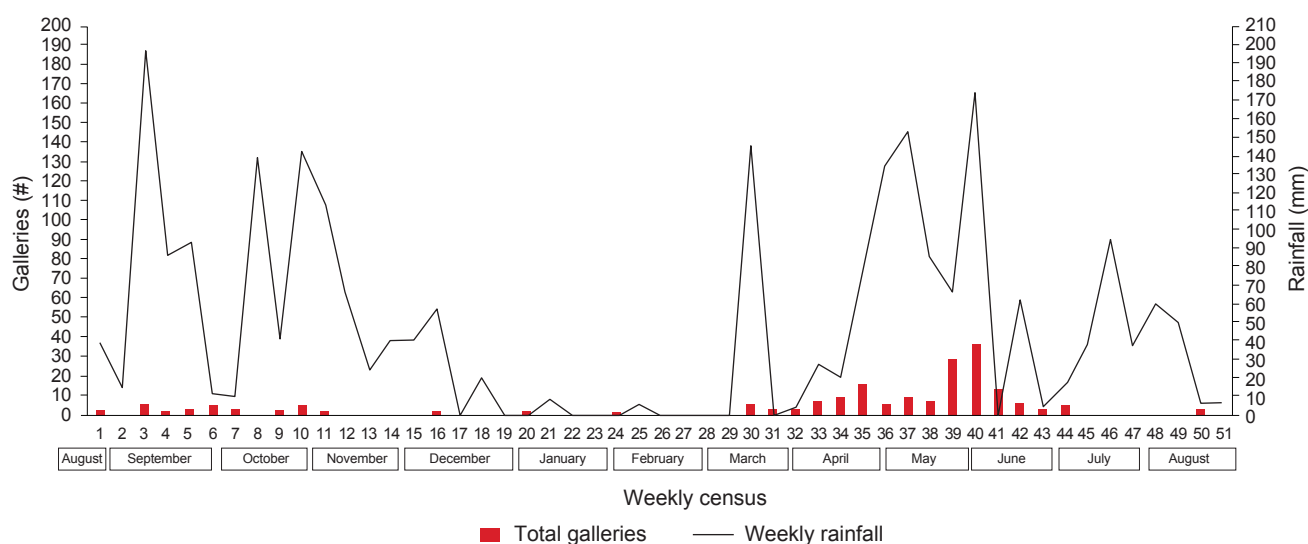


Figure 5. Total number of galleries of *Strategus aloeus* and rainfall, recorded weekly in plot 1 (P1) of Coarí × La Mé hybrid oil palm cultivar, which was planted at the start of the study.

was a negative correlation between the number of galleries and palm age ($\rho = -0.93$; $p < 0.0001$; $n = 105$). The last gallery was recorded 183 weeks (3 years 6 months) after the establishment of the crop (Figure 6).

In the analysis of all evaluation period from the establishment to the first 3 years 7 months of the crop in both plots, there was no correlation found between the number of galleries and the rainfall ($\rho = 0.12$; $p = 0.1453$; $n = 156$); and there was a negative correlation between the number of galleries and palm age ($\rho = -0.16$; $p = 0.0422$; $n = 156$). However, there was a difference in the abundance of the *S. aloeus* adult population in both plots. At 51 weeks after establishment, there were no galleries found in P1 compared to 85 galleries found in P2 at 52 weeks after establishment. These two plots had the

same cultivar and similar replanting management conditions. The only difference between them was the age and its *S. aloeus* natural infestation.

In the Coarí × La Mé hybrid cultivar, as in non-hybrid cultivars, *S. aloeus* infestations were reported in young palms during the first few years of cultivation (Aldana-De La Torre *et al.*, 2017; Bustillo-Pardey *et al.*, 2016). Therefore, the monitoring and control of this insect should be conducted starting from when the crop was established, up to three and a half years of the crop.

The positive correlation between the number of galleries constructed by adult *S. aloeus* and the rainfall recorded during the first few years of the establishment of the Coarí × La Mé hybrid oil palm (Figure 5) was consistent with that reported in *E. guineensis* oil palms, where an increase in

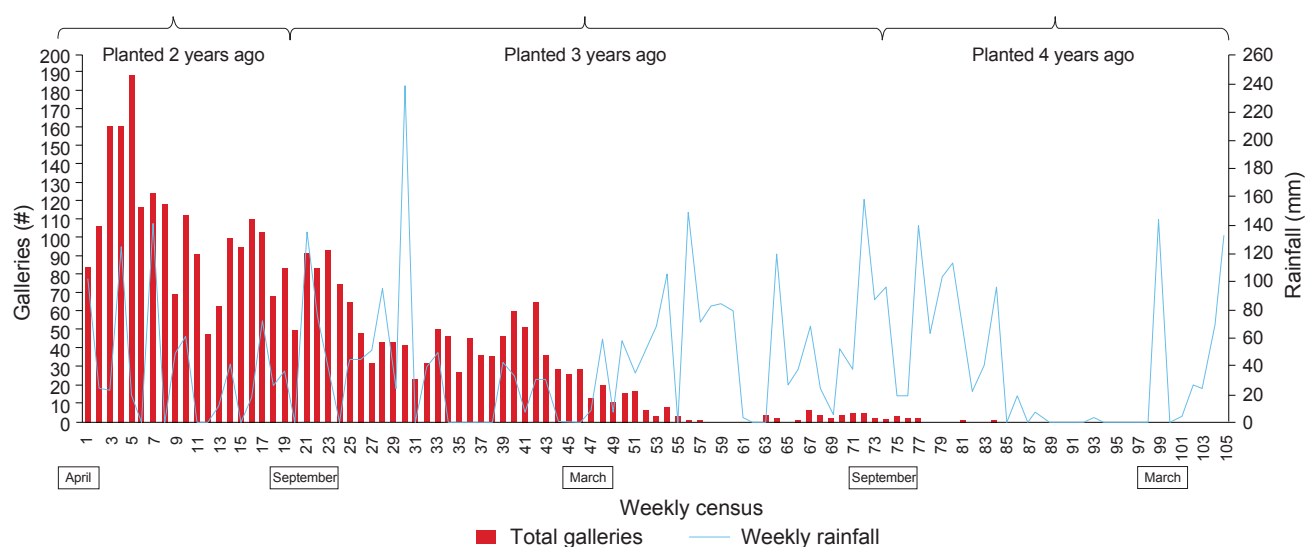


Figure 6. Total number of galleries of *Strategus aloeus* and rainfall recorded weekly in plot 2 (P2) of Coarí × La Mé hybrid oil palm cultivar, which was 18 months old at the start of the study.

the number of galleries after the start of the rainy season was observed (Ahumada *et al.*, 1995; Genty *et al.*, 1978). Furthermore, the results of this study are in agreement with other studies that show a positive correlation between *Diastocera trifasciata* (Fabricius) (Coleoptera: Cerambycidae) populations and rainfalls registered in cashew orchards (Akessé *et al.*, 2018) or between *Cosmopolites sordidus* Germar (Coleoptera: Curculionidae) and the rainfall registered in banana plantation in Africa (Uzakah and Olorunfemi, 2019).

However, after 18 months of the establishment of the crop, there was no association in the Coarí × La Mé hybrid cultivar between the number of galleries made by *S. aloeus* and rainfall (Figure 6), owing to the presence of overlapping generations of the insect where the females are permanently ovipositing in the waste. In other pest like *Oryctes owariensis* Beavois, 1807 (Coleoptera: Scarabaeidae: Dynastinae) or wood-boring Coleoptera [Bostrichidae and Curculionidae (Scolytinae and Platypodinae)], the population seem to increase during the dry season (less rainfalls) (Sanguansub *et al.*, 2020; Ukaroije and Abalis, 2020). In the same way, the rainfall can also cause larval mortality as in *Anthonomus pomorum* L. (Coleoptera: Curculionidae) (Popov and Dmitrieva, 2020), but according to this study, after 18 months of the hybrid palm crop establishment, the abundance of *S. aloeus* adults did not seem to be affected by the rainfall variation. The age of the palms (between 18 and 30 months) and high levels of *S. aloeus* populations seem to be the most important factors associated with damage in plots of the Coarí × La Mé hybrid oil palm cultivar.

CONCLUSION

The damage caused by *S. aloeus* in Coarí × La Mé hybrid cultivars was observed at eight days after the arrival of the adult males to the palm. The damage caused by *S. aloeus* in Coarí × La Mé hybrid cultivars was not lethal to the palm as it does not affect the meristem, even after 15 days of arrival of the adults to the palm. The monitoring and control of *S. aloeus* in Coarí × La Mé hybrid cultivars should be performed on a weekly basis since the establishment of the crop up to the first three and a half years of the crop, in order to avoid the pest from causing damage to the palm.

ACKNOWLEDGEMENT

The authors would like to thank Nestor Pulido, Agronomic Director and Enerilson Torrecillas (RIP), Chief of Plant Health of the Palmas Monterrey plantation for their support in developing this

study. They also thank the Oil Palm Development Fund and the Ministry of Agriculture and Rural Development of Colombia for financing this study.

REFERENCES

- Ahumada, F M L; Calvache, G H H; Cruz, C M A and Luque, Z J E (1995). *Strategus aloeus* (L.) (Coleoptera: Scarabaeidae): *Biología y comportamiento en Puerto Wilches (Santander)*. Palmas (Colombia), 16(3): 9-16.
- Akessé, E; Ouali-N'Goran, S-W; N'Dépo, O R and Tano, D K C (2018). Population fluctuation of *Diastocera trifasciata* (Fabricius, 1775) (Coleoptera: Cerambycidae), cashew branches girdler in the Brobo locality (Central Côte d' Ivoire). *J. Entomol. Zool. Stud.*, 6(5): 1064-1070.
- Aldana-De La Torre, R C; Aldana, J; Calvache, H and Franco, P (2010). *Manual de plagas de la palma de aceite en Colombia Bogota, Cenipalma*.
- Aldana-De La Torre, R C; Montes-Bazurto, L G; Barrios, C; Matabanchoy, J; Beltran, I J; Rosero, M and Bustillo-Pardey, A E (2017). *Guía de bolsillo para el reconocimiento de las plagas más frecuentes en la palma de aceite Bogota, Cenipalma*.
- Aldana, J; Pallares, C and Correa, N (2000). *Control químico de Strategus aloeus* (L.) (Coleoptera: Scarabaeidae). *Ceniavances* (Colombia), 67: 1-4.
- Ávila, R A; Bayona, C; Rincón, Á and Romero, H M (2014). Effect of replanting systems on populations of *Strategus aloeus* (L.) and *Rhynchophorus palmarum* (L.) associated with the oil palm O×G interespecific hybrid (*Elaeis oleifera* × *Elaeis guineensis*) in Southwestern Colombia. *Agron. Colomb.*, 32(2): 224-231.
- Bedford, G O (1980). Biology, ecology and control of palm Rhinoceros beetles. *Annu. Rev. Entomol.*, 25: 309-339.
- Bustillo-Pardey, A E (2014). *Manejo de insectos-plaga de la palma de aceite con énfasis en el control biológico y su relación con el cambio climático*. *Revista Palmas* (Colombia), 35(4): 66-77.
- Bustillo-Pardey, A E; Sarria, G; Aldana-De La Torre, R C and Arango, M (2016). *Guía de bolsillo para el reconocimiento y manejo de las principales enfermedades e insectos plaga en el cultivo de la palma de aceite Bogota, Fedepalma*.
- Calvache, H and Gomez, P L (1991). *Comportamiento de las plagas de la palma de aceite en Colombia durante 1990*. *Revista Palmas* (Colombia), 12(3): 7-14.

- Chinchilla, C (2003a). *Manejo Integrado de problemas fitosanitarios en palma aceitera Elaeis guineensis en América Central. Manejo Integr. Plagas y Agroecol.*, 67: 69-82.
- Chinchilla, C (2003b). *Manejo integrado de problemas fitosanitarios en palma de aceite Elaeis guineensis en America Central. Manejo Integr. Plagas y Agroecol.*, 67: 69-82.
- Genty, P; Desmier de Chenon, R and Morin, J P (1978). Oil palm pest in Latin America. *Oleagineux*, 33(7): 334-335.
- Gómez, A and Sáenz, A (2015). Susceptibility variation to different entomopathogenic nematodes in *Strategus aloeus* L. (Coleoptera: Scarabaeidae). *Springerplus*, 4(620): 1-5.
- Kim, J and Brou, V (2018). The genus *Strategus* Kirby (Coleoptera: Scarabaeidae: Dynastinae) in Louisiana. *South. Lepid. News*, 40(2): 100-105.
- Lomer, C J (1987). Infection of *Strategus aloeus* (L.) (Coleoptera: Scarabaeidae) and other Dynastinae with *Baculovirus oryctes*. *Bull. Entomol. Res.*, 77(1): 45-51.
- Morales Ipuz, L C; Neira Segura, Á L and Becerra-Encinales, J F (2017). *Aplicación de mejores prácticas fitosanitarias en el cultivo de la palma de aceite Bogota, Cenipalma*.
- Pallares, C; Aldana, J; Calvache, H; Ramírez, P; Rochat, D; Luque, E and Correa, N (2000). *Análisis del comportamiento y comunicación química intraespecífica en Strategus aloeus* (L.) (Coleoptera, Scarabaeidae - Dynastinae). *Revista Palmas (Colombia)*, 21(especial toml): 185-194.
- Popov, S Y and Dmitrieva, S V (2020). Life tables of the apple blossom weevil populations (*Anthonomus pomorum* L.) (Coleoptera, Curculionidae) on apple and pear trees in urbanized territories of Moscow and Tver provinces. *Entomol. Rev.*, 100(5): 577-586.
- Ratcliffe, B C (1976). A revision of the Genus *Strategus* (Coleoptera: Scarabaeidae). *Bull. Univ. Nebraska State Museum*, 10(3): 93-204.
- Rivera-Gasperín, S and Escobar-Hernández, F (2020). *Especies de Scarabaeidae (Coleoptera) del Cicolma, Veracruz, Mexico. Acta Zoológica Mex.*, 36: 1-19.
- Rochat, D; Ramirez-Lucas, P; Malosse, C; Aldana-De La Torre, R C; Kakul, T and Morin, J P (2000). Role of solid-phase microextraction in the identification of highly volatile pheromones of two Rhinoceros beetles *Scapanes australis* and *Strategus aloeus* (Coleoptera, Scarabaeidae, Dynastinae). *J. Chromatogr. A*, 885(1-2): 433-444.
- Sanchez, S and Ortiz, C (1997). *Presencia de Strategus aloeus L. (Scarabaeidae) en el estado de Tabasco, México Strategus aloeus L. (Scarabaeidae) en el estado de Tabasco, México. ASD Oil Palm Pap. (Costa Rica)*, 16: 31-34.
- Sanguansub, S; Buranapanichpan, S; Beaver, R A; Saowaphak, T; Tanaka, N and Kamata, N (2020). Influence of seasonality and climate on captures of wood-boring Coleoptera [Bostrichidae and Curculionidae (Scolytinae and Platypodinae)] using ethanol-baited traps in a seasonal tropical forest of Northern Thailand. *J. For. Res.*, 25(4): 223-231.
- Ukaroiye, R B and Abalis, R O (2020). Population dynamics and seasonal abundance of the beetle *Oryctes owariensis* beavois, UK 1807 (Coleoptera: Scarabaeidae: Dynastinae) in Raphia palms in Bayelsa State, Nigeria. *Asian J. Biol.*, 9(3): 14-27.
- Ulloa, A; Moya-Murillo, O M; Rincón, Á; Aldana-De La Torre, R C; Gomes de Oliveira, H; Ruiz, R and Ávila, R A (2010). *Dinámica de reproducción de Strategus aloeus bajo diferentes métodos de erradicación y daño causado en la nueva siembra. Ceniavance*, 166: 1-4.
- Uzakah, R P and Olorunfemi, D I (2019). Population dynamics of the plantain-banana weevil *Cosmopolites sordidus* Germar (Coleoptera: Curculionidae) in Bayelsa State, Southern Nigeria. *African Sci.*, 20(4): 193-199.
- Valencia, C; Pérez, S; Aldana-De La Torre, R C; Mesa, E and Gomes de Oliveira, H (2011). *Patogenicidad de hongos entomopatógenos del género Metarhizium sobre larvas de Strategus aloeus L. (Coleoptera: Scarabaeidae), en condiciones de laboratorio. Revista Palmas (Colombia)*, 32(4): 30-40.
- Wang, Y; Van Oers, M M; Crawford, A M; Vlak, J M and Jehle, J A (2007). Genomic analysis of *Oryctes rhinoceros* virus reveals genetic relatedness to *Heliothis zea* virus 1. *Arch. Virol.*, 152(3): 519-531.

CELL WALL-GLYCOLIPIDS PROFILING OF OIL PALM ROOTS DURING *Ganoderma boninense* INFECTION USING GAS CHROMATOGRAPHY-MASS SPECTROMETRY

ALEXANDER, A¹; ABDULLAH, S²; DAYOU, J³ and CHONG, K P^{4*}

ABSTRACT

Better understanding of the cell wall (CW)-glycolipids changes associated with basal stem rot (BSR) disease is essential for identifying the pathogen-host interaction to improve management and diagnostic measures. The aim of this study was to investigate the CW changes in the glycolipids profile of oil palm roots during *G. boninense* infection. We carried out lipidomic analysis of glycolipids fractionated from CW-lipids of oil palm seedlings artificially infected (AI) with *G. boninense*. Oil palm roots were harvested at three (first interval) and six months (second interval) post-AI from infected and control (uninfected) seedlings and were subjected to gas chromatography-mass spectrometry (GC-MS) based global lipidomic analysis. Principal component analysis (PCA) and partial least square-discriminant analysis (PLS-DA) confirmed 11 impaired glycolipids (six in the first and five in the second interval) associated to cell-signalling and break down of energy. Pyruvate metabolism and glycolysis or gluconeogenesis are the most perturbed pathways during the pathogenesis as revealed by pathway impact analysis. The possible utilisation of the glycolipids as biomarkers for diagnostic of *Ganoderma* infection was authenticated using the receiver operating characteristic (ROC) curve analysis. The current research suggests five glycolipids [Phosphatidylcholine (PC)(6:0/0:0), PC(2:0/2:0), Phosphatidic Acid (PA)(18:4(6Z,(Z,12Z,15Z)/0:0), PA(14:0/ 0:0) and γ -linolenic acid] as the potential biomarkers which may be further investigated for the early detection of BSR.

Keywords: cell wall-glycolipids profile, *Ganoderma boninense*, GC-MS, oil palm root.

Received: 2 June 2020; **Accepted:** 3 December 2021; **Published online:** 15 February 2022.

¹ Agrifert Malaysia Sdn. Bhd., Unit 11.01, Level 11, Mercu 2, No. 3, Jalan Bangsar, KL Eco City, 59200 Kuala Lumpur, Malaysia.

² Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia.

³ Vibration and Sound Research Group (e-VIBS), Faculty of Science and Natural Resources, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia.

⁴ Biotechnology Programme, Faculty of Science and Natural Resources, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia.

* Corresponding author e-mail: chongkp@ums.edu.my

INTRODUCTION

Worldwide oil palm production has seen a steady increase in the past years. Not only the production contribute to the majority of vegetable oils and fats, it also helps to alleviate poverty in developing countries especially in Indonesia and Malaysia. However, one of the major concerns of oil palm plantation in Southeast Asia is the basal stem rot (BSR) disease caused by *Ganoderma boninense* which could result in great loss (Tay and Chong, 2016). BSR is caused by white rot fungi, *Ganoderma* sp. The fungus grows within the palm, degrades the lignin and cellulose components of the oil palm (Mohd Aswad *et al.*, 2011).

Early detection and diagnosis of BSR disease is rather difficult with the current knowledge and technologies, due to late emergence of the BSR disease symptoms. The current detection technique was done by isolation of the fruiting bodies appearing on the oil palm trunks or primary roots near soil level followed by molecular identification of the isolated fungus (Madiah *et al.*, 2018). However, it is time consuming and by the time the foliar symptoms are observed, most of the tissues will have been killed by the fungus (Ariffin *et al.*, 2000). Nonetheless, the most common detection method in the field by planters is by observing the appearance of foliar symptoms or the presence of *Ganoderma* fruiting bodies. Other diagnostic tools for early detection of *G. boninense* include using *Ganoderma* Selective Medium (GSM) (Ariffin and Idris, 1992), some lab-based molecular and biochemical detection methods such as ergosterol analysis and immunoassay (Chong *et al.*, 2017). Recently, many researchers attempted to develop field detection methods, which include tomography (Idris *et al.*, 2009), Molecularly Imprinted Polymer (MIP) Sensors (Markon *et al.*, 2008) and hyperspectral reflectal data (Lelong *et al.*, 2010). Some emerging detection methods include the usage of infrared spectroscopy (Dayou *et al.*, 2014), ultrasonic (Ishaq *et al.*, 2014; Najmie *et al.*, 2011) and acoustics computed assisted tomography (Michael *et al.*, 2020). However, most of these detection methods are still at the experimental stage, and are not readily available to serve for the earlier detection purpose.

It is important to point out that early detection of BSR is crucial for better management of BSR in oil palm. An effective and robust approach is essential to achieve such objectives. The emerging lipidomics research could offer a great option in determining possible biomarkers and biochemical pathways for BSR early detection purpose. Meanwhile, plant and fungal non-cytoplasmic lipids compositions, such as those that reside in CW and cell membrane were continuously and dynamically change according to the biological, chemical and physical stresses. In oil palm-*Ganoderma* interaction, one of the promising examples is the utilisation of ergosterol. Ergosterol is the main component of the fungal membrane that is useful as biomarker for the quantification of fungal biomass (Chong *et al.*, 2014; Toh Choon *et al.*, 2012). The membrane lipid ergosterol is found almost exclusively in fungi and is frequently used by microbiologists as an indicator of living fungal biomass, based on the assumption that ergosterol is labile, therefore, rapidly degraded after the death of fungal hyphae (Mille-Lindblom *et al.*, 2004). The main advantage with ergosterol compared to other biomarkers, such as chitin and ATP is

its specific association with fungi. *G. boninense* biomass is reported to be directly correlated with ergosterol concentration; therefore, provides a reliable diagnostic method for the BSR detection (Alexander *et al.*, 2017; Chong *et al.*, 2012a; Mohd Aswad *et al.*, 2011). However, in the early stage of infection, ergosterol is difficult to be quantified due to the very small amount of fungal biomass that is present inside oil palm with addition of its labile properties. It is believed that other lipid composition, such as glycolipids are more stable than sterols, and unique due to their role in cell-cell signalling. If researchers are able to exploit the glycolipids information in plant CW and cell membrane especially during oil palm-*Ganoderma* interaction, they might be able to identify the presence of specific glycolipids compounds that are produced during early stage of infection. Therefore, the present work aimed to investigate further into glycolipids composition during oil palm-*Ganoderma* interaction. The emerging lipidomics field of research could offer a great option for determining possible biomarkers and biochemical pathways that can discriminate infected palms from those that are not infected, thus potentially providing important fundamental information for BSR early detection approach.

MATERIALS AND METHODS

Plant Material

Eight months-old oil palm commercial seedlings (*Dura* x *Pisifera*) were purchased from Sawit Kinabalu Sdn. Bhd., Beaufort, Sabah, Malaysia. The seedlings were grown in polybags (38 cm x 45 cm) with soil and sand mixture (2:1) and all seedlings were watered and fertilised using Granular NPK 15:15:6:4 following the standard agronomic practices.

Artificial Inoculation of Oil Palm Seedling using Rubber Wood Block (RWB) Technique

RWB were prepared from rubber wood. *G. boninense* UMSBR1a strain (Chong *et al.*, 2013) was inoculated to the RWB as inoculum for the artificial inoculation. The method of Khairudin (1991) was adopted for preparing the RWB (size of 6 x 6 x 12 cm) with *Ganoderma* inoculum. RWB were washed with clean water, kept in autoclavable polypropylene plastic bags and autoclaved for 2 hr at 121°C. Each of the plastic bags was added with 100 mL of sterile molten malt extract agar (MEA), and left to solidify after some time. Mycelial plugs with the size of 6 mm were excised from the edge of 14 days old *G. boninense* culture and inoculated on the RWB.

The *G. boninense*-inoculated RWB were tightly sealed and kept for eight weeks in the dark at a temperature of $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$ until fully colonised. Artificial inoculation was conducted by following Khairudin (1991). Oil palm seedlings were carefully removed from polybags and later kept seated on the *G. boninense*-inoculated RWB to ensure the roots were in direct contact with the inoculum. Twenty oil palm seedlings were inoculated with *Ganoderma* RWB while another 20 uninoculated served as healthy control. Extreme care was taken to minimise injury during inoculation and planting. All oil palm seedlings were placed and arranged in complete randomised blocks under shed house conditions with 70% of shade protection and regular watering.

Assessment of Disease Infection and Development

For the assessment of disease infection and development, method described by Japanis *et al.* (2021) was adopted with a slight modification. All the inoculated oil palm seedlings were destructively sampled to determine the colonisation and establishment of *G. boninense* at two intervals: first interval [three months after artificial inoculation (AI)] and second interval (six months after AI). Ten seedlings were destructively sampled for each of the sampling period. Root tissues from the seedlings were harvested and washed under tap water, soaked in 99% ethanol for 30 s before being dried under ambient air stream. The infection of *G. boninense* was detected based on the isolation and *Ganoderma* growth on GSM (Ariffin and Idris, 1992) and the presence of ergosterol (Chong *et al.*, 2012b). Disease incidence was calculated using the Equation (1) (Masood *et al.*, 2010):

$$\text{Disease incidence (DI)} = \frac{\text{Number of infected plants}}{\text{Total number of plants assessed}} \times 100 \quad (1)$$

Disease symptoms such as presence of fungal mass or fruiting bodies, yellowing or browning of leaf were determined using disease severity index (DSI) formula. DSI adopted from Abdullah *et al.* (2003) was used to determine the severity of the disease with score of 0 to 4 scales (Table 1). The DSI was calculated using the Equation (2):

$$\text{DSI} = \frac{\text{Number of seedlings in the rating} \times \text{rating number}}{\text{Total number of seedlings assessed} \times \text{highest rating}} \times 100 \quad (2)$$

To assess the internal symptoms of *Ganoderma* infection, the seedlings were cut longitudinally across the bole region. Bole damage was assessed based on the scale as described by Nursabrina *et al.* (2012) (Table 2).

The bole damage index was calculated based on the internal symptoms of the bole tissues using the Equation (3):

$$\text{Bole damage index} = \frac{\text{Number of seedlings in the rating} \times \text{rating number}}{\text{Total number of seedlings assessed} \times \text{highest rating}} \times 100 \quad (3)$$

TABLE 1. THE SIGNS AND SYMPTOMS OF DISEASE SEVERITY INDEX SCORED BASED ON DISEASE SCALE 0-4

Disease class	Signs and symptoms
0	Healthy plants with green leaves without appearance of fungal mycelium on any part of plants
1	Appearance of white fungal mass on any part of plants, with or without chlorotic leaves
2	Appearance of basidioma on any part of plants with chlorotic leaves (1 to 3 leaves)
3	Formation of basidioma of any part of plants with chlorotic leaves (> 3 leaves)
4	Formation of a well-developed basidioma and the plants dried

Source: Abdullah *et al.* (2003).

TABLE 2. SIGNS AND SYMPTOMS OF BOLE INDEX SCORED BASED ON 0-4

Disease class	Signs and symptoms
0	Healthy
1	up to 20% rotting of bole tissues
2	21% to 50% rotting of bole tissues
3	51% to 90% rotting of bole tissues
4	over 90% rotting of bole tissues

Source: Nursabrina *et al.* (2012).

Extraction of Cell Wall (CW)-Lipids

Oil palm root materials (100 g of fresh weight) were lyophilised for 48 hr. Then the lyophilised materials were homogenised using a commercial blender until fine, followed by CW isolation as described by Canut *et al.* (2016) with a slight modification. CW materials were subjected to lipids extraction. Samples of *G. boninense*, healthy and infected CW (1 g) were added to 50 mL centrifuge tubes together with 10 mL of chloroform and methanol mixture with a volume ratio of 2:1. The tubes were agitated in temperature-controlled incubator shaker (Thermo Scientific Orbital and

Incubator Shaker) for 120 min at 30°C. The liquid phase was recovered with centrifugation of 1000 g for 10 min. A 0.2 volume of miliQ® water was used to wash the solvent mixtures with additional agitation for another few seconds. The two phases were further separated by centrifugation at 1000 g. The lower chloroform phase which contained the lipids was kept for further use while the aqueous upper phase was removed. A stream of N₂ at temperature of 25 ± 2°C was used to dry the extracts.

Fractionation of CW-Lipids Using Solid Phase Extraction (SPE)

Fractionation of CW lipids extracts were performed using a Strata SI-1 silica disposable cartridge column (500 mg/6 mL) (Phenomenex) as described by Stobiecki *et al.* (1997) with slight modifications. The cartridge column was equilibrated by rinsing twice with 2 mL of chloroform using SPE manifold. Crude CW lipids dissolved in 2 mL of chloroform were loaded onto the column and the solvent was pulled through. Thereafter, the column was eluted with 10 mL of chloroform to elute sterol and neutral lipids fractions. Then, 10 mL of acetone and methanol mixture (9:1) was applied to elute glycolipid fraction. Finally, free fatty acids and phospholipid were eluted using 10 mL of methanol. Each fraction collected was dried under stream of nitrogen (N₂) prior to high-performance thin layer chromatography (HPTLC) analysis.

Permethylation of Glycolipids

Glycolipids permethylation was performed as described by Longo *et al.* (2013). Under N₂ stream, glycolipids were dried and stored overnight at 20°C with silica gel. The glycolipids were then suspended in 150 µL of anhydrous dimethyl sulphoxide (DMSO), agitated with few milligrams of anhydrous sodium hydroxide (NaOH) and 80 µL of iodomethane before incubated and shaken for 1 hr at a temperature of 25 ± 2°C. Dichloromethane (2 mL) and deionised water (2 mL) were added to the mixture, agitated and centrifuged for removal of the aqueous upper layer. Two mL of deionised water was used to wash the organic phase for three times before dried under N₂ stream.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis of Glycolipids

The GC-MS analysis of glycolipids was performed using GC-MS (Agilent, 5975C) with J&W GC column (30 m x 250 µm x 0.25 µm; Agilent) according to method described by Nyberg (1986) with some modifications. For GC-MS detection, an electron ionisation system with ionisation energy

of 70 eV was used. The injector temperature was maintained at 280°C. The carrier gas (helium) was set at the flow rate of 1.0 mL/min before the sample (1.0 µL) was introduced using the splitless mode. Three different temperature gradients were used for glycolipid analysis, 90°C (initial); 200°C at 20°C/min (10 min); 230°C at 10°C/min (10 min) and 265°C/min (final 5 min). Spectra which was generated at 50-650 *m/z* range at 20 scans per second with the solvent delay of 5 min were collected.

Multivariate Data Analysis

All raw spectrometric data were processed using open-source software MZmine (version 2.2.3). Chromatogram builder, peak detection, spectrum deconvolution, isotope filtering and peak grouping were used for data processing. The detected peaks were subjected to compound identification by searching NIST MS Search 2.0, METLIN database (<http://metlin.scripps.edu/>), MassBank (<http://www.massbank.jp/>), LipidMaps (<http://www.lipidmaps.org/>) and KEGG database (<http://www.genome.jp/kegg>) using exact molecular weights or MS fragmentation pattern data. The data were further normalised using the total summed metabolites' intensities and transformed by taking the natural log of the intensities' values of the analysed metabolites to make each feature comparable in magnitude to each other. Unsupervised principal component analysis (PCA) was used to analyse the pattern depicting metabolite differences and general clustering. While significantly altered metabolites between groups were identified using supervised partial least squares discriminant analysis (PLS-DA) generated. The values of R² and Q² parameters were used to verify the fitness and predictive ability of the model. VIP analysis with VIP-score >1.5 was used to determine specific metabolites backing most significantly to the differences recognised by PLS-DA. Significantly the different metabolites were then used to generate a correlation heatmap identifying metabolites that cluster according to sample groups (healthy or infected). Distance measure was generated by Pearson correlations and clustering using the Ward algorithm.

Identification of Altered Metabolic Pathways

To identify the most relevant metabolic pathways involved in *G. boninense*-oil palm interaction, metabolic pathway analysis and pathway topology analysis were performed using MetPA, a web-based metabolomics tool for pathway analysis and visualisation (<http://metpa.metabolomics.ca/MetPA>) according to method described by Chen *et al.* (2015). The functional pathway analysis of potential biomarkers was based

on the database source of the Kyoto Encyclopedia of Genes and Genomes (KEGG) (<http://www.genome.jp/kegg/>). The pathway topology analysis performed was as described by Fan *et al.* (2018) where the impact-value threshold was set as 0.10 and calculated from the analysis. Therefore any values above the threshold were rejected as potentially significant pathways.

Potential Biomarkers Identification

Potential biomarkers identification using Receiver Operating Characteristic (ROC) curve analysis was adopted from Barros *et al.* (2018) with some adjustments, by focusing on biomarkers that significantly altered in their metabolite's pathway. ROC analysis of the ROC curve analysis was conducted using the online ROCcET (<http://www.roccet.ca>). The area under the ROC curve (AUC) was used to calculate the sensitivity and specificity trade-offs. Areas under the ROC curve (AUC) is the method of choice for evaluating the performance of potential biomarkers: the greater the AUC (>0.7), the better the prediction of the model. Potential biomarkers with high sensitivity when infected with *Ganoderma* and low sensitivity when disease is absent were selected.

RESULTS AND DISCUSSION

Assessment of Infection

In the present work, successful inoculation was achieved using RWB technique as similar symptoms were developed at lower stem and bole tissues of inoculated oil palm seedlings, besides the presence of small white button (*Figure 1*)

as described by Alizadeh *et al.* (2011); Naher *et al.* (2011); Nur Ain Izzati and Abdullah (2008). All *Ganoderma*-inoculated seedlings showed symptoms of infection after three (first interval) and six months (second interval) post-AI. To further confirm the success of colonisation of the pathogen in roots, *G. boninense* was isolated from the infected oil palm seedling roots using GSM. Positive result on GSM was also correlated with the detection of ergosterol content extracted from roots of inoculated seedlings according to the method demonstrated by Chong *et al.* (2012a). Ergosterol is a sterol component largely restricted to fungi and is absent in other living biomass. No ergosterol was detected in the healthy seedlings. Symptoms of *Ganoderma* infection and signs of the presence of *Ganoderma* on inoculated seedlings of the eight months old OP seedlings were noticed as early as three months after inoculation. The uninoculated seedlings did not show any symptoms of BSR infection and remained healthy until the end of the experiment. At first interval, concentration of ergosterol was 39.17 µg/g, DI=100% and DSI=30%, meanwhile at second interval, the concentration of ergosterol increased significantly to 186.74 µg/g, DI=100% parallel to the increased of DSI=77.5% (*Table 3*) [data and calculation for ergosterol were obtained according to Chong *et al.* (2012a)]. Although some of the infected palms showed symptoms of BSR, DSI percentage at first interval was only 30% as most of the infected seedlings had lower score. Thus, this stage was regarded as the early infection due to the non-synchronous nature of BSR development (Rees *et al.*, 2009). At second interval, progression of DSI was also in accordance with bole damage index of 47.5% (*Table 3*). All symptoms appeared at second interval, thus, regarded as the 'late infection' stage of BSR disease.

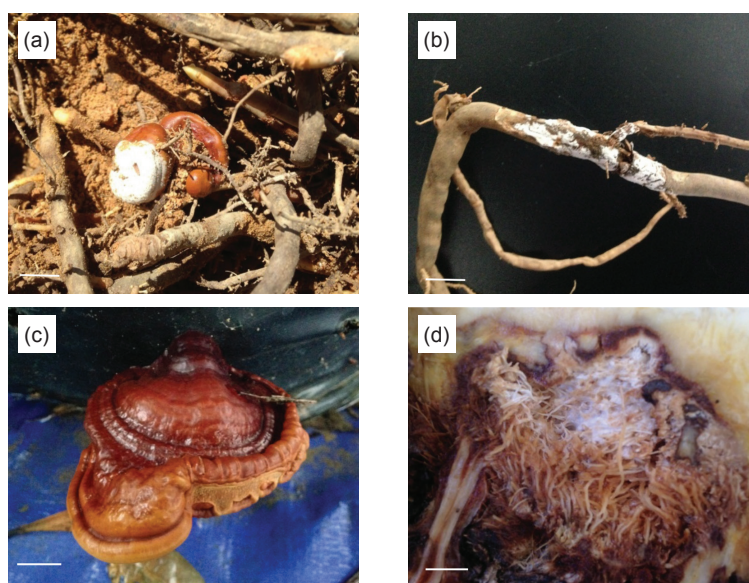


Figure 1. Physical evidence of successful artificial inoculation using Rubber Wood Block technique during first interval (a-b) and second interval (c-d). Bar=1 cm.

TABLE 3. DISEASE ASSESSMENT OF TWO-TIME INTERVALS OF OIL PALM SEEDLINGS INOCULATED WITH *Ganoderma*

Item	First interval	Second interval
Disease incidence (DI)	100%	100%
Ergosterol ($\mu\text{g/g}$)	$39.17 \pm 0.75^*$	$186.74 \pm 0.84^*$
Disease severity index (DSI; %)	30 [*]	77.5 [*]
Bole damage index (%)	0 [*]	47.5 [*]

Note: *Significantly different at $p \leq 0.05$ by Tukey comparison test in their respective assessment. Values are the means of 10 replicates, where each replicate consists of a seedling.

Differential Expression and Profile of Glycolipids Composition Related to *Ganoderma* Infection

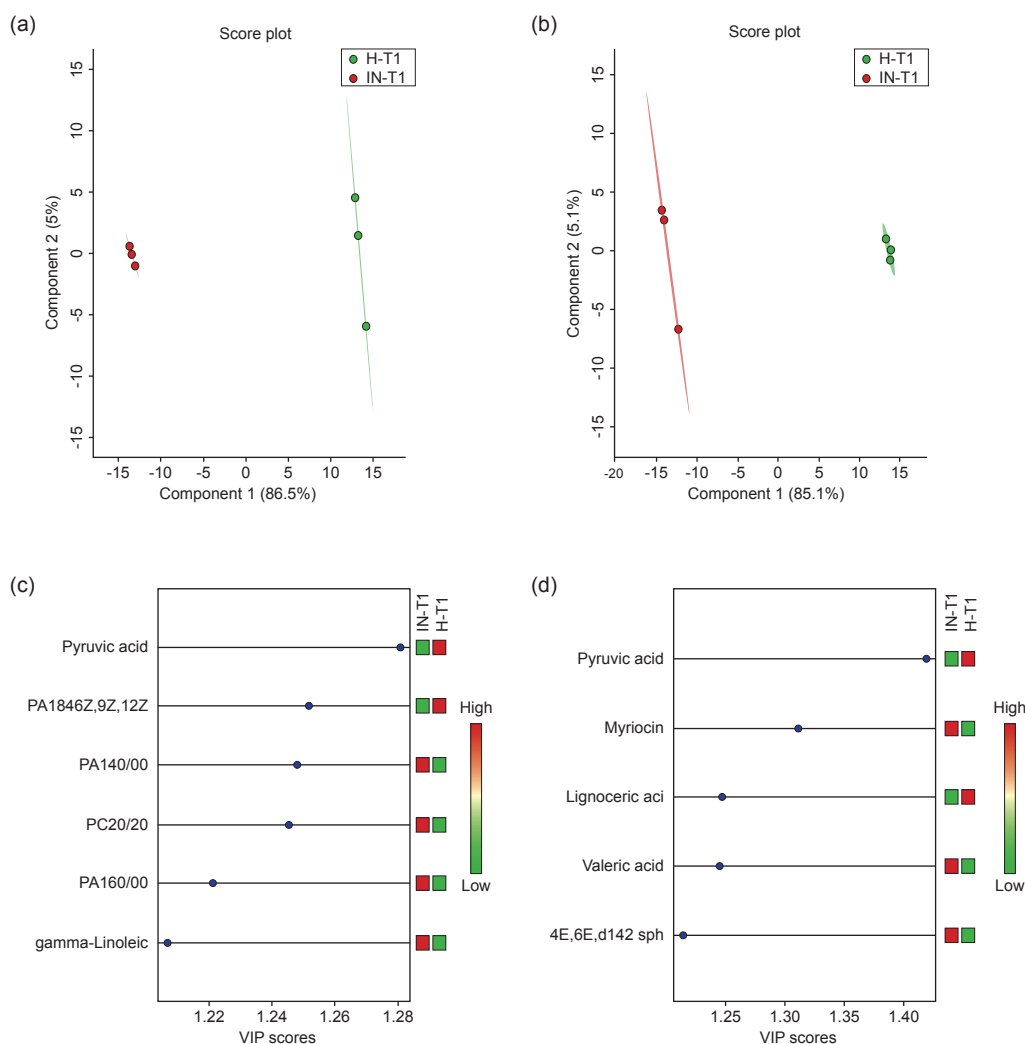
The differential metabolites between different sample groups were categorised according to the stage of infection assessed at two different intervals. Univariate statistical analysis performed on H-T1 (Healthy-First Interval) and IN-T1 (Infected-First Interval) datasets identified 31 glycolipids were significantly affected at first interval ($\log_2$ (FC) > 2 and p -value < 0.05). Evaluation by PCA showed a clearly different distribution along PC1 covering 86.5% of the total variance (data not shown). In PLS-DA analysis, the components also showed the separation pattern similar to PCA analysis (Figure 2a). Using a VIP score of larger than 1.2 as a cut-off, six metabolites were found highly important to the separation (Figure 2). In IN-T1 dataset, pyruvic acid was down-regulated, while phosphatidic acid (PA) [18:4(6Z,9Z,12Z,15Z)/0:0], PA (14:0/0:0), phosphatidylcholine (PC) (2:0/2:0), PA (16:0/0:0) and γ -linoleic acid shows up-regulation trend in relation to H-T1. The glycolipids produced by various microorganisms contain different mono- or disaccharides, conjugated with long-chain fatty acids or hydroxyl fatty acids through either acetyl or glycosidic linkages, and some of them exhibit antibiotic (Teichmann *et al.*, 2011). The preferential loss of a fatty acid molecule from the glycerol backbone is most likely due to kinetic control of the gas-phase fragmentation process, thus, leading to the detection of fatty acids in the glycolipids analysis (Zianni *et al.*, 2013).

At second interval, 48 metabolites were significantly regulated upon *G. boninense* infection. PCA model for the classification of the H-T2 (Healthy-Second Interval) and IN-T2 (Infected-Second Interval) groups obtained satisfactory validation with 85.1% variance along PC1 (data not shown). The PLS-DA model was used to elucidate the most reliable class-discriminating metabolites that were highly diagnostic for group separation (Figure 2b). Together with the VIP scores (>1.2) from the PLS-DA model, five metabolites with the highest values were identified, out of which, two metabolites (pyruvic acid and lignoceric acid) were

down-regulated, while valeric acid, myriocin and (4E,6E,d14:2) sphingosine were found up-regulated in IN-T2 groups (Figure 2b). Glycolipids profiling in the infected samples changed most significantly from their healthy controls at second interval, compared to first interval suggesting regulation of primary metabolism also occurs during plant-pathogen interactions. Using univariate analysis, IN-T1 *vs.* H-T1 and IN-T2 *vs.* H-T2 shared one metabolite, pyruvic acid that was down-regulated in infected samples at both intervals. Evidence for the role of glycolipids in plant defense responses has been provided by experiments showing the induction of genes involved in carbohydrate metabolism upon challenge by pathogens or pathogen-derived elicitors (Rojas *et al.*, 2014).

Apparently, fatty acid consisting of odd number of carbon atom chain, such as valeric acid might contribute to the regulation of systemic induced resistance (Kuć and Tuzun, 1990). Detection of myriocin, fungal secondary metabolites is one of most widely studied extraordinary sphingoid bases. Intriguingly, in spite of increased accumulation of myriocin, a long-chain sphingoid base, (4E,6E,d14:2) sphingosine was also increased simultaneously. These long-chain bases are important intermediates and signalling molecules in the sphingolipid catabolic pathway (Speigel and Milstien, 2002). The levels of long-chain bases in healthy tissues are usually low, however the study found that the treatment of the corn tissue with mycotoxins can increase their level dramatically (Wright *et al.*, 2003). Plant-pathogen interactions have implicated disruption of sphingolipid synthesis and build-up of long-chain bases during the hypersensitive response in programmed cell death associated with the plant defense response. The toxins myriocin resulting in an accumulation of sphingocine associated with plant necrosis promotion (Tanaka *et al.*, 1993) and programmed cell death (apoptosis) in plant tissues (Wang *et al.*, 1996) is related to their detection in the second interval. The response to these toxins may be complex and apparently involves the participation of several signalling pathways (Asai *et al.*, 2000), leading to long-chain base accumulation and cell death. But if long-chain base synthesis is concurrently inhibited, long-chain base build-up is prevented and the toxic effects are at least partly blocked (Spassieva *et al.*, 2002). These findings propose that sphingoid long-chain bases may be significant in signalling or regulatory roles in plant defense, and higher levels in plant tissues lead to cell death.

To identify the changes in glycolipid composition between infected and healthy samples at first and second interval, the multivariate statistical analysis was performed. To answer a question which the first two t-tests was unable to address, an analysis of variance (ANOVA) test was conducted



Note: H - Healthy seedlings; IN - *Ganoderma*-inoculated seedlings; T1 - First interval (three months post-artificial inoculation (AI)); T2 - Second interval (six months post-AI).

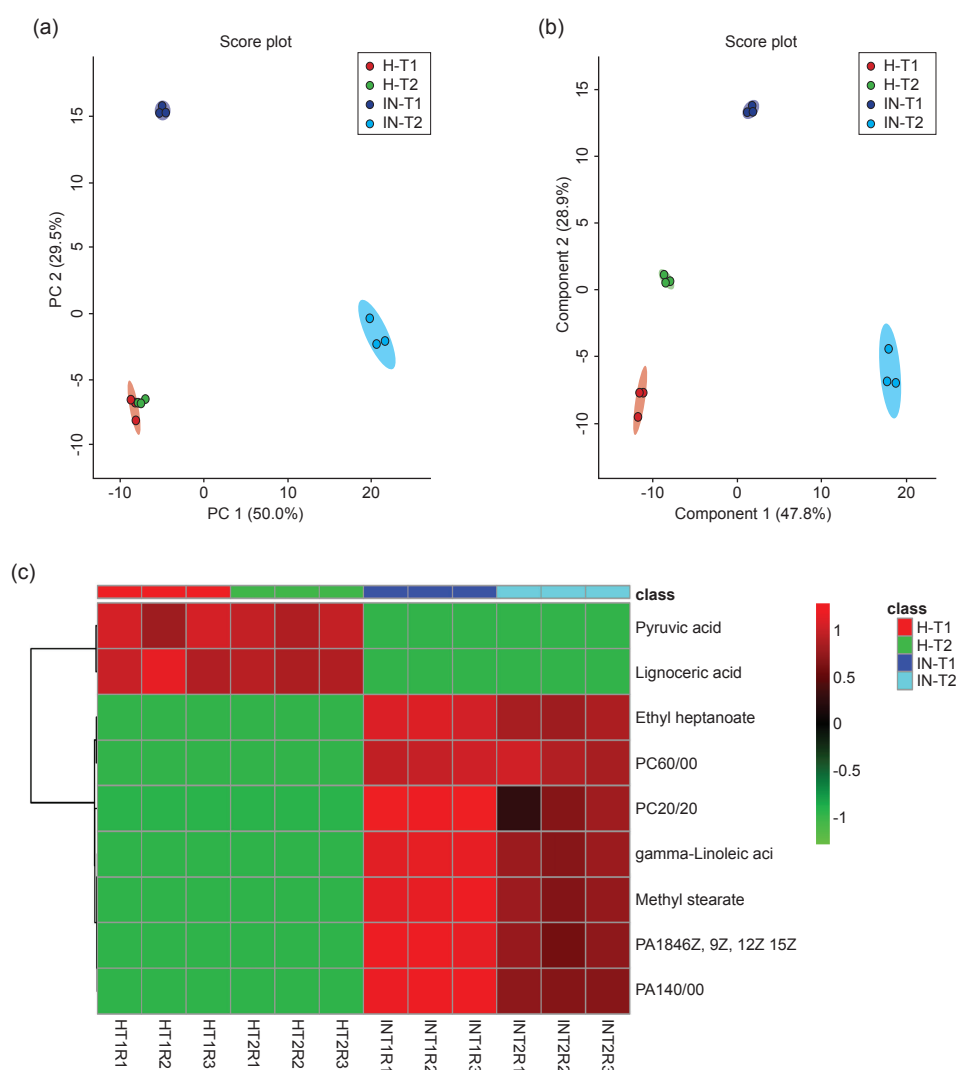
Figure 2. Partial least square-discriminant analysis (PLS-DA) of (a) IN-T1 vs. H-T1 and (b) IN-T2 vs. H-T2; variable importance in projection (VIP) score plot selected from PLS-DA model with $p > 0.05$ and (VIP) scores > 1.2 of (c) IN-T1 vs. H-T1 and (d) IN-T2 vs. H-T2. Colour key indicates metabolite expression value, green: Lowest, red: highest. Analysis by Metaboanalyst consists $n=3$ replicates for each group.

to identify the common changes in glycolipids profiles. ANOVA test revealed a total of 31 significant features with p -value < 0.05 . PCA showed that 79.5% of the total variance in the data was represented by the first two principal components (Figure 3a). PCA scores plot show H-T1, H-T2 and IN-T1 were separated from IN-T2 along PC1, indicating that IN-T1 and IN-T2 profiles were very distinct to each other. To obtain a more reliable statistical analysis and the specific loadings, a PLS-DA model was used to discriminate healthy and infected groups. PLS-DA was able to discriminate lipid samples based on the treatment they received with a good cross-validated model performance using the first two latent variables ($R^2 = 0.972$; $Q^2 = 0.945$). PLS-DA additionally informed upon which metabolites were most important for the classification model

via their VIP scores and identified nine metabolites (VIP values > 1.5) (Figure 3b). Further analysis using heatmap recognised two metabolites (Pyruvic acid and lignoceric acid), from healthy (H-T1 and H-T2) groups showed highly significant of differences from infected groups, suggesting that these two metabolites might significantly perturbed during *G. boninense* infection (Figure 3c). Pyruvic acid, a product of glycolysis and a very-long-chain of fatty acids such as lignoceric acid exert crucial functions in plant developmental processes, thus, when their levels are perturbed, is marked phenotypic consequences in plant growth (De Bigault Du Granrut and Cacas, 2016). Meanwhile, ethyl heptanoate, PC (6:0/0:0), PC (2:0/2:0), γ -linoleic acid, methyl stearate, PA [18:4(6Z, 9Z, 12Z, 15Z)/0:0] and PA (14:0/0:0) were significantly up-regulated in

IN-T1 and IN-T2 samples. These metabolites could be used as potential biomarkers to discriminate infected and healthy oil palm seedlings. Production of biologically active lipids, such as PA and PC has been identified as a major source of lipid second messenger and play an important role in signal transduction. Increases in PA concentration have been observed under various environmental stresses and were detected during early response in the *Cladosporium fulvum*-4/Avirulence-4 interaction (de Jong *et al.*, 2004). Induction of reactive oxygen species (ROS) formation is also linked to PA (Park *et al.*, 2004). Signal-induced production of PA in the cell was accompanied by the increases of PC, in contrast with the other study by Xie *et al.* (2015). Accumulation of PC might derive from the pathogen's membrane cell (Siebers *et al.*, 2016). Increased level of methyl stearate

was also in parallel to the findings in vegetative tissues of sunflower mutants (Cantisán *et al.*, 1999). This compound is converted into linoleic acid, a precursor for the defense signalling molecules jasmonic acid (Wang *et al.*, 2017). Jasmonic acid functions as a trigger to the phenylpropanoid pathway to increase the synthesis of phenolic compounds (Gundlach *et al.*, 1992). Significant increment of γ -linoleic acid was also consistently observed in *Arabidopsis thaliana* infected with *Sclerotinia sclerotiorum* at an all-time point (3, 6 and 24 hr after infection). Linoleic acid was also associated in pathogenesis and resistance-related metabolites of wheat, upon infection with *Fusarium* head blight disease (Hamzehzarghani *et al.*, 2008). No previous evidence on accumulation of ethyl heptanoate has been reported elsewhere.



Note: H - Healthy seedlings; IN - *Ganoderma*-inoculated seedlings; T1 - First interval [three months post-artificial inoculation (AI)]; T2 - Second interval (six months post-AI).

Figure 3. Multivariate cluster analysis of glycolipids profiles of H-T1 vs. IN-T1 vs. H-T2 vs. IN-T2 at first and second intervals. (a) Score-plot of principal component analysis (PCA), (b) Score-plot of partial least square-discriminant analysis (PLS-DA), and (c) Heatmap visualisation analysis of metabolites (VIP score >1.5; $p > 0.05$) identified from PLS-DA model with a potential identity to distinguish between infected and healthy groups. Colour key indicates metabolite expression value, green: Lowest, red: highest. Analysis by Metaboanalyst consists $n=3$ replicates for each group.

Metabolite Pathways Related to *G. boninense* Infection

Metabolic pathway analysis was performed to reveal the most relevant pathways related to *Ganoderma* infection. The impact score (IS) of these pathways calculated from pathway topology analysis above 0.10 was screened out as potential target pathway. Significant features with p -value < 0.05 and PLS-DA VIP score > 1.5 from multivariate analyses were pooled and further subjected to pathway analysis. The result in Figure 4 shows that two pathways; pyruvate metabolism (IS: 0.1129) and glycolysis or gluconeogenesis (IS: 0.1091) were associated with *G. boninense* throughout the whole experiment. As a hemibiotrophic fungal pathogen, *G. boninense* first establishes a biotrophic interaction with the host plant and later switches to a destructive necrotrophic lifestyle. This study suggests that when the fungus breaches into the host CW to access nutrients that are available in the starch reservoir (biotroph stage) (Rees *et al.*, 2009), it causes the host plant to induce the signaling pathway and its defensive environment. Fascinatingly however, *G. boninense* seems to be able to avoid direct contact with the defense compounds produced by the defensive signalling pathway, causing the infection to progress to the latent and the later stage of BSR disease as demonstrated in Glucosinolate metabolites for *Arabidopsis thaliana* defense mechanism against several pathogens (Clay *et al.*, 2009). When *G. boninense* initiates the necrotrophic program, it causes interference with the carbohydrate metabolism pathways (Su *et al.*, 2021). *Ganoderma boninense* invasion substantially

alters the primary metabolism of oil palm roots seedlings as seen by the downregulation of pyruvic acid and carbohydrate metabolism at first and second intervals. Pyruvic acid declines early in the infection process and continues to decline during the biotrophic stage. In this study, it was suggested that *G. boninense* avoided effective defenses by hijacking pyruvic acid-associated pathways as shown in Figure 4. During *G. boninense* colonisation, OP seedling induced the downregulation of pyruvic acid; involving itself in carbohydrate catabolism processes such as glycolysis or gluconeogenesis and pyruvate metabolism. It has been suggested that the energy saved by down-regulation of primary metabolism is diverted and used for defense responses (Rojas *et al.*, 2014). As plant defense responses require an abundant supply of energy, primarily derived from primary metabolic processes (Bolton, 2009). However, the ability of *G. boninense* to utilise the plant-carbohydrate upon colonisation decreases this metabolite, thus, weakening the oil palm into 'starvation', consequently exposing it to *G. boninense* colonisation (Divon and Fluhr, 2007; Li *et al.*, 2019).

Identification of Biomarkers Associated with *G. boninense* Infection

Biomarkers were identified using a subset of metabolites from the multivariate statistical analyses outlined earlier, as these metabolites were capable of discriminating between sample groups. ROC analysis was performed to further characterise the predictive value of selected metabolites independently. Disease analysis employs the ROC

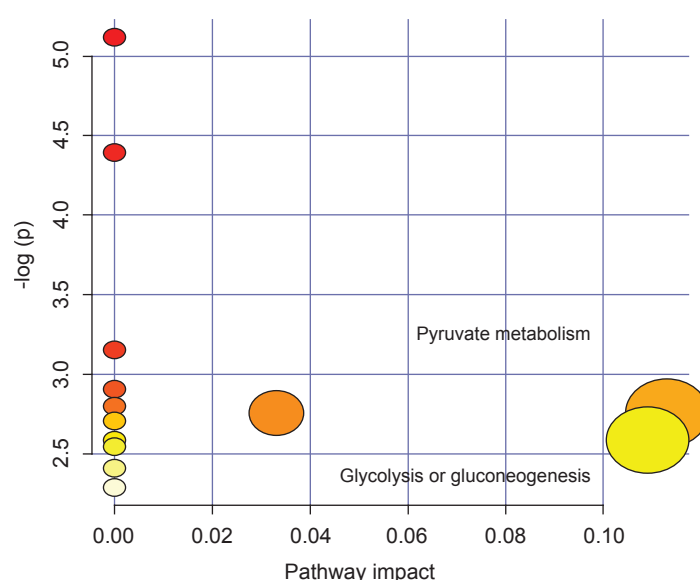


Figure 4. The pathway impact analysis of cell-wall lipid metabolites selected from multivariate analysis (from whole experiment) using MetPA. Each circle represents a matched pathway. Metabolic pathways with impact values > 0.05 were considered to be perturbed.

curve and the area under the ROC curve (AUC) to obtain a numerical value of the relationship between the specificity and sensitivity of a biomarker. The sensitivity and specificity indicate the probability tests for correctly identifying infected palms with the disease and without the disease, respectively (Lalkhen and McCluskey, 2008). AUC value of 1.0 shows a perfect prediction in term of the sensitivity and specificity of the diagnostic test (Greiner *et al.*, 2000), thus, these metabolites could be used as biomarkers to differentiate the infected and healthy tissues. To confirm the prediction of *Ganoderma*-infected tissue, a combination of more than one discriminatory metabolite was developed via a

logistic regression analysis and showed that the combination of metabolite also resulted in a better discriminator with AUC equal to 1 (Figure 5a), and a clear separation and discrimination were observed between the infected and healthy samples in the probability view (Figure 5b). The average accuracy based on 100 cross validations (CV) was 1 in this study (Figure 5c). Having an average accuracy close to 1 indicates a more valid CV prediction. Box-whisker plots revealed that all five biomarkers exhibited significantly higher levels in infected samples than in healthy ($p < 0.05$) (Figure 6). Since the five biomarkers showed the AUC value of 1.0, they were selected as biomarkers of infected palms.

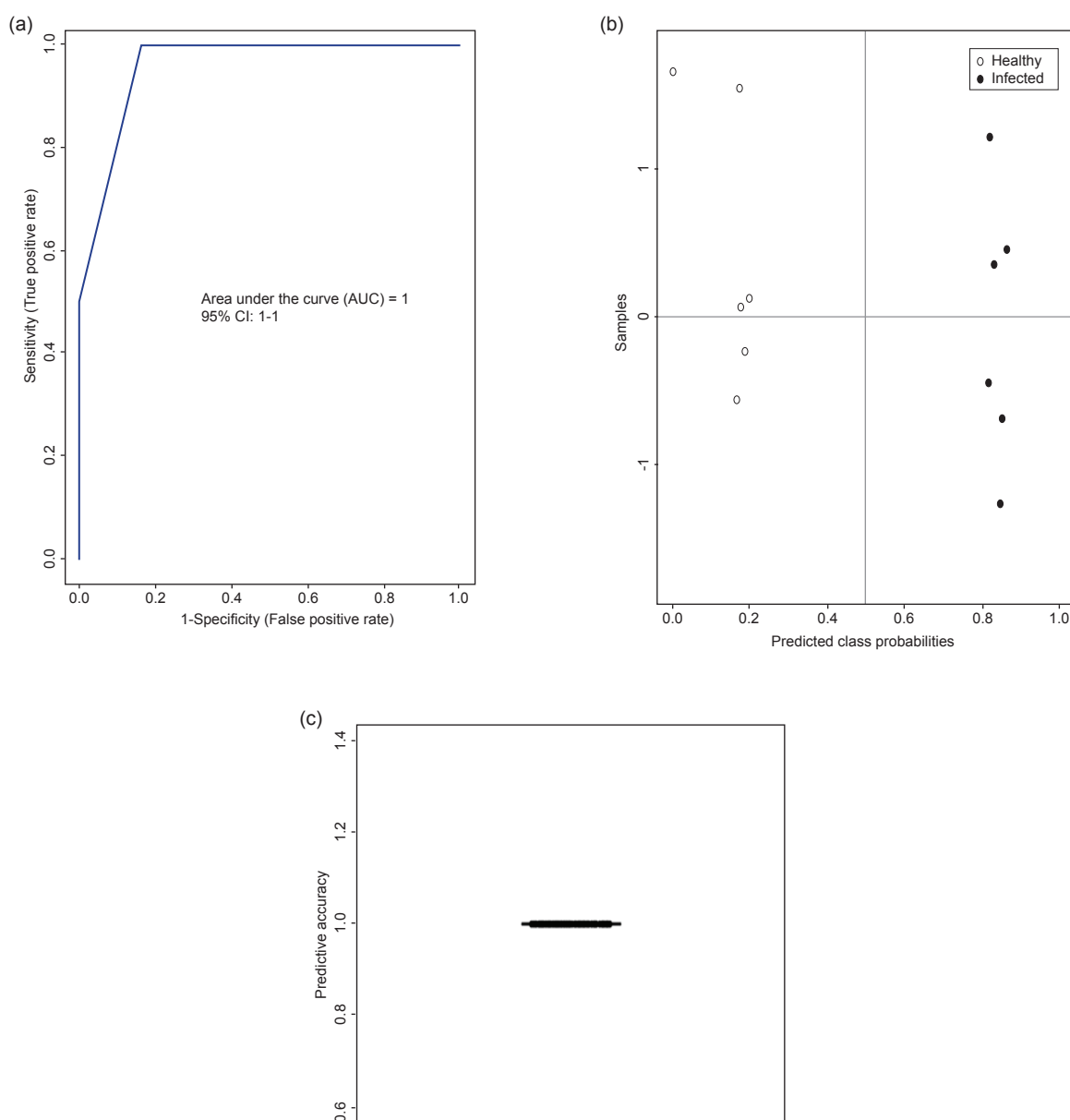


Figure 5. Biomarker analysis results (ROC view). Statistical method to evaluate infected and healthy discrimination using selected potential metabolites. (a) Probability view, (b) cross validation prediction, and (c) accuracy of selected metabolites, PC (6:0/0:0), PC(2:0/2:0), PA(18:4(6Z,9Z,12Z,15Z)/0:0), PA(14:0/0:0) and γ -linolenic acid.

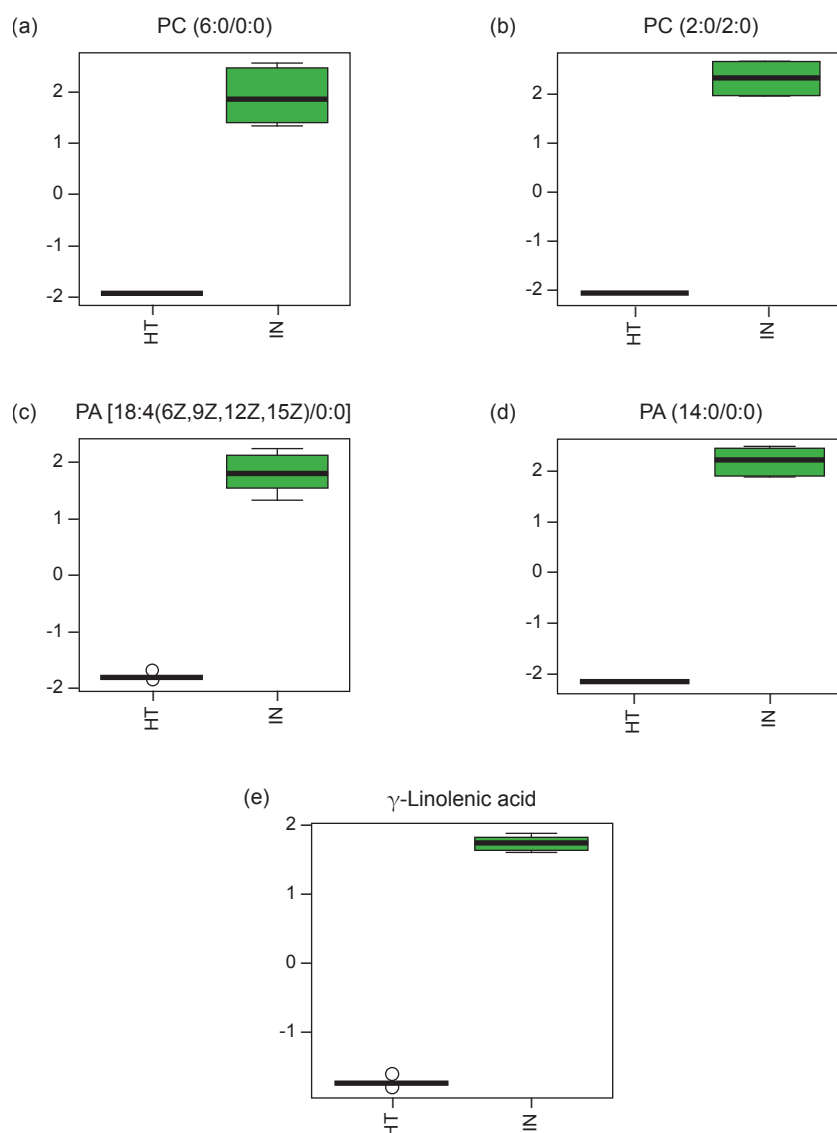


Figure 6. Box-and-whiskers plots representing relative abundance of (a) PC(6:0/0:0); (b) PC(2:0/2:0); (c) PA(18:4(6Z, 9Z, 12Z, 15Z)/0:0); (d) PA(14:0/0:0); (e) γ -linolenic acid in *Ganoderma*-infected and healthy oil palm seedlings. The relative abundance of each metabolite in lipid profiles of *Ganoderma*-infected was significantly higher than healthy groups using Student's *t*-test (*p*-value <0.05).

CONCLUSION

The current study indicates that lipid-profiling techniques combined with the chemometric analysis can develop a detailed picture and provide a broader assessment of the glycolipid change that occurs in oil palm roots CW in response to the BSR disease. The study provides an insight into the complex host-pathogen interactions, and also discovers altered lipidomics/metabolomics pathways involved in disease pathogenesis. Pyruvate metabolism and glycolysis or gluconeogenesis are the most perturbed pathways during the pathogenesis. Simulation of defense pathway using synthesised metabolites in oil palm may probably lead to the enhancement of defense reactions, leading to the formation of physiochemical reactions barrier which prevents the invasion of *G. boninense*. The five metabolites: PC(6:0/0:0), PC(2:0/2:0), PA[18:4(6Z, 9Z, 12Z,

15Z)/0:0], PA(14:0/ 0:0) and γ -linolenic acid which had been identified and analysed in the present work and could be used as diagnostic biomarkers to identify *G. boninense* infection for an earlier control. Future research using genetic approach on these biomarkers will deepen the understanding of their importance in defense/cell signaling and the role of glycolipid in plant-pathogen interaction in facilitating the development of the BSR early detection approach.

ACKNOWLEDGEMENT

The authors wish to thank the Ministry of Higher Education of Malaysia for funding the project through the Fundamental Research Grant Scheme (FRG0342-ST-2/2013). Support and service provided by the Universiti Malaysia Sabah are also gratefully acknowledged.

REFERENCES

- Abdullah, F; Ilias, G N M; Nelson, M; Nur Ain Izzati, M Z and Umi Kalsom, Y (2003). Disease assessment and the efficacy of *Trichoderma* as a biocontrol agent of basal stem rot of oil palm. *Res. Bull. Sci. Putra*, 11: 31-33.
- Alexander, A; Abdullah, S; Rosall, S and Chong, K P (2017). Evaluation of the efficacy and mode of action of biological control for suppression of *Ganoderma boninense* in oil palm. *Pak. J. Bot.*, 49(3): 1193-1199.
- Alizadeh, F; Abdullah, S N A; Khodavandi, A; Abdullah, F; Yusuf, U K and Chong, P P (2011). Differential expression of oil palm pathology genes during interactions with *Ganoderma boninense* and *Trichoderma harzianum*. *J. Plant Physiol.*, 168: 1106-1113.
- Ariffin, D and Idris, A S (1992). The *Ganoderma* selective medium (GSM). *PORIM Information Series No. 8*.
- Ariffin, D; Idris, A S and Singh, G (2000). Status of *Ganoderma* in oil palm. *Ganoderma Diseases of Perennial Crops* (Flood, J; Bridge, P D and Holderness, M eds.). CABI Publishing, Wallingford. p. 49-68.
- Asai, T; Stone, J M; Heard, J E; Kovtun, Y; Yorgrey, P; Sheen, J and Ausubel, F M (2000). Fumonisin B1-induced cell death in *Arabidopsis* protoplasts requires jasmonate-, ethylene-, and salicylate-dependent signaling pathways. *Plant Cell*, 12: 1823-1835.
- Barros, R P C; da Cunha, E V I; Catão, R M R; Scotti, L; Souza, M S R; Brás, A A Q and Scotti, M T (2018). Virtual screening of secondary metabolites of the genus *Solanum* with potential antimicrobial activity. *Rev. Bras. Farmacogn.*, 28: 686-691.
- Bolton, M D (2009). Primary metabolism and plant defense - Fuel for the fire. *Mol. Plant Microbe Interact.*, 22: 487-497.
- Cantisán, S; Martínez-Force, E; Álvarez-Ortega, R and Garcés, R (1999). Lipid characterization in vegetative tissues of high saturated fatty acid sunflower mutants. *J. Agric. Food Chem.*, 47(1): 78-82.
- Canut, H; Albenne, C and Jamet, E (2016). Isolation of the cell wall. *Isolation of Plant Organelles and Structures. Methods in Molecular Biology*. (Taylor, N and Millar, A eds.). Vol. 1511: Humana Press, New York, USA. p. 171-185.
- Chen, H H; Tseng, Y J; Wang, S Y; Tsai, Y S; Chang, C S; Kuo, T C; Yao, W J; Shieh, C C; Wu, C H and Kuo, P H (2015). The metabolome profiling and pathway analysis in metabolic healthy and abnormal obesity. *Int. J. Obes.*, 39(8): 1241-1248.
- Chong, K P; Atong, M and Rossall, S (2012a). The role of syringic acid in the interaction between oil palm and *Ganoderma boninense*, the causal agent of Basal Stem Rot. *Plant Pat.*, 61: 953-963. DOI: 10.1111/j.1365-3059.2011.02577x.
- Chong, K P; Atong, M and Rossall, S (2012b). The roles of syringic, caffeic and 4-hydroxybenoic acid in *Ganoderma*-oil palm interaction. *Asian J. Microbiol. Biotechnol. Environ. Sci.*, 14(2): 157-166.
- Chong, K P; Abdullah, S and Ng, T L (2013). Molecular fingerprint of *Ganoderma* spp. from Sabah, Malaysia. *Int. J. Agric. Biol.*, 15: 1112-1118.
- Chong, K P; Dayou, J and Alexander, A (2017). *Detection and Control of Ganoderma Boninense in Oil Palm Crop*. Springer Briefs in Agriculture. Springer, Cham. 77 pp.
- Chong, K P; Eldaa, P A and Dayou, J (2014). Relation of *Ganoderma* ergosterol content to basal stem rot disease severity index. *Adv. Environ. Biol.*, 8[14 (Special)]: 14-19.
- Clay, N K; Adio, A M; Denoux, C; Jander, G and Ausubel, F M (2009). Glucosinolate metabolites required for an *Arabidopsis* innate immune response. *Science*, 323(5910): 95-101.
- Dayou, J; Alexander, A; Sipaut, C S; Chong, K P and Lee, P C (2014). On the possibility of using FTIR for detection of *Ganoderma boninense* in infected oil palm tree. *Int. J. Adv. Agric. Environ. Eng.*, 1(1): 161-163.
- De Bigault Du Granrut, A and Cacas, J L (2016). How very-long-chain fatty acids could signal stressful conditions in plants? *Front. Plant Sci.*, 7: 1490.
- de Jong, C F; Laxalt, A M; Bargmann, B O; de Wit, P J; Joosten, M H and Munnik, T (2004). Phosphatidic acid accumulation is an early response in the Cf-4/Avr4 interaction. *Plant J.*, 39: 1-12.
- Divon, H H and Fluhr, R (2007). Nutrition acquisition strategies during fungal infection in plants. *FEMS Microbiol. Lett.*, 266 (1): 65-74.
- Fan, W; Ge, G; Liu, Y; Wang, W; Liu, L and Jia, Y (2018). Proteomics integrated with metabolomics: Analysis of the internal causes of nutrient changes in alfalfa at different growth stages. *BMC Plant Biol.*, 18(1): 1-15.
- Greiner, M; Pfeiffer, D and Smith, R D (2000). Principles and practical application of the receiver-

operating characteristic analysis for diagnostic tests. *Prev. Vet. Med.*, 45: 23-41.

Gundlach, H; Müller, M J; Kutchan, T M and Zenk, M H (1992). Jasmonic acid is a signal transducer in elicitor-induced plant cell cultures. *Proc. Natl. Acad. Sci. USA*, 89: 2389-2393.

Hamzehzarghani, H; Paranidharan, V; Abu-Nada, Y; Kushalappa, A C; Mamer, O and Somers, D (2008). Metabolic profiling to discriminate wheat near isogenic lines, with quantitative trait loci at chromosome 2DL, varying in resistance against *Fusarium* head blight. *Can. J. Plant Sci.*, 88: 789-797.

Idris, A S; Mazliham, M S and Madihah, A Z (2009). Current technologies for detection of *Ganoderma* in oil palm. *Proc. Agric. Biotechnol. Sustain. Conf. PIPOC. MPOB, Bangi*. p. 81-98.

Ishaq, I; Alias, M S; Kadir, J and Kawawani, I (2014). Detection of basal stem rot disease at oil palm plantations using sonic tomography. *J. Sustain. Sci. Manag.*, 9(2): 52-57.

Japanis, F G; Chan, Y S and Chong, K P (2021). Evaluation on the effectiveness of combination of biocontrol agents in managing *Ganoderma boninense* of oil palm. *Malays. J. Microbiol.*, 17(1): 1-10.

Khairudin, H (1991). Pathogenicity of three *Ganoderma* species on oil palm seedlings. *J. Perak Planters Assoc.* p. 43-49.

Kuč, J and Tuzun, S (1990). Metabolic regulation of resistance genes in tobacco for the control of blue mold. *Blue Mold Disease of Tobacco Literature Service* (Main, C and Spurr, H eds.). North Carolina State University, Raleigh. p. 35-46.

Lalkhen, A G and McCluskey, A (2008). Clinical tests: Sensitivity and specificity. *Cont. Edu. Anaesth. Critical Care Pain*, 8: 221-223.

Lelong, C C; Roger, J M; Brégand, S; Dubertret, F; Lanore, M; Sitorus, N; Raharjo, D and Caliman, J P (2010). Evaluation of oil-palm fungal disease infestation with canopy hyperspectral reflectance data. *Sensors (Basel)*, 10(1): 734-747.

Li, P; Liu, W; Zhang, Y; Xing, J; Li, J; Feng, J; Su, X and Zhao, J (2019). Fungal canker pathogens trigger carbon starvation by inhibiting metabolism in poplar stems. *Sci. Rep.*, 9: 10111.

Longo, L V G; Nakayasu, E S; Lopes, F G; Vallejo, M C; Matsuo, A L; Almeida, I C and Puccia, R (2013). Characterization of cell wall lipids from the pathogenic phase of *Paracoccidioides brasiliensis*

cultivated in the presence or absence of human plasma. *PLoS ONE*, 8(5): e63372.

Madihah, A Z; Maizatul-Suriza, M; Idris, A S; Bakar, M F A; Kamaruddin, S; Bharudin, I; Abu Bakar, F D and Murad, A M A (2018). Comparison of DNA extraction and detection of *Ganoderma*, causal of basal stem rot disease in oil palm using loop-mediated isothermal amplification. *Malays. Appl. Biol. J.*, 47(5): 119-127.

Markon, M A; Shakaff, A Y; Adom, A H; Ahmad, M N and Abdullah, A H (2008). The feasibility study of utilising electronic nose and ANN for plant malaise detection. *Proc. MUCET Malaysian Universities Conf. Eng. Technol. (MUCET)*. Putra Brasmana, Perlis, Malaysia. p. 1-6.

Masood, A; Saeed, S; Iqbal, N; Malik, M T and Kazmi, M R (2010). Methodology for the evaluation of symptoms severity of mango sudden death syndrome in Pakistan. *Pak. J. Bot.*, 42(2): 1289-1299.

Michael, M L; Chong, K P; Zenian, S; Lo, A S V and Dayou, J (2020). On the propagation of *Ganoderma boninense* infection of basal stem rot in oil palm with the aid of acoustics computed assisted tomography. *Tran. Sci. Tech.*, 7(3-2): 165-171.

Mille-Lindblom, C; von Wachenfeldt, E and Tranvik, L J (2004). Ergosterol as a measure of living biomass: Persistence in environmental samples after fungal death. *J. Microbiol. Methods*, 59: 253-262.

Mohd As'wad, A W; Sariah, M; Paterson, R R M; Zainal Abidin, M A and Lima, N (2011). Ergosterol analyses of oil palm seedlings and plants infected with *Ganoderma*. *Crop Prot.*, 30: 1438-1442.

Naher, L; Ho, C L; Tan, S G; Yusuf, U K and Abdullah, F (2011). Cloning of transcripts encoding chitinases from *Elaeis guineensis* Jacq. and their expression profiles in response to fungal infections. *Physiol. Mol. Plant Pathol.*, 76: 96-103.

Najmie, M M K; Khalid, K; Sidek, A A and Jusoh, M A (2011). Density and ultrasonic characterization of oil palm trunk infected by *Ganoderma boninense* disease. *Meas. Sci. Rev.*, 11(5): 160-164.

Nur Ain Izzati, M Z and Abdullah, F (2008). Disease suppression in *Ganoderma*-infected oil palm seedlings treated with *Trichoderma harzianum*. *Plant Protect. Sci.*, 44: 101-107.

Nursabrina, A A; Sariah, M and Zaharah, A R (2012). Suppression of basal stem rot disease progress on oil palm (*Elaeis guineensis*) after copper and calcium

supplementation. *Pertanika J. Trop. Agric. Sci.*, 35: 13-24.

Nyberg, H (1986). GC-MS methods for lower plant glycolipid fatty acids. *Gas Chromatography/Mass Spectrometry* (Linskens, H F and Jackson, J F eds.). Modern Methods of Plant Analysis (New Series). Vol. 3. Springer, Berlin, Heidelberg. p. 67-99.

Park, J; Gu, Y; Lee, Y; Yang, Z and Lee, Y (2004). Phosphatidic acid induces leaf cell death in *Arabidopsis* by activating the Rho-related small G protein GTPase- mediated pathway of reactive oxygen species generation. *Plant Physiol.*, 134: 129-136.

Rees, R W; Flood, J; Hasan, Y; Potter, U and Cooper, R M (2009). Basal stem rot of oil palm (*Elaeis guineensis*); mode of root infection and lower stem invasion by *Ganoderma boninense*. *Plant Pathol.*, 58: 982-989.

Rojas, C M; Senthil-Kumar, M; Tzin, V and Mysore, K S (2014). Regulation of primary plant metabolism during plant-pathogen interactions and its contribution to plant defense. *Front. Plant Sci.*, 5(17): 1-11.

Siebers, M; Brands, M; Wewer, V; Duan, Y; Georg, H and Peter, P (2016). Lipids in plant-microbe interactions. *Biochem. Biophys. Acta Mol. Cell Biol. Lipids*, 1861(9): 1379-1395.

Spassieva, S D; Markham, J E and Hille, J (2002). The plant disease resistance gene *Asc-1* prevents disruption of sphingolipid metabolism during AAL-toxin induced programmed cell death. *Plant J.*, 32: 561-572.

Spiegel, S and Milstien, S (2002). Sphingosine 1-phosphate, a key cell signaling molecule. *J. Biol. Chem.*, 277: 25851-25854.

Stobiecki, M; Wojtaszek, P and Gulewicz, K (1997). Application of solid phase extraction for profiling quinolizidine alkaloids and phenolic compounds in *Lupinus albus*. *Phytochem. Anal.*, 8(4): 153-158.

Su, T; Zhou, B; Cao, D; Pan, Y; Hu, M; Zhang, M; Wei, H and Han, M (2021). Transcriptomic profiling of *Populus* roots challenged with *Fusarium* reveals differential responsive patterns of invertase and invertase inhibitor-like families within carbohydrate metabolism. *J. Fungi*, 7(2): 89.

Tanaka, T; Abbas, H K and Duke, S O (1993). Structure-dependent phytotoxicity of fumonisins and related compounds in duckweed bioassay. *Phytochemistry*, 33: 779-785.

Tay, Z H and Chong, K P (2016). The potential of papaya leaf extract in controlling *Ganoderma boninense*. *IOP Conf. Series: Earth and Env. Sci.*, 36(1): 012027.

Teichmann, B; Lefebvre, F; Labbé, C; Böcker, M; Linne, U and Bélange, R R (2011). Beta hydroxylation of glycolipids from *Ustilago maydis* and *Pseudozyma flocculosa* by an NADPH-dependent β -hydroxylase. *Appl. Environ. Microbiol.*, 77: 7823-7829.

Toh Choon, R L; Sariah, M and Siti Mariam, M N (2012). Ergosterol from the soilborne fungus *Ganoderma boninense*. *J. Basic Microbiol.*, 52: 608-612.

Wang, H; Li, J; Bostock, R M and Gilchrist, D G (1996). Apoptosis: A functional paradigm for programmed plant cell death induced by a host selective phytotoxin and invoked during development. *Plant Cell*, 8: 375-391.

Wang, X; Li, J and Zhu, P (2017). Effect of *Sclerotinia sclerotiorum* on lipid metabolism in *Arabidopsis thaliana*. *J. Plant Dis. Prot.*, 124(5): 421-426.

Wright, B S; Snow, J W; O'Brien, T C and Lynch, D V (2003). Synthesis of 4-hydroxysphinganine and characterization of sphinganine hydroxylase activity in corn. *Arch. Biochem. Biophys.*, 415: 184-192.

Xie, L J; Chen, Q-F; Chen, M X; Yu, L J; Huang, L; Chen, L; Wang, F Z; Xia, F N; Zhu, T R; Wu, J X; Yin, J; Liao, B; Shi, J; Zhang, J H; Aharoni, A; Yao, N; Shu, W and Xiao, S (2015). Unsaturation of very-long-chain ceramides protects plant from hypoxia-induced damages by modulating ethylene signaling in *Arabidopsis*. *PLoS Genetic*, 11(3): e1005143.

Zianni, R; Bianco, G; Lelario, F; Losito, I; Palmisano, F and Cataldi, T R (2013). Fatty acid neutral losses observed in tandem mass spectrometry with collision-induced dissociation allows regiochemical assignment of sulfoquinovosyl-diacylglycerols. *J. Mass Spectrom.*, 48(2): 205-215.

OIL PALM SSR RESOURCE INTERFACE (OPSRI) – WEB-BASED BIOINFORMATIC ANALYSIS PIPELINE FOR SSR MINING

ROZANA ROSLI^{1*}; MOHD AMIN AB HALIM¹; TING NGOOT-CHIN¹; NOORHARIZA MOHD ZAKI¹; JAYANTHI NAGAPPAN¹; RAJINDER SINGH¹; LESLIE LOW ENG-TI¹ and ZETI-AZURA MOHAMED-HUSSEIN^{2,3}

ABSTRACT

Co-dominant simple sequence repeat (SSRs) are popular DNA markers, widely applied in oil palm genetic studies, especially in genetic mapping, marker-trait association, diversity analysis and detecting illegitimacy. A repository having detailed information on SSRs and their inheritance profiles in specific breeding lines is lacking in Malaysia. Such a database enables prioritising polymorphic SSRs for screening, identification of which otherwise can be expensive and time consuming. As such, to facilitate and accelerate development of informative SSRs, oil palm SSR resource interface (OPSRI) was established and currently contains information on 1983 markers that were genotyped successfully across several oil palm breeding lines. OPSRI is a well-structured database that can expedite marker development and reduce redundancy in the on-going efforts at developing DNA markers for genetic analysis of oil palm. The system has been integrated with MISA, Primer3, ORF search script and BLAST to enable SSR identification. This study also characterised a large number of expressed sequence tags (EST)-SSRs using an in-silico approach. The information within the OPSRI database can help accelerate research in oil palm as well as in crops such as coconut and date palm which have a high level of synteny and marker-transferability with oil palm.

Keywords: *Elaeis guineensis*, ESTs, SSR database.

Received: 6 January 2021; **Accepted:** 9 December 2021; **Published online:** 28 January 2022.

INTRODUCTION

Projects related to the generation of expressed sequence tags (ESTs) for understanding the transcribed space in the genomes of many plant species have aided gene identification and

the discovery of simple sequence repeat (SSR) markers. SSRs are short sequences of DNA that are repeated 1-6 times (Bhattarai *et al.*, 2021), and their identification and utilisation have been reported for many plants (Al-Faifi *et al.*, 2016; Song *et al.*, 2016; Vieira *et al.*, 2016). SSR markers have contributed immensely to the development and construction of genetic and physical maps. Among the wide repertoire of markers available for oil palm, SSRs have advantages as they are the most polymorphic co-dominant markers, high reproducibility, highly abundant in the genome and can be assayed using high resolution agarose gel electrophoresis systems that can be implemented in-house by many oil palm research stations (Gupta *et al.*, 1999; Singh *et al.*, 2007; Tautz and Renz, 1984; Zolkafli *et al.*, 2021). Interestingly, the location of an SSR locus in the genome has an influence on its level of

¹ Malaysian Palm Oil Board,
6 Persiaran Institusi, Bandar Baru Bangi,
43000 Kajang, Selangor, Malaysia.

² Department of Applied Physics,
Faculty of Science and Technology
Universiti Kebangsaan Malaysia,
43600 UKM Bangi, Selangor, Malaysia.

³ Centre for Bioinformatics Research,
Institute of Systems Biology (INBIOSIS)
Universiti Kebangsaan Malaysia,
43600 UKM Bangi, Selangor, Malaysia.

* Corresponding author e-mail: lizana@mpob.gov.my

polymorphism and specific function in a species (Lebedev *et al.*, 2020), where for example SSRs in the 5'UTR are more polymorphic compared to those in the 3'UTR (Wan *et al.*, 2020). SSRs found in the 5'UTR have an effect on gene transcription and regulation, whereas SSRs found in the 3'UTR are involved in gene silencing and transcription slippage (Li *et al.*, 2004; Varshney *et al.*, 2005). Furthermore, SSR markers located in non-coding introns (genomic SSRs), although not transcribed, are known to be involved in regulatory functions that influence plant development (Bagshaw, 2017; Lebedev *et al.*, 2020; Tranbarger *et al.*, 2012), thus, increasing their potential utility and informativeness. As such, determining the position of the SSR, whether it is in the 3'UTR, 5'UTR, coding or non-coding region is useful to further categorise the SSR. The information can be used to preselect and prioritise SSR markers for use in genetic analysis of the selected crop. Moreover, SSRs in genic regions are abundant and especially useful for tagging specific traits using the candidate gene approach.

In oil palm, SSRs are popular choice, and several studies have demonstrated the utilisation and efficiency of these markers. SSR markers have proven to be effective for use in studies such as genetic mapping, marker-trait association, as well as for analysing diversity of germplasm and advanced breeding lines (Bhagya *et al.*, 2020; Sunilkumar *et al.*, 2020; Ting *et al.*, 2013). More recently, Sarimana *et al.* (2021) demonstrated the effectiveness of these markers as tools for DNA fingerprinting, while Zolkafli *et al.* (2021) identified a core set of SSR markers that can be routinely used for the same purpose in a wide variety of oil palm genetic backgrounds. Previous studies have also established cross transferability of SSR (including EST-SSR) markers across closely related plants species, which enables a better understanding of their evolutionary history. Zaki *et al.* (2012) demonstrated that the SSR markers from oil palm could amplify across species and genera of the family Arecaceae. Meanwhile Bazzo *et al.* (2018), also observed a high transferability rate (>70%) of macauba palm-derived EST-SSR markers across six palm species in the Arecaceae family, namely; *Acrocomia intumescens*, *Acrocomia totai*, *E. guineensis*, *Bactris gasipaes*, *Euterpe edulis* and *Sabal causiarum*. Similarly reported for other plant species such as *Linum* (Soto-Cerda *et al.*, 2011), banana (Backiyarani *et al.*, 2013), *Prunus* (Sorkheh *et al.*, 2016) and chrysanthemum (Fan *et al.*, 2019). Transferability across closely related species and within genera facilitates genetic research, especially in understanding the mutational processes that have taken place within these regions among closely related plant species (Zaki *et al.*, 2012).

Although SSR markers have been widely utilised in oil palm research due to their ease of use at relatively low cost and availability from difference sources, a comprehensive database resource is still lacking. Two SSR databases are available in the public domain for oil palm: OpSatdb <https://ssr.icar.gov.in/index.php> (Babu *et al.*, 2019) and TropGENE-DB <https://tropgenedb.cirad.fr/tropgene/JSP/index.jsp> (Hamelin *et al.*, 2012), but these databases are limited to only listing the SSR markers. An expanded database that integrates experimental information related to the SSR markers and the potential utility of the SSR markers, will be more useful and desirable to researchers. The resource is required to help mine and characterise these markers more efficiently from the ever-growing repertoire of genomic resources that are becoming available. The huge amounts of data already available and new information being generated from the sequencing of additional oil palm breeding and germplasm lines have led to challenges in *de novo* mining of SSR and their utilisation. The existing EST collection and increasing availability of transcriptome and other related sequences, suggest that mining of SSRs from this enormous resource requires considerable technical skills, time and cost for successful execution. A number of software and scripts are available to assist in identifying SSR in sequences such as FullSSR (Metz *et al.*, 2016), GMATA (Wang and Wang, 2016), Krait (Du *et al.*, 2018) and recently reported Simple Sequence Repeat Molecular Marker Developer (SSRMMD) (Gou *et al.*, 2020). Nevertheless, data mining of SSRs would be more practical and cheaper when retrieved from an organised database (Vieira *et al.*, 2016), especially one with detailed information on the polymorphism of the SSR markers in selected breeding lines. The main limitation currently is that the SSR data and other relevant information, especially for oil palm, are mostly archived using spreadsheets, which are not robust and data retrieval can be difficult and messy. It is also difficult to integrate analytical tools to the spreadsheets. The availability of an automated database system, with easy-to-use analysis tools will enable researchers to more effectively retrieve relevant SSRs from ESTs and other collections of oil palm sequences for the development of SSR (including EST-SSR) markers. The availability of a web-based data management system will also make it more practical to manage information related to these markers such as motif type, repeat length, and position in the genome. Adding experimental information related to the specific SSRs, such as polymorphism rates and inheritance patterns in specific genetic backgrounds can expedite future research activities utilising these markers, especially in studies related to genetic mapping, QTL and diversity analysis.

MATERIALS AND METHODS

Data Source and Analysis

The dataset used in this study includes information on the sequence of the EG5.1 scaffold as well as amplification and segregation patterns of SSR markers in specific oil palm families. The information is stored in the database, where the user-friendly web interface facilitates data browsing. The data can also be downloaded easily through query interface. The experimental marker data-sets were generated from three mapping families of different genetic backgrounds namely, P2, T128 and OxG. P2 is a mapping family consisting of 87 *tenera* palms from Ulu Remis Deli *dura* (ENL48) and a Yangambi *pisifera* (ML161) (Ting *et al.*, 2013; 2014). T128 is a family consisting of 241 individual palms generated by self-pollination of a Nigerian *tenera* palm coded as 0.151/128, which is part of MPOB's germplasm collection (Singh *et al.*, 2013). The 0.151/128 was reported to have very high unsaturated oil with an iodine value (IV) of 63.4 (Kushairi *et al.*, 2011), which is higher than that observed in commercial *E. guineensis* palms. The OxG interspecific family consists of 108 F₁ hybrids generated by crossing the Colombian *E. oleifera* (UP1026) and palm 0.151/128 (Singh *et al.*, 2009; Ting *et al.*, 2014).

Development of the Oil Palm SSR Resource Interface (OPSRI)

OPSRI was developed using HTML5, PHP scripting language, CSS3 styling code, Javascript and Bootstrap framework packages. The MariaDB database is used to manage the SSR data. Figure 1 shows an Entity-Relationship Diagram (ERD) representing the database module. The ERD clearly shows the two main independent entities, namely the SSR markers in the public (published) and private (unpublished) databases, with two normalised tables, namely the library and clone_mypalmviewer tables. Markers from public and private entities may have been derived from the sequencing of the same library information which is available in the library table. The information which is available in the library table describes library details such as breeding line, genotype, species and methylation status. All SSR markers were mapped to oil palm genome build EG5.1 and their genome locations are stored in the clone_mypalmviewer table. A collection of experimental results obtained from the testing of the oil palm SSR primers on specific populations and/or breeding lines is deposited in primer_exp_res table. The specific populations and/or breeding lines for which marker data is available currently are the P2, T128 and OG families, which have been described above. In addition, the information on the location of the SSR containing sequences on the published

oil palm genome build (EG5.1) is available in clone_mypalmviewer and can be visualised in Oil Palm Genome Browser: MyPalmViewer, <http://gbrowse.mpob.gov.my> (Low *et al.*, 2020). The architecture of the system is important as it provides information on the dataflow and shows efficiency of data handling. The architecture is flexible and can accommodate integration of new systems or any restructuring required in the future. Apache servers are used to enable interaction between users and applications. This system is not restricted to any one operating system (OS) as it was tested on Windows, LINUX and MacOS. The analysis pipeline in OPSRI was developed using open-source bioinformatics tools such as NCBI BLAST (Altschul *et al.*, 1997), MISA scripts (Thiel *et al.*, 2003) and Primer3 programmes (Rozen and Skaletsky, 2000) and ORF search scripts (Figure 2).

In silico Mining of SSRs

Oil palm EST sequences, downloaded from NCBI GenBank database were assembled using the CAP3 assembly programme (Huang and Madan, 1999), resulting in contigs with consensus sequences and singletons, which help to avoid redundancy. The batch FASTA file of consensus and singleton sequences was used to mine for SSRs using MISA (MicroSatellite) (Thiel *et al.*, 2003). Criteria used for the SSR mining were minimum of 10 repeats for mononucleotide motifs, six repeats for dinucleotide, five repeats for trinucleotide, four for tetranucleotide and five for pentanucleotide repeat motifs. The maximum number of interrupting bases between two SSRs in a compound microsatellite was less than 100 bp. Additional bash scripts used the MISA output file as an input file to design primer pairs using Primer3 (Rozen and Skaletsky, 2000). Parameters set for primer design were as follows: length range from 18-24 bp, melting temperature 57°C-62°C and expected fragment size of 100-300 bp.

Classification of SSR Distribution

The SSR sequences were further classified into three categories, based on their genomic positions, 5'UTR, 3'UTR and coding region. The classifications were done by identifying the full-length open reading frames (ORF) of the unique gene datasets. SSRs located before the start codon were classified as 5' UTR whereas, SSRs found after the stop codon were considered to be in the 3' UTR. Finally, repeat motifs located between the start and stop codons were considered as SSRs located within the coding sequence (CDS) region. Blast analysis was also conducted on the SSR containing sequences, against the RefSeq protein database (O'Leary *et al.*, 2016) using NCBI BLAST (Altschul *et al.*, 1997). All positive hits with an e-value lower than 1×10^{-6} were included in the analysis.



Figure 1. ERD of OPSRI database.

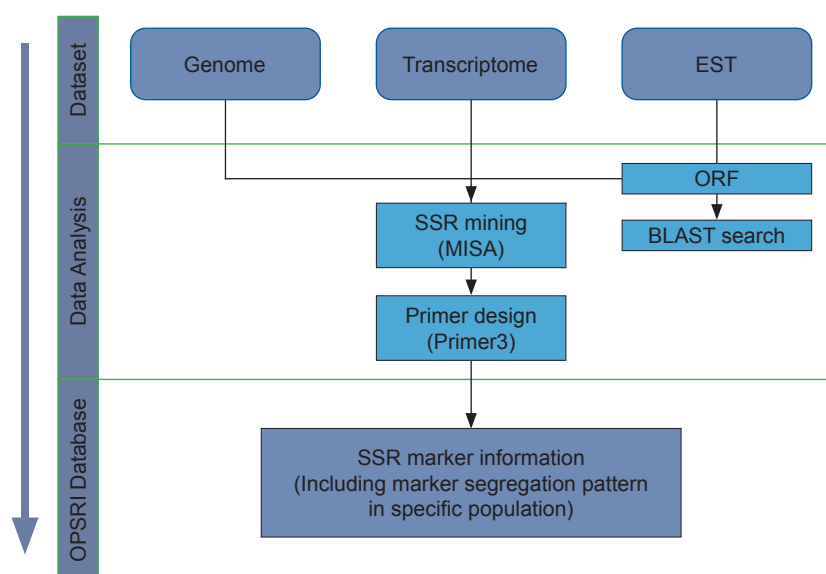


Figure 2. OPSRI analysis pipelines workflow.

RESULTS AND DISCUSSION

The Organisation of OPSRI

OPSRI comprises three main modules - database, analysis and query (Figure 3). The OPSRI homepage is divided into three panels, namely the top, navigation and results panels. The top panel includes the Home, Sitemap, Change Password, Logout and Query Search (Figures 4a-i). The navigation panel (bottom left panel) contains File Manager, Analysis Tools, Analysis Pipeline, SSR Database, Contact Us and Manual (Figures 4a-ii). The results panel (bottom right panel) displays results of the queries or analysis (Figures 4a-iii). Users can perform the data query in SSR database navigation panel. In a single webpage, the user can analyse, explore and perform the data query or view the results of a search. The list of publications associated with the data is also shared with users in a graphic link form. A manual pocket book, which is accessible after login, provides detailed instructions to guide users on the analysis module and also to help them get familiar with the system. To facilitate easy and fast interaction, the module "Contact Us" is available to provide support to users. User feedback is included as part of the efforts to improve the system.

Database Features

The OPSRI database at present contains information on a total of 1983 published SSR markers (Ting *et al.*, 2016). These SSRs were developed from various genomic, transcriptome and EST libraries as well as from selective regions in the published oil palm EG5 genome build (Low *et al.*, 2018). The

SSR markers from genomic libraries are given the nomenclature sEG, sMg, sMo, sMh, sPSc, sOleiSc and PA while those from transcriptomes are sTEg and those from ESTs are labelled as sEG. The SSRs were utilised for construction of genetic linkage maps for several mapping populations, coded as P2 [Ulu Remis Deli *dura* (ENL48) × Yangambi *pisifera* (ML161)], T128 (self-crossing of a Nigerian *tenera*, T128) and OxG [Colombian *E. oleifera* (UP1026) × Nigerian *tenera* (T128)]. A total of 191, 92 and 156 SSR markers were polymorphic in the three mapping populations, namely P2, T128 and OG, respectively as summarised in Table 1. The results showed that a high proportion of the polymorphic SSRs were di(p2)- repeats at 64%, followed by tri(p3)- repeat motifs at 15%. These two repeat motifs were also found to be the most abundant in the oil palm genome (Babu *et al.*, 2019). The identification and recording in the database, of SSR markers that are polymorphic in a specific family is useful to researchers, as they can prioritise these SSR markers for the genetic analysis of specific populations. The utilisation of these SSR markers will also facilitate comparison across different studies especially if they are linked to the same trait in a QTL analysis, which will add confidence to the marker-trait association observed. These are among the main advantages of the present database.

OPSRI Usage

A number of studies have reported on the availability of bioinformatics pipelines for mining of SSRs from sequencing data, examples of which include a system packaged in the tool Galaxy (Griffiths *et al.*, 2016) and a system known as



TABLE 1. SUMMARY OF SSR MARKERS GENOTYPED IN THREE MAPPING POPULATIONS

No.	SSR type	Library	Polymorphic SSR		
			P2	T128	OxG
1	Compound (C)	EST	1	1	2
		<i>Pisifera</i> V3*	1	-	-
		<i>Pisifera</i> V4*	4	-	2
		<i>Pisifera</i> V5*	2	-	3
		233 [§] (<i>Dura</i> _MF) ⁺	5	-	2
		282 [§] (<i>Columbian Oleifera</i> _MF) ⁺	4	1	3
		Total	17	2	12
2	Imperfect compound (C*)	EST	-	1	1
		<i>Pisifera</i> V3*	1	1	1
		233 [§] (<i>Dura</i> _MF) ⁺	8	2	5
		282 [§] (<i>Columbian Oleifera</i> _MF) ⁺	4	1	1
		Total	13	5	8
3	Mono-repeats (p1)	EST	-	2	2
		Transcriptome	1	1	1
		233 [§] (<i>Dura</i> _MF) ⁺	1	-	1
		280 [§] (<i>Dura</i> _MF) ⁺	1	-	-
		282 [§] (<i>Columbian Oleifera</i> _MF) ⁺	-	-	1
		Total	3	3	5
4	Di-repeats (p2)	EST	8	9	12
		Transcriptome	-	2	2
		<i>Pisifera</i> V1*	6	8	8
		<i>Pisifera</i> V3*	8	6	4
		<i>Pisifera</i> V4*	-	-	2
		<i>Pisifera</i> V5*	5	-	3
		213 [§] (<i>Dura</i> _UF) [#]	1	-	-
		233 [§] (<i>Dura</i> _MF) ⁺	24	8	20
		280 [§] (<i>Dura</i> _MF) ⁺	1	-	-
		281 [§] (<i>Pisifera</i> _MF) ⁺	31	13	28
		282 [§] (<i>Columbian Oleifera</i> _MF) ⁺	37	12	20
		302 [§] (<i>Columbian Oleifera</i> _MF) ⁺	1	1	1
		Total	122	59	100
5	Tri-repeats (p3)	EST	5	9	9
		<i>Oleifera</i>	-	2	2
		<i>Pisifera</i> V1*	2	3	4
		<i>Pisifera</i> V3*	1	1	1
		<i>Pisifera</i> V4*	2	-	2
		<i>Pisifera</i> V5*	2	-	-
		233 [§] (<i>Dura</i> _MF) ⁺	1	1	1
		281 [§] (<i>Pisifera</i> _MF) ⁺	1	-	-
		282 [§] (<i>Columbian Oleifera</i> _MF) ⁺	10	1	6
		Total	24	17	25
6	Tetra-repeats (p4)	EST	2	1	1
		<i>Pisifera</i> V1*	1	2	1
		234 [§] (<i>Dura</i> _UF) [#]	1	-	1
		282 [§] (<i>Columbian Oleifera</i> _MF) ⁺	2	-	1
		Total	6	3	4
7	Penta-repeats (p5)	<i>Pisifera</i> V3*	2	2	-
		233 [§] (<i>Dura</i> _MF) ⁺	1	-	-
		282 [§] (<i>Columbian Oleifera</i> _MF) ⁺	2	-	-
		Total	5	2	0
8	Hexa-repeats (p6)	233 [§] (<i>Dura</i> _MF) ⁺	-	1	1
		282 [§] (<i>Columbian Oleifera</i> _MF) ⁺	1	-	1
		Total	1	1	2

Note: **Pisifera* V1, V2, V3, V4 and V5 denotes the assembly version of the *Pisifera* genome. #UF standard (no methylation filter) genomic library, +MF methyl filtered genomic library, § Information for the library is available in Low *et al.* (2014), - no polymorphic markers genotyped.

ESAP Plus (Ponyared *et al.*, 2016). More recently, an approach was introduced which can directly identify polymorphic SSRs. The system known as IDSSR, facilitates mining of polymorphic SSRs within regions that were pre-classified as insertion/deletions in a single sequenced genome (Guang *et al.*, 2019). However, apart from the fact that IDSSR is a Perl script-based pipeline which requires some level of computational expertise to execute, the SSRs are mined only from specific regions of the genome, resulting in potential loss of informative markers in other parts of the genome. In addition, SSRome, a web-based database that mined SSRs from 6533 different organisms and further classified them as either originating from genic/non-genic regions among other characteristics, was also reported (Mokhtar and Atia, 2019). However, in the databases described above, experimental details of the SSR markers are not included. In this respect, the main advantage of the OPSRI web-based pipeline reported here compared to the other systems is that it is integrated with the oil palm SSR genotyping database, which facilitates identification of the most informative markers, based on actual experimental data obtained from diverse genetic backgrounds, namely the P2, T128 and O_xG families.

Generally, the OPSRI pipelines developed in this study allow for a wider application, ranging from SSR search to primer design (combinations of MISA, Primer3, ORF search script and BLAST). In addition, users can directly search and compare the primers designed with those available within the OPSRI database, to avoid redundancy in assaying for a particular SSR locus. This is a clear advantage over other web-based tools that only focus on specific steps in the analysis *e.g.*, MISA-web which was designed to only search for SSRs (Beier *et al.*, 2017). The OPSRI analysis module also provides two options to users, who can either key in the fasta format sequence into the query box (under File Manager) or upload the sequence file (Figure 4b). In OPSRI, users also have the option to analyse the sequence(s) either by selecting a specific programme or using the analysis pipeline linking all the four programmes *i.e.*, MISA, Primer3, ORF search script and BLAST. Results generated from the pipeline can be viewed (via hover or by clicking on the file name) or downloaded.

The query module in the database has been designed with graphical user interface to facilitate data archiving (Figure 4c). This is particularly helpful to users who are not familiar with Structural Query Language (SQL) scripting. Search for marker information such as SSR type, motif, primer information and marker genotype profile can be performed by selecting any query option such as marker name (*e.g.*, sMg00026), SSR type (*e.g.*, p3), primer sequence or library (*e.g.*, EST) (Figure 4d). Additionally, a free text search for SSR markers

linked to specific genes such as the shell and fruit colour genes is also available. The database allows easy retrieval of information related to these markers.

Information available on the motif types and the experimental data facilitates identification of polymorphic SSR primers. Therefore, markers informative in one population can be prioritised to analyse oil palm derived from different genetic backgrounds, enabling a wide range of genetic studies, such as the saturation of genetic linkage maps. Users can register and access the OPSRI database at no cost at <http://opsri.mpob.gov.my>. Standard users will be able to view and download publicly available SSR primers, while OPSRI pre-registered users at MPOB will be able to access both public and private databases.

Distribution of Repeat Motifs in Oil Palm EST to Enrich the Information in OPSRI

In order to enhance the information available in OPSRI, a subset of EST data from a NCBI Genbank was mined for SSR markers and the positions of the repeat motifs were either located in the UTR or CDS regions. EST cluster analysis revealed 13 600 consensus and 7631 singletons giving a total of 21 231 unique sequences. Twenty sequences with fewer than 100 nucleotides were excluded from further analysis. The subsequent SSR search showed that 2465 SSRs were found in 2014 EST clones. The 2465 SSRs consisted of 983 (39.9%) mononucleotides, 794 (32.2%) dinucleotides, 650 (26.4%) trinucleotides, 31 (1.25%) tetranucleotides, 4 (0.16%) pentanucleotides and 3 (0.12%) hexanucleotides. The distribution of the number of repeats observed across the SSR motifs is shown in Table 2. The high number of dinucleotides observed in the ESTs utilised in this study compared to trinucleotides is consistent with that observed by Babu *et al.* (2019) in oil palm. Among the dinucleotide repeats, the most common motif is AG/CT (75.5%), whereas CG/CG (0.1%) is the least abundant motif. The AG/CT repeats are also the most copious motifs in many plants (Guo *et al.*, 2017; Liu *et al.*, 2018; Rabeh *et al.*, 2018; Wan *et al.*, 2020). With respect to the tri-nucleotide repeats, AGG/CCT motif was the most common, followed by AAG/CTT and CCG/CGG. The A/T motif was the most abundant repeat motif observed in this study. Tóth *et al.* (2000) also observed an abundance of A/T motifs in various eukaryotic genomes, which the authors reported was likely due to the poly (A/T) tails of specific retrotransposon's (especially ALU and LINE-1) scattered across the genome. Interestingly the number of A/T repeats observed declined with increase in repeat length (Table 1) as was observed for monocotyledons by Qin *et al.* (2015).

TABLE 2. FREQUENCY AND DISTRIBUTION OF THE DIFFERENT SSR TYPES IDENTIFIED IN 2014 OIL PALM ESTs

Repeats	No. of repeats												Total
	5	6	7	8	9	10	11	12	13	14	15	>15	
A/T	-	-	-	-	-	215	130	88	86	63	52	240	874
C/G	-	-	-	-	-	40	29	7	11	4	7	11	109
AC/GT	-	7	19	10	12	5	3	1	1	2	1	5	66
AG/CT	-	33	133	110	110	49	34	16	12	15	10	70	592
AT/AT	-	11	21	19	28	14	9	9	3	2	2	14	132
CG/CG	-	3	-	1	-	-	-	-	-	-	-	-	4
AAC/GTT	8	6	2	2	-	1	-	-	-	-	-	-	19
AAG/CTT	55	29	17	9	6	1	2	-	-	-	-	1	120
AAT/ATT	15	8	5	-	4	2	1	2	-	-	-	1	38
ACC/GGT	38	17	4	-	1	1	-	1	-	1	-	-	63
ACG/CGT	7	1	3	2	-	-	-	-	-	-	-	-	13
ACT/AGT	2	-	1	-	-	-	-	-	1	-	1	-	5
AGC/CTG	36	21	11	11	1	2	-	-	-	-	-	1	83
AGG/CCT	78	32	12	8	6	1	-	-	-	-	-	-	137
ATC/ATG	34	14	5	3	3	2	-	-	-	-	-	-	61
CCG/CGG	65	24	13	4	4	1	-	-	-	-	-	-	111
AAAG/CTTT	2	1	-	-	-	-	-	-	-	-	-	-	3
AAAT/ATTT	6	1	-	-	-	-	-	-	-	-	-	-	7
AACC/GGTT	-	1	-	-	-	-	-	-	-	-	-	-	1
AAGC/CTTG	-	1	-	-	-	-	-	-	-	-	-	-	1
AAGG/CCTT	-	-	1	-	-	-	-	-	-	-	-	-	1
ACAT/ATGT	4	2	-	-	-	1	-	-	-	-	-	-	7
ACGC/CGTG	1	-	-	-	-	-	-	-	-	-	-	-	1
ACGT/ACGT	1	-	-	-	-	-	-	-	-	-	-	-	1
AGAT/ATCT	-	-	-	-	1	-	-	-	-	-	-	-	1
AGCG/CGCT	1	-	-	-	-	-	-	-	-	-	-	-	1
AGGC/CCTG	1	-	-	-	-	-	-	-	-	-	-	-	1
AGGG/CCCT	1	1	-	-	-	-	-	-	-	-	-	-	2
ATGC/ATGC	2	1	-	-	-	-	-	-	-	-	-	-	3
CCCG/CGGG	1	-	-	-	-	-	-	-	-	-	-	-	1
AAAAT/ATTTT	2	-	-	-	-	-	-	-	-	-	-	-	2
AAGGG/CCCTT	1	-	-	-	-	-	-	-	-	-	-	-	1
AATAT/ATATT	1	-	-	-	-	-	-	-	-	-	-	-	1
ACCTCG/AGGTCG	1	-	-	-	-	-	-	-	-	-	-	-	1
ACGCCG/CGGCGT	1	-	-	-	-	-	-	-	-	-	-	-	1
AGCCTG/AGGCTC	-	1	-	-	-	-	-	-	-	-	-	-	1
N													983
NN													794
NNN													650
NNNN													31
NNNNN													4
NNNNNN													3

Note: - No SSR identified.

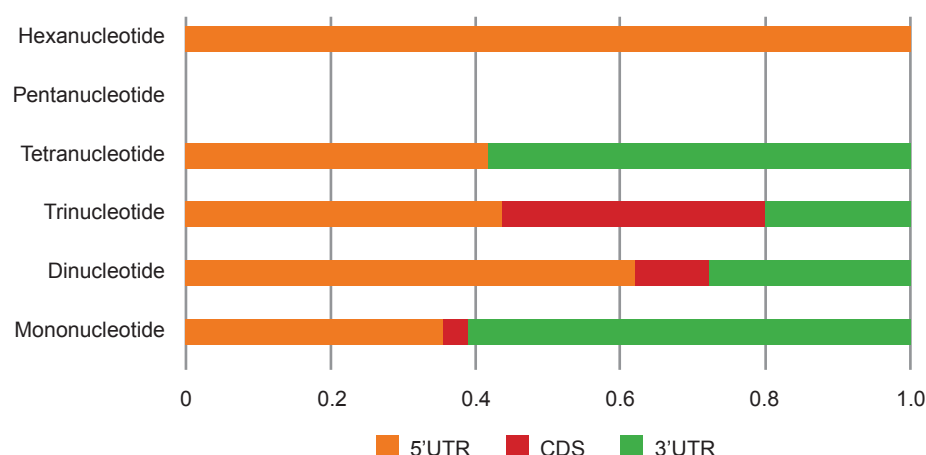


Figure 5. A comparison of the distribution of different SSR repeat motifs in the 5'UTR, 3'UTR and CDS.

The location of each SSR, whether in the UTR or coding region, was identified and validated through the Blast programme. The results revealed 481 ESTs with 544 SSRs in the 5'UTR, 316 ESTs with 372 SSRs in the 3'UTR and 150 ESTs with 175 SSRs in the coding region. An analysis of the SSR motifs in the 5'UTR, 3'UTR and coding regions revealed that 84, 70 and 14 ESTs, respectively have more than one SSR. The analysis also showed that the UTRs contained more SSRs compared to coding regions, similar to the pattern observed in rice and *Arabidopsis* (Lawson *et al.*, 2006). The distribution of different repeat type classes also showed that the 5'UTR had higher dimeric repeats (247/397) and trinucleotides (144/331), while the 3'UTR showed more mono repeats (188/350). In CDS, the large number of trinucleotide motifs (121/331) are probably involved in increasing the protein size due to the repeat domains. For the dinucleotide repeats, there was a bias towards the AG/CT motif in both UTRs, with 221 found in the 5'UTR and 67 in the 3'UTR. Similarly, in the CDS, 37 dinucleotide repeats contained the AG/CT motif. The abundance of the dinucleotide AG/CT SSR motifs was also reported in other oil plants such as *Arachis hypogaea* (Wang *et al.*, 2017) and *Elaeagnus mollis* (Liu *et al.*, 2020). Interestingly, the 5' UTR contained more trinucleotides than the 3' UTR (44% *vs.* 20% SSR). Figure 5 shows a comparison of the distribution of the different SSR repeat motifs in the 5'UTR, 3'UTR and CDS.

These observations have been incorporated into the database and may assist in selecting and designing primers flanking the appropriate motifs for experimental purposes. Selecting dinucleotide motifs in UTR and trinucleotides in CDS, especially those with a long repeat type may improve chances of identifying polymorphic SSR markers in oil palm populations. However, it is important to note that the association between the polymorphism

level of an SSR and the length of SSR motif is species dependent (Hou *et al.*, 2017; Sigang *et al.*, 2021), and as such, when the information becomes available for oil palm it will be updated on the database. For example, polymorphism rates were not associated with the length of the SSR in peanut, where the polymorphism decreased as motif repeat number increased (Zhao *et al.*, 2012). In addition, Scott *et al.* (2000) observed that in grapes, the level of polymorphism for the SSR uncovered from the three different regions of an EST varied at the taxonomy level (genera, cultivar and species). The study further revealed that SSRs from the 3'UTR were most polymorphic when screening cultivars, while SSRs at the 5'UTR were informative when comparing cultivar of different species. The SSRs in CDS were most polymorphic between species and samples from related genera. Thus, this and other relevant information can serve as a guide to researchers to select and prioritise EST-SSR markers from the OPSRI database for use in their own research programmes.

CONCLUSION

In this study, the development of a database integrated with bioinformatics tools (MISA, Primer3, ORF search script and BLAST), facilitates the mining of SSRs from a collection of oil palm sequences. The database is further enriched with experimental data, and information on frequency and distribution of repeat motifs as well as location of the SSR motifs in a gene. Such information is highly useful for selecting candidate SSR markers for use in research. As such, the systematic archiving of information on the SSRs markers in this web-based tool, should prove useful for oil palm genetic studies. More importantly, researchers will also have the opportunity to select SSR markers-

based on their previous performance (especially polymorphism rate in specific families), length of repeat motifs or position in the genome, which will accelerate implementation of genomics guided breeding programmes in oil palm. The database facilitates data-sharing among researchers, where the benefit extends beyond oil palm to other plants from closely related genera and taxa such as coconut and date palm.

ACKNOWLEDGEMENT

We thank the Director-General of MPOB for permission to publish this article. We would also like to acknowledge colleagues of the Bioinformatics and Genomics Units for their kind assistance. Special thanks to Prof. Denis Murphy from the University of South Wales, Dr. Chan Pek Lan and Dr. S. Ravigadevi for valuable comments on the article.

REFERENCES

- Al-Faifi, S A; Migdadi, H M; Algamdi, S S; Khan, M A; Ammar, M H; Al-Obeed, R S; Al-Thamra, M I; El-Harty, E H and Jakse, J (2016). Development, characterization and use of genomic SSR markers for assessment of genetic diversity in some Saudi date palm (*Phoenix dactylifera* L.) cultivars. *Electron. J. Biotechnol.*, 21: 18-25.
- Altschul, S F; Madden, T L; Schäffer, A A; Zhang, J; Zhang, Z; Miller, W and Lipmann, D J (1997). Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. *Nucleic Acids Res.*, 25(17): 3389-3402.
- Babu, K; Mary Rani, K L; Sahu, S; Mathur, R K; Naveen Kumar, P; Ravichandran, G; Anitha, P and Bhagya, H P (2019). Development and validation of whole genome-wide and genic microsatellite markers in oil palm (*Elaeis guineensis* Jacq.): First microsatellite database (OpSatdb). *Sci. Rep.*, 9(1): 1899.
- Backiyarani, S; Uma, S; Varatharaj, P and Saraswathi, M S (2013). Mining of EST-SSR markers of *Musa* and their transferability studies among the members of order the Zingiberales. *Appl. Biochem. Biotechnol.*, 169(1): 228-238.
- Bagshaw, A T M (2017). Functional mechanisms of microsatellite DNA in eukaryotic genomes. *Genome Biol. Evol.*, 9(9): 2428-2443.
- Bazzo, B R; de Carvalho, L M; Carazzolle, M F; Pereira, G A G and Colombo, C A (2018). Development of novel EST-SSR markers in the macaúba palm (*Acrocomia aculeata*) using transcriptome sequencing and cross-species transferability in Arecaceae species. *BMC Plant Biol.*, 18(1): 276.
- Beier, S; Thiel, T; Münch, T; Scholz, U and Mascher, M (2017). MISA-web: A web server for microsatellite prediction. *Bioinformatics*, 33(16): 2583-2585.
- Bhagya, H P; Kalyana Babu, B; Gangadharappa, P M; Naika, M B N; Satish, D and Mathur, R K (2020). Identification of QTLs in oil palm (*Elaeis guineensis* Jacq.) using SSR markers through association mapping. *J. Genet.*, 99(1): 19.
- Bhattarai, G; Shi, A; Kandel, D R; Solís-Gracia, N; da Silva, J A and Avila, C A (2021). Genome-wide simple sequence repeats (SSR) markers discovered from whole-genome sequence comparisons of multiple spinach accessions. *Sci. Rep.*, 11(1): 9999.
- Du, L; Zhang, C; Liu, Q; Zhang, X and Yue, B (2018). Krait: An ultrafast tool for genome-wide survey of microsatellites and primer design. *Bioinformatics*, 34(4): 681-683.
- Fan, M; Gao, Y; Gao, Y; Wu, Z; Liu, H and Zhang, Q (2019). Characterization and development of EST-SSR markers from transcriptome sequences of chrysanthemum (*Chrysanthemum × morifolium* Ramat.). *HortScience*, 54(5): 772-778.
- Gou, X; Shi, H; Yu, S; Wang, Z; Li, C; Liu, S; Ma, J; Chen, G; Liu, T and Liu, Y (2020). SSRMMD: A rapid and accurate algorithm for mining SSR feature loci and candidate polymorphic SSRs based on assembled sequences. *Front. Genet.*, 11: 706.
- Griffiths, S M; Fox, G; Briggs, P J; Donaldson, I J; Hood, S; Richardson, P; Leaver, G W; Truelove, N K and Preziosi, R F (2016). A galaxy-based bioinformatics pipeline for optimised, streamlined microsatellite development from Illumina next-generation sequencing data. *Conserv. Genet. Resour.*, 8(4): 481-486.
- Guang, X M; Xia, J Q; Lin, J Q; Yu, J; Wan, Q H and Fang, S G (2019). IDSSR: An efficient pipeline for identifying polymorphic microsatellites from a single genome sequence. *Int. J. Mol. Sci.*, 20(14): 3497.
- Guo, R; Landis, J B; Moore, M J; Meng, A; Jian, S; Yao, X and Wang, H (2017). Development and application of transcriptome-derived microsatellites in *Actinidia eriantha* (Actinidiaceae). *Front. Plant Sci.*, 8: 1383.
- Gupta, P K; Varshney, R K; Sharma, P C and Ramesh, B (1999). Molecular markers and their applications in wheat breeding. *Plant Breed.*, 118(5): 369-390.

- Hamelin, C; Sempere, G; Jouffe, V and Ruiz, M (2012). TropGeneDB, the multi-tropical crop information system updated and extended. *Nucleic Acids Res.*, 41(D1): D1172-D1175.
- Hou, B; Feng, S and Wu, Y (2017). Systemic identification of *Hevea brasiliensis* EST-SSR markers and primer screening. *J. Nucleic Acids*, 2017: 1-9.
- Huang, X and Madan, A (1999). CAP3: A DNA sequence assembly program. *Genome Res.*, 9(9): 868-877.
- Kushairi, A; Mohd Din, A and Rajananidu, N (2011). Oil palm breeding and seed production. *Further Advances in Oil Palm Research (2000-2010)* (Mohd Basri, W; Choo, Y M and Chan, K W eds.). Vol. 1. MPOB, Bangi. p. 47-101.
- Lawson, M J and Zhang, L (2006). Distinct patterns of SSR distribution in the *Arabidopsis thaliana* and rice genomes. *Genome Biol.*, 7(2): 1-11.
- Lebedev, V G; Subbotina, N M; Maluchenko, O P; Lebedeva, T N; Krutovsky, K V and Shestibratov, K A (2020). Transferability and polymorphism of SSR markers located in flavonoid pathway genes in *Fragaria* and *Rubus* species. *Genes*, 11(1): 11.
- Li, Y C; Korol, A B; Fahima, T and Nevo, E (2004). Microsatellites within genes: Structure, function and evolution. *Mol. Biol. Evol.*, 21(6): 991-1007.
- Liu, Y; Guo, Y; Xing, D and Long, C (2018). Development and characterization of genomic simple sequence repeats for *Colocasia gigantea* (Blume) Schott using 454 sequencing. *Chilean J. Agric. Res.*, 78(1): 23-29.
- Liu, Y; Li, S; Wang, Y; Liu, P and Han, W (2020). De novo assembly of the seed transcriptome and search for potential EST-SSR markers for an endangered, economically important tree species: *Elaeagnus mollis* diels. *J. For. Res.*, 31(3): 759-767.
- Low, E-T L; Rosli, R; Jayanthi, N; Mohd-Amin, A H; Azizi, N; Chan, K L; Maqbool, N J; Maclean, P; Brauning, R; McCulloch, A; Moraga, R; Ong-Abdullah, M and Singh, R (2014). Analyses of hypomethylated oil palm gene space. *PLoS ONE*, 9(1): p.e86728.
- Low, E-T L; Jayanthi, N; Chan, K-L; Sanusi, N S N M; Ab Halim, M A; Rosli, R; Azizi, N; Amiruddin, N; Angel, L P L; Ong-Abdullah, M; Singh, R; Manaf, M A A; Sambanthamurthi R; Parveez, G K A and Kushairi, A (2018). The oil palm genome revolution. *J. Oil Palm Res.*, 29(4): 456-468.
- Low, E-T L; Azizi, N; Halim, M A A; Sanusi, N S N M; Chan, K-L; Amiruddin, N; Jayanthi, N; Ong-Abdullah, M; Singh, R; Sambanthamurthi, R; Manaf, M A A A and Kushairi, A (2020). Oil palm genome: Strategies and applications. *The Oil Palm Genome* (Ithnin, M and Din, A K eds.). *Compendium of Plant Genomes*. Springer, Cham. p. 83-115.
- Metz, S; Cabrera, J M; Rueda, E; Giri, F and Amavet, P (2016). FullSSR: Microsatellite finder and primer designer. *Adv. Bioinform.*, 2016: 1-4.
- Mokhtar, M M and Atia, M A M (2019). SSRome: An integrated database and pipelines for exploring microsatellites in all organisms. *Nucleic Acids Res.*, 47(D1): D244-D252.
- O'Leary, N A; Wright, M W; Brister, J R; Ciufo, S; Haddad, D; McVeigh, R; Rajput, B; Robbertse, B; Smith-White, B; Ako-Adjei, D; Astashyn, A; Badretdin, A; Bao, Y; Blinkova, O; Brover, V; Chetvernin, V; Choi, J; Cox, E; Ermolaeva, O; Farrell, C M; Goldfarb, T; Gupta, T; Haft, D; Hatcher, E; Hlavina, W; Joardar, V S; Kodali, V K; Li, W; Maglott, D; Masterson, P; McGarvey, K M; Murphy, M R; O'Neill, K; Pujar, S; Rangwala, S H; Rausch, D; Riddick, L D; Schoch, C; Shkeda, A; Storz, S S; Sun H; Thibaud-Nissen, F; Tolstoy, I; Tully, R E; Vatsan, A R; Wallin, C; Webb, D; Wu, W; Landrum, M J; Kimchi, A; Tatusova, T; DiCuccio, M; Kitts, P; Murphy, T D and Pruitt, K D (2016). Reference sequence (RefSeq) database at NCBI: Current status, taxonomic expansion, and functional annotation. *Nucleic Acids Res.*, 44(D1): D733-D745.
- Ponyared, P; Ponsawat, J; Tongsim, S; Seresangtakul, P; Akkasaeng, C and Tantisuwichwong, N (2016). ESAP plus: A web-based server for EST-SSR marker development. *BMC Genomics*, 17(13): 163-173.
- Qin, Z; Wang, Y; Wang, Q; Li, A; Hou, F and Zhang, L (2015). Evolution analysis of simple sequence repeats in plant genome. *PLoS ONE*, 10(12): p.e0144108.
- Rabeh, K; Gaboun, F; Belkadi, B and Filali-Maltouf, A (2018). In silico development of new SSRs primer for aquaporin linked to drought tolerance in plants. *Plant Signal. Behav.*, 13(11): 1-7.
- Rozen, S and Skaletsky, H (2000). Primer3 on the WWW for general users and for biologist programmers. *Bioinformatics Methods Protocols* (Misener, S and Krawetz, S A eds.). *Methods in Molecular Biology*TM. Vol. 132. Humana Press, Totowa, NJ. p. 365-386.

- Sarimana, U; Herrero, J; Erika, P; Indarto, N; Wendra, F; Santika, B; Ritter, E; Sembiring, Z and Asmono, D (2021). Analysis of genetic diversity and discrimination of oil palm DxP populations based on the origins of *pisifera* elite parents. *Breeding Sci.*, 71(2): 134-143.
- Scott, K D; Eggler, P; Seaton, G; Rossetto, M; Ablett, E M; Lee, L S and Henry, R J (2000). Analysis of SSRs derived from grape ESTs. *Theor. Appl. Genet.*, 100: 723-726.
- Sigang, F; Hao, H; Yong, L; Pengfei, W; Chao, Z; Lulu, Y; Xiuting, Q and Qiu, L (2021). Genome-wide identification of microsatellite and development of polymorphic SSR markers for spotted sea bass (*Lateolabrax maculatus*). *Aquac. Rep.*, 20: 100677.
- Singh, R; Nagappan, J; Tan, S G; Panandam, J M and Cheah, S C (2007). Development of simple sequence repeat (SSR) markers for oil palm and their application in genetic mapping and fingerprinting of tissue culture clones. *Asia Pac. J. Mol. Biol. Biotechnol.*, 15(3): 121-131.
- Singh, R; Tan, S G; Panandam J M; Rahimah, A R; Leslie Ooi, L C L; Low, E T L; Sharma, M; Jansen, J and Cheah, S C (2009). Mapping quantitative trait loci (QTLs) for fatty acid composition in an interspecific cross of oil palm. *BMC Plant Biol.*, 9: 114.
- Singh, R; Ong-Abdullah, M; Low, E T L; Abdul Manaf, M A; Rosli, R; Nookiah, R; Leslie Ooi, C L; Ooi, S-E; Chan, K-L; Halim, M A; Azizi, N; Nagappan, J; Bacher, B; Lakey, N; Smith, S W, He, D; Hogan, M; Budiman, M A; Lee, E K; DeSalle, R; Kudrna, D; Goicoechea, J L; Wing, R A; Wilson, R K; Fulton, R S; Ordway, J M; Martienssen, R A and Sambanthamurthi, R (2013). Oil palm genome sequence reveals divergence of interfertile species in old and new worlds. *Nature*, 500(7462): 335-339.
- Song, X Y; Zhang, C Z; Li, Y; Feng, S S; Yang, Q and Huang, S W (2016). SSR analysis of genetic diversity among 192 diploid potato cultivars. *Hortic. Plant J.*, 2(3): 163-171.
- Sorkheh, K; Prudencio, A S; Ghebinejad, A; Dehkordi, M K; Erogul, D; Rubio, M and Martínez-Gómez, P (2016). *In silico* search, characterization and validation of new EST-SSR markers in the genus *Prunus*. *BMC Res. Notes*, 9(1): 1-11.
- Soto-Cerda, B J; Saavedra, H U; Navarro, C N and Ortega, P M (2011). Characterization of novel genic SSR markers in *Linum usitatissimum* (L.) and their transferability across eleven *Linum* species. *Electron. J. Biotechnol.*, 14(2): 4-4.
- Sunilkumar, K; Murugesan, P; Mathur, R K and Rajesh, M K (2020). Genetic diversity in oil palm (*Elaeis guineensis* and *Elaeis oleifera*) germplasm as revealed by microsatellite (SSR) markers. *Indian J. Agric. Sci.*, 90(4): 69-73.
- Tautz, D and Renz, M (1984). Simple sequences are ubiquitous repetitive components of eukaryotic genomes. *Nucleic Acids Res.*, 12(10): 4127-4138.
- Thiel, T; Michalek, W; Varshney, R and Graner, A (2003). Exploiting EST databases for the development and characterization of gene-derived SSR-markers in barley (*Hordeum vulgare* L.). *Theor. Appl. Gen.*, 106(3): 411-422.
- Ting, N C; Jansen, J; Nagappan, J; Ishak, Z; Chin, C W; Tan, S G; Cheah, S C and Singh, R (2013). Identification of QTLs associated with callogenesis and embryogenesis in oil palm using genetic linkage maps improved with SSR markers. *PLoS ONE*, 8(1): p.e53076.
- Ting, N C; Jansen, J; Mayes, S; Massawe, F; Sambanthamurthi, R; Ooi, L C L; Chin, C W; Arulandoo, X; Seng, T Y; Alwee, S S R S and Ithnin, M (2014). High density SNP and SSR-based genetic maps of two independent oil palm hybrids. *BMC Genom.*, 15(1): 1-11.
- Ting, N C; Yaakub, Z; Kamaruddin, K; Mayes, S; Massawe, F; Sambanthamurthi, R; Jansen, J; Low, L E T; Ithnin, M; Kushairi, A; Arulandoo, X; Rosli, R; Chan, K L; Amiruddin, N; Sritharan, K; Lim, C C; Nookiah, R; Amiruddin, M D and Singh, R (2016). Fine-mapping and cross-validation of QTLs linked to fatty acid composition in multiple independent interspecific crosses of oil palm. *BMC Genom.*, 17(1): 1-17.
- Tóth, G; Gáspári, Z and Jurka, J (2000). Microsatellites in different eukaryotic genomes: Survey and analysis. *Genome Res.*, 10(7): 967-981.
- Tranbarger, T J; Kluabmongkol, W; Sangsrakru, D; Morcillo, F; Tregear, W J; Tragoonrung, S and Billotte, N (2012). SSR markers in transcripts of genes linked to post-transcriptional and transcriptional regulatory functions during vegetative and reproductive development of *Elaeis guineensis*. *BMC Plant Biol.*, 12(1): 1-12.
- Varshney, R K; Graner, A and Sorrells, M E (2005). Genic microsatellite markers in plants: Features and applications. *Trends Biotechnol.*, 23(1): 48-55.

- Vieira, M L C; Santini, L; Diniz, A L and Munhoz, C D F (2016). Microsatellite markers: What they mean and why they are so useful. *Genet. Mol. Biol.*, 39(3): 312-328.
- Wan, Y; Zhang, M; Hong, A; Zhang, Y and Liu, Y (2020). Characteristics of microsatellites mined from transcriptome data and the development of novel markers in *Paeonia lactiflora*. *Genes*, 11(2): 214.
- Wang, X and Wang, L (2016). GMATA: An integrated software package for genome-scale SSR mining, marker development and viewing. *Front. Plant Sci.*, 7: 1350.
- Wang, H; Lei, Y; Yan, L; Wan, L; Cai, Y; Yang, Z; Lv, J; Zhang, X; Xu, C and Liao, B (2017). Development and validation of simple sequence repeat markers from *Arachis hypogaea* transcript sequences. *Crop J.*, 6(2): 172-180.
- Zaki, N M; Singh, R; Rosli, R and Ismail, I (2012). *Elaeis oleifera* genomic-SSR markers: Exploitation in oil palm germplasm diversity and cross-amplification in Arecaceae. *Int. J. Mol. Sci.*, 13(4): 4069-4088.
- Zhao, Y; Prakash, C S and He, G (2012). Characterization and compilation of polymorphic simple sequence repeat (SSR) markers of peanut from public database. *BMC Res. Notes*, 5(1): 1-7.
- Zolkafli, S H; Ithnin, M; Chan, K-L; Zainol Abidin, M I; Ismail, I; Ting, N C; Ooi, L C-L and Singh, R (2021). Optimal set of microsatellite markers required to detect illegitimate progenies in selected oil palm (*Elaeis guineensis* Jacq.) breeding crosses. *Breed. Sci.*, 71(2): 253-260.

FIELD EVALUATION OF OIL PALM GENETIC MATERIALS FOR PARTIAL RESISTANCE IN *Ganoderma* HOTSPOTS OF TROPICAL PEAT SOIL

MOHD DIN, A^{1*}; MARHALIL, M¹; MOHD MUSTAKIM, M¹; NORZIHA, A¹; KUSHAIRI, A² and RAJANAIDU, N³

ABSTRACT

The Malaysian Palm Oil Board's (MPOB) oil palm genetic materials were evaluated for *Ganoderma* infection in *Ganoderma* hotspots at the MPOB Research Station in Bagan Datuk, Perak, Malaysia. The objectives of the study were to investigate the development of the disease among oil palm genetic materials [dura x dura (DxD), dura x pisifera (DxP) and D-selfed] and subsequently to identify progenies having partial resistance to the basal stem rot (BSR) disease caused by *Ganoderma*. The progenies were planted in 2008 in a completely randomised design in an area formerly cultivated with oil palm but devastated by *Ganoderma*, hence regarded as a *Ganoderma* hotspot. Field census for symptoms of palms with *Ganoderma* infection was conducted for eight years, four years after field planting. The study showed a low infection rate of less than 10%, hence potential partial resistance on 31 (10 DxD, 13 DxP and eight D-selfed) progenies. The partial resistance of the 31 progenies will need to undergo further reconfirmation via screening in the nursery using established techniques prior to being utilised for the production of partial resistance planting materials. Upon confirmation of the partial resistance, utilising parents linked to the DxD, D-selfed and DxP progenies could undergo further breeding and improvement before being utilised as maternal parents for seed production. Individual palms from the DxP progenies could also be directly mass propagated via cloning for immediate utilisation.

Keywords: *Ganoderma* hotspots, oil palm genetic materials, partial resistance, tropical peat soil.

Received: 28 September 2021; **Accepted:** 7 March 2022; **Published online:** 12 May 2022.

INTRODUCTION

The oil palm (*Elaeis guineensis* Jacq.) is an important perennial oil crop for many countries. Malaysia is one of the leading producers and exporters of palm oil in the world. Oil palm breeding plays a key role in the development of the oil palm industry, especially in the production of quality planting materials.

Oil palm breeding populations, exploited for commercial seed production, have an extremely narrow genetic base (Arasu and Rajanaidu, 1975). A bulk of the current planting material is produced using maternal palms that are descendants of the four 'Bogor' palms that were planted at the Bogor Botanical Gardens in 1848. These four palms, which formed the basic breeding stock, were planted at Deli, Sumatra, Indonesia and evolved into the 'Deli dura' population in oil palm breeding. There are Deli *dura* sub-populations such as Serdang, Elmina, Ulu Remis, Johor Labis and Dumpy (Kushairi and Rajanaidu, 2000; Yong, 1992).

In view of the narrow genetic base of current oil palm planting materials, oil palm germplasm collection has gained importance to broaden the gene pool of current breeding materials. Germplasm conservation is important to sustain

¹ Malaysian Palm Oil Board,
6 Persiaran Institusi, Bandar Baru Bangi,
43000 Kajang, Selangor, Malaysia.

² No. 2 Jalan TH 1, Tiara Heights,
43900 Sepang, Selangor, Malaysia.

³ 10-14-3 Danau Permai Condo, Jalan 3/109F
58100 Kuala Lumpur, Malaysia.

* Corresponding author e-mail: mohddin@mpob.gov.my

oil palm genetic variation for future exploitation. MPOB has collected oil palm germplasm from the centres of oil palm origin in Africa and Latin America (Rajanaidu, 1994) since 1973.

The basal stem rot (BSR) disease, which is caused by the fungus *Ganoderma boninense*, is a major destructive disease of the oil palm. Among devastating diseases, the *Ganoderma* disease is the only pathogenic disease, causing serious losses of field palms in Southeast Asia (SEA), especially in Malaysia and Indonesia and also other oil palm growing countries such as Colombia, Cameroon and Papua New Guinea. Flood *et al.* (2002) reported that in severely infected areas in the East Asia, over 50% of palms may be infected by *Ganoderma*. Yield reduction may occur both from the death of palms and from reduced yield in infected but still living palms (Corley and Tinker, 2016). Detection and control of this oil palm disease are therefore of great importance to maintain high productivity.

Today, Integrated *Ganoderma* Management (IGM) is practised at varying degrees in the oil palm industry and smallholdings. Implementation of IGM still faces many challenges and a concerted effort that focuses on policy, research, training and communication approaches, is needed in order to attain the full potential of IGM (Idris *et al.*, 2019). In 2016, MPOB published the standard operating procedure (SOP) guidelines to control *Ganoderma* as part of an awareness campaign to manage and mitigate this disease more effectively (Idris *et al.*, 2016). Some BSR disease control methods in existing plantings and management strategies at replanting have been achieved and are being implemented in several oil palm estates and smallholdings in Malaysia (Idris, 2011; Mohd Shukri *et al.*, 2019). Research to develop advanced technologies and the use of the mobile application, remote sensing and Geographical Information System (GIS) application to facilitate *Ganoderma* census/monitoring, biology/epidemiology/etiology, early detection, control and management are necessary for future improvement of the present IGM practices. The exploitation of modern biotechnology, nanoscience and nanotechnology could lead to new innovations in IGM technologies.

The development of breeding materials for *Ganoderma* tolerant or partial resistant traits has been placed under high priority in Malaysia (Mohd Din *et al.*, 2005; 2014). Plant pathologists and breeders do make a clear distinction between total resistance (specific) and partial resistance (nonspecific) (Durand-Gasselin *et al.*, 2011). Total resistance generally results from a gene for gene interaction between the plant and the pathogen. However, such a specific resistance can be bypassed easily by the pathogen, which is not suitable for oil palm. The appropriate strategy would be to select multiple defense genes involved in partial resistance. This

selection will encourage and provide sustainable nonspecific resistance to a larger diversity of isolates of the pathogen, rather than a single gene for gene resistance. Selecting palms for multiple partial disease resistances will not result in the disappearance of sick palms in the field, but it will be more efficient to limit their number.

In the longer term, breeding for disease resistance offers the greatest hope for the future control of BSR disease. In Malaysia, efforts are being made to develop more productive planting materials through various breeding programs implemented by MPOB and privately owned oil palm plantations. It was reported that breeding materials collected from Cameroon revealed a high genetic diversity as did those of Nigeria and Sierra Leone (Hayati *et al.*, 2004). Cameroon and the Democratic Republic of the Congo (ex-Zaire) germplasms have shown high tolerance to *Ganoderma* disease in field planting (Rajanaidu, 1994). Field observations in North Sumatra disclosed that *E. guineensis* of Deli origin from Malaysia and Indonesia were more susceptible than African material (Durand-Gasselin *et al.*, 2005), and other trials have revealed differences in susceptibility, indicating possible genetic resistance within host populations (Breton *et al.*, 2006; Idris *et al.*, 2004). The existence of resistant genotypes has also been indicated in trials of 20 *dura* x *pisifera* (DxP) crosses in Indonesia (Purba *et al.*, 1994) and in *E. oleifera* x *E. guineensis* hybrids in Malaysia (Chung *et al.*, 1994). Thus, resistant materials are viewed as an outstanding promise for future management of BSR in oil palm in SEA (Breton *et al.*, 2006; Durand-Gasselin *et al.*, 2005; Idris *et al.*, 2004) and there are ongoing works in Sumatera and Socfindo in Indonesia. At MPOB, 15 oil palm progenies with different crosses [*viz.* *dura* x *dura* (DxD), DxP, *tenera* x *pisifera* (TxP), and *tenera* x *tenera* (TxT)], were screened for resistance to *G. boninense* using three methods, namely rubber woodblock (RWB) sitting technique on germinated seeds (RWB-GS), RWB sitting technique on 4-months old seedlings (RWB-S) and root inoculation technique (RIT) on 12-months old seedlings (Idris, 1999; Idris *et al.*, 2006; Rahamah *et al.*, 2015). There was found to be no significant difference among the progenies screened for resistance to *G. boninense* infection using RWB-GS but had significant differences using RWB-S and RIT. Based on the dead seedlings, the progeny PK 4493 (DxP, Zaire x Cameroon) with 50.0% dead seedlings using RWB-GS while 42.5% and 15.0% dead seedlings for RWB-S and RIT, respectively. Of these materials, the most susceptible was a Deli (Elmina) x Deli (Elmina) (DxD), whilst the most partial resistant was a Zaire x Cameroon (DxP). The identified genetic materials are being used in MPOB breeding programme to develop oil palm resistance to BSR disease.

Screening of oil palm progenies in the field, particularly in *Ganoderma* hotspots, is gaining importance and results have been reported by various researchers. Akbar *et al.* (1971) observed differences in incidence between West African and Deli materials in the field in Indonesia. de Franqueville *et al.* (2001) from Bangun Bandar, Indonesia discovered sources of oil palm genetic resistance and susceptibility to *Ganoderma* sp. They showed significant differences between DP/DT crosses in *Ganoderma* incidence in eight of 12 breeding trials and between clones in six of seven clonal trials. Durand-Gasselin *et al.* (2005) reported lower incidence among Deli x Yangambi crosses than pure Deli *dura*. Selfed Deli *dura* progenies were also found to be more susceptible to *Ganoderma* infection than inter-crossed Deli *dura* progenies in Kluang, Malaysia (Norziha *et al.*, 2012) whereas the Deli material was more susceptible than AVROS, with the crosses being intermediate (Rahmaningsih *et al.*, 2013). Nurul Fatiha *et al.* (2019) did a study involving nursery and field screenings. In nursery screening, the artificial *Ganoderma* fungi inoculum using woodblocks from rubber were used. Selected germinated seeds and clonal seedlings were planted with *Ganoderma* artificial inoculum for 12 months, purposely to screen for their tolerance level against BSR. The field study involved 20 progenies of both DxP and clonal planting materials, planted in 12.78 ha (an area previously identified as high BSR incidence hotspot), for field screening and selection of potential BSR tolerant material. Based on the two screening methodologies, they were able to observe the correlation for planting material with high tolerance in both nursery and field study. This article deliberates the results of a field study in *Ganoderma* hotspots at the MPOB Research Station Bagan Datuk, Perak, Malaysia to investigate the development of the BSR disease among different oil palm progenies.

MATERIALS AND METHODS

The planting materials used in this study involved three breeding trials: Trial 0.501 (21 DxP progenies), Trial 0.502 (23 DxP progenies) and Trial 0.504 (14 D-selfed progenies). The DxP genetic materials in Trial 0.501 (Table 1) were grouped into Deli x Deli and Germplasm x Germplasm progenies. The DxP genetic materials in Trial 0.502 (Table 2), on the other hand, were grouped into Deli x AVROS, Deli x Yangambi, Deli x Germplasm, Germplasm x Germplasm and Germplasm x AVROS. In Trial 0.504 (Table 3), the D-selfed were grouped into Deli selfed and Germplasm selfed. The Deli *duras* were from sub-populations Ulu Remis, Serdang, Johor Labis, Banting and Elmina. The germplasm

progenies, on the other hand, were derived from crosses involving germplasm from Tanzania (TZA), Angola (AGO) and Nigeria (NGA).

Intermediate partial resistant standard (Deli *dura* selfed and Deli *dura* x Cameroon *pisifera*) progenies and partial resistant standard (Zaire *dura* x Cameroon *pisifera* and Deli *dura* x AVROS *pisifera*) progenies (Idris *et al.*, 2004; Nurazah *et al.*, 2013) were also included in the trial.

Historically, the land was planted with oil palm (DxP planting materials), which were devastated by *Ganoderma*. The trials were laid in the *Ganoderma* hotspots with tropical peat soil type in 2008 at the MPOB Research Station Bagan Datuk, Perak, Malaysia in a Completely Randomised Design (CRD) with two to four replications at 16 palms per progeny per replication. A total number of 3952 palms were evaluated in this study. Census on the palms for *Ganoderma* infection was done on a quarterly basis from 2013 to 2019.

Based on field observations, the number of palms that showed BSR symptoms such as mottling of the lower fronds, necrosis, retarded growth, desiccation of fronds, presence of fruiting bodies or sporophores on the stem base, frond bases or roots (Idris *et al.*, 2016) were recorded and the data analysed using Statistical Analysis System (SAS version 9.2).

RESULTS

DxD (Trial 0.501)

The purpose of this trial was to determine if there were any potential DxD families with a low infection rate (less than 10%) towards *Ganoderma*. Selected agronomic traits as per Malaysian Standard (MS157:2017) would be observed thereafter. For families that fulfil MS157:2017, selected *duras* from that family may be selfed or cloned. The selfed *duras* or clonal *duras* may subsequently be progeny tested with *pisiferas* from the advanced breeding population. The resultant *teneras* from the progeny test may be utilised as ortet for the production of *Ganoderma* partial resistant clones.

The percentage of *Ganoderma* infection in each cross was monitored over seven years (Figure 1). The initial infection rate in 2013 was 1.26% for Deli x Deli progenies and 0.40% for the Germplasm x Germplasm progenies. The infection rate (%) of the disease increased over the years. By 2019, the Deli x Deli progenies recorded 40.05% infection whereas a much lower infection of 8.53% was observed in the Germplasm x Germplasm progenies. Overall, the Deli x Deli progenies recorded a higher percentage of *Ganoderma* infection in each year of the evaluation, compared to the Germplasm x Germplasm progenies. The initial infection rate in

TABLE 1. PEDIGREE OF DXD PROGENIES IN TRIAL 0.501

No.	Progeny code	Pedigree no.	Material	Cross type	No. of palms	Remarks
1	ECP HP 429	0.338/209 x 0.338/89	Deli Ulu Remis x Deli Ulu Remis	DxD	32	Deli x Deli
2	ECP HP 551	0.338/332 x 0.281/44	Deli Ulu Remis x Deli Serdang	DxD	64	Deli x Deli
3	ECP HP 578	0.338/63 x 0.278/318	Deli Ulu Remis x Deli Johor Labis	DxD	64	Deli x Deli
4	ECP HP 590	0.338/373 x 0.281/44	Deli Ulu Remis x Deli Serdang	DxD	64	Deli x Deli
5	ECP HP 592	0.338/422 x 0.278/318	Deli Ulu Remis x Deli Johor Labis	DxD	64	Deli x Deli
6	ECP HP 607	0.338/63 x 0.281/57	Deli Ulu Remis x Deli Serdang	DxD	48	Deli x Deli
7	ECP HP 629	0.338/422 x 0.281/64	Deli Ulu Remis x Deli Serdang	DxD	64	Deli x Deli
8	PK 4638	0.256/650 x 0.256/1030	TZA x TZA	DxD	64	Germplasm x Germplasm
9	PK 4263	0.256/2995 x 0.312/99	TZA x AGO	DxD	64	Germplasm x Germplasm
10	PK 4271	0.256/1926 x 0.312/99	TZA x AGO	DxD	48	Germplasm x Germplasm
11	PK 4288	0.256/2995 x 0.312/941	TZA x AGO	DxD	64	Germplasm x Germplasm
12	PK 4294	0.256/1926 x 0.312/941	TZA x AGO	DxD	64	Germplasm x Germplasm
13	PK 4349	0.312/99 x 0.256/2995	AGO x TZA	DxD	32	Germplasm x Germplasm
14	PK 4352	0.312/941 x 0.256/2995	AGO x TZA	DxD	32	Germplasm x Germplasm
15	PK 4375	0.256/2995 x 0.256/1926	TZA x TZA	DxD	64	Germplasm x Germplasm
16	PK 4384	0.312/99 x 0.256/1926	AGO x TZA	DxD	48	Germplasm x Germplasm
17	PK 4429	0.312/36 x 0.311/405	AGO x AGO	DxD	64	Germplasm x Germplasm
18	PK 4442	0.312/941 x 0.256/1926	AGO x TZA	DxD	64	Germplasm x Germplasm
19	PK 4525	0.256/984 x 0.256/1030	TZA x TZA	DxD	48	Germplasm x Germplasm
20	PK 4526	0.256/1926 x 0.256/2995	TZA x TZA	DxD	64	Germplasm x Germplasm
21	PK 4580	0.311/1 x 0.311/262	AGO x AGO	DxD	48	Germplasm x Germplasm
22	PK 4009	0.212/438 x 0.212/438	Deli Banting self	DxD	64	Deli self (intermediate std)
23	PK 4841	0.212/6 x 0.219/1371	Deli Elmina x CMR	DxP	64	Deli x Cameroon (intermediate std)
24	PK 4454	0.212/203 x 0.174/480	Deli Ulu Remis x AVROS	DxP	64	*Deli x AVROS (partial resistant std)
Total number of seedlings					1 360	

Note: Shaded grey are intermediate and partial resistant standards.

*DxP standard cross; TZA - Tanzania; AGO - Angola.

TABLE 2. PEDIGREE OF DXP PROGENIES IN TRIAL 0.502

No.	Progeny code	Pedigree no.	Material	Cross type	No. of palms	Remarks
1	ECP HP 415	0.279/24x0.394/456	Deli Banting x Avros	DxP	64	Deli x Avros
2	ECP HP 550	0.279/24 x 0.394/234	Deli Banting x Avros	DxP	64	Deli x Avros
3	ECP HP 618	0.281/44 x 0.394/234	Deli Johor Labis x Avros	DxP	64	Deli x Avros
4	PK 4118	0.254/191 x 0.174/480	Deli Ulu Remis x Avros	DxP	64	Deli x Avros
5	PK 4535	0.332/100 x 0.394/24	Deli Ulu Remis x Avros	DxP	64	Deli x Avros
6	PK 4550	0.332/278 x 0.395/419	Deli Ulu Remis x Avros	DxP	64	Deli x Avros
7	PK 4591	0.332/340 x 0.395/419	Deli Ulu Remis x Avros	DxP	48	Deli x Avros
8	PK 4674	0.332/116 x 0.395/372	Deli Ulu Remis x Avros	DxP	32	Deli x Avros
9	PK 4529	0.332/451 x 0.395/204	Deli Ulu Remis x Yangambi	DxP	64	Deli x Yangambi
10	PK 4548	0.332/45 x 0.395/204	Deli Ulu Remis x Yangambi	DxP	48	Deli x Yangambi
11	ECP HP 500	0.338/361 x 0.337/552	Deli Ulu Remis x NGA	DxP	64	Deli x Germplasm
12	PK 4540	0.332/218 x 0.337/1092	Deli Ulu Remis x NGA	DxP	64	Deli x Germplasm
13	PK 4621	0.332/220 x 0.337/554	Deli Ulu Remis x NGA	DxP	64	Deli x Germplasm
14	PK 4648	0.332/116 x 0.337/291	Deli Ulu Remis x NGA	DxP	64	Deli x Germplasm
15	PK 4679	0.312/1241 x 0.337/291	AGO x NGA	DxP	64	Germplasm x Germplasm
16	PK 4474	0.256/2058 x 0.337/1092	TZA x NGA	DxP	48	Germplasm x Germplasm
17	PK 4539	0.312/682 x 0.337/1092	AGO x NGA	DxP	64	Germplasm x Germplasm
18	PK 4651	0.256/2425 x 0.337/1092	TZA x NGA	DxP	64	Germplasm x Germplasm
19	PK 4465	0.311/405 x 0.174/480	AGO x Avros	DxP	48	Germplasm x Avros
20	PK 4482	0.311/405 x 0.394/24	AGO x Avros	DxP	32	Germplasm x Avros
21	PK 4504	0.312/99 x 0.174/247	AGO x Avros	DxP	64	Germplasm x Avros
22	PK 4505	0.311/269 x 0.174/211	AGO x Avros	DxP	48	Germplasm x Avros
23	PK 4570	0.256/2313 x 0.394/24	TZA x Avros	DxP	64	Germplasm x Avros
24	PK 4009	0.212/438 x 0.212/438	Deli Banting self	DxD	64	Deli self (intermediate std)
25	PK 4841	0.212/6 x 0.219/1371	Deli Elmina x CMR	DxP	64	Deli x Cameroon (intermediate std)
26	PK 4454	0.212/203 x 0.174/480	Deli Ulu Remis x AVROS	DxP	64	*Deli x AVROS (partial resistant std)
Total number of seedlings					1 520	

Note: Shaded grey are intermediate and partial resistant standards.

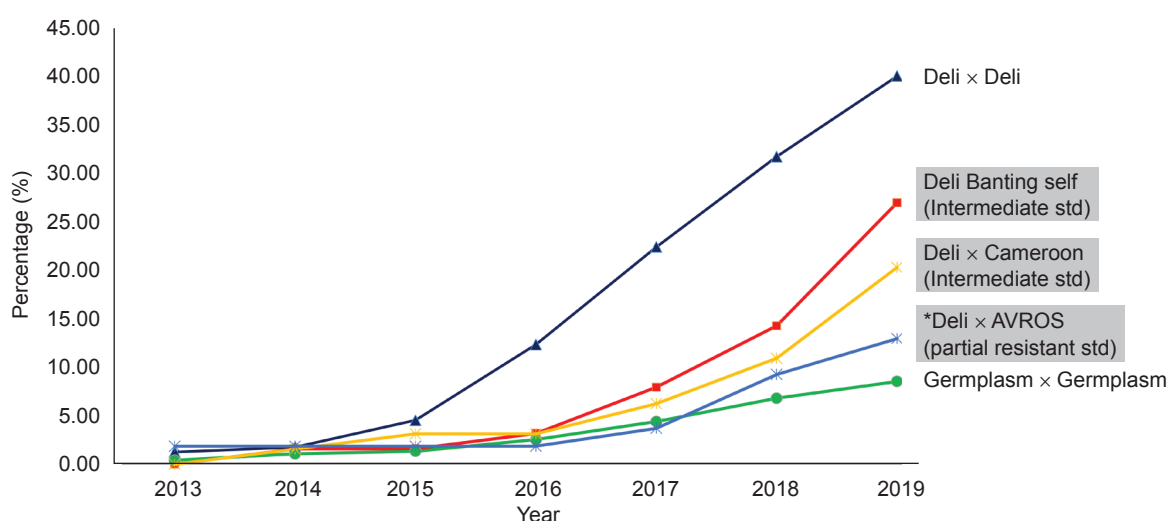
*DxP standard cross; TZA - Tanzania; AGO - Angola; NGA - Nigeria.

TABLE 3. PEDIGREE OF D-SELFED PROGENIES IN TRIAL 0.504

No.	Progeny code	Pedigree no.	Material	Cross type	No. of palms	Remarks
1	ECP HP 594	0.281/57 x 0.281/57	Deli Serdang x Deli Serdang	DxD	64	Deli self
2	ECP HP 609	0.338/460 x 0.338/460	Deli Ulu Remis x Deli Ulu Remis	DxD	64	Deli self
3	PK 4074	0.212/306 x 0.212/306	Deli Elmina x Deli Elmina	DxD	64	Deli self
4	ECP HP 401	0.282/978 x 0.282/978	NGA x NGA	DxD	64	Germplasm self
5	ECP HP 411	0.282/1065 x 0.282/1065	NGA x NGA	DxD	64	Germplasm self
6	ECP HP 413	0.282/935 x 0.282/935	NGA x NGA	DxD	64	Germplasm self
7	PK 4233	0.311/1022 x 0.311/1022	AGO x AGO	DxD	48	Germplasm self
8	PK 4364	0.256/2995 x 0.256/2995	TZA x TZA	DxD	48	Germplasm self
9	PK 4410	0.312/36 x 0.312/36	AGO x AGO	DxD	64	Germplasm self
10	PK 4414	0.311/405 x 0.311/405	AGO x AGO	DxD	64	Germplasm self
11	PK 4481	0.256/1926 x 0.256/1926	TZA x TZA	DxD	64	Germplasm self
12	PK 4485	0.256/2058 x 0.256/2058	TZA x TZA	DxD	64	Germplasm self
13	PK 4532	0.256/2125 x 0.256/2125	TZA x TZA	DxD	64	Germplasm self
14	PK 4542	0.311/1 x 0.311/1	AGO x AGO	DxD	32	Germplasm self
15	PK 4009	0.212/438 x 0.212/438	Deli Banting x Deli Banting	DxD	48	Deli self (intermediate std)
16	PK 4841	0.212/6 x 0.219/1371	Deli Elmina x CMR	DxP	64	Deli x Cameroon (intermediate std)
17	PK 4493	0.221/1340 x 0.219/1371	ZRE x CMR	DxP	64	Zaire x Cameroon (partial resistant std)
18	PK 4427	0.212/3 x 0.174/211	Deli Elmina x AVROS (S.C)	DxP	64	*Deli x AVROS (partial resistant std)
Total number of seedlings					1 072	

Note: Shaded grey are intermediate and partial resistant standards.

*DxP standard cross; TZA - Tanzania; AGO - Angola; NGA - Nigeria.



Note: Shaded grey is intermediate and partial resistant standards.

*DxP standard cross.

Figure 1. *Ganoderma* infection rate (%) of DxD progenies in Trial 0.501 from 2013 to 2019.

2013 for both intermediate standards was 0.00% but eventually rose to more than 20.00% by 2019. According to Mior *et al.* (2009), the development of disease incidence is not a function of time but rather a sign of the individual palm responding to the *Ganoderma* infection. The higher response of the Deli x Deli progenies to *Ganoderma* infection as compared to Germplasm x Germplasm progenies in this study supported the above testimony.

ANOVA showed significant differences ($p \leq 0.01$) in the infection rate among the five types of crosses. There were significant differences between Deli x

Deli and Germplasm x Germplasm progenies. The infection rate on the Deli x Deli was significantly higher than both intermediate infection standards. On the other hand, the infection rate on the Germplasm x Germplasm was significantly lower than both intermediate standards. The infection rate on the partial resistant standard (Deli x AVROS) was significantly lower than the Deli x Deli but was not significantly different from the infection rate on Germplasm x Germplasm.

Ten (PKs 4638, 4271, 4294, 4375, 4384, 4429, 4442, 4525, 4526 and 4580) of the 14 Germplasm

x Germplasm progenies gave low infection rate (less than 10.00%), ranging from 0.00% to 9.68%. However, all of the seven Deli x Deli progenies gave high infection rates (more than 10.00%), ranging from 18.75% to 53.97% (Table 4). Both intermediate standards and the partial resistant standard gave a high infection rate (more than 10.00%). Only one progeny (PK 4442) fulfilled the Malaysian Standard (MS157:2017) used as a benchmark for agronomic performance to be eligible for utilisation as a maternal parent. For the other progenies, further introgression to *duras* from the advanced breeding population may be required to improve the agronomic performance.

DxP (Trial 0.502)

The purpose of this trial was to determine if there were any potential DxP families with a low infection rate (less than 10.00%) towards *Ganoderma*. Selected agronomic traits as per Malaysian Standard (MS157:2017) would be observed thereafter. The resultant *teneras* from the progeny test may be utilised as ortet for the production of *Ganoderma* partial resistant clones.

The initial infection in 2013 was 0.23% for Deli x AVROS progenies, 0.00% for both Deli x Yangambi and Germplasm x Germplasm progenies (Figure 2). Similar to trial 0.501, the infection rate (%) of the disease increased over the years. In 2019, the Deli x AVROS progenies recorded 22.43% infection while the Deli x Yangambi progenies recorded 12.73% infection. The lowest infection rate was observed in the Germplasm x Germplasm progenies with 4.41%. Meanwhile, infection rates for the Deli x Germplasm progenies were similar to Deli x Yangambi progenies, increasing from 0.00% in 2013 to 13.62% in 2019. Germplasm x AVROS progenies, on the other hand, displayed infection rates close to Germplasm x Germplasm progenies, increasing from 0.00% in 2013 to 4.46% in 2019.

ANOVA showed significant differences ($p \leq 0.01$) between the eight groups of progenies. By Duncan New Multiple Range Test, significant differences were observed in the infection rates between Deli x AVROS and Deli x Yangambi progenies. However, there was no significant difference in the infection rates between Germplasm x Germplasm and Germplasm x AVROS.

Thirteen progenies, two Deli x AVROS (PKs 4550 and 4591), two Deli x Germplasm (ECH HP 500 and PK 4621), four Germplasm x Germplasm (PKs 4679, 4474, 4539 and 4651) and five Germplasm x AVROS (PKs 4465, 4482, 4504, 4505 and 4570) gave low infection rates (less than 10.00%) (Table 5). Both intermediate standards

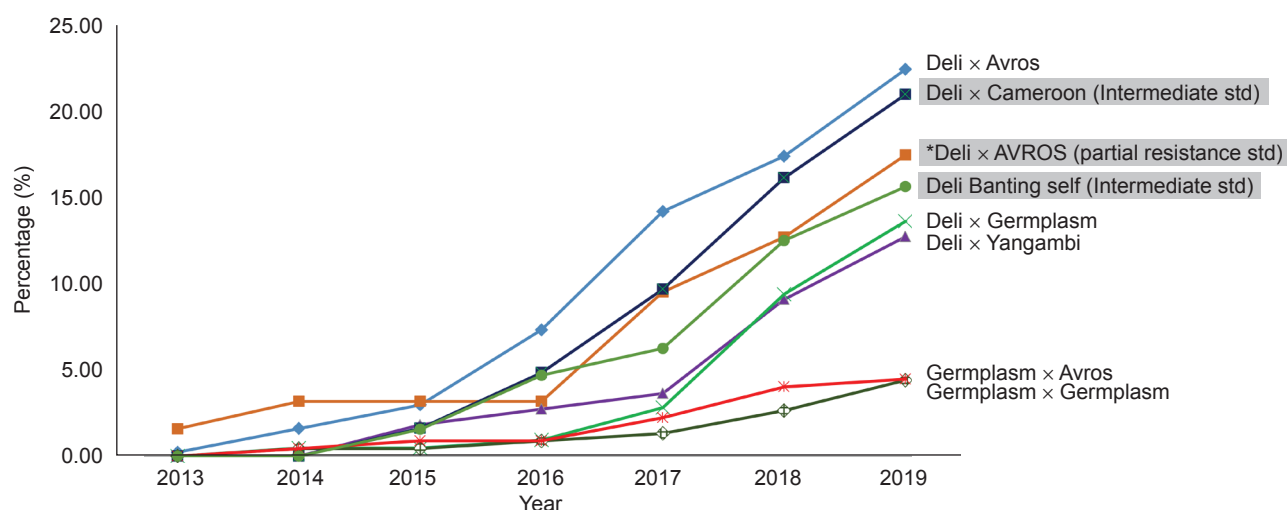
TABLE 4. AGRONOMIC PERFORMANCE OF DxP PROGENIES WITH LOW INFECTION RATE (< 10%) TO *Ganoderma* IN TRIAL 0.501

No.	Crosses	Progeny code	Pedigree no.	Material	n	Cross	Infection % (2019)	MFFB	M/F	K/F	S/F	O/DM	O/B	K/B	OY	TEP
1	Germplasm x Germplasm	PK4638	0.256/650x0.256/1030	TZA x TZA	61	DxD	1.64	124.70	60.13	14.81	25.06	75.80	14.72	7.54	23.95	31.72
2	Germplasm x Germplasm	PK4271	0.256/1926x0.312/99	TZA x AGO	48	DxD	8.33	174.41	50.61	16.23	33.16	77.70	14.01	9.10	25.35	35.66
3	Germplasm x Germplasm	PK4294	0.256/1926x0.312/941	TZA x AGO	62	DxD	8.06	155.29	50.48	15.81	33.70	78.81	14.26	9.15	23.89	33.10
4	Germplasm x Germplasm	PK4375	0.256/2995x0.256/1926	TZA x TZA	62	DxD	9.68	161.40	46.82	17.08	36.11	76.22	10.62	8.53	18.58	27.48
5	Germplasm x Germplasm	PK4384	0.312/99x0.256/1926	AGO x TZA	48	DxD	8.33	167.48	45.05	18.36	36.59	78.18	13.71	11.47	27.16	40.78
6	Germplasm x Germplasm	PK4429	0.312/36x0.311/405	AGO x AGO	60	DxD	1.67	117.44	55.07	9.32	35.61	78.93	16.75	5.92	23.41	28.31
7	Germplasm x Germplasm	PK4442	0.312/941x0.256/1926	AGO x TZA	63	DxD	4.76	163.03	58.13	17.90	23.98	77.27	13.86	9.11	23.54	32.60
8	Germplasm x Germplasm	PK4525	0.256/984x0.256/1030	TZA x TZA	48	DxD	0.00	127.82	54.21	16.45	29.34	71.97	11.85	8.60	20.47	29.32
9	Germplasm x Germplasm	PK4526	0.256/1926x0.256/2995	TZA x TZA	64	DxD	4.69	151.11	50.04	16.83	33.14	77.00	12.69	9.48	25.13	36.36
10	Germplasm x Germplasm	PK4580	0.311/1x0.311/262	AGO x AGO	47	DxD	4.26	134.06	49.70	12.52	37.79	79.32	13.54	6.71	18.56	24.01
Partial resistant std		PK4454	0.212/203x0.174/480	*Deli x AVROS	54	DxP	12.96	152.57	71.39	11.72	16.89	79.47	22.05	7.34	35.64	42.97
MS157:2017								150.00	57.00	5.00	33.00	75.00	19.00			

Note: Shaded yellow are traits which fulfilled MS157:2017.

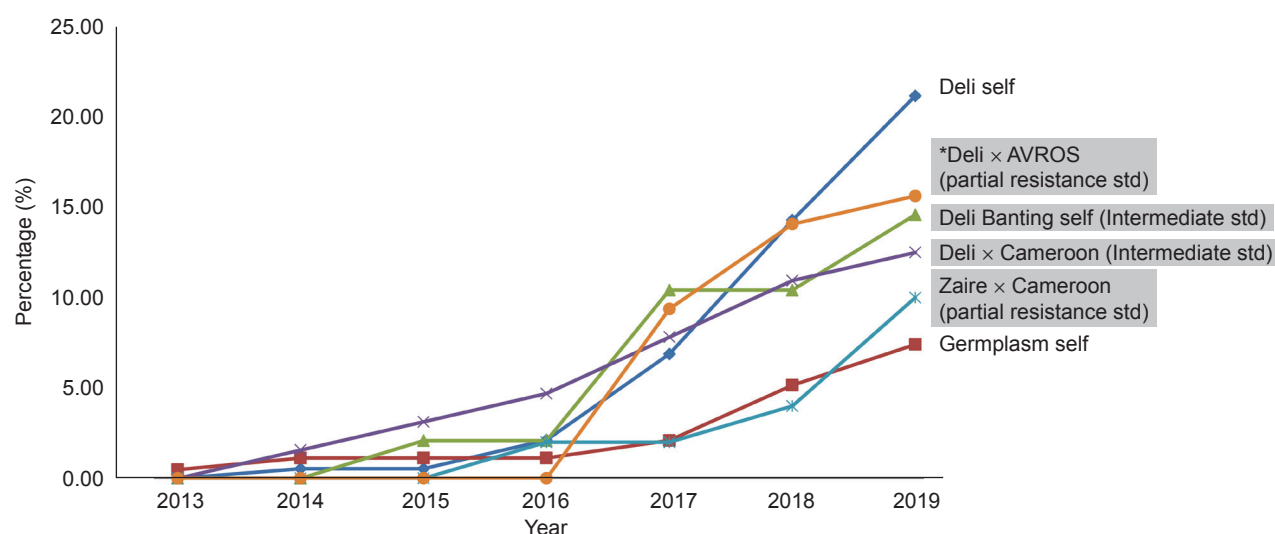
Shaded grey is partial resistant standard cum DxP standard cross.

FFB - fresh fruit bunch (kg palm⁻¹ yr⁻¹); M/F - mesocarp to fruit (%); K/F - kernel to fruit (%); O/DM - oil to dry mesocarp (%); O/B - oil to bunch (%); K/B - kernel to bunch (%); OY - oil yield (kg palm⁻¹ yr⁻¹); TEP - total economic product (kg palm⁻¹ yr⁻¹).



Note: Shaded grey is intermediate and partial resistant standards.
*DxP standard cross.

Figure 2. *Ganoderma* infection rate (%) of DXP progenies in Trial 0.502 from 2013 to 2019.



Note: Shaded grey is intermediate and partial resistant standards.
*DxP standard cross.

Figure 3. *Ganoderma* infection rate (%) of D-selfed progenies in Trial 0.504 from 2013 to 2019.

and the partial resistant standard displayed high infection rates (more than 10.00%). Benchmarking the agronomic performance of the 13 selected progenies (with low infection rate) against MS157:2017, none of the DXP or *tenera* progenies fulfilled all the four traits in order to be eligible for immediate selection and utilisation as planting materials.

Since none of the 13 DXP families fulfilled MS157:2017, no *teneras* can be selected for cloning and utilised as *Ganoderma* partial resistant clonal planting material. Selected agronomic traits as per Malaysian Standard (MS157:2017) would be observed thereafter.

D-selfed (Trial 0.504)

The purpose of this trial was to determine if there were any potential D-selfed families with a low infection rate (less than 10.00%) towards *Ganoderma*. The next step after that would be to look at selected agronomic traits as per Malaysian Standard (MS157:2017). For families that fulfil MS157:2017, selected *duras* from that family may be selfed or cloned. The selfed *duras* or clonal *duras* may subsequently be progeny tested with *pisiferas* from the advanced breeding population. The resultant *teneras* are potential candidates for *Ganoderma* partial resistant clones.

TABLE 5. AGRONOMIC PERFORMANCE OF DxP PROGENIES WITH LOW INFECTION RATE (< 10%) TO *Ganoderma* IN TRIAL 0.502

No.	Crosses	Progeny code	Pedigree no.	Material	Cross type	n	Infection % (2019)	MFFB	M/F	K/F	S/F	O/DM	O/B	K/B	OY	TEP
1	Deli x AVROS	PK4550	0.332/278 x 0.395/419	Deli Ulu Remis x AVROS	DxP	58	5.17	157.01	80.53	8.66	10.81	81.96	23.79	4.71	42.72	47.70
2	Deli x AVROS	PK4591	0.332/340 x 0.395/419	Deli Ulu Remis x AVROS	DxP	47	6.38	179.57	79.80	7.60	12.60	79.73	23.18	3.92	44.92	49.52
3	Deli x Germplasm	ECP HP500	0.338/361 x 0.337/552	Deli Ulu Remis x NGA	DxP	53	7.55	184.71	80.01	4.56	15.43	79.43	26.21	2.75	48.50	51.55
4	Deli x Germplasm	PK4621	0.332/220 x 0.337/554	Deli Ulu Remis x NGA	DxP	32	6.25	93.49	75.48	11.52	13.01	79.55	21.74	6.80	30.68	36.43
5	Germplasm x Germplasm	PK4679	0.312/1241 x 0.337/291	AGO x NGA	DxP	64	0.00	159.39	71.27	9.78	18.96	77.88	19.32	4.70	34.88	39.99
6	Germplasm x Germplasm	PK4474	0.256/2058 x 0.337/1092	TZA x NGA	DxP	48	4.17	137.60	76.74	10.77	12.49	80.45	21.54	5.66	41.01	47.64
7	Germplasm x Germplasm	PK4539	0.312/682 x 0.337/1092	AGO x NGA	DxP	53	7.55	146.27	79.38	8.24	12.37	80.15	24.59	4.58	39.23	43.63
8	Germplasm x Germplasm	PK4651	0.256/2425 x 0.337/1092	TZA x NGA	DxP	62	6.45	137.46	71.48	12.44	16.09	76.91	17.78	6.68	28.40	34.71
9	Germplasm x AVROS	PK4465	0.311/405 x 0.174/480	AGO x AVROS	DxP	46	8.70	133.93	76.35	11.44	12.22	81.50	26.67	6.89	40.20	46.47
10	Germplasm x AVROS	PK4482	0.311/405 x 0.394/24	AGO x AVROS	DxP	29	3.45	89.22	74.90	11.91	13.20	78.41	23.23	7.26	37.74	44.98
11	Germplasm x AVROS	PK4504	0.312/99 x 0.174/247	AGO x AVROS	DxP	54	0.00	155.93	77.75	11.71	10.53	80.91	20.53	5.65	46.41	54.01
12	Germplasm x AVROS	PK4505	0.311/269 x 0.174/211	AGO x AVROS	DxP	46	4.35	173.98	74.02	11.87	14.11	80.41	20.41	5.80	33.82	39.56
13	Germplasm x AVROS	PK4570	0.256/2313 x 0.394/24	TZA x AVROS	DxP	49	6.12	114.97	70.57	12.91	16.52	80.57	16.29	5.07	23.61	28.23
Partial resistant std		PK4454	0.212/203 x 0.174/480	*Deli x AVROS	DxP	63	17.46	151.50	78.55	8.73	12.72	81.37	24.14	4.67	42.87	47.82
MS157:2017								170.00					25.00	3.00	42.50	

Note: Shaded yellow is traits which fulfilled MS157:2017.

Shaded grey is partial resistant standard cum DxP standard cross.

FFB - fresh fruit bunch (kg palm⁻¹ yr⁻¹); M/F - mesocarp to fruit (%); K/F - kernel to fruit (%); S/F - shell to fruit (%); O/DM - oil to dry mesocarp (%); O/B - oil to bunch (%); K/B - kernel to bunch (%); OY - oil yield (kg palm⁻¹ yr⁻¹); TEP - total economic product (kg palm⁻¹ yr⁻¹).

TABLE 6. AGRONOMIC PERFORMANCE OF D-SELFED PROGENIES WITH LOW INFECTION RATE (< 10%) TO *Ganoderma* IN TRIAL 0.504

No.	Crossing programme	Progeny code	Pedigree no.	Material	Cross type	n	Infection % (2019)	MFFB	M/F	K/F	S/F	O/DM	O/B	K/B	OY	TEP
1	Germplasm selfed	PK4233	0.311/1022 x 0.311/1022	AGO x AGO	DxD	48	6.25	122.69	56.62	10.51	32.88	79.19	14.85	5.39	20.73	25.19
2	Germplasm selfed	PK4364	0.256/2995 x 0.256/2995	TZA x TZA	DxD	48	6.25	91.70	64.16	10.46	25.38	76.74	14.48	5.07	10.56	13.20
3	Germplasm selfed	PK4410	0.312/36 x 0.312/36	AGO x AGO	DxD	56	1.79	74.60	61.05	9.79	29.17	80.44	15.67	5.19	14.26	16.98
4	Germplasm selfed	PK4414	0.311/405 x 0.311/405	AGO x AGO	DxD	64	6.25	55.75	53.06	11.94	35.00	79.05	14.67	6.05	13.64	17.02
5	Germplasm selfed	PK4481	0.256/1926 x 0.256/1926	TZA x TZA	DxD	62	0.00	88.76	54.87	15.00	30.13	75.51	15.23	8.64	20.47	27.34
6	Germplasm selfed	PK4485	0.256/2058 x 0.256/2058	TZA x TZA	DxD	59	8.47	63.72	61.92	6.77	31.32	77.30	18.76	4.31	21.52	24.48
7	Germplasm selfed	PK4532	0.256/2125 x 0.256/2125	TZA x TZA	DxD	63	3.17	77.93	49.09	12.12	38.78	77.12	14.25	7.21	20.37	26.72
8	Germplasm selfed	PK4542	0.311/1 x 0.311/1	AGO x AGO	DxD	32	3.13	88.46	60.14	12.49	27.38	77.57	17.67	7.85	20.28	25.69
Partial resistant std		PK4493	0.221/1340 x 0.219/1371	Zaire x Cameroon	DxP	50	10.00	66.01	59.09	15.21	25.71	75.50	14.31	7.74	19.31	25.60
Partial resistant std		PK4427	0.212/3 x 0.174/211	*Deli x AVROS	DxP	64	15.63	148.77	79.66	8.77	11.56	80.00	22.94	4.94	34.75	39.20
MS157:2017								150.00	57.00	5.00	33.00	75.00	19.00			

Note: Shaded yellow is traits that fulfilled MS157:2017.

Shaded grey is partial resistant standards. * DxP standard cross.

FFB - fresh fruit bunch (kg palm⁻¹ yr⁻¹); M/F - mesocarp to fruit (%); K/F - kernel to fruit (%); S/F - shell to fruit (%); O/DM - oil to dry mesocarp (%); O/B - oil to bunch (%); K/B - kernel to bunch (%); OY - oil yield (kg palm⁻¹ yr⁻¹); TEP - total economic product (kg palm⁻¹ yr⁻¹).

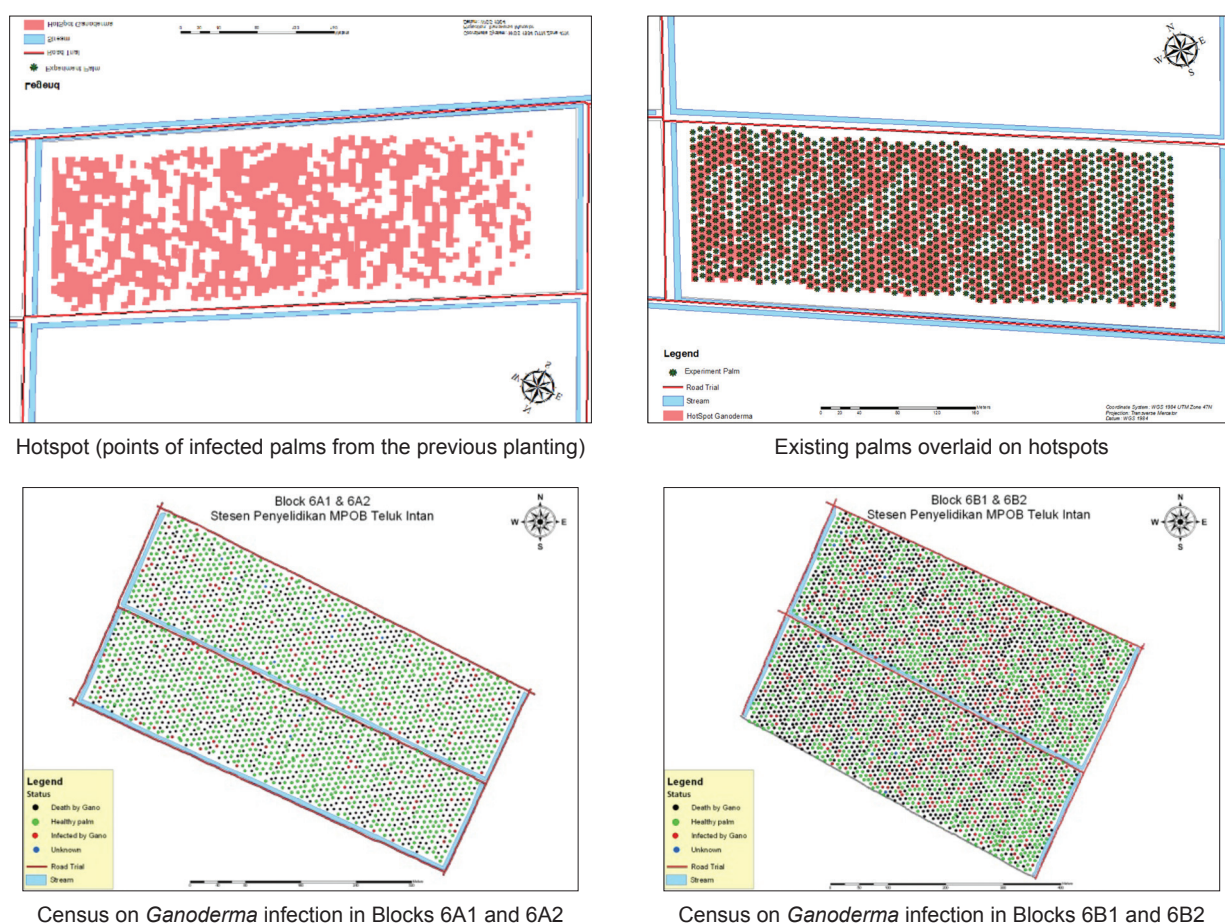


Figure 4. Establishment and development of trial plots in the *Ganoderma* hotspot of Bagan Datuk, Perak, Malaysia.

The initial infection in 2013 was 0.47% for Germplasm selfed and 0.00% for Deli selfed progenies (Figure 3). The infection (%) of the disease increased over the years. In 2019, the Deli selfed progenies recorded a 21.16% infection rate whereas the Germplasm selfed progenies recorded a lower infection rate at 7.41%.

ANOVA showed significant differences ($p \leq 0.01$) in infection rates among the six groups of progenies. By Duncan New Multiple Range Test the infection rate on Deli selfed palms was significantly higher than Germplasm selfed progenies.

Eight germplasm selfed progenies involving four Angola selfed and four Tanzania selfed had low infection rates ranging from 0.00% to 8.47%. However, all the three Nigerian selfed progenies gave high infection rates ranging from 11.11% to 16.13%. Both intermediate standards and one partial resistant standard showed high infection rates (more than 10.00%). However, the other partial resistant standard (Zaire x Cameroon) had an infection rate of 10.00% (Table 6). Benchmarking agronomic performance, none of the D-selfed progenies from the eight selected Germplasm selfed progenies (with low infection rate) fulfilled the required six traits to be eligible for immediate selection as potential maternal parents. This is expected since these

progenies are still rather wild genetic materials, being derived from selfed germplasm palms. Furthermore, selfing may also have led to inbreeding depression among the agronomic traits as can be seen from the inferior FFB yield performance among the selfed progenies. Nevertheless, selfing did not appear to have any negative influence on the bunch quality traits, especially for K/F and O/DM. Hence, further introgression to *duras* from the advanced breeding population would be the way to move forward to improve the agronomic performance. The establishment and development of trial plots in this study are shown in Figure 4.

DISCUSSION

Breeding for disease resistance may provide a long term solution for future control of BSR disease. In Malaysia, efforts on this are being pursued by oil palm breeders from MPOB and the industry, usually jointly with plant pathologists. Results from the three trials (DxD, DxP and D-selfed) on the susceptibility of the Deli *dura* and Deli-based crosses as reported in this study appeared to support earlier field observations. For instance, in North Sumatra *E. guineensis* of Deli origin from

Malaysia and Indonesia was found to be more susceptible than African material (Durand-Gasselin *et al.*, 2005). The performance of germplasm and germplasm-based crosses in the three trials also supported earlier field observations for partial resistance. It was reported that oil palm germplasm collected from Cameroon, Nigeria and Sierra Leone revealed high genetic diversity (Hayati *et al.*, 2004). Germplasm from Cameroon and Zaire also showed partial resistance to *Ganoderma* (Rajanaidu, 1994). Other trials have also revealed differences in susceptibility, indicating possible genetic resistance within host populations (Breton *et al.*, 2006; Idris *et al.*, 2004). The existence of resistant genotypes has also been indicated in trials of 20 DxP crosses in Indonesia (Purba *et al.*, 1994) and in *E. oleifera* x *E. guineensis* hybrids in Malaysia (Chung *et al.*, 1994). Thus, resistant materials view as an outstanding promise for future management of BSR in oil palm in SEA (Breton *et al.*, 2006; Durand-Gasselin *et al.*, 2005; Idris *et al.*, 2004). There was some work on this with Socfindo in Sumatera, Indonesia.

CONCLUSION

The study showed low infection rate of less than 10%, hence potential partial resistance to *Ganoderma* for 10 DxD (Germplasm x Germplasm) progenies in Trial 0.501, 13 DxP (two Deli x AVROS, two Deli x Germplasm, four Germplasm x Germplasm and five Germplasm x AVROS) progenies in Trial 0.502 and eight D-selfed (Germplasm selfed) progenies in Trial 0.504. The partial resistance of the 31 progenies may need to be reconfirmed in the nursery using established screening techniques. Upon confirmation of the partial resistance, parents linked to the DxD, D-selfed and DxP progenies could be utilised for further breeding and improvement before being utilised as a maternal parent for production of partial resistant planting material. Individual palms from the DxP progenies, on the other hand, could also be directly mass propagated via cloning for immediate utilisation as partial resistant planting material.

ACKNOWLEDGEMENT

The authors would like to thank the Director-General of MPOB for permission to publish this article.

REFERENCES

Akbar, U; Kusnadi, M and Ollaonier, M (1971). Influence of the type of planting materials and of mineral nutrients on oil palm stem rot due to *Ganoderma*. *Oleagineux*, 26: 527-534.

Arasu, N T and Rajanaidu, N (1975). Conservation and utilisation of genetic resources in the oil palm (*Elaeis guineensis* Jacq.). *South East Asia Genetic Resources*. p. 182-186.

Breton, F; Hassan, Y; Hariadi; Lubis, Z and de Franqueville, H (2006). Characterization of parameters for the development of an early screening test for basal stem rot tolerance in oil palm progenies. *J. Oil Palm Res., Special Issue*: 24-36.

Chung, G F; Pow, K W; Musa, B and Ho, C Y (1994). Preliminary results of land clearing practices on *Ganoderma* incidence in *Elaeis guineensis* and its hybrid with *Elaies oleifera*. *Proc. of the First International Workshop on Perennial Crop Diseases Caused by Ganoderma*. 28 November - 3 December 1994. UPM, Serdang, Selangor. 9 pp.

Corley, R H V and Tinker, P B (2016). *The Oil Palm*. 5th edition. Wiley Blackwell. 639 pp.

de Franqueville, H; Asmady, H; Jacquemard, J.C; Hayun, Z and Durand-Gasselin, T (2001). Indications on sources of oil palm (*Elaeis guineensis* Jacq.) genetic resistance and susceptibility to *Ganoderma* sp., the cause of basal stem rot. *Proc. 2001 PIPOC International Palm Oil Congress (Agriculture)*. p. 420-431.

Durand-Gasselin, T; Asmady, H; Flori, A; Jacquemard, J C; Hayun, Z; Breton, F and de Franqueville, H (2005). Possible sources of genetic resistance in oil palm (*Elaeis guineensis* Jacq.) to basal stem rot caused by *Ganoderma boninense* - Prospects for future breeding. *Mycopathologia*, 159: 93-100.

Durand-Gasselin, T; de Franqueville, H; Breton, F; Amblard, P; Jacquemard, J C; Syaputra, I; Cochard, B; Louise, C; Nouy, B. (2011). Breeding for sustainable palm oil. *Proc. of the Seminar Breeding for Sustainability in Oil Palm*. ISOPB. p. 178-193.

Flood, J; Hasan, Y and Foster, H L (2002). *Ganoderma* diseases of oil palm - An interpretation from Bah Lias Research Station. *Planter*, 78: 689-710.

Hayati, A; Wickneswari, R; Maizura, I and Rajanaidu, N (2004). Genetic diversity of oil palm (*Elaeis guineensis* Jacq.) germplasm collections from Africa: Implications for improvement and conservation of genetic resource. *Theor. Appl. Genet.*, 108: 1274-1284.

Idris, A S (1999). Basal stem rot of oil palm (*Elaeis guineensis* Jacq.) in Malaysia: Factors associated with variation in disease severity. Ph.D thesis, Wye College, University of London, United Kingdom.

- Idris, A S; Kushairi, A; Ismail, S and Ariffin, D (2004). Selection for partial resistance in oil palm progenies to *Ganoderma* basal stem rot. *J. Oil Palm Res.*, 16(2): 12-18.
- Idris, A S; Kushairi, D; Ariffin, D and Basri, M W (2006). A technique for inoculation of oil palm germinated seeds with *Ganoderma*. *MPOB Information Series*, 314.
- Idris, A S (2011). Other devastating diseases of oil palm. *Further Advances in Oil Palm Research (2000-2010)* (Basri, M W; Choo, Y M and Chan, K W eds.). MPOB, Bangi. p. 522-542.
- Idris, A S; Nurrasyeda, R; Rusli, M H; Sundram, S and Norman, K (2016). *Standard Operating Procedures (SOP) Guidelines for Managing Ganoderma Disease in Oil Palm*. MPOB, Bangi. 41 pp.
- Idris, A S; Rusli, M H; Sundram, S; Maizatul Suriza, M; Mohd Din, A and Meilina Ong, A (2019). *Ganoderma* disease - Research status and challenges in integrated *Ganoderma* management. *Proc. International Seminar on Breeding for Ganoderma Tolerance in Oil Palm*. ISOPB. 4-19.
- Kushairi, A and Rajanaidu, N (2000). Breeding populations, seed production and nursery management. *Adv. in Oil Palm Res.* p. 39-96.
- Mior, M H; Idris, A S; Wahid, O and Kushairi, A (2009). Spatial, temporal and hotspot analysis of basal stem rot disease caused by *Ganoderma* in oil palm planted on inland soil at Kluang, Johor. *Proc. of the PIPOC 2009 International Palm Oil Congress*. MPOB, Bangi. p. 1371-1382.
- Mohd Din, A; Kushairi, A; Mohd Isa, Z A; Noh, A and Rajanaidu, N (2005). MPOB strategic plan for fast track breeding programmes. *Proc. 2005 Nat. Sem. Breed. & Clonal Tech.* 7 March 2005. Hotel Palace of The Golden Horses, Kuala Lumpur. p. 43-53.
- Mohd Din, A; Rajanaidu N; Kushairi, A; Noh, A; Norziha, A; Meilina, O A and Ravigadevi, S (2014). Oil palm breeding programme for *Ganoderma* tolerance. *Proc. Workshop on Integrated Management of Ganoderma Disease on Oil Palm*. Promenade Hotel, Kota Kinabalu, Sabah.
- Mohd Shukri, I; Idris, A S; Izzuddin Anuar; Rusli, M H and Norman, K (2019). Implementation of technologies in management of *Ganoderma* disease in Malaysia. *Proc. of the PIPOC 2019 International Palm Oil Congress*. MPOB, Bangi.
- Norziha, A; Noh, A; Fadila, A M; Zulkifli, Y; Mohd Din, A; Rajanaidu, N and Kushairi, A (2012). *Ganoderma* infection studies in selfed and intercrossed Deli *Dura* progenies. *Proc. International Seminar on Breeding for Oil Palm Disease Resistance*. Venue Hotel AR Salitre, Bogota Colombia.
- Nurazah, Z; Idris, A S; Kushairi, A and Umi Salamah, R (2013). Metabolite profiling of oil palm towards understanding basal stem rot (BSR) disease. *J. Oil Palm Res.*, 25 (1): 58-71.
- Nurul Fatiha Farhana, H; Noramiza, S; Hamdan, I; Muhammad Nazmi, B; Rafidah, M K; Raja Bahiyah Nur, R H; Mohd Nasruddin, M; Siti Habsah, R and Tan, J S (2019). FGV screening program for *Ganoderma* tolerant planting materials. *Proc. International Seminar on Breeding for Ganoderma Tolerance in Oil Palm*. ISOPB. 50-59.
- Purba, R Y; Purba, A R and Sipayung, A (1994). Uji resistensi beberapa persilangan kelapa sawit DxP terhadap *Ganoderma boninense* PAT. *Bulletin PPKS*, 2: 81-88.
- Rahamah, M S H; Idris, A S; Maizatul Suriza, M; Kushairi, A; Mohd Din, A and Marhalil, M (2015). Three methods for screening of oil palm resistance to *Ganoderma* disease. *Proc. of the PIPOC 2015 International Palm Oil Congress (Agriculture, Biotechnology and Sustainability)*. Kuala Lumpur, Malaysia. 11 pp.
- Rahmaningsih, M; Setiawati, U; Breton, F and Nelson, S (2013). Results from Deli x AVROS (and reciprocal crosses) for *Ganoderma* partial resistance. *Proc. of the PIPOC 2013 International Palm Oil Congress*. MPOB, Bangi.
- Rajanaidu, N (1994). *PORIM Oil Palm Genebank: Collection, Evaluation, Utilisation of Oil Palm Genetic Resources*. PORIM, Bangi.
- Yong, Y Y (1992). The oil palm breeding programme in Malaysia. *Proc. of the Seminar on the Science of Oil Palm Breeding*. PORIM, Bangi. p. 141-164.

MECHANICAL DESIGN ANALYSIS AND IMPROVEMENT OF ALUMINIUM ALLOY CANTAS SICKLE BLADE USING FINITE ELEMENT METHOD SIMULATION

AHMAD SYAZWAN RAMLI^{1*}; MOHD AZWAN MOHD BAKRI¹; MOHD RIZAL AHMAD¹
and MOHD IKMAL HAFIZI AZAMAN¹

ABSTRACT

This article explores the possibility of improving the design of Cantas, by focusing on the failure analysis and the design optimisation of its sickle blade. The sickle blade material was replaced with 6262-T8 aluminium alloy. An analysis to inspect the sickle's mechanical strength against 300 N of cutting force was simulated using the finite element method (FEM) software. The total stress experienced by the new design (i.e., 1204 MPa) exceeded the material's ultimate tensile strength of 390 MPa; therefore, would break due to mechanical failure. The dimension of the weak point on the sickle was then increased to 25.0%. The improved sickle design only experienced 314 MPa of stress and did not exceed its ultimate tensile strength. The design was further optimised to reduce its weight. A reduction of 14.0% was achieved compared to the previous improved design and 69.7% compared to the original spring steel sickle with only 352 MPa of stress experienced. It also can withstand any directional impact force in drop test simulation. The aluminium alloy sickle is also 48.0% cheaper than spring steel sickle. However, a cost per tonne of productivity calculation shows that the performance of both sickle materials is very much the same.

Keywords: agriculture, design, FEM analysis, palm oil, sickle.

Received: 24 May 2021; **Accepted:** 22 November 2021; **Published online:** 28 January 2022.

INTRODUCTION

Oil palm has been cultivated due to its high oil yield per unit area of plantation (Basiron, 2007). It is the main commodity crop of Malaysia, with export revenue reaching more than RM67.12 billion in 2018 (Parveez, 2020). In palm oil plantations, harvesting fresh fruit bunch (FFB) from palm trees is still a major challenge that needs to be solved. Therefore, a motorised oil palm cutter named Cantas has been developed to address this issue (Figure 1) (Ahmad *et al.*, 2020). By using Cantas, the productivity of an oil palm plantation can be increased; therefore,

reducing the number of labours required for the plantation's operation. The cutter consists of a sickle on a pole that vibrates using a petrol engine, and was later substituted with an electric motor to ease and facilitate FFB cutting during harvesting.

The latest version of Cantas is named Cantas Electro, and it runs on a fully electric power supplied by two units of lithium-ion batteries. However, further optimisation and improvement measures can be done to enhance the efficiency of Cantas. Jelani *et al.* (2008) improved the Cantas technology by introducing Cantas Evo version, which has a new cutting head design, engine, pole gripper and shaft guider. However, the sickle design improvement was not addressed in his study. The Cantas's sickle improvement design was done a few years earlier by the introduction of the 'C-shaped' sickle profile, which was found to be more efficient and able to

¹ Malaysian Palm Oil Board,
6 Persiaran Institusi, Bandar Baru Bangi,
43000 Kajang, Selangor, Malaysia.

* Corresponding author e-mail: ahmad.syazwan@mpob.gov.my

reduce vibrations during harvesting (Jelani *et al.*, 2008). However, the study only focused on the performance of the new sickle profile and did not consider other materials for it. The sickle has to be as light as possible to reduce its vibration because a high momentum is generated by a heavier sickle (Jelani *et al.*, 2008). Lighter sickle also translates to less inertia, and therefore, less effort is needed to provide the motion for the sickle's vibration; thus, increasing its cutting efficiency (Jelani *et al.*, 2008; Winn, 2010). In addition, too much vibration increases tool wear, and decreases geometric accuracy and surface finish (Rusinek *et al.*, 2014). Other than that, Hand Arm Vibration Syndrome (HAVS) is a common illness that is associated with regular and frequent exposure to vibration tools, such as Cantas (Salleh *et al.*, 2013). Hence, the sickle blade used for FFB harvesting needs to be robust and strong enough against wear and tear. This is because the oil palm frond, for example, contains a very high amount of lignin and silica (Dahlan, 2000). These elements found in palm oil fronds have the potential to wear out the sickle blade.

So far, there has been little discussion about sickle design optimisation. Therefore, this article intends to investigate the possibility of further improving the sickle blade design. In this research, the Cantas sickle blade made of AISI 5160 high carbon steel, also commonly known as spring steel, was replaced by aluminium alloy with the goal of reducing the weight of the Cantas to improve its ergonomic design, as well as increasing its durability.

MATERIALS AND METHODS

Material Selection

The Cantas sickle blade is currently made of AISI 5160 high carbon steel material. This material is also commonly known as spring steel due to its application in the automotive industry for various

heavy duty spring applications (The Material World, 2020). Besides, spring steel is also commonly used as a material to produce cutting tools, such as sickles, machetes, and various forms of knives used in the agriculture industry.

However, similar to other types of steel, spring steel is prone to corrosion due to rust. The rust is developed via oxidation of elements, such as the iron content in spring steel with oxygen and water in the surrounding environment. Steel corrosion due to rust weakens the structural strength of a steel tool; hence, making the tool prone to mechanical failure. Moreover, the high density of steel means that any tools made of it would be heavy. A heavy sickle on a pole like the Cantas design would cause a stability issue when the operator handles the Cantas during harvesting. The operator would also require extra energy to handle the high momentum force and inertia generated by the weight of the sickle multiplied with the length of the pole. This situation would also cause difficulties in manoeuvring the Cantas efficiently during operation. Therefore, it would be advantageous if a lightweight material could be used as the Cantas sickle blade, in order to reduce the stability and manoeuvring issues due to the heavier weight of a steel sickle.

To solve these issues, aluminium alloy is suggested as a replacement material for the sickle blade. Aluminium is a common metal used for lightweight applications due to its high strength-to-weight ratio compared to other metals. Aluminium is also ductile, which is the opposite property of brittle; therefore, aluminium deforms first before material failure occurs (Gunturi *et al.*, 2019). A brittle material does not deform, but breaks and cracks instantly when the stress exceeds its tensile strength (Andreoni and Yip, 2020). The crack initiation and propagation resulted from the fatigue of the material, is a major source of a component's failure (Lin and Smith, 1998).

For the sickle application, the 6262-T8 aluminium alloy grade was chosen due to its high tensile ultimate strength (*i.e.*, 390 MPa) compared to



Figure 1. Cantas, a motorised oil palm tree harvester.

other aluminium alloy grades (MakeItFrom, 2019). Besides, aluminium is resistant to corrosion. The oxidation of aluminium produces an aluminium oxide layer that protects the material from further oxidation through a chemical process known as passivation (Kuznetsova *et al.*, 2001). Therefore, aluminium will not corrode or face any structural weakening issues like rust. These advantages are the main reasons for selecting aluminium alloy as the replacement material for the spring steel of the Cantas sickle blade.

Analysis Method

Finite element method (FEM) simulation. In order to determine the mechanical strength of the sickle blade using aluminium alloy, a failure analysis with the help of FEM simulation was conducted using ANSYS software. The failure simulation analysis was conducted because this kind of analysis is able to identify any failure that might have occurred if such parameter (*i.e.*, material replacement) is applied to the current design, as well as enabling any suggestion to avoid the failure to be verified in an efficient way (Zerbst *et al.*, 2015). The simulation can predict the mechanical structural behaviour of a defined structure or an object based on the assigned loads, physical surroundings, and physical design shape using partial differential equations (Harish, 2019).

This method enables engineers and designers to check the suitability and rigidity of their designed object via computer simulation instead of building a prototype first. This approach can also reduce the initial cost of building the prototype by producing an almost identical design and reducing any possible occurrence of mechanical failure.

The first step in conducting FEM simulation is preparing the three-dimensional (3D) computer-aided drawing (CAD) of the sickle blade. *Figure 2*

shows the dimension of the sickle and the thickness of the blade which is set to 1.2 mm. Once the sickle blade was designed, the CAD was uploaded and generated in the pre-processing stage of the software. At this stage, a mesh generation tool was used to convert the sickle design into smaller elements. This step is done so that the software can assign the FEM algorithm to each element of the sickle. The mesh size is chosen to be 1 mm which generates 698 460 nodes and 364 030 elements due to the factor of computer processing power limitation. Then, all loads exerted on the sickle, as well as the physical and mechanical properties of the chosen materials (*i.e.*, AISI 5160 high carbon spring steel and 6262-T8 aluminium alloy), were assigned to the 3D sickle model. The simulation results for the spring steel were set as the benchmark for comparison with the simulation results of the aluminium alloy. For this analysis, a cutting force of 300 N was assigned at the sharp edge of the sickle blade and the fixed support at the bottom neck of the sickle, as shown in *Figure 2* (Jelani *et al.*, 1998).

After that, the computational tool in the software performed an engineering analysis on the mechanical structural strength of the sickle blade once the simulation was run. The sickle design was then further optimised with the aid of FEM simulation to determine the mechanical structural strength of each sickle design improvement.

Design improvement. Once the simulation result is obtained, the design is further assessed to detect any potential failure or damage. If failure is detected, new design improvements using 3D CAD software can be done to modify the geometry and profile of the sickle design, so that the identified weak point can be eliminated. The new improved design is again verified using the FEM simulation analysis to detect any existence of weak points and possible failure regions.

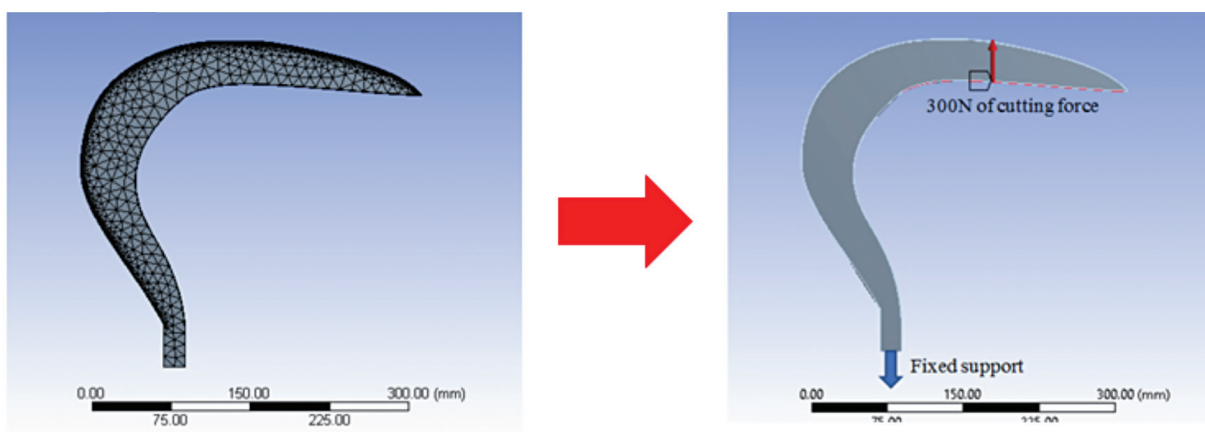


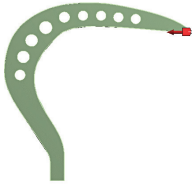
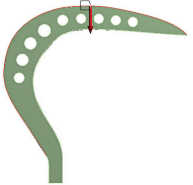
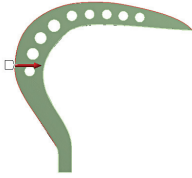
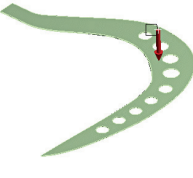
Figure 2. A meshed 3D model of sickle blade design and loads assignment.

Drop test. Drop test simulation was also performed to verify the quality of the new sickle design. In this test, the impact load exerted on the sickle is assumed to be solely generated by the potential energy of the Cantas based on its height when used. The impact force, F , was calculated using Equation (1) (Lüders *et al.*, 2017):

$$F = m \cdot g \cdot h \quad (1)$$

The formula equates the gravitational potential energy generated from the initial height of the sickle. The potential energy is based on the formula of the multiplication of the sickle's weight, m , with the gravitational acceleration, g , and the initial height of the sickle during operation, h . The gravitational force, g is maintained at a constant value of 9.81 m/s^2 , whereas the height, h , is set at 7 m, which is the reach of Cantas sickle blade, with a sickle weight of 68.98 g. If the sickle falls and hits the ground in the first three directions as specified in Table 2, it is assumed that the impact force is generated only by the weight of the sickle. However, if the sickle falls horizontally flat to the side of the sickle, then the height, h is set to be 2 m. The software calculated the amount of stress experienced by the sickle due to each impact load direction. The stress values were also compared with the material's ultimate tensile strength value to ensure the stresses generated do not break the sickle. Table 1 summarises the directions of the possible loads exerted during the drop test.

TABLE 1. POSSIBLE EXERTED LOAD DIRECTIONS DURING DROP TEST

Load positions and directions	
90°	180°
	
270°/-90°	90° horizontal
	

Economic analysis. Once the structural quality of the new and optimised design of the sickle has been confirmed, an economic analysis of the new aluminium alloy sickle design was carried out to verify its cost-efficiency compared to the conventional spring steel sickle. An economic analysis was done to calculate the operating cost

required to harvest a ton of FFB from the plantation, and this calculation was used to assess the efficiency of the technology. If the calculated cost of the proposed technology is lower than the current operational cost per tonne of FFB of the plantation, then the proposed technology is deemed to have better cost efficiency than the current technology (Azwan *et al.*, 2017). To estimate the price of the sickle, the price of a spring steel sickle in the current market (*i.e.*, RM150) is considered as a benchmark price. The price of steel and aluminium generally differs based on the current global commodity price of these metals, grades, metallurgy process, and manufacturer's quotation.

Therefore, to estimate the price of this theoretical aluminium sickle, the price difference between steel and aluminium was compared using the global commodity price. On the 19 August 2020, the steel price was USD642/t, and the aluminium price was USD1749/t. Thus, the price of aluminium is 135% higher than steel. By dividing the price of the spring steel sickle with its weight (*i.e.*, 199.58 g), the value of the spring steel sickle is RM0.75/g. Based on this value, the estimated price for the aluminium sickle is RM1.01/g.

RESULTS AND DISCUSSION

Design Simulation

The stress and deformation values are among the simulation results that need to be analysed in this study. The stress value represents the amount of stress experienced by the sickle when subjected to the assigned load of the defined cutting force. If the stress value experienced by the sickle exceeds the sickle material's ultimate tensile strength value; thus, the load has sufficient force to cause mechanical failure to the structure. The cutting force exerted on the sickle is also high enough to break the sickle; hence, making the material unsuitable to be used for Cantas. The deformation value of the sickle also needs to be verified. If the deformation value of the sickle blade is too high, the cutting efficiency is then reduced, and the harvesting process becomes more difficult. A material with high deformation value means that the material is highly ductile and soft, and these properties are not favourable as a cutting tool. In addition, the weight of the sickle can also be determined with the help of the 3D CAD software. Hence, by changing the material assigned to the 3D sickle design, the weight of the sickle with different materials can be determined and compared. Figure 3 compares the simulation results of the amount of stress experienced by the spring steel and aluminium alloy sickles, whereas Figure 4 compares the deformation experienced by both sickles based on the simulation results.

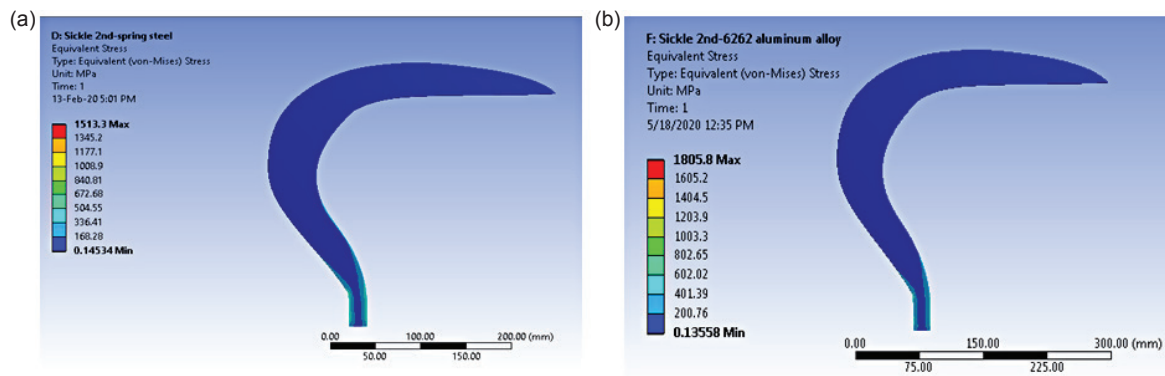


Figure 3. Comparison of stress simulation results between (a) spring steel and (b) aluminium alloy sickles.

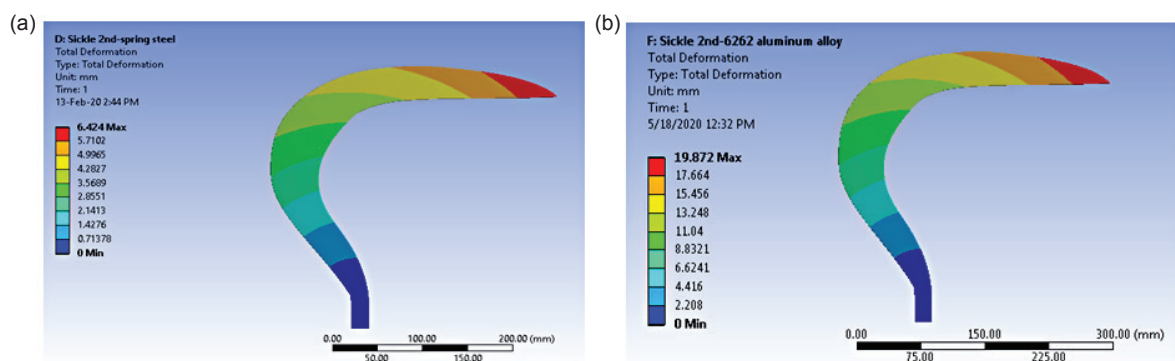


Figure 4. Comparison of deformation simulation results between (a) spring steel and (b) aluminium alloy sickles.

The amount of stress experienced by the aluminium sickle was 1203 MPa, which exceeded its ultimate tensile strength value of 390 MPa. Therefore, the aluminium sickle would break when cutting force is exerted to the sickle. Therefore, aluminium is unsuitable as sickle material for Cantas if this design is used. The deformation value of the aluminium sickle is very high (*i.e.*, 19.87 mm), which is more than three times higher than the spring steel sickle (*i.e.*, 6.42 mm); hence, highlighting the unsuitability of this material as a sickle blade material. However, an aluminium sickle blade is very light (*i.e.*, 68.98 g), which is 65% lighter than a spring steel sickle blade, *i.e.* 199.58 g, and this property is very advantageous if the material can be adapted into the design of the Cantas sickle.

Design Improvement

Based on the graphical results, the area where mechanical failure may occur at the aluminium sickle is concentrated at the bottom neck of the sickle. It is possible to improve the design at that weak point, so that mechanical failure can be avoided. Once the design of the 3D model was improved, another FEM

simulation was performed on the model to verify whether the design improvement is sufficient to avoid any mechanical failure and prevent the sickle from breaking.

The 3D CAD model of the sickle design was modified by increasing the width of the sickle neck by 25%, from 20 mm to 25 mm (*Figure 5*). Then, the same methodology for the previous mechanical structural simulation of the sickle was implemented using the new sickle design.

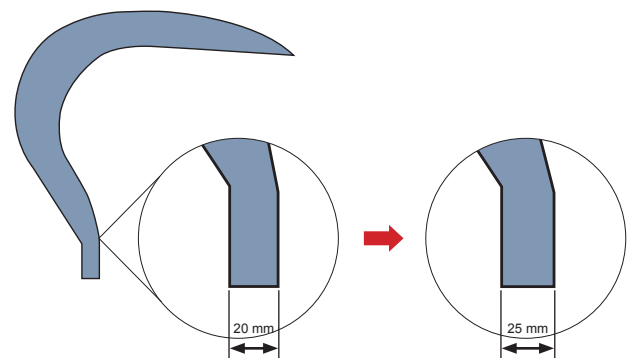


Figure 5. Design improvement to strengthen the weak point of the aluminium sickle.

Figure 6 depicts the simulation for the stress value experienced by the improved design of the aluminium sickle. By implementing this improvement, the stress experienced by the sickle (*i.e.*, 314 MPa) did not exceed its ultimate tensile strength value. Therefore, the aluminium sickle blade would not break under the defined cutting force when using the new and improved sickle blade design. However, the new design is slightly heavier (*i.e.*, 70.45 g).

In order to improve the weight of the sickle blade, its design was further improved by adding several relief holes. The holes were added accordingly at the area that did not experience any stress, as marked by the blue colour in the simulation graphic. By adding the relief holes, the weight of the sickle was reduced to 62.36 g, which represents approximately 11% of further weight reduction.

Next, the mechanical structural strength of the further improved sickle design needs to be verified by the FEM simulation. The same method

was applied to this design, similar to the previous simulation. The results in Figure 7 show that the stress experienced for this sickle design is only 356.92 MPa. Thus, the sickle did not experience any mechanical failure and it would not break when the cutting force is exerted during operation.

Simulation Validation

In order to verify the simulation result, a validation is made by a comparison with the literature. The Ramberg-Osgood (R-O) model is chosen as a comparison with the stress-strain description of the sickle model simulation (Castaldo *et al.*, 2017). The R-O model is mostly used to estimate the non-linear relationship between strain and stress, as well as the stress-strain curve in materials near the material's yield point (Gardner and Ashraf, 2006). Figure 8 depicts a graphical representation of a R-O model of the Aluminium 6262-T8 stress-strain curve and the stress-strain curve of the FEM simulation model of the aluminium sickle.

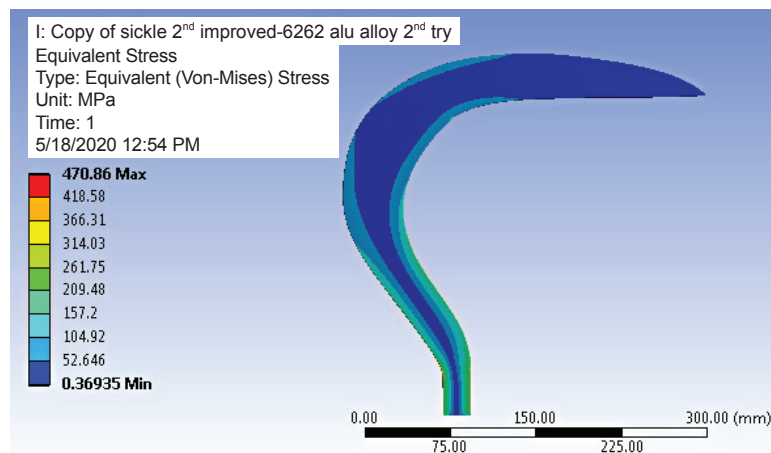


Figure 6. Simulation results of the stress value for the sickle with improved design.

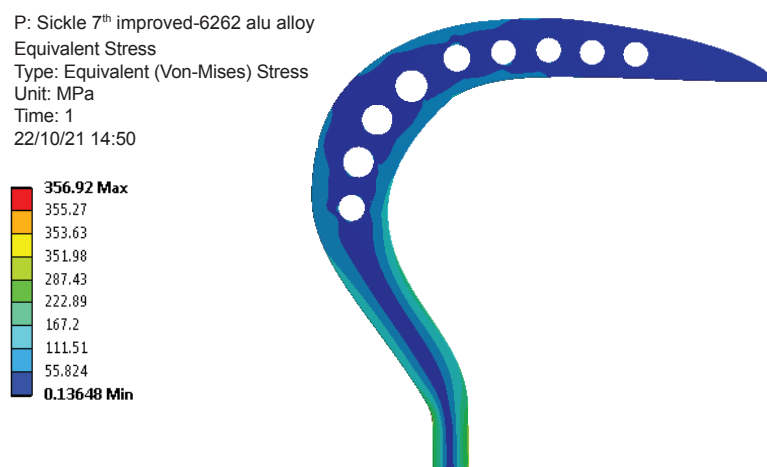


Figure 7. Simulation results of the stress value for the sickle with relief holes.

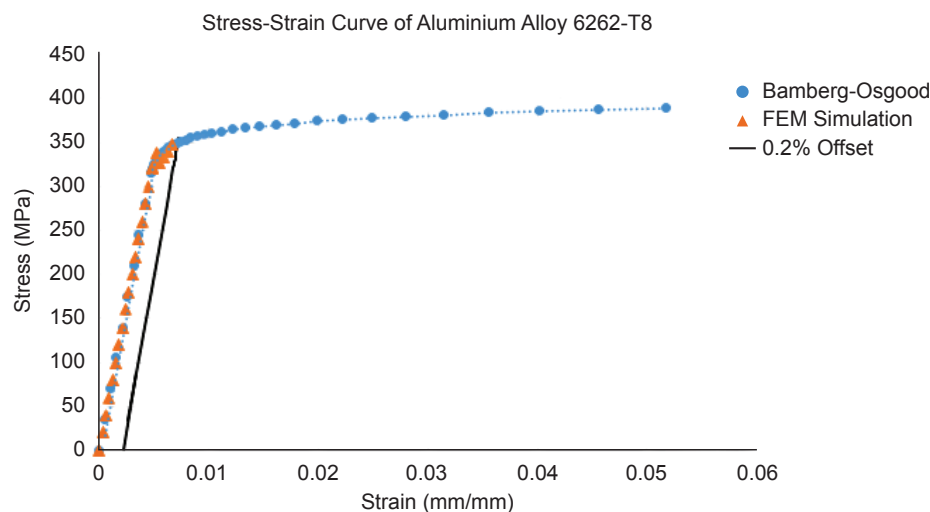


Figure 8. FEM simulation result comparison with Bamberg-Osgood model.

As shown in Figure 8, the stress-strain curve of the aluminium sickle model generated through the FEM simulation fits into the R-O model. In addition, the curve of the FEM simulation does not exceed the 0.2% offset yield strength, which means that the sickle only experiences recoverable elastic deformation. Hence, there is no irreversible elastic deformation occurring when the sickle is subjected to the cutting load.

Drop test. The result of the drop test simulation is summarised in Table 2 below by comparing the direction of the impact load exerted to the simulated stress experienced by the optimised sickle design.

As shown in Table 2, the stress generated due to the impact forces from different directions ranged from 1.17 to 131.03 MPa and did not exceed the value of the material's ultimate tensile strength. Therefore, it can be assumed from this drop test that the aluminium alloy sickle has enough strength to handle the drop impact without causing any damage to its structure. However, a full test with a real aluminium alloy sickle prototype is needed in order to verify the simulation data result. This is because the analysis of the real failures is the main credible data that can provide important information on how the design should exactly be optimised based on available theories, fabrication details, and concept (Alonso-Martinez *et al.*, 2019). However, with the help of FEM simulation, the number of prototypes required for the real test can be as minimal as possible, as well as identifying and narrowing down the number of weak points in the design. The results were also compared with the work conducted by Jelani *et al.* (2008) where they conducted a drop test at a height of 2 m with different dropping angles on the whole Cantas. However, only test results with similar dropping angles were selected so that the comparison is relevant. Table 3 shows the comparison of results from Jelani *et al.* (2008) and this study.

As seen in Table 3, the Cantas sickle is able to handle the drop test without causing any damage, either being dropped at 2 m for spring steel sickle or even at 7 m for aluminium alloy sickle. This means that aluminium alloy is suitable to be adopted for the Cantas sickle design based on the FEM simulation results.

Economic analysis. Based on the price per gram chosen for this study, an aluminium sickle that weighs 68.98 g would be priced at RM69.84, which is 53% cheaper than the current price of spring steel sickle. However, due to the fabrication cost of extra relief holes, it is estimated that the price of the sickle would be 10% higher. Therefore, the estimated price of the aluminium sickle is approximately RM76.82, which is 48% cheaper than the spring steel sickle. For this estimated calculation, it is assumed that both sickles are fabricated using conventional machining to cut the metal into the desired sickle shape and both sickles have similar costs of additional hardening treatment. Besides, the productivity of cost per tonne of harvesting FFB using the aluminium sickle in the plantation was also calculated. The calculation is summarised in Table 4.

Based on the calculation in Table 4, the cost per tonne of FFB incurred by using the aluminium sickle is slightly cheaper than using a common spring steel sickle, which is approximately a 0.6% reduction. Even though the price difference is not very significant, other benefits of using the aluminium sickle have provided more merits and advantages when using this material, such as its lightweight property, high resistance to corrosion, and reduction of vibration generated during Cantas operation. Therefore, using 6262-T8 aluminium alloy as the Cantas sickle blade is more preferable and highly recommended than steel.

TABLE 2. DROP TEST SIMULATION OF THE OPTIMISED SICKLE DESIGN

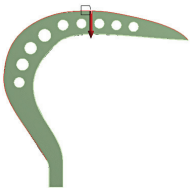
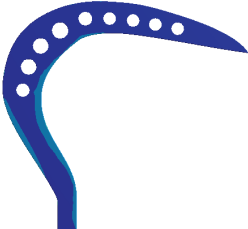
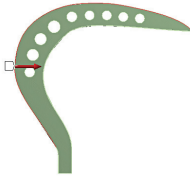
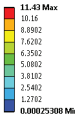
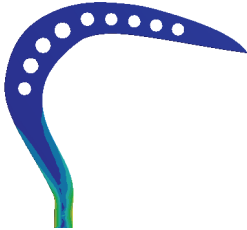
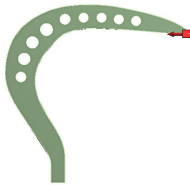
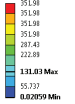
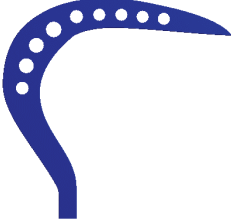
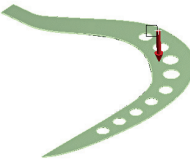
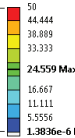
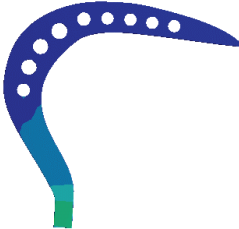
Load positions and directions	Simulation results	Stress (MPa)
180° 	T: DROP TEST 3 Equivalent Stress Type: Equivalent (von-Mises) Stress Unit: MPa Time: 1 03/11/21 16:18  	1.17
270°/-90° 	S: DROP TEST 2 Equivalent Stress Type: Equivalent (von-Mises) Stress Unit: MPa Time: 1 03/11/21 16:14  	11.43
90° 	Q: Drop Test Sickle 7th improved 6262 alu alloy Equivalent Stress Type: Equivalent (von-Mises) Stress Unit: MPa Time: 1 03/11/21 16:03  	131.03
90° horizontal 	U: DROP TEST 4 Equivalent Stress Type: Equivalent (von-Mises) Stress Unit: MPa Time: 1 03/11/21 17:33  	24.56

TABLE 3. COMPARISON OF SIMULATED DROP TEST WITH

Research	Height (m)	Dropping angle	Result
Jelani <i>et al.</i> (2008)	2	90° 1 st drop	No damage
	2	90° 2 nd drop	No damage
	2	90° 3 rd drop	No damage
This study	2	90° horizontal	No damage
	7	90°	No damage
	7	-90°/270°	No damage

Source: Jelani *et al.* (2008).

TABLE 4. THE PRODUCTIVITY COST PER TONNE OF FFB USING ALUMINIUM AND SPRING STEEL SICKLES

Description	Calculation	
	Aluminium sickle	Spring steel sickle
Sickle price	RM76.82	RM150.00
Economic life	1 year	
Working days per year	300 days	
Depreciation (Price/(Life span × Working days))	RM0.25	RM0.50
Labour cost	RM50/day	
Productivity	6 t/day	
Total cost per day (Labour cost + Depreciation)	RM50.25	RM50.50
Cost per tonne = Total cost/Productivity	RM8.37/t FFB	RM8.42/t FFB

CONCLUSION

Based on the simulation done using the FEM software, it can be concluded that 6262-T8 aluminium alloy is not suitable as a material for Cantas sickle blade if the original sickle design is applied. The amount of stress generated by the cutting load of 300 N (*i.e.*, 1203 MPa) exceeds the material's ultimate tensile strength value. Hence, mechanical failure will occur due to high stress and the sickle will break.

However, aluminium alloy can still be used as the sickle blade material if the design is improved by strengthening the weak points on the sickle blade. By increasing the width of the sickle bottom neck by 25%, the stress value experienced by the sickle had reduced up to 314 MPa, which did not exceed its material ultimate tensile strength value. This showed that strengthening the neck of the sickle was able to eliminate the weak point identified earlier. However, by applying this improvement, the weight of the sickle had increased slightly by 2% to 70.45 g, from the initial weight of 68.98. To further reduce its weight, several relief holes have been strategically placed in areas that does not experience any stress. Consequently, the weight of the sickle blade was reduced to 62.36 g (*i.e.*, 68.6% of weight reduction compared to spring steel sickle).

Another FEM simulation was conducted to verify that the latest design improvement (*i.e.*, addition of relief holes) does not weaken the structure. The simulation showed that the latest sickle design experienced only up to 356.92 MPa of stress which still did not exceed the material's ultimate tensile strength value. Hence, the failure potential for the sickle material is still avoidable. Finally, a drop test analysis was performed using the sickle to prove that the sickle would not be damaged if it drops to the ground. The drop test simulation results showed that the sickle only experienced stress load between 1.117 to 131.03 MPa only, and therefore, confirmed that the aluminium alloy sickle could withstand the drop

impact. An economic analysis was also carried out to determine its cost-efficiency had indicated that, the cost per tonne required by the plantation is RM8.37/t of FFB when using the aluminium alloy, compared to RM8.42/t of FFB when using the conventional spring steel. Although the cost reduction is only about 0.6%, 6262-T8 aluminium alloy sickle is still considered advantageous in terms of being lightweight, and resistant to corrosion and vibration. Therefore, based on this study, an optimised Cantas sickle blade design using 6262-T8 aluminium alloy has better advantages in comparison to the conventional spring steel sickle and it is suitable for application in an oil palm plantation.

ACKNOWLEDGEMENT

The authors would like to express their sincere gratitude to the management of Malaysian Palm Oil Board (MPOB) to allow this article to be published, as well as to those who have involved and contributed in this research either directly or indirectly.

REFERENCES

- Ahmad, M R; Jamaludin, N; Jelani, A R; Bakri, Azwan and Shuib, A R (2020). The effect of design parameters on force and energy requirements for cutting oil palm fronds. *Jurnal Teknologi*, 82(4): DOI: 10.11113/jt.v82.14236.
- Alonso-Martinez, M; Adam, J M; Alvarez-Rabanal, F P and del Coz Díaz, J J (2019). Wind turbine tower collapse due to flange failure: FEM and DOE analyses. *Eng. Fail. Anal.*, 104: 932-949. DOI: 10.1016/j.engfailanal.2019.06.045.
- Andreoni, W and Yip, S (2020). *Handbook of Materials Modeling. Applications: Current and Emerging Materials*. 2nd edition. Springer. p. 74-76.

- Azwan, M B; Norasikin, A L; Sopian, K; Abd Rahim, S; Norman, K; Ramdhan, K and Solah, D (2017). Assessment of electric vehicle and photovoltaic integration for oil palm mechanisation practise. *J. Clean. Prod.*, 140: 1365-1375. DOI: 10.1016/j.jclepro.2016.10.016.
- Basiron, Y (2007). Palm oil production through sustainable plantations. *Eur. J. Lipid Sci. Technol.*, 109(4): 289-295.
- Castaldo, P; Natri, E and Piluso, V (2017). FEM simulations and rotation capacity evaluation for RHS temper T4 aluminium alloy beams. *Compos. B Eng.*, 115: 124-137. DOI: 10.1016/j.compositesb.2016.10.026.
- Dahlan, I (2000). Oil palm frond, a feed for herbivores. *Asian-Australas. J. Anim. Sci.*, 13: 300-303.
- Gardner, L and Ashraf, M (2006). Structural design for non-linear metallic materials. *Eng. Struct.*, 28(6): 926-934.
- Gunturi, C; Escobedo, J P and Islam, M A (2019). Comparative analysis of dynamic impact tests between the charpy V-notch test and the drop tower test. *Characterization of Minerals, Metals and Materials 2019*. Springer, Cham. p. 553-560. DOI: 10.1007/978-3-030-05749-7.
- Harish, A (2019). Simscale. <https://www.simscale.com/blog/2016/10/what-is-finite-element-method/>, accessed on 3 February 2020.
- Jelani, A R; Ahmad, D; Hitam, A; Yahya, A and Jamak, J (1998). Force and energy requirements for cutting oil palm frond. *J. Oil Palm Res.*, 10(2): 10-24.
- Jelani, A R; Hitam, A; Jamak, J; Noor, M; Gono, Y and Ariffin, O (2008). Cantas™ – A tool for the efficient harvesting of oil palm fresh fruit bunches. *J. Oil Palm Res.*, 20: 548-558.
- Kuznetsova, A; Yates, J T; Zhou, G; Yang, J C and Chen, X (2001). Making a superior oxide corrosion passivation layer on aluminum using ozone. *Langmuir*, 17(7): 2146-2152. DOI: 10.1021/la001300x.
- Lin, X B and Smith, R A (1998). Fatigue growth simulation for cracks in notched and unnotched round bars. *Int. J. Mech. Sci.*, 40(5): 405-419.
- Lüders, K and Pohl, R O (2017). *Pohl's Introduction to Physics. Volume 1: Mechanics, Acoustics and Thermodynamics* (Klaus, L; Robert, O P and Williams, D B eds.). Springer. p. 19.
- MakeItFrom (2019). MakeItFrom.com. <https://www.makeitfrom.com/material-properties/6262-T8-Aluminum>, accessed on 3 February 2020.
- Parveez, G K A; Elina, H; Soh, K L; Meilina, O A; Kamalrudin, M S; Bidin, M N I Z; Sundram, S; Hasan, Z A A and Idris, Z (2020). Oil palm economic performance in Malaysia and R&D progress in 2019. *J. Oil Palm Res.*, 32(2): 159-190. DOI: 10.21894/jopr.2020.0032.
- Rusinek, R; Wiercigroch, M and Wahi, P (2014). Modelling of frictional chatter in metal cutting. *Int. J. Mech. Sci.*, 89: 167-176.
- Salleh, S M; Rahim, E A; Roslan, M N; Ismon, M; Madlan, M A; Masrol, S R; Jelani, A R and Ahmad, M R (2013). Hand-arm vibration of unskilled oil palm motorised cutter operator. *Appl. Mech. Mater.*, 315: 695-699. DOI: 10.4028/www.scientific.net/AMM.315.695.
- The Material World (2020). The Material World. <https://www.theworldmaterial.com/sae-aisi-5160-high-carbon-spring-steel/>, accessed on 3 February 2020.
- Winn, W (2010). *Introduction to Understandable Physics: Modern and Frontier Physics: Volume IV - Modern and Frontier Physics*. AuthorHouse. 444 pp.
- Zerbst, U; Klinger, C and Clegg, R (2015). Fracture mechanics as a tool in failure analysis - Prospects and limitations. *Eng. Fail. Anal.*, 55: 376-410. DOI: 10.1016/j.engfailanal.2015.07.001.

CHARACTERISATION OF PALM PYROLIGNEOUS ACID AND ITS EFFECTIVENESS AS ANTIFUNGAL AGENT FOR OIL PALM TRUNK

FAZLIANA ABDUL HAMID^{1*}; NAHRUL HAYAWIN ZAINAL¹; NUR ELIYANTI ALI OTHMAN¹;
FATIHA ISMAIL¹; NOORSHAMSIANA ABDUL WAHAB¹ and ASTIMAR ABDUL AZIZ¹

ABSTRACT

Pyroligneous acid (PA) is a by-product from carbonisation of palm kernel shell (PKS) with more than 200 organic compounds, including phenolic and organic acids. The purpose of this study was to characterise and evaluate the effectiveness of PA to protect oil palm trunk (OPT) against fungal attack. Wood vinegar was produced at four carbonisation temperatures (i.e., 500°C, 600°C, 700°C and 800°C). The chemical properties of PA were measured using Fourier transform infrared (FTIR) spectroscopy and gas chromatography-mass spectroscopy (GC-MS). The antifungal assay of PA from carbonisation at 600°C was applied on OPT against a white-rot fungus, *Trametes versicolor*, and a brown-rot fungus, *Fomitopsis palustris*. The effectiveness of PA against both fungi was evaluated by soaking OPT in the PA solution for 24 hr at 25°C, followed by exposure to the media containing the fungi, as stipulated in ASTM D-4455 standard. The lowest mean weight loss (9.33%) was obtained after eight weeks of exposure. The OPT-PA-treated specimen was highly resistant to the growth of *T. versicolor*. It was assumed that acid and phenolic components contributed to enhanced growth control of fungi, as indicated from the GC-MS results. The chemical compositions of PA proved that the antifungal activities could be controlled using a high concentration of PA.

Keywords: brown-rot and white-rot fungi, oil palm trunk, oil palm trunk treatment, pyroligneous acid.

Received: 19 July 2021; **Accepted:** 30 November 2021; **Published online:** 28 January 2022.

INTRODUCTION

Palm kernel shell (PKS) is one of the abundant biomass components of oil palm and more than 4 million tonnes of PKS are produced based on fresh fruit bunch processing in palm oil mills in Malaysia, and the value is expected to increase by 2020 (MPOB, 2020). PKS contains high carbon content with a high density of 0.6 g cm⁻³ and a tough structure due to the high composition of lignin and carbon (Nahrul Hayawin *et al.*, 2018). PKS is currently used as a combustion boiler

fuel with very limited efficiency. The current practice produces oil palm charcoal and activated carbon in a two-step process (i.e., carbonisation, followed by activation at high temperatures). This method requires high energy usage and extended duration, and it is also uneconomical (Rashidi *et al.*, 2014). Nahrul Hayawin *et al.* (2018) developed a system of simultaneous carbonisation-activation to produce charcoal and activated carbon. This system also produced by-products (e.g., PA, tar and gases) that were condensed and trapped in the cold traps.

PA also known as wood vinegar, has a yellowish or dark brown colour with acidic pH. The acid originates from a complex highly oxygenated aqueous liquid fraction obtained from the condensation of carbonised products or

¹ Malaysian Palm Oil Board,
6 Persiaran Institusi, Bandar Baru Bangi,
43000 Kajang, Selangor, Malaysia.

* Corresponding author e-mail: fazliana@mpob.gov.my

slow pyrolysis of wood and other lignocellulosic raw materials in the absence or limited oxygen (Zheng *et al.*, 2018). The heavier fraction called wood tar decanted at the bottom of the container and separates from PA after some days, even a few months. PA comprises a high amount of water (10%-20%), a mixture of carboxylic acids with acetic acid as the major compound (10%), phenols and derivatives, aldehydes, and pyrolytic lignin. The major compounds obtained in pyroligneous extract include formic acid, acetic acid, propionic and valeric acids; methanol, butanol, and amyl alcohol; phenol, cresols, guaiacol, and syringol derivatives; and organic compounds (*e.g.*, formaldehyde, acetone, furfural and valerolactone) (de Souza *et al.*, 2017; Lee *et al.*, 2011).

The production of PA from PKS has gained interest due to high lignin compounds that are correlated to the yield of phenolic compounds (Seri Elyanie *et al.*, 2021). Furthermore, PA has broad applications as antimicrobial, antioxidant, anti-inflammatory, soil enhancer, coagulant for natural rubber, and termiticidal and pesticidal agents. In addition, it is a source of valuable chemicals and smoky flavour for food (Mathew and Zakaria, 2015). PA has been used as an alternative medicine to promote greater body healing in Asian countries, such as China, Korea and Japan (Theapparath *et al.*, 2018). The effectiveness and characteristics of PA may vary slightly depending on its chemical composition, which can be affected by different parameters, such as duration, heating rate, temperature, particle size and biomass feedstock used (Fačková *et al.*, 2020).

The phenolic and acid compounds obtained in PA have the potential as a source of valuable chemicals for wood preservation against biological degradation (Lee *et al.*, 2011). According to Mohan *et al.* (2008), PA can be used as a preservative for timber in wet conditions. There are very few studies being conducted on the potential of bioactive compounds from PKS, particularly PA. Furthermore, the utilisation of PA in commercial products for protecting wood against fungal attack is still limited.

PA was obtained by Nahrul Hayawin *et al.* (2018) in the production of charcoal and activated carbon from oil PKS using a two-in-one activation process, which was conducted simultaneously in the same instrument. It is the most economically viable process with lower production costs than conventional processes. The PA from this carbonisation process can be used in the bio-preservation of oil palm biomass during storage. This research aims to characterise the chemical composition of PA derived from PKS and to evaluate the effectiveness of PA for oil palm trunk (OPT) treatment against fungal attack under laboratory conditions.

MATERIALS AND METHODS

Materials

Raw PKS was obtained from Ulu Kanchong Palm Oil Factory, Ulu Kanchong Estate, Negeri Sembilan, Malaysia. Carbonisation was performed according to the methods described in Nahrul Hayawin *et al.* (2018). Raw PKS was carbonised at different temperatures (*i.e.*, 500°C, 600°C, 700°C and 800°C).

Methods

Collection of PA from charcoal production. Approximately 3 kg of PKS per batch was carbonised using a double-insulated carbonisation-activation system for 2-3 hr. The details of the experimental setup were referred to the system developed by Nahrul Hayawin *et al.* (2018). The smoke released during the process was transported via a pipe at the bottom of the reactor to a condensation unit with the assistance of an exhaust fan. Condensed gases that passed through the condenser were collected as wood vinegar. The operating system was controlled automatically using a locally fabricated programmable logic controller (PLC) (Imetronic Sdn. Bhd.). The collected wood vinegar was filtered to remove dust and tar from the liquid.

Physical and chemical properties of pyroligneous acid. The pH value and density of PA were measured by a pH meter (Sartorius) and a pycnometer (Mettler Toledo), respectively. The chemical component of PA produced at different temperatures was analysed using Fourier transform infrared (FTIR) spectroscopy (TG-IR Hypernation System, Perkin-Elmer, USA) and gas chromatography-mass spectroscopy (GC-MS) (Agilent 7890, USA). The FTIR analysis was performed by dripping 1 mL of liquid PA onto the liquid sample holder. The IR spectrum was recorded in the wavelength range of 4000-400 cm^{-1} with a resolution of 4 cm^{-1} and four scans per sample using a Perkin-Elmer Spectrum (Perkin-Elmer, Waltham, MA, USA). PA waste needs to be pre-treated prior to GC-MS analysis (Wei *et al.*, 2010). Therefore, 10 mL of PA was washed by 3 mL of diethyl ether six times, in which 30% (v/v) of ether was used each time. The remaining ether in the liquid at the bottom layer was removed. About 0.1 g of magnesium sulphate was added to the sample, which was then purged with nitrogen for 20 min to remove excess solvent. The GC-MS method was described in Oramahi *et al.* (2018).

Decay fungi test. Decay resistance test for determining antifungal activity on OPT was carried out according to ASTM D-2017 (2008) involving two types of fungi: Brown-rot fungi (*Fomitopsis palustris*)

and white-rot fungi (*Trametes versicolor*) (ASTM, 1978). Both fungi were grown on potato dextrose agar (PDA) and incubated in a sealed petri dish using an incubator at 27°C with 70% environment humidity. The test was carried out for a period of eight weeks with standard weekly observation until fungi started to grow on the test OPT block.

The fungi used in the decay resistance test (*F. palustris* and *T. versicolor*) were supplied by MyEnzyme Sdn. Bhd. The isolation of fungi was repeated several times until pure fungi were obtained. The fungal resistance test was carried out according to ASTM D-4445 using OPT samples (length, width, and thickness of 5, 20 and 10 mm, respectively) treated with PA, which were placed onto the PDA inoculated with brown-rot and white-rot fungi (ASTM, 2003; Khoirun Nisa *et al.*, 2016). Untreated OPT samples were used as a control for both experiments and eight pieces of OPT samples were used for each treatment including control sample. All treated samples were soaked at 25°C in selected PA for 24 hr. After that, the treated OPT samples were removed and excess solution was washed off and allowed to dry to constant weight for three weeks at room temperature. The samples were sterilised at 100°C after the OPT samples reached a constant weight, weighed, and exposed to PDA inoculated with both fungi in a sealed petri dish at 27°C for eight weeks. After eight weeks of incubation, the surface covered with fungi was removed. The samples were then dried at 105°C to a constant weight. Mass loss (ML) was determined for the control and impregnated wood blocks gravimetrically using the following equation:

$$ML = \frac{m_{dw1} - m_{dw2}}{m_{dw1}} \times 100\%$$

where m_{dw1} (g) is the mass of the wood block before being exposed to the fungi and m_{dw2} (g) is the mass of the decayed wood block.

Statistical analysis. The statistical significant test (ANOVA single factor, Fischer LSD test) was conducted on the PA treatment on the OPT test block.

RESULTS AND DISCUSSION

Effect of Carbonisation Temperature on PA Yield

The PA yield was 30% (w/v) at 500°C and achieved the maximum value of 58% at 600°C. After that, the yield decreased at 650°C (Figure 1). The increment in PA yield is mainly attributed to the decomposition of cellulose and lignin components, which are correlated to the major components in PKS. Besides the decomposition of cellulose and

lignin, many volatile materials also decomposed at this temperature range, resulting in the release of several gases, such as carbon monoxide, carbon dioxide, and methane. The results also showed that the yield of PA decreased at the temperature range of 700°C-800°C. Less PA was produced and more tar was formed due to the decomposition of lignin at high temperatures. Another explanation is the secondary degradation of some ingredients found in PA at higher carbonisation temperatures as they are thermally unstable products (Demiral and Ayan, 2011).

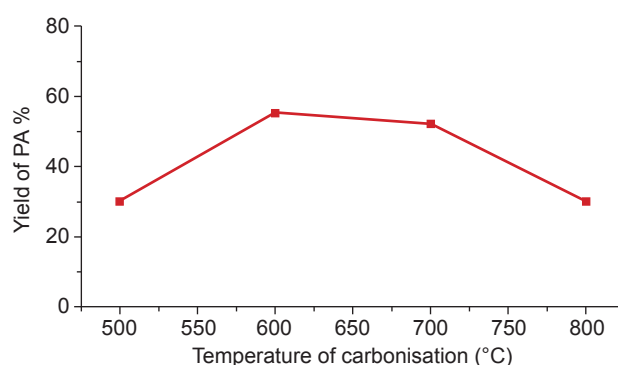


Figure 1. Amount of PA yield vs. carbonisation temperature.

Chemical Properties of Pyroligneous Acid

The chemical properties of PA were analysed and reported based on the highest yield at 600°C (Table 1). Based on the observation of carbonised PKS, the PA was yellowish-brown with a density of approximately 1.021 g mL⁻¹. The FTIR spectra of PA at different temperatures are shown in Figure 2. The broad bands of the hydroxyl stretching and carbonyl groups at 3327 cm⁻¹ and 1637 cm⁻¹, respectively, indicate the presence of water and other polymeric O-H in PA. The C-H stretching from the methylene group, C-O ether, and aromatic compounds are overlapping absorption bands at 1637 cm⁻¹. The spectrum also showed the overlapping peaks of C-H, indicating the presence of alkanes in the PA group at the range of 3200-2800 cm⁻¹ (Tsai *et al.*, 2007).

TABLE 1. PHYSICAL PROPERTIES OF PYROLIGNEOUS ACID

Parameter	Result
pH	3.5
Density	1.021 g mL ⁻¹
Colour	Yellowish-brown

The decomposition of cellulose and hemicellulose produced several products, such as carboxylic acids, ketones, phenols, aldehydes and alcohols. The band at 1800-1600 cm⁻¹ can be

attributed to C=O, which represents the existence of ketones, aldehydes, and carboxylic acids. Meanwhile, the band at 2122 cm^{-1} belongs to C=C stretching vibration, indicating the presence of alkanes and aromatic compounds in PA.

Chemical characterisation using GC-MS identified about 20 main components in the PA produced at 600°C , including their derivatives (Table 2). The major compounds obtained were carboxylic acids, phenols, ketones, aldehydes, alcohols, alkenes, furans, and esters with other derivatives, such as acetic acid (43.5%), glyceraldehyde (16.90%), and propanoic acid (13.98%). The higher amount of acid in PA is due to the decomposition of hemicellulose and volatile matter during carbonisation at 250°C - 350°C

(Demirbas, 2007). The degradation of hemicellulose and cellulose at 250°C - 275°C will lead to the production of light oxygenates, acetic acid, furfural and water. The thermal degradation of cellulose occurred in two stages, depending on the pyrolysis temperature, where some cellulose degrades below 320°C and the remaining cellulose degrades at higher temperatures (Wang *et al.*, 2021). Phenolic compound (12.48%) was obtained from the decomposition of lignin at 500°C - 600°C , which is relatively high, followed by furans, esters, alkanes and ketones. The decomposition of lignin occurs at intermediate degradation temperatures from 250°C to 500°C , producing various hydroxyl and methoxy substituted phenylpropane compounds (Theapparat *et al.*, 2018).

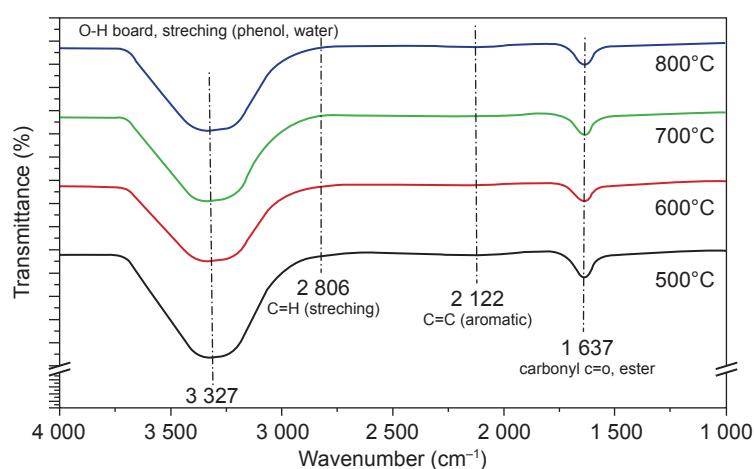


Figure 2. FTIR spectrum of PA produced at different temperature.

TABLE 2. MAIN COMPONENTS IN THE PALM BASED PYROLIGNEOUS ACID AT 600°C

Chemical category	Retention time (min)	Compounds	Relative contents (%)
Nitrogenated and derivates	7.44	Pyridine	9.05
Organics acid	24.54	Acetic acid	43.59
	10.56	Formic acid	0.81
	11.65	Propanoic acid	13.98
	13.77	4-hydroxybutanoic acid	4.92
	9.07	Glyceraldehyde	16.90
Aldehyde	29.05	3-methylpentanal	0.21
	20.22	Phenol	12.48
Furan and derivatives		Tetrahydro-3-furanol	4.92
	17.79	Tetrahyrdo-2H-pyran-2-one	4.25
	33.64	2 (5H) furanone	0.57
		4-methyl -5H-furan -2 -one	0.26
	35.35	4H-pyran -4 one	0.24
Alkanes	22.62	Ethenylmethylene cyclopropane	0.09
Esters	10.9	Cyclohexan-1,4,5-triol 3-one -1-carboxylic acid	1.66
	19.88	Hexadecanoic acid , 2 (octadecyloxy)ethyl ester	0.79
	22.38	Hexadecanoic acid, 1-hydroxymethyl)1-2-ethanediyl ester	1.05
Ketones	13.80	Butyrolactone	5.79

Antifungal Activity of Palm Pyroligneous Acid on Oil Palm Trunk Treatment

Figure 3 shows the fungal colonisation obtained on the surface of OPT test blocks after eight weeks of exposure to white-rot and brown-rot fungi. All the OPT test blocks treated with PA showed promising results against fungal growth compared to the untreated OPT samples. The highest antifungal activity of PA on white-rot fungi is probably due to the presence of acid and phenolic compounds in PA. The phenol content in PA is responsible for antifungal activity (Oramahi *et al.*, 2018). As the carbonisation temperature increased, phenolic compounds were obtained from the decomposition of lignin at 300°C-500°C of pyrolysis temperature, and this approach is effective for preventing fungal or mould growth (Lee *et al.*, 2011).

After five days of exposure, all OPT test blocks treated with PA showed that less than 10% of the surface area was covered with brown-rot and white-rot fungi compared to non-treated OPT samples. The untreated OPT test blocks were used as control sets. The formation of brown-rot fungi could be observed after one week of treatment. Almost 75% of the surface area of the OPT-PA test blocks was covered by brown-rot fungi after 30 days of exposure. The increase in water activity would enhance the growth of fungi (Khairon Nisa *et al.*, 2016). Surprisingly, the treated OPT test blocks exposed to white-rot fungi showed less than 10% surface coverage after 30 days of exposure. The resistance of wood against fungi might be due to the inhibition of

enzymatic activity by wood vinegar (Oramahi *et al.*, 2018). Temperature is the most important factor in the characterisation of the components and the effectiveness of PA in controlling wood degradation (Nakai *et al.*, 2007). However, it must be considered that the experiments are soil-free bioassays.

The effectiveness of the treatment was observed based on the resistance to rot biodegradation without ground contact. The wood exposed to the outdoor testing without any treatment showed less resistance to fungi attack, which was not comparable to the short-term durability in the laboratory testing. With the exposure to ground contact, the deterioration occurred rapidly in the shade (Brischke and Meyer-Veltrup, 2016). The phenolic and acid components available in PA would be able to treat the white-rot fungi but were less inhibitory to the brown-rot fungi. The soaking method is not suitable to prevent fungal attack. Improved methods should be developed to enhance the prevention of OPT from fungal growth during storage and before further process.

Decay Fungi Test

There was a significant difference in the mean weight loss value of the three different OPT test blocks (Figure 3). The untreated OPT sample recorded the highest mean weight loss value of 37.14%, followed by OPT-PA BR (21.68%). The treated OPT-PA sample exposed to white-rot fungi indicated the lowest mean weight loss of 9.33%. Resistance classes are based on the mean weight value according to the standard ASTM D-2017-71

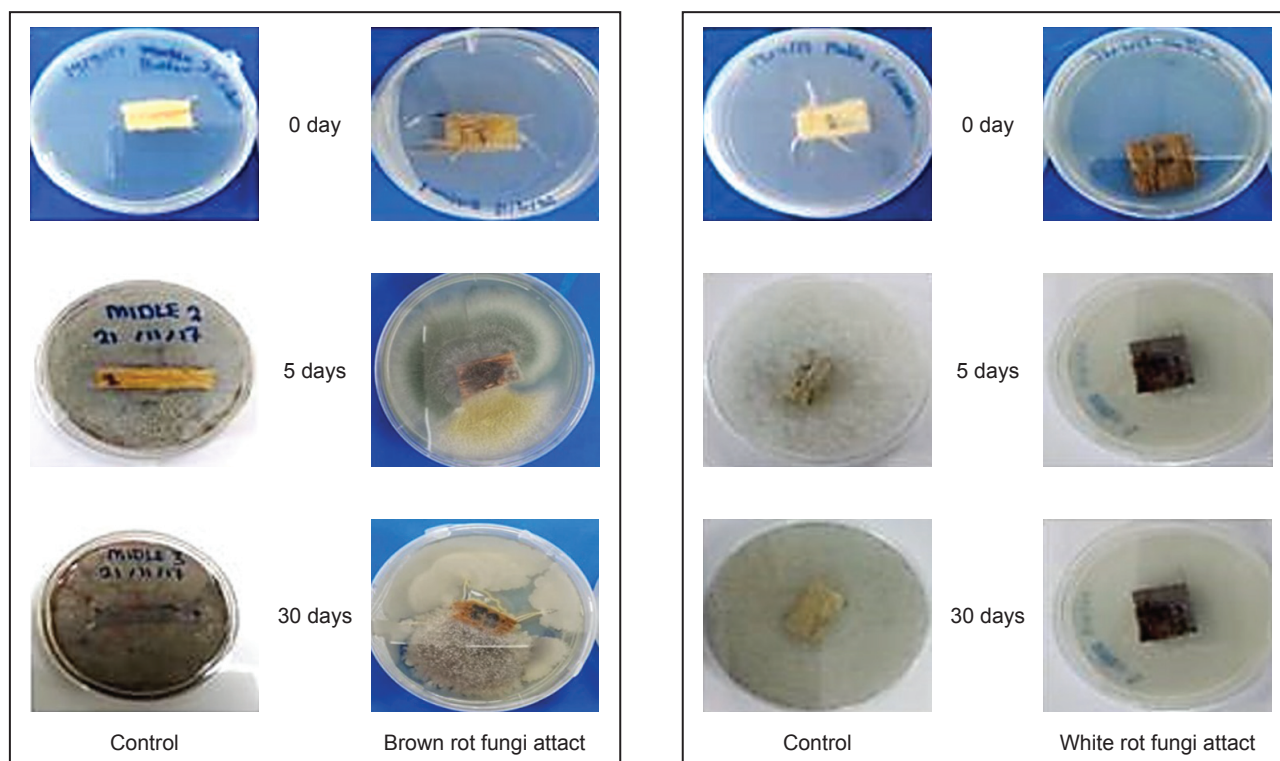


Figure 3. OPT control and OPT-PA exposed to brown and white rot fungi.

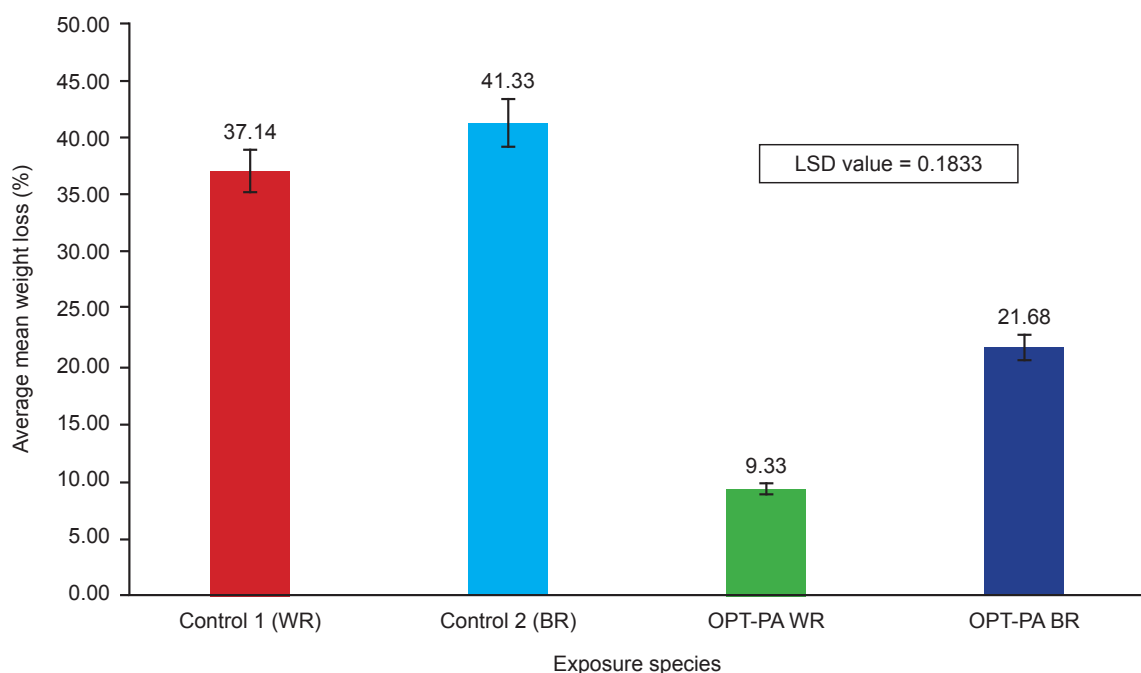


Figure 4. Mean weight loss of untreated and treated OPT-PA against white-rot (WR) and brown-rot (BR) fungi.

(ASTM, 1978). The OPT-PA treated blocks were the most resistant to fungal attack due to the blocks' low mean weight loss value (Table 3). The exposure of the treated OPT to brown-rot fungi was categorised as resistance class only. Meanwhile, the untreated OPT sample indicated the highest mean weight loss and showed moderate resistance against both fungi. The details of resistance classes can be referred to Table 4. The moisture content above 25% and the temperature between 20°C and 35°C are the factors influencing the decay of wood by fungi (Andrade *et al.*, 2012).

TABLE 3. MEAN WEIGHT LOSS AND RESISTANT CLASS OF UNTREATED AND TREATED OPT TEST BLOCKS AGAINST WHITE AND BROWN ROT FUNGI AFTER 8 WEEKS

Sample	Mass loss (%)	Class resistant
Untreated OPT	37.14	Moderate resistant
OPT-PA BR	21.68	Resistant
OPT-PA WR	9.33	Highly resistant

TABLE 4. CLASS OF DECAY-RESISTANT

Class of resistance	Mass loss (%)	Residual loss (%)
Highly resistant (HR)	0-10	90-100
Resistant (R)	11-24	76-89
Moderate Resistant (MR)	25-44	56-75
Not resistant (NR)	>45	<55

Source: ASTM D-2017-71, ASTM (1978); Wong *et al.*, (2012).

In wood delignification, lignin and hemicellulose are most degraded. This can be observed from the mean weight loss value of the untreated and treated OPT samples after 30 days of exposure to both fungi. The results showed that the decay process was inhibited by the fungicidal agent if the wood is not protected well. The hydrolytic activities and enzymes produced by both fungi are vital in the degradation of chemical components of wood (Andrade *et al.*, 2012). The degradation of wood structure, especially lignin and carbohydrate, is due to white-rot fungi, whereas brown-rot fungi only attack carbohydrates (Pandey and Nagveni, 2007). In general, white-rot and brown-rot fungi require 60 and 120-150 days for the degradation process, respectively. The high acid and phenolic compounds in PA act as preservatives, which are suitable for protecting OPT by enhancing durability towards decay, and this is in agreement with Edi Suhaimi *et al.* (2013).

CONCLUSION

A high yield of PA (55%) was successfully produced at 600°C. As the carbonisation temperature increased, the yield of PA decreased due to the production of tar. The results highlighted the potential of PA derived from PKS as an antifungal agent for OPT treatment during storage. The PA showed good antifungal properties, which can be attributed to the presence of phenolic and carboxylic acids, such as acetic acid and propanoic acid, as

well as aldehydes and ketones. The treated OPT-PA samples demonstrated high resistance against the degradation of white-rot fungi even for 30 days of exposure. An improved method to protect OPT and the use of PA as a biopreservative can be effective for OPT treatment during storage.

ACKNOWLEDGEMENT

The authors would like to thank the support from the MPOB for financial support in completing this project.

REFERENCES

- Andrade, F A; Calonego, F W; Severo, E T D and Furtado, E L (2012). Selection of fungi for accelerated decay in stumps of *Eucalyptus* spp. *Bioresour. Technol.*, 110: 456-461. DOI: 10.1016/j.biortech.2012.01.069.
- ASTM (1978). Standard Method of Accelerated Laboratory Test of Natural Decay Resistance of Wood. *American Society for Testing and Material ASTM D2017-71*. Philadelphia. p. 638.
- ASTM (2003). Standard Test Method for Fungicides for Controlling Sapstain and Mold on Unseasonable Lumber (Laboratory Method). *American Society for Testing and Material*. USA. p. 4.
- Brischke, C and Meyer-Veltrup, L (2016). Performance of thermally modified wood during 14 years of outdoor exposure. *Int. Wood Prod. J.*, 7(2): 89-95.
- Demiral, I and Ayan, E A (2011). Pyrolysis of grape bagasse: Effect of pyrolysis conditions on the product yields and characterization of the liquid product. *Bioresour. Technol.*, 102: 3946-3951. DOI: 10.1016/j.biortech.2010.11.077.
- Demirbas, A (2007). The influence of temperature on the yields of compounds existing in bio-oils obtained from biomass samples via pyrolysis. *Fuel Process. Technol.*, 88: 591-597. DOI: 10.1016/j.fuproc.2007.01.010.
- de Souza Araujo, E; Pimenta, A S; Feijo, F M C; Castro, R V O; Fasciotti, M; Monteiro, T V C and de Lima, K M G (2017). Antibacterial and antifungal activities of pyroligneous acid from wood of *Eucalyptus urograndis* and *Mimosa tenuiflora*. *J. Appl. Microbiol.*, 124(1): 85-96. DOI: 10.1111/jam.13626.
- Edi Suhaimi Bakar; Hao, J; Ashaari, Z and Choo, A C Y (2013). Durability of phenolic-resin-treated oil palm wood against subterranean termites a white-rot fungus. *Int. Biodeter. Biodegr.*, 85: 126-130.
- Fačkovcová, Z; Vannini, A; Monaci, F; Grattacaso, M; Paoli, L and Loppi, S (2020). Effects of wood distillate (pyroligneous acid) on sensitive bioindicators (lichen and moss). *Ecotoxicol. Environ. Saf.*, 204: 111117. DOI: 10.1016/j.ecoenv.2020.111117.
- Khoirun Nisa, M; Maizatulkmal, Y; Siti Hajar, M S; Nurul Husna, R; Chai, B M; Nurul Farhana, M; Sulaiman, N; Salmiah, U and Zainul Akmar, Z (2016). Evaluation on efficiency of pyroligneous acid from palm kernel shell as antifungal and solid pineapple biomass as antibacterial and plant growth promoter. *Sains Malays.*, 45(10): 1423-1434.
- Lee, S H; H'ng, P S; Chow, M J; Sajap, A S; Tey, B T; Salmiah, U and Sun, Y L (2011). Effectiveness of pyroligneous acids from vapour released in charcoal industry against biodegradable agent under laboratory condition. *J. Appl. Sci.*, 11(24): 3848-3853.
- Mathew, S and Zakaria, Z A (2015). Pyroligneous acid-the smoky acidic liquid from palm biomass. *Appl. Microbiol. Biotechnol.*, 99: 611-622. DOI: 10.1007/s00253-014-6242-1.
- Mohan, D; Shi, J; Nicholas, D D; Pittman Jr, C U; Steele, P H and Cooper, J E (2008). Fungicidal values of bio-oils and their lignin-rich fractions obtained from wood/bark fast pyrolysis. *Chemosphere*, 71: 456-465.
- MPOB (2020). MPOB Statistic 2020. <https://bepi.mpob.gov.my/index.php/en/area/area-2020/oil-palm-planted-area-as-at-dec-2020>, assessed on 14 September 2021.
- Nahrul Hayawin, Z; Astimar, A A; Juferi, I; Nor Faizah, J; Ropandi, M; Mohamad Faizal, I; Mohd Ali, H; Ezyana, K B; Nur Sulihatimarsyila, A W and Suraini, A A (2018). Carbonisation-activation of oil palm kernel shell to produce activated carbon and methylene blue adsorption kinetics. *J. Oil Palm Res.*, 30(3): 495-502. DOI: 10.21894/jopr.2018.0039.
- Nakai, T; Kartal, S N; Hata, T and Imamura, Y (2007). Chemical characterization of pyrolysis liquids of wood-based composites and evaluation of their bio-efficiency. *Build. Environ.*, 42: 1236-1241. DOI: 10.1016/j.buildenv.2005.11.022.
- Oramahi, H A; Yoshimura, T; Farah Diba; Dina Setyawati and Nurhaida (2018). Antifungal and antitermitic activities of wood vinegar from oil

palm trunk. *J. Wood Sci.*, 64: DOI: 10.1007/s10086-018-1703-2.

Pandey, K K and Nagveni, H C (2007). Rapid characterisation of brown and white rot degraded chir pine and rubberwood by FTIR spectroscopy. *Holz Roh Werkst*, 65(6): 477-481. DOI: 10.1007/s00107-007-0181-9.

Rashidi, N A; Yusup, S; Borhan, A and Loong, L H (2014). Experimental and modelling studies of carbon dioxide adsorption by porous biomass derived activated carbon. *Clean Tech. Environ. Policy*, 16: 1353-1361. DOI: 10.1007/s10098-014-0788-6.

Seri Elyanie Zulkifli; Mohd Amir Asyraf Mohd Hamzah; Maizatulakmal Yahayu; Astimar Abd Aziz; Najihah Mohd Hashim and Zainul Akmar Zakaria (2021). Optimisation of microwave assisted production of acid condensate from palm kernel shell and its biological activities. *Biomass Conv. Bioref.* DOI: 10.1007/s13399-021-01631-6.

Theapparatt, Y; Chandumpai, A and Faroongsarng, D (2018). Physicochemistry and utilization of wood vinegar from carbonization of tropical biomass waste. *Tropical Forests - New Edition* (Sudarshana, P; Nageswara-Rao and Soneji, J R eds.). *IntechOpen*. p. 163-183. DOI: 10.5772/intechopen.77380.

Tsai, W T; Lee, M K and Chang, Y M (2007). Fast pyrolysis of rice husk: Product yields and compositions. *Bioresour. Technol.*, 98: 22-28. DOI: 10.016/j.biortech.2005.12.005.

Wang, J; Minami, E; Asmadi, M and Kawamoto, H (2021). Effect of delignification on thermal degradation reactivities of hemicellulose and cellulose in wood cell walls. *J. Wood Sci.*, 67: 19. DOI: 10.1186/s10086-021-01952-0.

Wei, Q; Ma, X and Dong, J (2010). Preparation, chemical constituents and antimicrobial activity of pyroligneous acids from walnut tree branches. *J. Anal. Appl. Pyrolysis*, 87: 24-28. DOI: 10.1016/j.jaap.2009.09.006.

Wong, A H H; Jem, J M E and Lai, J K (2012). Classifying white rot decay resistance of some hardwoods from Sarawak and Peninsular Malaysia and correlations with their tropical in-ground durability. *The International Research Group on Wood Protection. International Union of Forest Research Organizations All Division 5 Conference*. <http://ir.unimas.my/id/eprint/16023>.

Zheng, H; Sun, C; Hou, X; Wu, M; Yao, Y and Li, F (2018). Pyrolysis of *Arundo donac* L. to produce pyrolytic vinegar and its effect on the growth of dinoflagellate *Karenia brevis*. *Bioresour. Technol.*, 247: 273-281.

EMULSIFYING PROPERTIES AND ANTIOXIDANT ACTIVITY OF PHOSPHOLIPIDS FRACTIONS FROM PALM PRESSED FIBRE

KGS AHMADI^{1*}; TETI ESTIASIH² and AHMAD DIAUL KHULUQ³

ABSTRACT

Palm pressed fibre (PPF) is a by-product of crude palm oil (CPO) extraction and has great potential as a source of phospholipids (PL). PPF was extracted from chloroform:methanol to extract total lipid, and polar lipid was separated using methanol. PL from PPF was fractionated by ethanol, and subsequently, the ethanol-soluble fraction was fractionated by acetone. Four fractions were obtained: ethanol-soluble, ethanol-insoluble, ethanol-acetone soluble, ethanol soluble-acetone insoluble. All fractions had different PL molecular species compositions. Different compositions of PL in the fractions resulted in different hydrophilic-lipophilic balance (HLB) values, emulsifying properties, and antioxidant activity. HLB value of crude PL from PPF was 6.07 that was suitable for water in oil (w/o) emulsion indicated by low emulsifying activity index (EAI) and emulsion stability index (ESI) in oil-water (o/w) emulsion systems. Ethanol-soluble and ethanol-acetone soluble fractions exhibited better emulsifying properties and higher HLB values of around 10. Crude PL and its fractions showed a potential antioxidant activity to prevent bulk soybean oil in a single antioxidant system. Synergism with α -tocopherol appeared in a binary antioxidant system indicated by low peroxide value compared to the single one. PL composition affected the ability of PL fractions as antioxidants.

Keywords: binary antioxidant system, emulsifying properties, fractionation, HLB value, palm pressed fibre.

Received: 6 July 2021; **Accepted:** 17 January 2022; **Published online:** 23 February 2022.

INTRODUCTION

Phospholipids (PL) are a mixture of phosphate-containing lipids mainly used in the food industry as emulsifiers. Commercially, PLs are well known as lecithin, a term that is commonly used for a mixture of amphiphilic compounds that mainly

comprised of PL and other minor constituents of glycolipids, carbohydrates, triglycerides, and a trace amount of water, free fatty acids (FFA), and sterols (Arnold *et al.*, 2014). PL are also used as wetting agents, lubricants, texture modifiers, and dispersants to improve the characteristics of food products such as solubility of instant powder, less stickiness of candy, good rheology of chocolate and spreads, and others. PLs are the cell membrane constituents, and the human body recognises these compounds well, thus, PL is a safe emulsifier.

The primary source of PL is of vegetable origins, mainly oil-bearing seeds (Ahmad and Xu, 2015) such as soybean, sunflower (Cabezas *et al.*, 2012), canola (Xie and Dunford, 2019), rapeseed (Arnold *et al.*, 2014), camelina seed (Belayneh *et al.*, 2018) that are obtained during degumming in oil refining. Vegetable lecithin is a mixture of naturally occurring lipids containing more than 50% PL

¹ Department of Agroindustrial Technology, Tribhuwana Tungadewi University, Jalan Tlagawarna, Tlogomas, Malang, Indonesia.

² Department of Food Science and Technology, Universitas Brawijaya, Jalan Veteran, Malang, Indonesia.

³ Indonesian Sweetener and Fiber Crops Research Institute, Jalan Raya Karangploso, KM 4 Kotak Pos 199, Kepuh Utara, Kepuharjo, Kec. Karang Ploso, Malang, Indonesia.

* Corresponding author e-mail: kgs.ahmadi@yahoo.com

(Robert *et al.*, 2020). The most widely used lecithin in the food industry is soybean lecithin. PL of animal origins are the egg yolk (Bernardo *et al.*, 2019), bovine milk (Nagai, 2012), and fish (Malaplate *et al.*, 2019). Different sources of PL have different characteristics and emulsifying properties.

Some residual PL is also found in the industrial food wastes such as rice bran (Garba *et al.*, 2020; Sun *et al.*, 2020), fish discard (Topuz *et al.*, 2021) and palm pressed fibre (PPF) (Choo *et al.*, 2004). PPF is a by-product of the extraction process of CPO. The fibrous structure of PPF entraps 5%-7% of the residual oil (Ooi *et al.*, 2019) with good oxidative stability that is contributed by PL (Lau *et al.*, 2006). PPF residual oil contains 46 800 ppm of PL. The quantity of PPF PL is considerably high due to palm oil's huge world production (Choo *et al.*, 2004). Thus, the PPF PL utilisation as the source of PL is important as an alternative to conventional PL sources.

PL are amphiphilic in nature because they have a hydrophobic tail consisting of two fatty acid chains at the sn-1 and sn-2 positions of a glycerol moiety and a polar phosphate head group at sn-3 (Robert *et al.*, 2020) that are attached to other hydrophilic molecules such as ethanolamine (PE), choline (PC), inositol (PI, phosphatidylinositol), serine (PS, phosphatidylserine), and glycerol (PG, phosphatidylglycerol). The most common bioactive PL in biological vegetable cells are PC, PE, PI and PS (Robert *et al.*, 2020).

Lecithin is usually modified or fractionated to have tailor-made lecithin that is suitable for the processing and characteristics of food products. The chemical modification of PL is aimed to improve emulsifying properties by means of acid or base-catalysed hydrolysis, acetylation, hydroxylation, and hydrogenation (Li *et al.*, 2019). Modification by enzyme is used to liberate fatty acid from sn-1 or sn-2 of the glycerol backbone of PL, resulting in better emulsifying properties for o/w emulsion (Estiasih *et al.*, 2013). Fractionation is one way to improve the emulsifying properties of PL without modification of their chemical structures. The fractionation of PL is usually conducted by solvent to have different polarity of PL. PL are the main components of native lecithin and have different solubility in acetone or ethanol (Cabezas *et al.*, 2012; Xie and Dunford, 2019). Solvent fractionation is used to obtain PL fractions with different compositions compared to the origin (Cabezas *et al.*, 2012). The PPF PL is still limitedly explored, and its fractionation is important to evaluate as the alternative of commonly modified lecithin.

Lecithin fractionation aims to obtain fractions with desirable functionalities. Ethanol is commonly used as the solvent for fractionation (Xie and Dunford, 2019). PC has better solubility in alcohol than the other PL. The ethanol-soluble fraction

containing a high PC/PE ratio is found in the ethanol-soluble fraction, but the insoluble fraction has a low PC/PE ratio. PI, lysophosphatidylinositol (LPI), and phosphatidic acid (PA) are less soluble in alcohol and enriched in the ethanol-insoluble fraction (Cabezas *et al.*, 2012). PL fractionation using ethanol and acetone as solvents is compatible with the regulations required for subsequent food and pharmaceutical applications (Bernardo *et al.*, 2019).

The main function of PL for food, pharmaceutical, and cosmetic industries is as an emulsifier since PL naturally have amphiphilic properties. Lecithin has been widely applied in both o/w and w/o emulsions (Zembyla *et al.*, 2020) by facilitating small droplet formation (Wu *et al.*, 2019), positioning themselves at the interface to stabilise emulsions (Zembyla *et al.*, 2020), and also reducing the interfacial tension (Wu *et al.*, 2019). The advantage of lecithin over other emulsifiers is the ability to provide a strongly negative surface charge over a wide pH range (Warren *et al.*, 2019). Different PL fractions exhibit different emulsifying properties. The PC-enriched fraction has improved o/w emulsifying properties; meanwhile, the fraction enriched with PI is suitable for w/o emulsions (Xie and Dunford, 2019). It is important to elucidate the suitability of PPF PL and its fractions as an emulsifier for the o/w or w/o system.

PL has been reported to inhibit lipid oxidation in bulk oil and emulsions, but the stabilisation mechanism still remains controversial (Cardenia *et al.*, 2011). PL are sometimes used as antioxidants in foods. Several mechanisms have been proposed for the antioxidant activity of PL. PL are chelators that could bind prooxidative metals, participating in Maillard reaction during lipid oxidation to produce antioxidative compounds, altering the other antioxidants to o/w emulsions' interface, or in a bulk oil regenerating primary antioxidants (Cui and Decker, 2016). One mechanism of the phospholipid-tocopherol synergism is the donation of a hydrogen atom by PL to the tocopheryl radical (Doert *et al.*, 2012). The ternary mixture of PL, tocopherol and ascorbic acid are powerful antioxidants for stabilising highly unsaturated oils (Doert *et al.*, 2017). Antioxidant activity of PPF PL and its fractions and synergism with other antioxidants have not been evaluated yet.

The emulsifying properties and antioxidant activity of PL from PPF and its fractions are interesting to examine since PPF might be an alternative source of PL. Ethanol and acetone fractionation of PL from PPF are supposed to produce different PL molecular species compositions with different emulsifying properties and antioxidant activity. The emulsifying properties of crude PL from PPF and its fractions were evaluated in the o/w emulsion system as a food major emulsion

system. The antioxidant activities of crude PL and PL fractions in a single and synergistic binary system of bulk oil were also evaluated.

MATERIALS AND METHODS

Materials

PPF was kindly obtained from a palm oil milling, Surya Dumai Inc., Riau Province, Indonesia. The variety of oil palm was *tenera*. The PPF was stored at room temperature before being used. The chemicals for PL extraction from PPF, emulsifying properties, and antioxidant activity analysis were analytical grade from Merck (Germany). Commercial soybean oil was used for emulsifying properties and antioxidant activity analysis. The purity of acetone and ethanol for fractionation was 99.7% and 98.0%, respectively. Standards for PL analysis comprised of PE (purity $\geq 98.0\%$ by TLC), PC (purity $\geq 99.0\%$ by TLC), PI (purity $\geq 98.0\%$ by TLC), PA (purity $\geq 98.0\%$), PG (purity $\geq 99.0\%$ by TLC), DPG (purity $\geq 97.0\%$ by TLC), and TLC Silica Gel G60 plates were obtained from Sigma Aldrich (Singapore).

Extraction of Total Lipid from PPF

Yunoki *et al.* (2008) method was used to extract total lipids from PPF (Figure 1). A total of 100 g of fine and dry PPF was extracted twice with

100 mL chloroform:methanol (2:1 v/v), and once with 100 mL chloroform:methanol (1:2 v/v), subsequently. Each extraction was conducted for 1 hr at room temperature, and the solvent and the cake were separated by filtering through coarse filter paper. The solvent of each extract was removed with a rotary evaporator, and all lipid extracts were mixed.

Separation of Crude PL from Extracted Lipid

The Palacios and Wang (2005) method, with a slight modification, was used to separate crude PL from total lipid (Figure 2). Total lipid, 10 g, was dissolved in 40 mL chloroform for 10 min. The mixture was then centrifuged for 10 min at $1542 \times g$ to separate the chloroform-soluble (supernatant) and insoluble fractions (residue or sub-natant). Chloroform from the supernatant was removed by rotary evaporation. The residue after evaporation was then extracted again with 30 mL of chloroform. After centrifugation at $1542 \times g$ for 10 min, the chloroform from the supernatant of this second extraction was removed.

The chloroform-insoluble fraction from the first extraction was mixed with the chloroform-insoluble fraction from the second extraction. After removing the residual chloroform by rotary evaporation, the chloroform-insoluble fractions were extracted by methanol, 20 mL, to obtain methanol-soluble fraction. Methanol was removed from this fraction, and crude PL was obtained.

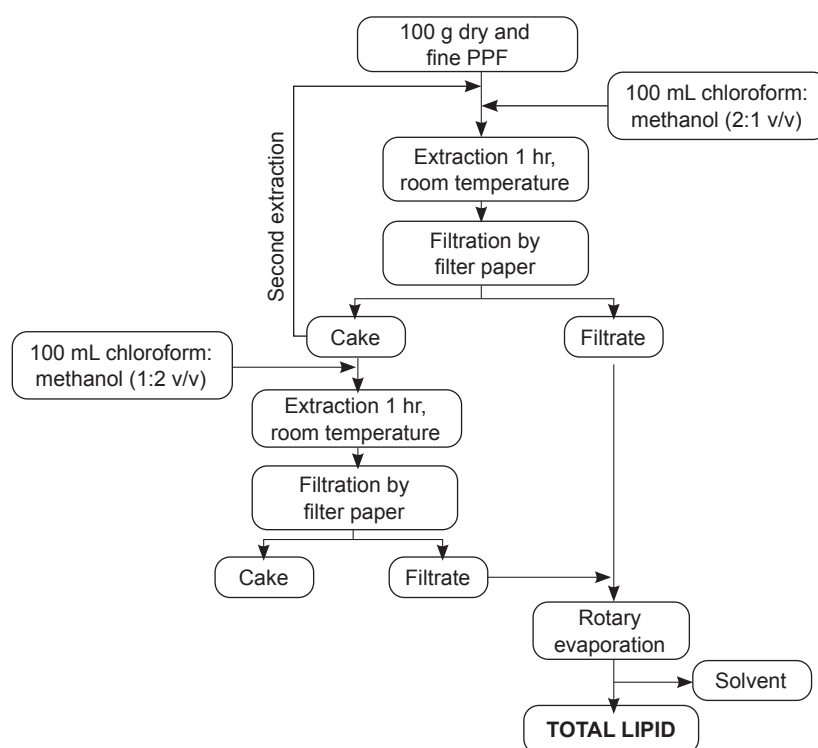


Figure 1. Total lipid extraction from PPF.

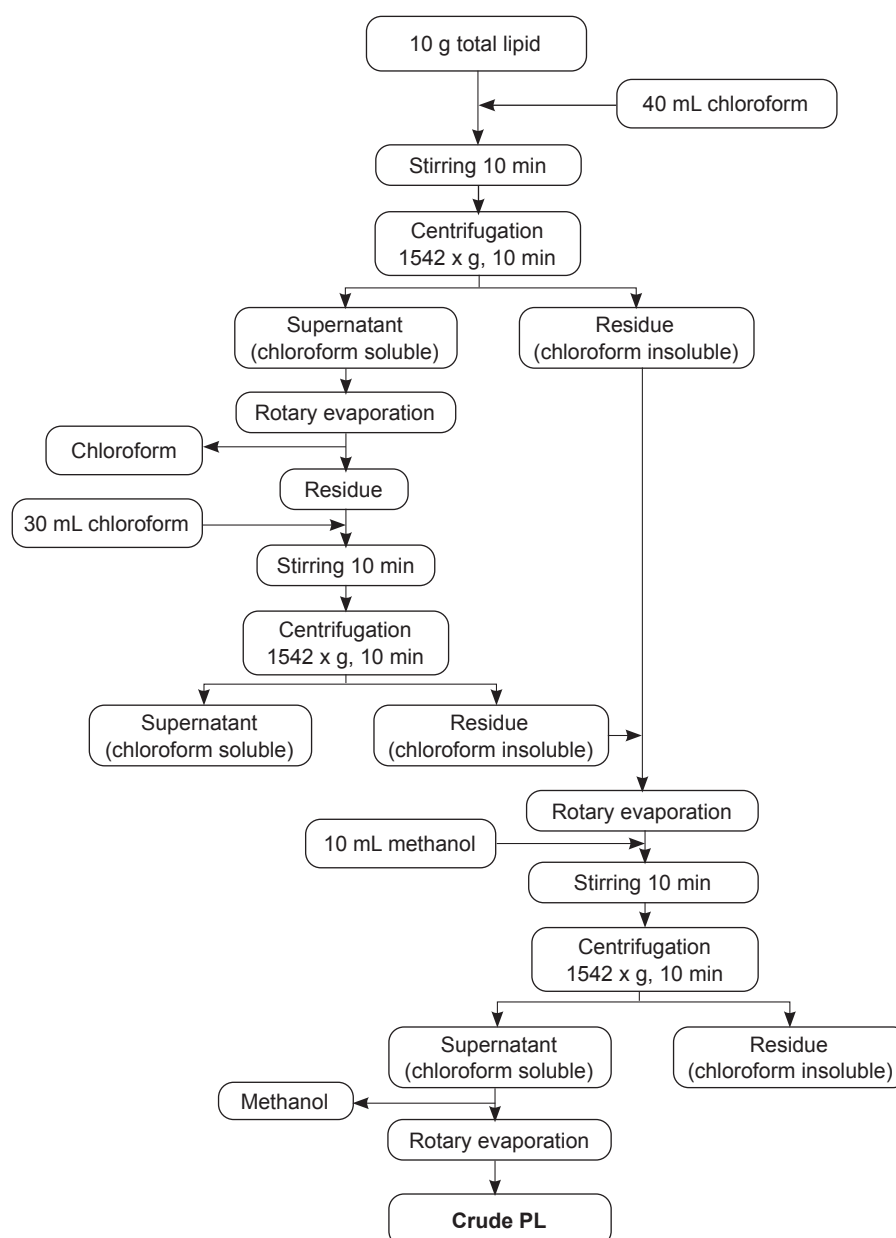


Figure 2. Separation of crude PL from the total lipid of PPF.

PL Fractionation

The fractionation of PL was conducted by using acetone and ethanol at room temperature (Figure 3). Extraction by ethanol was used as the first fractionation. Crude PL, 5 g, was dissolved in 20 mL ethanol and agitated for 60 min by using a magnetic stirrer. The mixture was centrifuged for 10 min at 15 000 $\times$ g. The residue was an ethanol-insoluble fraction, and the supernatant contained an ethanol-soluble fraction. A rotary evaporator removed ethanol from the supernatant and residual ethanol in an ethanol-insoluble fraction to obtain ethanol-soluble and ethanol-insoluble fractions.

Some of the ethanol-soluble fraction was taken and further fractionated by using acetone through dissolving in acetone at a ratio of the ethanol-soluble

fraction to acetone 1:4 (w:v). The mixture was agitated for 60 min by a magnetic stirrer. Acetone soluble fraction and acetone-insoluble fraction were separated by centrifugation at 15 000 $\times$ g for 10 min. A rotary evaporator removed the residual acetone from each fraction. By solvent fractionation, four fractions were obtained: Ethanol-soluble fraction, ethanol-insoluble fraction, ethanol-acetone soluble fraction, and ethanol soluble-acetone insoluble fraction; and crude PL without fractionation as a control.

Analysis of PL Profile

The analysis of the PL profile was according to the method of Nzai and Proctor (1998). This analysis used silica gel G60 plates with a

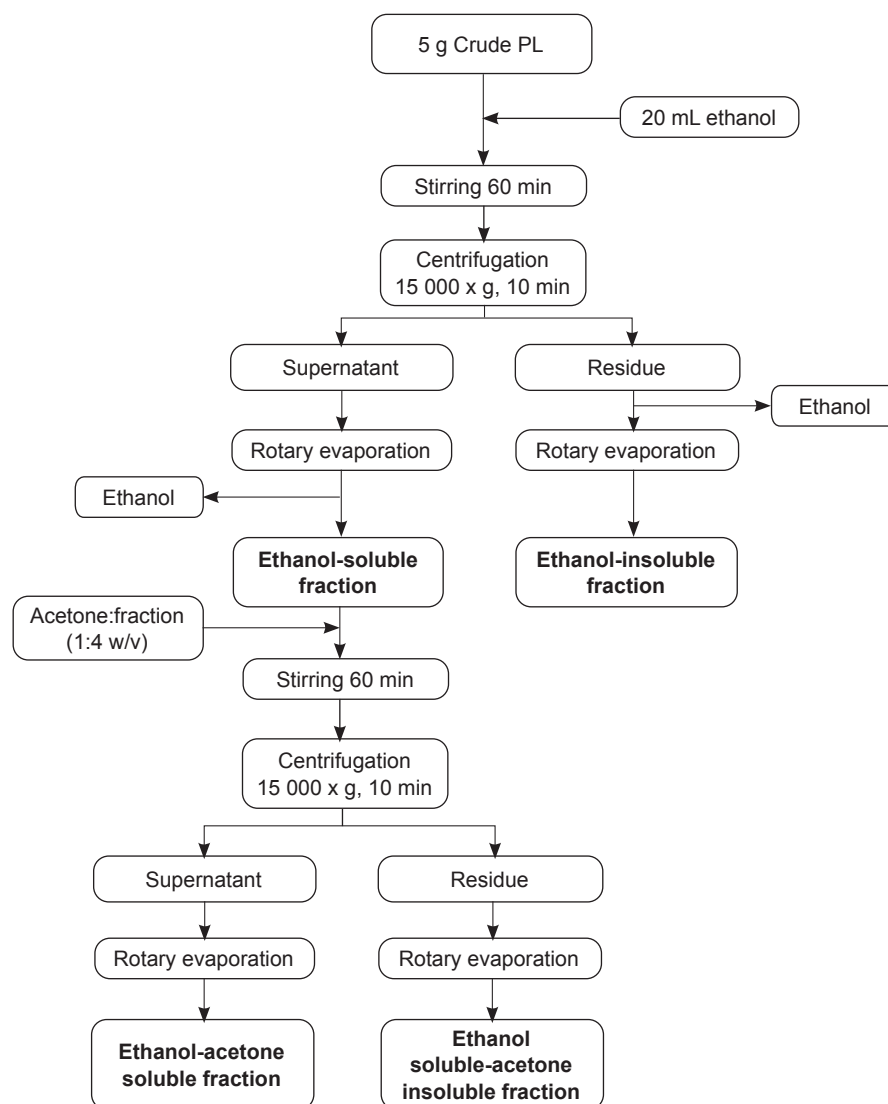


Figure 3. Fractionation of crude PL from PPF.

chloroform:methanol:water (75:25:3 v/v/v) system as a developing solution. Each standard PL, crude PL, and the fractions were dissolved separately in a chloroform:methanol solution (95:5 v/v) at 1 mg mL⁻¹ concentration. After activating the plates by drying for 20 min in an oven at 100°C, the PL standard, crude PL, and the fraction solution in a volume of 10 µL were spotted separately. The plate development was run for 40 min, and then the plates were dried at room temperature for 10 min, and heated in the oven for 10 min at 90°C. TLC scanner (CAMAG TLC Scanner 3) was used for the quantification of the molecular species of PL. PL concentration in the samples was calculated on the basis of area curves of each PL from the densitogram of the TLC scanner.

Analysis of Emulsifying Properties

Hydrophilic lipophilic balance (HLB). The suitability of PL from PPF and its fractions to

stabilise emulsions are measured by HLB value. HLB plays an essential role in balancing the interfacial tension between oil and water in an emulsion. The HLB value permits the ability of an emulsifier to stabilise w/o or o/w emulsions (Alam *et al.*, 2020). HLB value ranges from 0 to 20 and was measured based on saponification number (S) (AOAC, 1997) and acid value (Av) (AOAC, 1980) with the Equation (1) as follows:

$$HLB = 20 \left(1 - \frac{S}{Av} \right) \quad (1)$$

Emulsifying activity index (EAI) and emulsifying stability index (ESI). In this study, the capability of crude PL and its fraction in stabilising o/w emulsions was evaluated by EAI and ESI. The EAI represents the emulsifiers' ability to be absorbed in the interface during the emulsion formation, and a higher EAI represents a stronger emulsification capability (Chen *et al.*, 2020). The emulsifying

activity index represents the surface area covered by the emulsifier. Thus, the higher EAI value indicates the emulsifier's greater ability to produce smaller droplets of the dispersed phase. The turbidity method was used to measure EAI and ESI of crude PL from PPF and its fractions (Pearce and Kinsella, 1978). The emulsion was prepared by mixing 0.5 g of soybean oil with 9.5 mL of distilled water to have an oil fraction of 0.5%. As much as 0.05 g crude PL or its fractions was added to the mixture of oil and water. The mixture was homogenised by agitating with a homogeniser (Ultra Turrax) at a speed of 12 500 rpm for 1 min. EAI and ESI were measured by mixing 0.12 mL of emulsion with phosphate buffer (0.005 M, pH 7) containing sodium dodecyl sulphate (SDS) 0.3% to a volume of 10 mL, to have an oil volume fraction of 6×10^{-5} . When emulsion and phosphate buffer were mixed, the time was t_0 and the absorbance at 500 nm was A_0 . The mixture was allowed to stand for 10 min (t_{10}), and the absorbance at t_{10} was expressed as A_t . The following Equation (2) was used to calculate EAI ($\text{m}^2 \text{g}^{-1}$) and ESI (min):

$$\text{EAI} = \frac{(2.303)(2)(A_0 \times \text{dilution factor})}{C \times \Phi \times 10\,000} \quad (2)$$

A_0 = absorbance at min 0

C = emulsifier concentration (g/mL)

Φ = oil volume fraction

A_t = absorbance at min 10

Δt = 10 min

$$\text{ESI} = A_0 \times \frac{\Delta t}{A_0 - A_t}$$

Antioxidant Activity

The antioxidant activity measurement was based on the ability to inhibit lipid peroxidation of bulk soybean oil. The ability of crude PL and its fraction as a single antioxidant was measured in 5 mL of soybean oil that was added by crude PL or its fractions at concentration of 0.5% or 5000 ppm (w/v). The mixture was incubated at 37°C for five days, and every day a sample was removed to measure the peroxidation value by the ferric thiocyanate method (Hills and Thiel, 1946). For binary antioxidant systems, crude PL and its fraction were added separately to soybean oil. α -tocopherol of 1000 ppm was added to the system and homogenised by agitation with a homogeniser (Ultra Turrax) at the speed of 1500 rpm. The mixture was incubated for five days, and every day, the peroxide value was measured. Commercial antioxidant BHT at a concentration of 100 ppm and α -tocopherol at a 1000 ppm concentration were used as controls.

Statistical Analysis

Correlations between PL molecular species from PPF PL fractions and EAI, ESI, and the mean of peroxide values during five days incubation in single and antioxidant systems were conducted by multivariate Pearson Correlation using IBM SPSS Statistic 24. All the treatments were replicated three times. The data were analysed by one-way analysis of variance using SPSS software, and Duncan Multiple Range Test further analysed the differences among fractions at a significance level of 5%.

RESULTS AND DISCUSSION

PL Composition of Crude PL from PPF and its Fractions

Crude PL and its fractions had a different PL profile (Table 1). Predominant PL in the crude PL from PPF were PC, followed by PG, PE, DPG, PI and PA in decreasing order of concentration. Three species of PL and their concentrations from PPF extracted by hexane were PC>PE>PG, and four species of PL were found in the ethanol extract (PC>PE>PG>PA) (Choo *et al.*, 2004). Meanwhile, Choo *et al.* (2004) reported four species of PL in PPF, which were PG>PC>PE>LPC (lysophosphatidylcholine). The concentration of PL in the ethanol extract was much higher than in hexane extract. This study found more molecular species of PL. Gradual extraction using chloroform:methanol to extract total lipids and separation of polar lipids using methanol from chloroform-insoluble fraction resulted in more PL species. The purity of PL extract from PPF was 61.67%. Choo *et al.* (2004) reported that the concentration of PL in the ethanol extract was 4.68% and in the hexane extract was 0.14%. Chua *et al.* (2009) extracted the PL from PPF by chloroform:methanol 2:1 (v/v), and the concentration of PL in the extract ranged from 1.01% to 5.33%, depending on the ratio of dry PPF to solvent (w/v).

The FFA found in the extract were impurities. FFA were more polar than triglycerides that were soluble in the methanol during PL separation. Less polar PL, such as PG, DPG, PI and PA, were found in the extract. The extraction of total lipid from PPF used the mixture of chloroform and methanol. Polar and nonpolar lipids were extracted in this step. Separation of PL from total lipids was conducted by chloroform that PL was insoluble and more polar residue than chloroform soluble matters. Further extraction by methanol extracted more polar lipid such as PC. However, less polar PL including PE, PG, DPG, PI and PA were also found due to the suitable polarity with methanol.

Fractionation by ethanol resulted in an increased purity of the PL (*Table 1*). PC increased dramatically in the ethanol extract compared to the original crude PL. Lower PI, PE, PG and DPG were found meanwhile, PA concentration was similar to the origin. The concentration of PC increased in the ethanol-soluble fraction. On the other hand, the ethanol-insoluble fraction was enriched with PI and PE (Cabezas *et al.*, 2009). In this study, the purity of the ethanol-insoluble fraction was higher than that of the soluble one. A sharp increase of PE and PI was found in the ethanol-insoluble fraction. This finding is in accordance with the results of the study by Xie and Dunford (2019) that PE, PI and LPI increased in ethanol-insoluble fraction. This finding was in accordance with the Palacios and Wang (2005) report that PC increased after ethanol extraction. In this study, the PL quantification was based on the percentage of each PL peak area curve. The decrease in one species of PL would increase other species of PL. PE, PG and DPG concentration increased in ethanol insoluble fraction, indicating that the ethanol-insoluble fraction contained higher, less polar PL.

Further fractionation of ethanol-soluble fraction by acetone resulted in an acetone soluble fraction that was enriched with PC. Meanwhile, the ethanol soluble-acetone insoluble fraction was rich in PI, PE, PG and DPG. The concentration of more polar PL was found in the ethanol-acetone soluble. In contrast, the concentration of less polar PL was found in the ethanol soluble-acetone insoluble fraction. Acetone and ethanol have different polarities in that ethanol is more polar than acetone. Acetone is usually used to purify PL so that the acetone insoluble fraction matters indicate the PL's purity. Acetone is used to separate nonpolar lipids from the PL. Therefore, the less polar PL species were found in the acetone-insoluble fraction.

Further fractionation of ethanol-soluble fraction by acetone resulted in an acetone soluble fraction that was enriched with PC. Meanwhile, the ethanol soluble-acetone insoluble fraction was rich in PI, PE, PG and DPG. The concentration of more polar PL was found in the ethanol-acetone soluble. In contrast, the concentration of less polar PL was found in the ethanol soluble-acetone insoluble fraction. Acetone and ethanol have different polarities in that ethanol is more polar than acetone. Acetone is usually used to purify PL so that the acetone insoluble fraction matters indicate the PL's purity. Acetone is used to separate nonpolar lipids from the PL. Therefore, the less polar PL species were found in the acetone-insoluble fraction.

Emulsifying Properties of Crude PL from PPF and its Fractions

An emulsion contains two immiscible liquids that are thermodynamically unstable. One way to stabilise emulsions is to reduce interfacial tension in the boundary between the immiscible liquids by using emulsifiers. An emulsifier's effectiveness to stabilise emulsions might be affected by several factors, such as HLB value, concentration, and emulsifier flexibility to reposition at the interface (Feng *et al.*, 2018). The HLB values of crude PL from PPF and its fraction are shown in *Table 2*.

The crude PL from PPF had a low HLB value of 6.07 that might be contributed by PL molecular species composition (*Table 1*). The predominant PL in crude PL from PPF were less polar PL, such as PA, PG, DPG, PI and PE, which caused hydrophobic moieties to dominate, resulting in low HLB value. Some lecithin ingredients are not particularly good at stabilising o/w emulsions when used in isolation because of low or intermediate HLB numbers of 2 to 8 (Mc Clements and Gumus, 2016). Thus, the crude PL from PPF might be suitable for w/o emulsion.

TABLE 1. PL PROFILE (%) CRUDE PL FROM PPF AND ITS FRACTIONS

PL	Crude PL (%)	Fractions (%)			
		Ethanol soluble	Ethanol insoluble	Ethanol-acetone soluble	Ethanol soluble-acetone insoluble
Free fatty acid (FFA)	18.61 ± 0.87c	21.28 ± 0.98b	10.53 ± 0.46d	24.21 ± 1.04a	11.19 ± 0.54d
Phosphatidic acid (PA)	7.02 ± 0.51c	7.44 ± 0.24b	7.36 ± 0.36bc	7.65 ± 0.34ab	8.34 ± 0.56a
Phosphatidylglycerol (PG)	21.24 ± 1.65b	15.79 ± 0.98c	24.67 ± 1.23a	14.46 ± 0.88c	21.72 ± 1.02b
Diphosphatidylglycerol (DPG)	8.88 ± 0.26c	7.92 ± 0.56d	17.38 ± 0.32b	7.72 ± 0.44d	18.18 ± 0.56a
Phosphatidylethanolamine (PE)	13.21 ± 1.01b	3.31 ± 0.23d	13.13 ± 0.89b	7.47 ± 0.78c	16.31 ± 1.24a
Phosphatidylinositol (PI)	8.87 ± 0.47c	3.23 ± 0.28e	21.28 ± 0.89a	3.91 ± 0.13d	16.72 ± 1.12b
Phosphatidylcholine (PC)	22.17 ± 1.09c	41.03 ± 2.34a	3.65 ± 0.23e	34.58 ± 1.78b	7.54 ± 0.65d
Purity (%)	61.67 ± 1.05	65.78 ± 0.78	71.44 ± 0.67	67.06 ± 0.59	68.49 ± 0.98

Note: The data with different notification means different significantly at $\alpha < 0.05$ for the same row.

TABLE 2. EMULSIFYING PROPERTIES AND ANTIOXIDANT ACTIVITY OF CRUDE PL FROM PPF AND ITS FRACTIONS

PL and fraction	HLB value	EAI (m ² g ⁻¹)	ESI (min)	Average PV (meq kg ⁻¹) during 5-day storage	
				Single antioxidant system	Binary antioxidant system
Crude PL	6.07 ± 0.24c	197.80 ± 7.89c	62.51 ± 1.29c	5.20 ± 0.23c	4.18 ± 0.11cd
Ethanol soluble	10.85 ± 0.58a	282.36 ± 2.98b	78.43 ± 2.21b	6.70 ± 0.37a	5.12 ± 0.23b
Ethanol insoluble	5.68 ± 0.16b	196.88 ± 3.45c	52.70 ± 1.87d	5.35 ± 0.52bc	4.33 ± 0.16c
Ethanol-acetone soluble	10.11 ± 0.78a	309.81 ± 9.88a	94.07 ± 3.43a	5.80 ± 0.26b	4.27 ± 0.11c
Ethanol soluble-acetone insoluble	5.54 ± 0.22b	179.49 ± 4.56d	43.89 ± 2.01e	5.87 ± 0.32b	4.05 ± 0.11d
α-tocopherol				6.30 ± 0.66ab	6.55 ± 0.22a
Butylated hydroxytoluene				3.63 ± 0.40d	3.83 ± 0.21e

Note: The data with different notification means different significantly at $\alpha < 0.05$ for the same column.

The fractionation by ethanol increased the HLB value of the ethanol-soluble fraction to 10.85, which is suitable for the o/w emulsion (Alam *et al.*, 2020). Increasing HLB value was related to the dramatic increase in PC, meanwhile less polar PL decreased. Analysis of Pearson correlation showed that PC positively correlated with HLB value. The ethanol-insoluble fraction had a significantly lower HLB value of 5.68 ($\alpha < 0.05$) than the HLB value of the original crude PL from PPF. A sharp increase in concentration was found in PI and DPG.

Meanwhile, other less polar PL increased to some extent. Increasing PI and DPG might lower HLB values, as indicated by a negative Pearson correlation between both with HLB value. The HLB value of the ethanol soluble-acetone insoluble fraction was not significantly different from that of the ethanol-insoluble fraction. The composition of PL in both fractions was similar, which was lacking in PC but rich in the less polar PL. Meanwhile, the ethanol-acetone soluble fraction had an increased HLB value compared to the crude PL from PPF. This HLB value was not significantly different ($\alpha < 0.05$) from that of the ethanol-soluble fraction. Both fractions were rich in PC, and the composition of PL in the fractions was also almost similar. Based on the HLB value, fractionation of crude PL from PPF by ethanol and acetone produced fractions with different HLB values. However, the ethanol-soluble and ethanol-acetone soluble HLB values were not significantly different, and the same characteristics were found for ethanol insoluble and ethanol soluble-acetone insoluble fractions. Therefore, further acetone fractionation after ethanol fractionation was not required because no significant HLB values were produced.

Different fractions of PL from PPF showed different PL compositions that might result in different emulsifying properties. Data in Table 2 show that PL fractions which were able to create the highest specific surface area were found in the

ethanol-acetone soluble fraction. EAI of this fraction was significantly higher than that of the ethanol-soluble fraction, although the HLB values of both fractions were similar. Although the ethanol-acetone soluble fraction had a lower PC concentration (Table 1), it had a higher purity and lower concentrations of less polar PL such as DPG and PG, thus, revealing better emulsifying properties than the ethanol-soluble fraction. PL with a high PC level was reported to produce smaller oil droplets (Cabezas *et al.*, 2012). Analysis of Pearson correlation showed that PC attributed positively with EAI. Other PL had a negative correlation. Meanwhile, the presence of a high amount of PI corresponded to w/o emulsions (Belayneh *et al.*, 2018), which means the lower concentration of PI in ethanol-acetone soluble fraction resulted in better emulsifying properties for o/w emulsion.

The EAI of the ethanol-insoluble fraction was not significantly different from that of crude PL from PPF, although both PL compositions were also different. Crude PL had higher PC than the ethanol-insoluble fraction, but the levels of PI and PG, as less polar PL, were much lower. Thus, the EAI values of both were similar. PI, PG, DPG and PA are less polar PL suitable for w/o emulsions; therefore, the capability to create smaller droplets in o/w emulsion was lower. The ethanol soluble-acetone insoluble fraction showed the lowest EAI and also had the lowest HLB value. This fraction was suitable for w/o emulsion because PG and other less polar PL dominated the PL composition.

The ESI indicates the emulsifier capability to remain at the (o/w) interface in emulsions after a period of storage (Chen *et al.*, 2020). Emulsion stability is related to PL's molecular species composition (McClements and Gumus, 2016). The molecular composition of the PL affects emulsion stability (Guiotto *et al.*, 2013). Data in Table 2 show that the ESI of crude PL and its fraction was in accordance with the EAI. The highest EAI of PL fraction also

revealed the highest ESI. The smaller droplets produced during emulsification that was stabilised by PL fractions resulted in higher emulsion stability. The highest ESI was found in the ethanol-acetone soluble fraction, and the order pattern of ESI of each PL fraction was similar to the EAI. The PL molecular species composition similarly affected ESI and EAI. PL fractions containing a high level of PC produced o/w emulsions with high stability, and PL fractions containing less polar PL resulted in lower o/w emulsion stability. Analysis of Pearson correlation showed that PC had a positive contribution to ESI. Meanwhile, other PL showed a negative correlation.

Antioxidant Activity

Some studies revealed that PL has the ability to prevent oxidation in o/w emulsion (Cardenia *et al.*, 2011) and bulk oil (Cui *et al.*, 2014; Charanyaa *et al.*, 2019). In this study, crude PL capability from PPF and its fractions to inhibit oxidation was evaluated in bulk soybean oil. The primary oxidation products (peroxide value, pv) were monitored during 5 day-storage at 37°C. A commercial synthetic antioxidant butylated hydroxytoluene (BHT) and a natural antioxidant α -tocopherol were used as controls.

In a single antioxidant system, crude PL and its fractions revealed antioxidant activity (Figure 4). Their activity at a concentration of 5000 ppm was comparable to BHT's antioxidant activity at 100 ppm. α -tocopherol at a 1000 ppm concentration failed to retard oxidation indicated by a sharp increase of peroxide value after 4-day storage. The ethanol-insoluble fraction showed the highest antioxidant activity among PL fractions and always had the lowest daily peroxide value. Except for the ethanol-insoluble fraction, crude PL from PPF and its fractions did not show significantly different antioxidant activity. Fractionation by ethanol and

acetone did not produce fractions with better inhibition of peroxidation.

The groups of PE and PA in PL structures significantly contributed to the antioxidant activity, while inositol showed little effect (Doert *et al.*, 2017). The PL fraction with the highest PI (ethanol-insoluble fraction) showed the lowest antioxidant activity in this study. Cardenia *et al.* (2011) reported that PL's antioxidant activity was affected by the type of phosphate head group. PC with oleic or palmitic acids are the most effective antioxidant to inhibit hydroperoxide formation in an emulsion. The PL antioxidant activity is related to their ability to form a layer at the emulsion droplets interface or to chelate metals. A physical barrier to oxygen contributes to PC's antioxidant activity in the w/o emulsion (Choe and Choe, 2016). The different structures of emulsions and bulk oil might result in different mechanisms of PL inhibition to oxidation. In this study, PC and PA showed a positive correlation with average daily peroxide values; other PL were negatively correlated.

Some mechanisms have been proposed for the antioxidant activity of PL. Some PL effectively inhibits lipid oxidation in emulsion due to their capacity to scavenge free radicals (McClements and Gumus, 2016) by hydrogen donation (Choe and Choe, 2016). PL could chelate prooxidative metals, alter other antioxidant locations, regenerate primary antioxidants, and produce antioxidative compounds through Maillard reactions during lipid oxidation (Cui and Decker, 2016). In this study, the proposed mechanism of antioxidant activity of crude PL from PPF and its fractions was scavenging activity because no prooxidant metal and primary antioxidants were added to the bulk oil, and there was no protein or amine as precursors for Maillard reaction in the system.

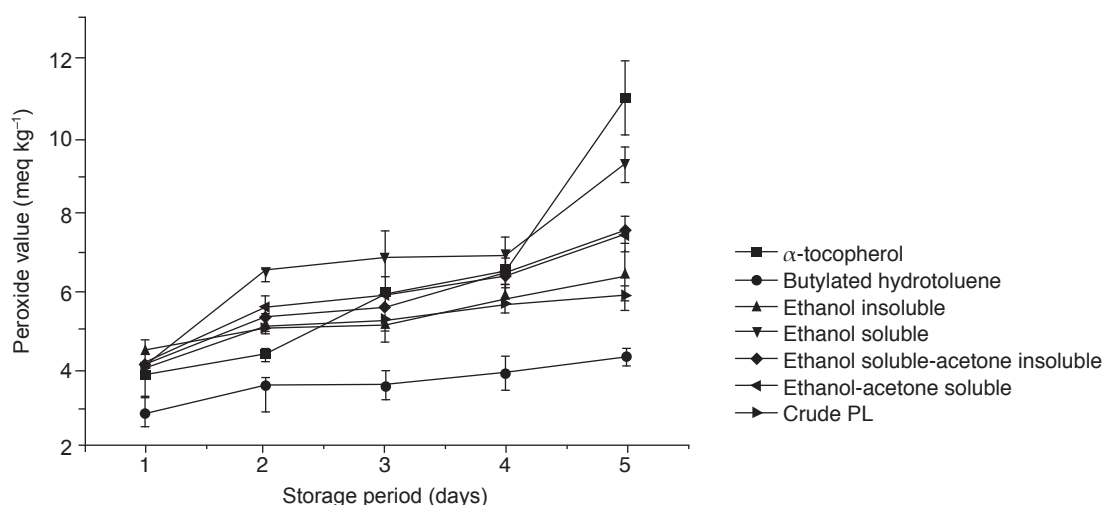


Figure 4. The effect of crude PL from PPF and its fractions in inhibiting peroxidation of bulk soybean oil during 5-day storage in a single antioxidant system. The concentration of crude PL and its fractions was 5000 ppm, BHT was 100 ppm, and α -tocopherol was 1000 ppm.

Crude PL and its fractions were added to soybean oil to evaluate their ability to regenerate the primary antioxidant α -tocopherol. Figure 5 shows that α -tocopherol in the bulk oil-PL system produced lipid oxidation of 4.30% to 35.27% lower than lipid oxidation of a single antioxidant system, depending on the PL fractions. All of the PL fractions and crude PL exhibited synergism with α -tocopherol, which was indicated by lower daily peroxide values of soybean oil added by the binary antioxidant system. The reductions were 4.30% for insoluble ethanol fraction, 35.27% for ethanol-soluble fraction, 26.32% for ethanol-acetone soluble fraction, 30.96% for ethanol soluble-acetone insoluble fraction, and 19.52% for crude PL. The lowest antioxidant activity was found in the binary system stabilised by the ethanol-soluble fraction, and the highest one was produced by the ethanol soluble-acetone insoluble fraction (Table 2). Other PL fractions did not show a significant difference in antioxidant activity.

PL molecular species composition affected the antioxidant activity in the binary system. The ethanol soluble-acetone insoluble fraction had the highest level of PE. PE is a PL species with an amine group that has the ability to donate H^+ to a tocopheryl radical. PL and phenolic antioxidants such as α -tocopherol synergistically inhibit lipid oxidation (Doert *et al.*, 2012). PE and PS can regenerate α -tocopheryl quinone radical to α -tocopherol. As a non-amine PL, PC does not show a synergistic effect on lipid oxidation inhibition (Doert *et al.*, 2017). The ethanol soluble-acetone insoluble fraction had the highest level of PE and showed the highest antioxidant activity. Meanwhile, the ethanol-soluble fraction had the highest PC level and exhibited the lowest antioxidant activity in the binary system compared to other fractions.

All of the PL fractions had similar levels of PA, and presumably, this PL had an equal contribution to the antioxidant activity of each fraction. Phosphoric moiety in PA contributes to the antioxidant activity of PL (Doert *et al.*, 2017). PA acts as an antioxidant synergist and acidity regulator (Silva and Lidon, 2016). PA from PL fractions might also regenerate α -tocopherol, thus having a role in lowering the peroxidation of soybean oil with a binary antioxidant system. Other PL species, PG and DPG, had not been reported for their role as antioxidants. Both had glycerol moiety, which might have no role in regenerating primary antioxidants, such as inositol in PI. PI has been reported to have no capability in regenerating primary antioxidants (Doert *et al.*, 2012).

Process for Scaling Up

Before scaling up, it is very important to consider the feasibility of PL production from PPF and its fractionation. Production of PL from PPL requires some equipment in the pilot plant scale, including a mixing tank to extract the total lipid, separation of PL from total lipid, and fractionation. A pilot-scale centrifuge is required to separate supernatant and sub-natant (residue) in total lipid extraction, PL separation, and fractionation. Evaporation solvent or residual solvent from total lipid, crude PL, and its fractions after centrifugation required a pilot-scale rotary evaporator. Scale-up is very important for commercial production of such products, including PL and its fractions from PPF. Some parameters should be established in scaling up, such as time for extraction, separation, centrifugation, and the speed for stirring and centrifuging. Therefore, further study is required to produce PL from PPF and its fractions on a pilot plant and commercial scale.

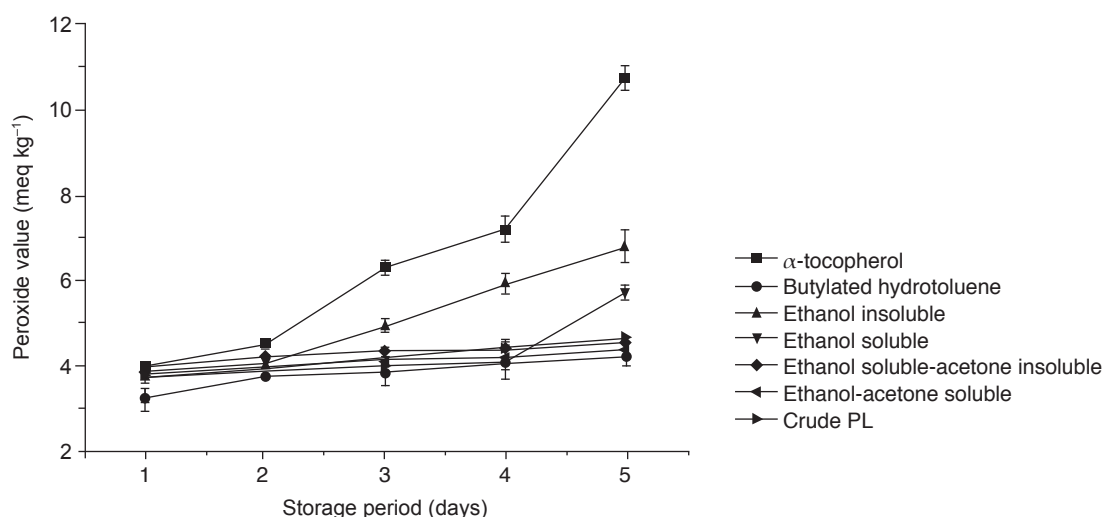


Figure 5. The effect of crude PL from PPF and its fractions (each was 5000 ppm) in inhibiting peroxidation of bulk soybean oil during 5-day storage in binary antioxidant systems with α -tocopherol of 1000 ppm. BHT of 100 ppm was used as control.

CONCLUSION

PPF could be used as the source of PL. Crude PL from PPF and its fractions had different PL profiles, and the predominant PL in crude was PG, followed by PC, PE, DPG, PI and PA. Fractionation by ethanol resulted in the increasing purity of the PL with PC as predominant PL in the ethanol-soluble fraction, and less polar PL was dominant in the ethanol-insoluble fraction. The crude PL from PPF had a low HLB, and fractionation by ethanol produced a soluble fraction with a higher HLB value, but a lower value was found in the insoluble fraction. Further fractionation by acetone did not produce significantly different HLB values. It was found that crude PL from PPF was not suitable to stabilise o/w emulsions. Ethanol and acetone fractionation resulted in better emulsifying properties and emulsion stability. Ethanol soluble fraction from PPF PL was a good candidate as an emulsifier for o/w food emulsion systems.

Meanwhile, crude PPF PL and ethanol insoluble fraction were better for the w/o system. Fractionation by ethanol was sufficient to stabilise o/w emulsion system. Crude PL from PPF and its fractions showed antioxidant activity in the bulk oil system and exhibited synergism with α -tocopherol. Antioxidant activity was affected by PL composition in that the high level of PC had a significant contribution at preventing lipid oxidation in a single antioxidant system. PPF PL was better to use as an antioxidant in binary rather than single systems. Fractionation by ethanol followed by acetone was required to have better antioxidant activity.

ACKNOWLEDGEMENT

The authors would like to thank the Ministry of Research and Technology, the Republic of Indonesia for funding this research through *Insentif Riset Dasar*.

REFERENCES

- Ahmad, M U and Xu, X (2015). *Polar Lipids: Biology, Chemistry and Technology*. AOCS Press, Urbana. 568 pp.
- Alam, S; Algahtani, M S; Ahmad, M Z and Ahmad, J (2020). Investigation utilizing the HLB concept for the development of moisturizing cream and lotion: *In-vitro* characterization and stability evaluation. *Cosmetics*, 7(2): 1-12. DOI: 10.3390/cosmetics7020043.
- AOAC (1980). Analytical Chemistry. *Official Methods of Analysis*. 16th edition. Association of Official Analytical Chemist (AOAC). Washington DC, USA.
- AOAC (1997). Official Method 920.160. Saponification Number of Oils and Fats. *Official Methods of Analysis*. Rockville, USA.
- Arnold, G; Schade, E; Schneider, Y; Friedrichs, J; Babick, F; Werner, C and Rohm, H (2014). Influence of individual phospholipids on the physical properties of oil-based suspensions. *J. Amer. Oil Chem. Soc.*, 91(1): 71-77. DOI: 10.1007/s11746-013-2361-6.
- Belayneh, H D; Wehling, R L; Cahoon, E and Ciftci, O N (2018). Lipid composition and emulsifying properties of *Camelina sativa* seed lecithin. *Food Chem.*, 242(7): 139-146. DOI: 10.1016/j.foodchem.2017.08.082.
- Bernardo, J; Videira, R A; Valentão, P; Veiga, F and Andrade, P B (2019). Extraction of phospholipid-rich fractions from egg yolk and development of liposomes entrapping a dietary polyphenol with neuroactive potential. *Food Chem. Toxicol.*, 133(8): 110749. DOI: 10.1016/j.fct.2019.110749.
- Cabezas, D M; Diehl, B W K and Tomás, M C (2009). Sunflower lecithin: Application of a fractionation process with absolute ethanol. *J. Amer. Oil Chem. Soc.*, 86(2): 189-196. DOI: 10.1007/s11746-008-1336-5.
- Cabezas, D M; Madoery, R; Diehl, B W K and Tomás, M C (2012). Emulsifying properties of different modified sunflower lecithins. *J. Amer. Oil Chem. Soc.*, 89(2): 355-361. DOI: 10.1007/s11746-011-1915-8.
- Cardenia, V; Waraho, T; Rodriguez-Estrada, M T; Julian McClements, D and Decker, E A (2011). Antioxidant and prooxidant activity behavior of phospholipids in stripped soybean oil-in-water emulsions. *J. Amer. Oil Chem. Soc.*, 88(9): 1409-1416. DOI: 10.1007/s11746-011-1807-y.
- Charanyaa, S; Belur, P D and Regupathi, I (2019). Effect of intrinsic and extrinsic factors on the storage stability of sardine oil. *Curr. Res. Nutr. Food Sci.*, 7(3): 749-760. DOI: 10.12944/CRNFSJ.7.3.14.
- Chen, J; Zhang, X; Xue, S and Xu, X (2020). Effects of ultrasound frequency mode on myofibrillar protein structure and emulsifying properties. *Int. J. Biol. Macromol.*, 163: 1768-1779. DOI: 10.1016/j.ijbiomac.2020.09.114.
- Choe, J and Choe, E (2016). Effect of soy-derived phospholipid on the autoxidation of canola oil in a water/oil emulsion. *J. Amer. Oil Chem. Soc.*, 93(8): 1085-1094. DOI: 10.1007/s11746-016-2855-0.
- Choo, Y M; Bong, S C; Ma, A N and Chuah, C H (2004). Phospholipids from palm-pressed fiber.

- J. Amer. Oil Chem. Soc.*, 81(5): 471-475. DOI: 10.1007/s11746-004-0925-4.
- Chua, S C; Tan, C P; Mirhosseini, H; Lai, O M; Long, K and Baharin, B S (2009). Optimization of ultrasound extraction condition of phospholipids from palm-pressed fiber. *J. Food Eng.*, 92(4): 403-409. DOI:10.1016/j.jfoodeng.2008.12.013.
- Cui, L and Decker, E A (2016). Phospholipids in foods: Prooxidants or antioxidants? *J. Sci. Food Agric.*, 96(1): 18-31. DOI: 10.1002/jsfa.7320.
- Cui, L; Kittipongpittaya, K; McClements, D J and Decker, E A (2014). Impact of phosphoethanolamine reverse micelles on lipid oxidation in bulk oils. *J. Amer. Oil Chem. Soc.*, 91(11): 1931-1937. DOI: 10.1007/s11746-014-2544-9.
- Doert, M; Jaworska, K; Moersel, J T and Kroh, L W (2012). Synergistic effect of lecithins for tocopherols: Lecithin-based regeneration of α -tocopherol. *Eur. Food Res. Technol.*, 235(5): 915-928. DOI: 10.1007/s00217-012-1815-7.
- Doert, M; Krüger, S; Morlock, G E and Kroh, L W (2017). Synergistic effect of lecithins for tocopherols: Formation and antioxidant effect of the phosphatidylethanolamine-l-ascorbic acid condensate. *Eur. Food Res. Technol.*, 243(4): 583-596. DOI: 10.1007/s00217-016-2768-z.
- Estiasih, T; Ahmadi, K; Ginting, E and Priyanto, A D (2013). Modification of soy crude lecithin by partial enzymatic hydrolysis using phospholipase A1. *Int. Food Res. J.*, 20(2): 843-849.
- Feng, J; Chen, Q; Wu, X; Jafari, S M and McClements, D J (2018). Formulation of oil-in-water emulsions for pesticide applications: impact of surfactant type and concentration on physical stability. *Environ. Sci. Pollut. Res.*, 25(22): 21742-21751. DOI: 10.1007/s11356-018-2183-z.
- Garba, U; Singanusong, R; Jiamyangyuen, S and Thongsook, T (2020). Extracting lecithin from water degumming by-products of rice bran oil and its physicochemical, antioxidant and emulsifying properties. *Food Biosci.*, 38(9): 100745. DOI: 10.1016/j.fbio.2020.100745.
- Guiotto, E N; Cabezas, D M; Diehl, B W K and Tomás, M C (2013). Characterization and emulsifying properties of different sunflower phosphatidylcholine enriched fractions. *Eur. J. Lipid Sci. Technol.*, 115(8): 865-873. DOI: 10.1002/ejlt.201200394.
- Hills, G L and Thiel, C C (1946). The ferric thiocyanate method of estimating peroxide in the fat of butter, milk and dried milk. *J. Dairy Res.*, 14(340-353). DOI: 10.1017/s0022029900004799.
- Lau, H L N; Choo, Y M; Ma, A N and Chuah, C H (2006). Quality of residual oil from palm-pressed mesocarp fiber (*Elaeis guineensis*) using supercritical CO₂ with and without ethanol. *J. Amer. Oil Chem. Soc.*, 83(10): 893-898. DOI: 10.1007/s11746-006-5043-9.
- Li, J; He, Y; Anankanbil, S and Guo, Z (2019). *Phospholipid-based surfactants in Biobased Surfactants: Synthesis, Properties and Applications* (Hayes, D G; Solaiman, D K Y and Ashby, R D eds.). Elsevier Inc, New York. p. 243-286.
- Malaplate, C; Poerio, A; Huguet, M; Soligot, C; Passeri, E; Kahn, C J F; Linder, M; Arab-Tehrany, E and Yen, F T (2019). Neurotrophic effect of fish-lecithin based nanoliposomes on cortical neurons. *Mar. Drugs*, 17(7): 1-13. DOI: 10.3390/md17070406.
- McClements, D J and Gumus, C E (2016). Natural emulsifiers - Biosurfactants, phospholipids, biopolymers and colloidal particles: Molecular and physicochemical basis of functional performance. *Adv. Colloid Interface Sci.*, 234: 3-26. DOI: 10.1016/j.cis.2016.03.002.
- Nagai, K (2012). Bovine milk phospholipid fraction protects Neuro2a cells from endoplasmic reticulum stress via PKC activation and autophagy. *J. Biosci. Bioeng.*, 114(4): 466-471. DOI: 10.1016/j.jbiosc.2012.05.009.
- Nzai, J M and Proctor, A (1998). Phospholipids determination in vegetable oil by thin-layer chromatography and imaging densitometry. *Food Chem.*, 63(4): 571-576. DOI: 10.1016/s0308-8146(98)00043-0.
- Ooi, J; Promentilla, M A B; Tan, R R; Ng, D K S and Chemmangattuvalappil, N G (2019). Alternative solvent design for oil extraction from palm pressed fibre via computer-aided molecular design. *Technologies for the Oil Palm Industry* (Foo, C Y D and Tun Abdul Aziz eds.). Green Springer, Singapore. p. 33-55.
- Palacios, L E and Wang, T (2005). Extraction of egg-yolk lecithin. *J. Amer. Oil Chem. Soc.*, 11: 565-569. DOI: 10.1007/s11746-005-1110-5.
- Pearce, K N and Kinsella, J E (1978). Emulsifying properties of proteins: Evaluation of a turbidimetric technique. *J. Agric. Food Chem.*, 26(3): 716-723.
- Robert, C; Couëdelo, L; Vaysse, C and Michalski,

- M C (2020). Vegetable lecithins: A review of their compositional diversity, impact on lipid metabolism and potential in cardiometabolic disease prevention. *Biochimie*, 169: 121-132. DOI: 10.1016/j.biochi.2019.11.017.
- Silva, M and Lidon, F (2016). An overview on applications and side effects of antioxidant food additives. *Emir. J. Food Agric.*, 28(12): 823. DOI: 10.9755/ejfa.2016-07-806.
- Sun, X; Zhang, L; Tian, S; Yang, K and Xie, J (2020). Phospholipid composition and emulsifying properties of rice bran lecithin from enzymatic degumming. *LWT - Food Sci. Technol.*, 117(8): 108588. DOI: 10.1016/j.lwt.2019.108588.
- Topuz, O K; Aygün, T; Alp, A C; Yatmaz, H A; Torun, M and Yerlikaya, P (2021). Characterization and emulsifying properties of aquatic lecithins isolated from processing discard of rainbow trout fish and its eggs. *Food Chem.*, 339(9): 128103. DOI: 10.1016/j.foodchem.2020.12810.
- Warren, P B; Shin, S and Stone, H A (2019). Diffusiophoresis in ionic surfactants: Effect of micelle formation. *Soft Matter*, 15(2): 278-288. DOI: 10.1039/c8sm01472h.
- Wu, D; Lu, J; Zhong, S; Schwarz, P; Chen, B and Rao, J (2019). Effect of chitosan coatings on physical stability, antifungal and mycotoxin inhibitory activities of lecithin stabilized cinnamon oil-in-water emulsions. *LWT - Food Sci. Technol.*, 106(1): 98-104. DOI: 10.1016/j.lwt.2019.02.029.
- Xie, M and Dunford, N T (2019). Fractionating of canola lecithin from acid degumming and its effect. *Food Chem.*, 300(6): 125217. DOI: 10.1016/j.foodchem.2019.125217.
- Yunoki, K; Kukino, O; Nadachi, Y; Fujino, T and Ohnishi, M (2008). Separation and determination of functional complex lipids from chicken skin. *J. Amer. Oil Chem. Soc.*, 85(5): 427-433. DOI: 10.1007/s11746-008-1228-8.
- Zembyla, M; Murray, B S and Sarkar, A (2020). Water-in-oil emulsions stabilized by surfactants, biopolymers and/or particles: A review. *Trends Food Sci. Technol.*, 104(7): 49-59. DOI: 10.1016/j.tifs.2020.07.028.

PROPERTIES OF SOLID POLYURETHANES MADE FROM PALM OLEIN-BASED POLYOLS PREPARED WITH VARIOUS TYPES OF NUCLEOPHILES

TUAN NOOR MAZNEE TUAN ISMAIL^{1*}; NOR AZOWA IBRAHIM²; KOSHEELA DEVI POO PALAM¹;
HOONG SENG SOI¹; MOHD AZMIL MOHD NOOR¹; YEONG SHOOT KIAN¹; EMILIA ABD. MALEK²;
IBRAHIM SENDIJAREVIC³ and VAHID SENDIJAREVIC³

ABSTRACT

Understanding on structure-property relationship between raw materials and resultant polyurethane (PU) products is very crucial in order to develop a product that is commercially feasible. This study is aimed to evaluate structure-property relationship of palm olein-based polyols in solid polyurethanes (SPUs). SPUs were prepared by reacting palm olein-based polyols (POoP) and 1,4-butanediol (BDO) with 4,4'-methylenebis(phenyl isocyanate) at a mole ratio of 1:1:2, respectively. Mechanical and thermal properties, phase interaction, crystallinity and solvent resistance of SPUs were evaluated. Mechanical properties and solvent resistance of SPUs were greatly influenced by hard segment concentration and crosslink density as witnessed in SPUs based on palm olein polyols made from water and methanol as nucleophiles. All SPUs are highly crystalline polymer (wide angle X-ray diffraction), thermally stable [thermogravimetric analysis (TGA)], exhibited phase-mixed morphologies between hard and soft segments [dynamic mechanical analyser (DMA)] and dimensionally stable in most solvents tested. In general, SPU made from POoP H (epoxide group of epoxidised palm olein ring-opened with water) offers high hardness and SPU made from POoP EG (epoxide group of epoxidised palm olein ring-opened with 1,2-ethanediol) offers good elongation and tear strength, exhibiting the most promising and economically feasible SPUs, depending on their target applications.

Keywords: mechanical and thermal properties, palm olein-based polyols, solid polyurethanes.

Received: 20 October 2021; **Accepted:** 31 January 2022; **Published online:** 2 March 2022.

INTRODUCTION

Vegetable oils have attracted significant attentions as one of the most important platform chemicals for

chemical industry due to their availability, relatively low cost and environmental sustainability (Caillol *et al.*, 2012; Chen *et al.*, 2014; Lligadas *et al.*, 2010). Vegetable oils, mainly triglycerides constituted by glycerol and three fatty acid chains, commonly contain unsaturated carbon-carbon double bonds ($-C=C-$) or alkene groups that are available for further modification to form more reactive functional groups (Chen *et al.*, 2014; Omonov *et al.*, 2016). Many modifications of vegetable oils have been described including epoxidation, carbonation, esterification and/or transesterification, ozonolysis, *etc.* (Armylisas *et al.*, 2017; Omonov *et al.*, 2016). There are several approaches available for epoxidation of

¹ Malaysian Palm Oil Board,
6 Persiaran Institusi, Bandar Baru Bangi,
43000 Kajang, Selangor, Malaysia.

² Department of Chemistry, Faculty of Science,
Universiti Putra Malaysia,
43400 Serdang, Selangor, Malaysia.

³ Troy Polymers Inc., 900 E. Mandoline Avenue,
Madison Heights, Michigan 48071, USA.

* Corresponding author e-mail: maznee@mpob.gov.my

the alkene to obtain the epoxide, however, the most widely used method is the classical epoxidation reaction proposed by Prileschajew (Omonov *et al.*, 2016). This reaction has two stages, whereby in the first stage, peroxy acid is generated *in-situ* using hydrogen peroxide and carboxylic acid, and in the second stage, the peroxy acid reacts with alkene to form epoxide. Indeed, epoxidation of vegetable oils is a matured industrial process, well controlled and less expensive (Caillol *et al.*, 2012; Omonov *et al.*, 2016).

The structure of vegetable oil with respect to type, composition and distribution of fatty acids in the triglycerides molecule is complex. The fatty acid profile of vegetable oil is a unique characteristic. Variability in the composition is expected even in the same type of oil, depending on the type of soil, plant and local weather conditions (Lligadas *et al.*, 2010; Zlatanić *et al.*, 2004). Generally, different vegetable oils have different degree of unsaturation, vegetable oils with high degree of unsaturation will have high iodine number, *e.g.*, soybean has iodine number of 126.6; canola, 114.1; linseed, 182.0; sunflower, 133.3; corn, 125.5 (Zlatanić *et al.*, 2004) and palm olein, 56.4 g I₂ 100 g⁻¹ (Calliau *et al.*, 2007). Therefore, it is expected that polyols prepared with vegetable oils may vary in hydroxyl numbers, viscosities, hydroxyl functionalities and thermal properties, depending on the degree of unsaturation present in the oil (Lligadas *et al.*, 2010; Zlatanić *et al.*, 2004).

Vegetable oil-based polyols could be considered a new class of polyol in polyurethane (PU). The use of vegetable oils such as palm, soybean and canola in the development of polyols has received substantial interest due to the environmental concerns such as petrochemical-based manufacturing activities which promote air pollution, global climate change and oil spills (Lligadas *et al.*, 2010; Petrović, 2008). PUs prepared with vegetable oil-based polyols exhibit a number of excellent properties which are attributable to hydrophobicity of vegetable oil moiety (Lligadas *et al.*, 2010) contributed by the presence of long hydrocarbon chains in the triglycerides backbone.

Palm oil, being the most abundant vegetable oil in the world, offers a good source of raw material for preparation of vegetable oil-based polyols. In 2020, about 5.87 million hectares of oil palm was planted in Malaysia, which produced about 19.14 million tonnes of crude palm oil (Parveez *et al.*, 2021). The development of palm oil-based polyols in Malaysia was first initiated by the Palm Oil Research Institute of Malaysia (PORIM) [now known as Malaysian Palm Oil Board (MPOB)] in early 1990s, whereby epoxidation and alcoholysis are the two main chemical reactions used to produce polyols.

Syntheses of palm olein-based polyols (POoP), in which the epoxide groups of epoxidised palm olein (EPOo) were ring-opened with water, methanol

and diols (C2 to C6) were successfully carried out in the presence of borontrifluoride in diethylether catalyst (Tuan Noor Maznee *et al.*, 2018a). It was found that the POoP had certain degree of oligomers, indicating occurrence of oligomerisation during ring-opening reaction. Epoxide group of EPOo ring-opened with water (H), methanol (M) and 1,2-propanediol (PG) resulted in POoP with 100% secondary hydroxyls, while, epoxide group ring-opened with 1,2-ethanediol (EG), 1,3-propanediol (PDO), 1,4-butanediol (BDO), 1,5-pentanediol (PTDO) and 1,6-hexanediol (HDO) resulted in POoP with a mixture of primary (26%-36%) and secondary hydroxyls (64%-74%). A similar observation was also reported for polyols made from fatty acid methyl ester or winter grade biodiesel as a feedstock (Tuan Noor Maznee *et al.*, 2018b).

Depending on their feedstock, palm oil-based polyols with variable functionalities, hydroxyl numbers and viscosities could be produced. Palm oil-based polyols have been evaluated for rigid PU foams (Tanaka *et al.*, 2008; Tuan Noor Maznee *et al.*, 2001). In flexible PU foam, literature reported that drop-in replacement of petrochemical-based polyol with palm oil-based polyols at certain percentage had resulted in more uniform cell size structure and improved physical properties of PU foam (Srihanum *et al.*, 2017). Additionally, POoP and fatty acid methyl ester-based polyol (PolyFAME) could be used to make soft PU elastomers with adhesion properties (Mohd Norhisham *et al.*, 2017).

Morphology, mechanical and thermal properties of PUs are greatly influenced by molecular weight of soft segment (SS) (Brokenbrow *et al.*, 1971; Costa *et al.*, 2014), chemical nature of repeating unit of SS (Drobny, 2007; Xu *et al.*, 2015), hard segment (HS) and SS concentrations (Costa *et al.*, 2014; Drobny, 2007) and incorporation of fillers (Cruz and Viana, 2015; Drobny, 2007). Most of the studies on the above-mentioned factors were conducted on solid polyurethanes (SPUs) because of their well-defined chemical structure (Hepburn, 1992), which effectively and in most cases, directly illustrate the structure-property relationship. SPUs are segmented linear block copolymers formed by alternating hard and soft segments. The HS is composed of urethane groups and chain extender(s), displaying strong hydrogen bonding between urethane groups, which form physical cross-linked networks, while the SS consists of a flexible polyol chain (Costa *et al.*, 2014; Cruz and Viana, 2015).

Despite all the palm-based PU products developed so far, there is a need for a comprehensive study on properties of SPUs made from POoP in order to understand better the structure-property relationship of palm olein-based PU products. Therefore, in this study, SPUs were prepared with various types of POoP (epoxide groups were ring-opened with various types of nucleophiles),

and their effect on the properties of SPUs were evaluated. This understanding will help to expedite the development of palm-based PU products, which have commercial viability.

MATERIALS AND METHODS

Materials

Syntheses and characterisations of palm olein-based polyols *i.e.*, POoP H, POoP M, POoP EG, POoP PG, POoP PDO, POoP BDO, POoP PTDO and POoP HDO have been described in details by Tuan Noor Maznee *et al.* (2018a). POoP H, POoP M, POoP EG, POoP PG, POoP PDO, POoP BDO, POoP PTDO and POoP HDO were made from epoxidised palm olein whereby the epoxide groups were ring-opened with H, M, EG, PG, PDO, BDO, PTDO and HDO, respectively. Properties of POoP are shown in Table 1. The same batches of polyols were used to make SPUs. In preparation of SPUs, 4,4'-methylenebis(phenyl isocyanate) (MDI, Mondur M from Covestro Baytown, Texas, USA) was used as an isocyanate, while BDO of 99% purity from Alfa Aesar (Ward Hill, Massachusetts, USA) was used as chain extender. For solvent resistance test of SPUs, deionised water, mineral oil (ultra grade 20, BOC Edwards Vacuum Technology, Mississauga, Canada), toluene of 99.9% purity, methyl ethyl ketone (MEK) of 99.5% purity, sodium hydroxide of 0.1 N (Merck, Darmstadt, Germany) and hydrochloric acid of 0.1 N (Fisher Chemical, Loughborough, United Kingdom) were used. All materials were used as received, unless otherwise stated.

Methods

Preparation of solid polyurethanes (SPUs). SPUs were prepared via one-shot method by reacting a mixture composed of POoP and BDO with MDI at a mole ratio of 1:1:2, respectively and an isocyanate index of 1.02. Sheet and button shaped samples of SPUs were prepared for evaluation of their mechanical properties. POoP and BDO were preheated at 80°C to 120°C prior to blending. The POoP and BDO were weighed into the speed mixer cup of 100 ml and were mixed for 60 s at 2200 rpm using a multiaxial mixer. It was subsequently placed in an air-circulating oven at 80°C to 120°C for at least 15 min. A melted MDI sample was weighed using a syringe and conditioned in an air-circulating oven at 70°C for at least 15 min. All components were mixed using a multiaxial mixer at 2200 rpm for 20 s. At gel time, the mixture was immediately poured into an aluminium mould covered with Teflon sheets (both top and bottom). Both Teflon sheets and metal moulds were preheated at 120°C in an air-circulating oven. Then, it was immediately transferred into a hot press, where it was pressed for 2 hr. Finally, the prepared SPU was post-cured in an oven for 20 hr at 100°C. The prepared SPUs were then left for seven days at room temperature prior to testing. For designation of the SPUs, S-H, S-M, S-EG, S-PG, S-PDO, S-BDO, S-PTDO and S-HDO were made from POoP H, POoP M, POoP EG, POoP PG, POoP PDO, POoP BDO, POoP PTDO and POoP HDO, respectively.

Characterisation of solid polyurethanes (SPUs). Thermal properties of SPUs were determined using differential scanning calorimetry (DSC)

TABLE 1. PROPERTIES OF PALM OLEIN-BASED POLYOLS

Sample designations	Carbon number of nucleophile	Properties								
		Hydroxyl number (mg KOH g ⁻¹)	Primary hydroxyl content (%)	Secondary hydroxyl content (%)	Functionality**	Acid number (mg KOH g ⁻¹)	Oxirane oxygen content (%)	Viscosity, 25°C (mPa.s)	Iodine number (g I ₂ 100 g ⁻¹)	Moisture content (%)
POoP H	-	158.9	0	100	3.50	2.30	0.03	solid	5.69	0.03
POoP M	C1	83.4	3	97	2.14	1.48	0.02	787	6.57	0.02
POoP EG	C2	110.5	33	67	4.74	1.42	0.03	5 416	4.45	0.03
POoP PDO	C3	105.4	29	71	4.73	0.43	0.05	7 789	6.55	0.02
POoP BDO	C4	121.3	26	74	4.38	0.32	0.02	4 241	3.64	0.04
POoP PTDO	C5	118.4	36	64	4.54	0.27	0.04	4 678	6.99	0.04
POoP HDO	C6	120.5	36	64	4.28	0.46	0.02	3 688	10.63	0.04
POoP PG	C3*Br	119.8	5	95	3.96	1.38	0.02	3 649	5.99	0.04

Source: Tuan Noor Maznee *et al.* (2018a).

Note: *Br - branched structural diol.

**Average functionality calculated from average equivalent weights of polyols (from measured hydroxyl numbers) and M_n measured via GPC.

(DSC 6000, Perkin Elmer, Massachusetts, USA), thermogravimetric analysis (TGA) (simultaneous thermal analyser, STA 6000, Perkin Elmer, Massachusetts, USA) and dynamic mechanical analyser (DMA) (Q800, TA Instruments, Delaware, USA). DSC analysis of SPU was carried out over the temperature range of -45°C to 255°C at a heating rate of 10°C min⁻¹. In the first run, the SPU sample was heated from room temperature to 150°C. The sample was then cooled down to -45°C and it was heated again to 255°C (second run). Thermal property from the second run was discussed in this study. For TGA analysis, the SPU sample was heated from room temperature to 800°C at a heating rate of 10°C min⁻¹ under N₂ gas with a flow rate of 20 mL min⁻¹. DMA analysis of SPU (35.5 mm x 12.5 mm x 2.5 mm) was carried out in multi-frequency-stress mode using a single cantilever clamp at a constant frequency of 1 Hz and a heating rate of 3°C min⁻¹.

Hardness (Shore A and D) of samples were measured using Durometer (Westop, Nishi Tokyo Seimitsu, Japan) according to ASTM D2240-05. Resiliency of SPU was measured using a Bashore Resiliometer following ASTM D2632-79. Tensile strength and elongation at break of SPU were determined using Instron 5965 following ASTM D412-98a at room temperature. Tensile recovery was measured as a percent of sample's length recovered after 10 min of tensile testing. Tear strength (Die C) of SPU was measured following ASTM D624-00. Wide angle X-ray diffraction (WAXD) analysis of SPU was conducted using a Diffractometer, Shimadzu XRD 6000 model (Kyoto, Japan). Data were collected between 2° and 50° at a scanning rate of 2°C min⁻¹.

with 2θ scanning, using nickel-filtered Cu Kα (λ = 1542 nm) at a set voltage and current of 30 kV and 30 mA, respectively. Degree of crystallinity of SPUs was calculated using formula as reported by Shetranjiwalla *et al.* (2017):

$$\text{Degree of crystallinity} = \frac{A_c}{A_c + A_a} \times 100$$

where A_c is the area under the crystal diffraction peaks and A_a is the area under the amorphous halo.

Solvent resistance of SPU was carried out on the specimen with a dimension of 10 × 40 × 2 mm, which was immersed in deionised water, mineral oil, toluene, MEK, hydrochloric acid (0.1 N) and sodium hydroxide (0.1 N) at room temperature (28°C). The weight change of the specimen was recorded after day 1 and day 7.

RESULTS AND DISCUSSION

SPUs were produced via reaction of POoP with MDI in the presence of BDO chain extender through a one-shot method. Schematic reaction between POoP using POoP EG as representation, BDO and MDI to form SPU. The POoP used in this study have been previously prepared through epoxidation and alcoholysis reactions (Tuan Noor Maznee *et al.*, 2018a), whereby the properties of the POoP are shown in Table 1. All SPUs had HS concentrations of 31% to 46% (Table 2), depending on the hydroxyl number of the POoP (Table 1).

TABLE 2. PROPERTIES OF SPUs

Composition	S-H	S-M	S-EG	S-PDO	S-BDO	S-PTDO	S-HDO	S-PG
Polyol	POoP H	POoP M	POoP EG	POoP PDO	POoP BDO	POoP PTDO	POoP HDO	POoP PG
# of C atoms in nucleophile	-	C1	C2	C3	C4	C5	C6	C3*Br
Hard segment concentration (%)	46	31	39	36	39	39	40	40
Properties								
Hardness (Shore A)	89 ± 2	69 ± 2	86 ± 2	89 ± 2	88 ± 2	89 ± 2	92 ± 2	89 ± 2
Hardness (Shore D)	29 ± 1	17 ± 1	25 ± 1	29 ± 2	28 ± 1	31 ± 1	34 ± 1	30 ± 1
Resilience (%)	9.2 ± 0.1	14.6 ± 0.2	11.4 ± 0.2	11.2 ± 0.4	11.6 ± 0.3	10.9 ± 0.2	11.8 ± 0.4	12.1 ± 0.6
Tensile strength at break (MPa)	22.4 ± 0.8	n/a	15.3 ± 0.7	11.5 ± 0.3	16.9 ± 0.6	15.0 ± 0.3	17.0 ± 0.7	17.1 ± 1.0
Tensile set (recovery of length after 10 min) (%)	8.0 ± 3.7	n/a	5.8 ± 0.6	3.3 ± 0.2	4.7 ± 0.6	4.1 ± 1.2	5.0 ± 0.6	4.9 ± 0.6
Elongation at break (%)	20.5 ± 8.4	n/a	26.0 ± 4.1	28.6 ± 2.0	26.2 ± 3.7	28.3 ± 2.1	33.3 ± 2.9	29.6 ± 4.5
Tear strength (N cm ⁻¹)	1906 ± 50	191 ± 3	1 017 ± 68	640 ± 38	1 006 ± 31	852 ± 104	986 ± 105	966 ± 30
Melt transition temperature (°C)	11	7	no melting peak	no melting peak	no melting peak	no melting peak	no melting peak	no melting peak
Enthalpy of fusion (J g ⁻¹)	3.14	7.28	n/a	n/a	n/a	n/a	n/a	n/a

Note: n/a - not available (SPU sample was soft); *Br - branched structural diol; ± - standard deviation.

Mechanical Properties of SPUs

Mechanical properties of SPUs were measured in terms of hardness, resilience, elongation at break, tensile and tear strengths. Generally, hardness of SPUs increased with increasing diol chain length (from C2 to C6). The hardness was highest in SPU made from POoP HDO of C6 diol (Table 2). In the case of S-H, high hardness observed was due to the relatively higher HS concentration in this SPU (46%) as a result of higher hydroxyl number (Table 1). Higher tensile and tear strengths and lower resilience and elongation at break in S-H were also observed due to the higher concentration of HS. Conversely, the SPU labelled as S-M, which was made from POoP M, with the lowest hydroxyl number and functionality, had exhibited the lowest hardness and tear strength, as expected, due to the lower cross-link density as indicated by lowest functionality of this polyol (Table 1) and lowest HS concentration (Table 2). The tensile strength and elongation at break of S-M could not be measured because the SPU was too soft (the sample failed at the grip point of the clamp). No significant effect between structure of diol [linear (PDO) *vs.* branched diol (PG)] was observed on the hardness, resilience and elongation at break of SPU. In general, there was no significant difference in the mechanical properties among SPUs made from POoPs, which were prepared with diols of different chain length. This might be due to the chemical structure of triglycerides, that is relatively larger than the chemical structure of diols used in this study, which minimise the influence of the diol's structure on the properties of SPUs.

Thermal Behaviour and Dynamic Properties of SPUs

Thermal behaviour of SPUs was analysed using DSC, TGA and DMA. Melt transitions measured by DSC were observed in S-H and S-M, which were made from POoP H and POoP M, respectively. The melt transitions of S-H and S-M were observed at 11°C and 7°C, with the enthalpy of fusion of 3.14 and 7.28 J g⁻¹, respectively. There was no thermal transition observed in SPUs made from other POoP, in which the epoxide groups were ring-opened with diols (Figure 1). This would suggest that the polyol SS had mixed sufficiently with the HS, which completely disrupted the crystallinity of the polyol segment. Similar observations were reported by Caillol *et al.* (2012) and Del Rio *et al.* (2010).

Thermal decomposition temperature (T_d) (onset) of SPUs was measured using TGA. The thermal decomposition of SPUs started at about 259°C to 304°C (Table 3, Figure 2) depending on the types of nucleophiles used during epoxide ring-opening reaction of the POoP, initiated by the decomposition of urethane groups at around 290°C (Datta and Kasprzyk, 2018) and 238°C–286°C (Tuan Noor Maznee *et al.*, 2019). T_d of SS is initiated by a random scission of the ester linkage at around 370°C (Datta and Kasprzyk, 2018) and 360°C to 386°C (Tuan Noor Maznee *et al.*, 2019). In this study, T_d of SS was observed around 350°C to 427°C due to a random scission of both ester and ether linkages as the POoP structurally have both ester and ether linkages (Tuan Noor Maznee *et al.*, 2018a). It was observed that SPUs made from POoP, prepared with epoxide groups ring-opened with diols, had higher T_d than epoxide groups ring-opened with H and M (Table 3).

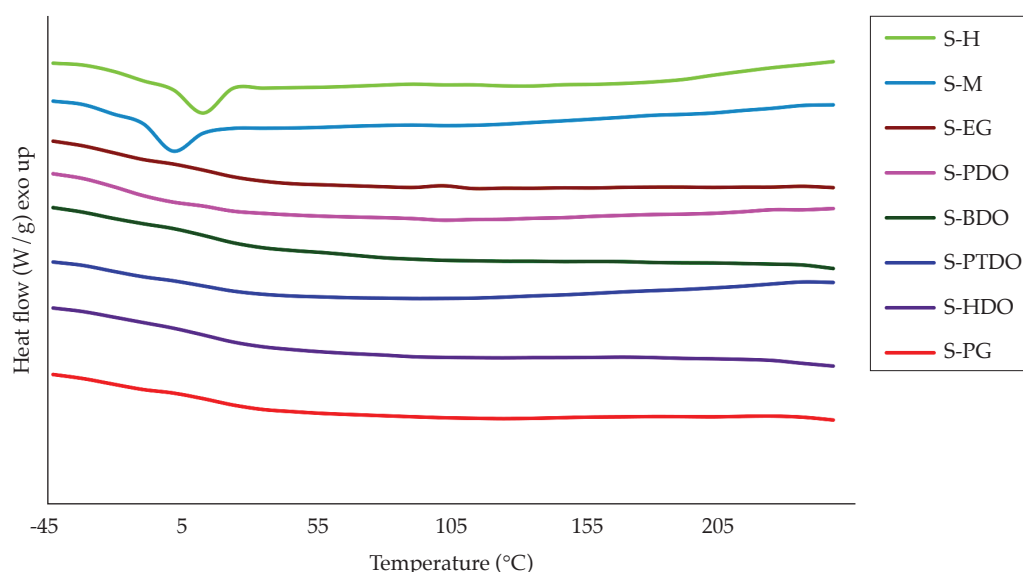


Figure 1. DSC thermograms of SPUs made from palm olein-based polyols.

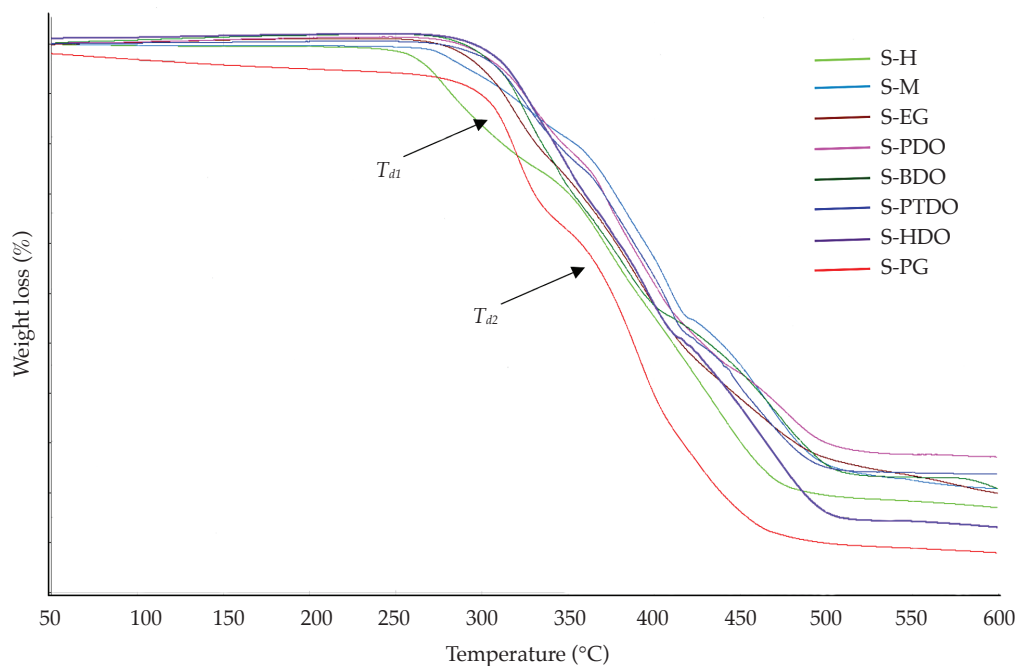


Figure 2. TGA thermograms of SPUs made from palm olein-based polyols.

TABLE 3. THERMAL DECOMPOSITION OF SPUs MADE FROM PALM OLEIN-BASED POLYOLS

SPU	Thermal decomposition, T_{d1} , °C	Thermal decomposition, T_{d2} , °C
S-H	259	351
S-M	271	364
S-EG	292	367
S-PDO	300	363
S-BDO	300	377
S-PTDO	298	366
S-HDO	304	427
S-PG	300	368

Thermodynamic properties of the SPUs determined using DMA are as shown in Figures 3 and 4. A gradual decrease in storage modulus of the SPUs was observed as the temperature was increased from -100°C to 100°C (Figure 3). At low temperature region (-100°C to 0°C), all SPUs displayed the same storage modulus, except S-M, which was made from POoP with almost 100% secondary hydroxyl groups which had the lowest hydroxyl number and functionality (Table 1). This indicates that the S-M had lower degree of crosslinking in comparison to other SPUs. At higher temperature region (0°C to 100°C), S-M exhibited the largest reduction in storage modulus due to the low degree of crosslinking, while S-H exhibited the least reduction, which could be due to the high HS concentration of this SPU, i.e., 46% (Table 2).

Other SPUs exhibited insignificant difference in storage modulus, indicating that different chain length of diols (C2 to C6) used in ring-opening reaction of epoxide groups to form POoP did not affect the storage moduli of the prepared SPUs. Broad $\tan \delta$ peaks of SPUs were observed (Figure 4), which could be attributed to the presence of amorphous and crystalline domains. It was observed that the $\tan \delta$ peak shifted to a higher temperature region in SPUs made from POoP, in which the epoxide groups were ring-opened with water and diols. The overall height of the $\tan \delta$ peak decreased due to the increased presence of the crystalline domains that impacted the dynamic properties of the SPUs. The $\tan \delta$ peak could also be ascribed to phase interaction, whereby a narrow $\tan \delta$ peak indicates phase separation between the HS and SS, while a broad $\tan \delta$ peak suggests phase mixing morphology (Hojabri *et al.*, 2010; Sonnenschein *et al.*, 2010; Xie *et al.*, 2009). In this study, all SPUs exhibited phase-mixed morphologies.

Diffraction Pattern and Crystallinity of SPUs

Diffraction pattern and crystallinity of SPUs were further evaluated using WAXD analysis at 25°C. Overall, the WAXD patterns of all SPUs showed that all the prepared samples were of highly crystalline polymers (Figure 5). There were also two main crystalline peaks observed at 2θ in the range of 13° and 16° with d -spacing in between 6.35 Å to 6.39 Å and 5.30 Å to 5.32 Å, respectively.

In terms of relative intensity of the diffraction peak at $2\theta = 13^\circ$, it had decreased with the increasing carbon chain length of the diol which was used to prepare POoP, from C1 (methanol in S-M) to C6 (1,6-HDO in S-HDO). Overall, with different

types of nucleophiles used during ring-opening reaction of epoxide groups to make POoPs, the SPU obtained did not exhibit significant difference in the d -spacing, crystallite size and degree of crystallinity of the SPU samples (Table 4).

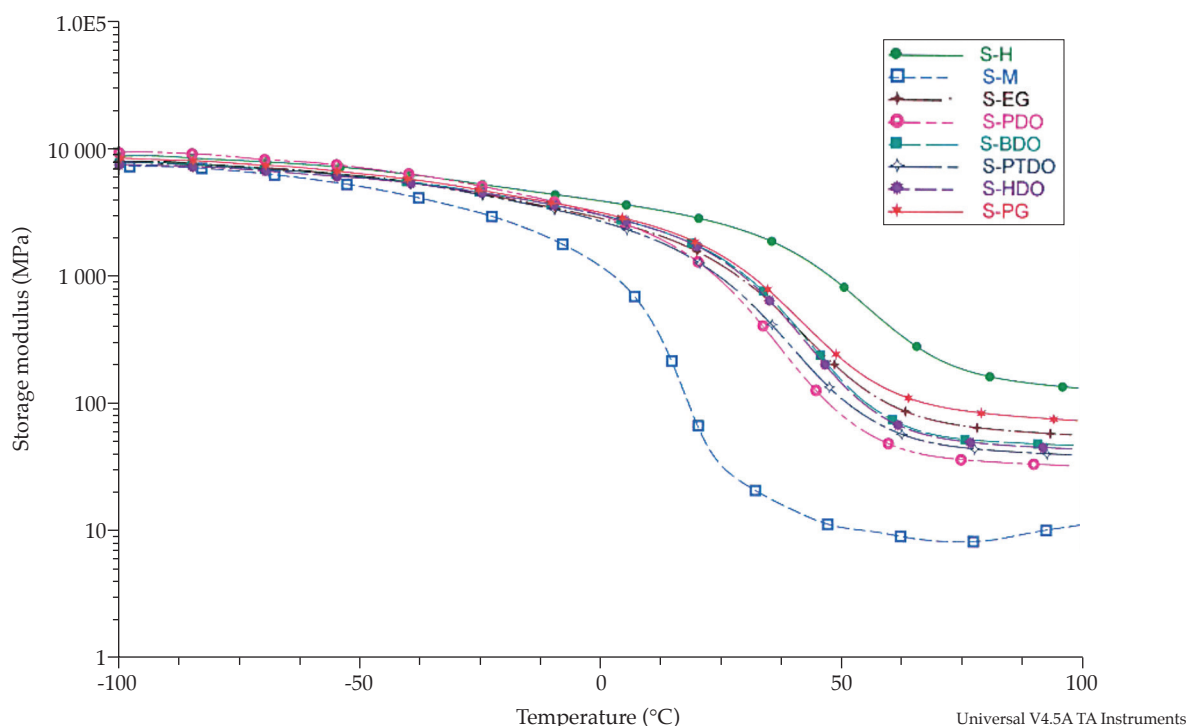


Figure 3. Temperature dependence of storage modulus for SPU made from palm olein-based polyols.

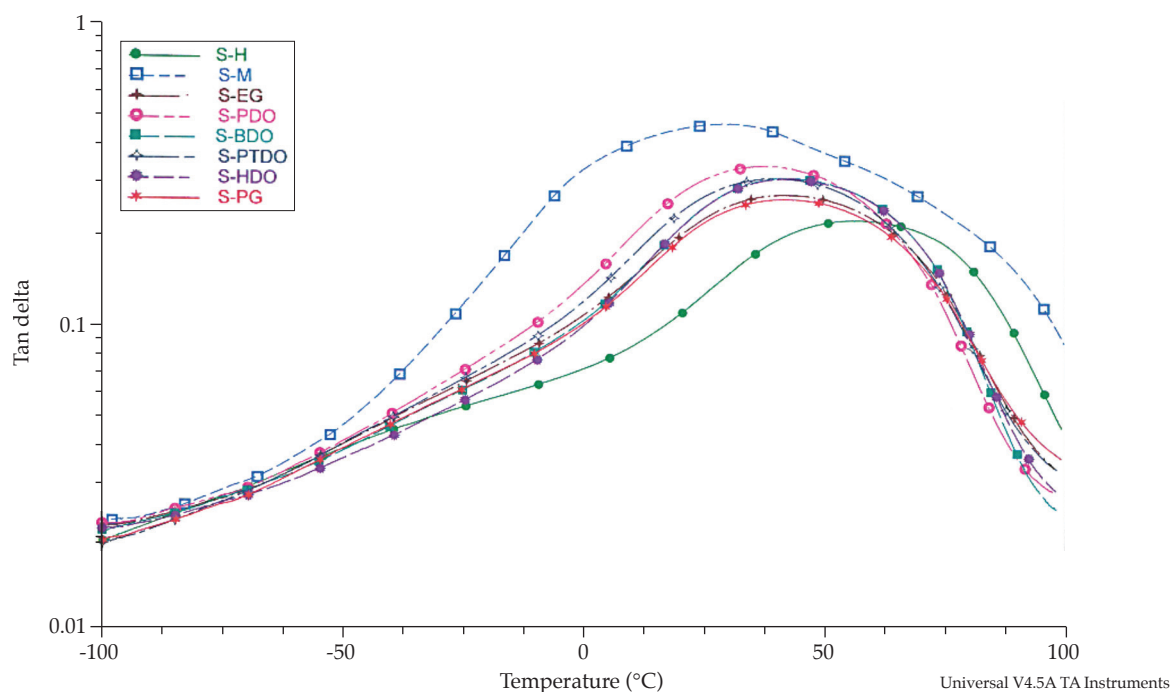


Figure 4. Temperature dependence of $\tan \delta$ for SPU made from palm olein-based polyols.

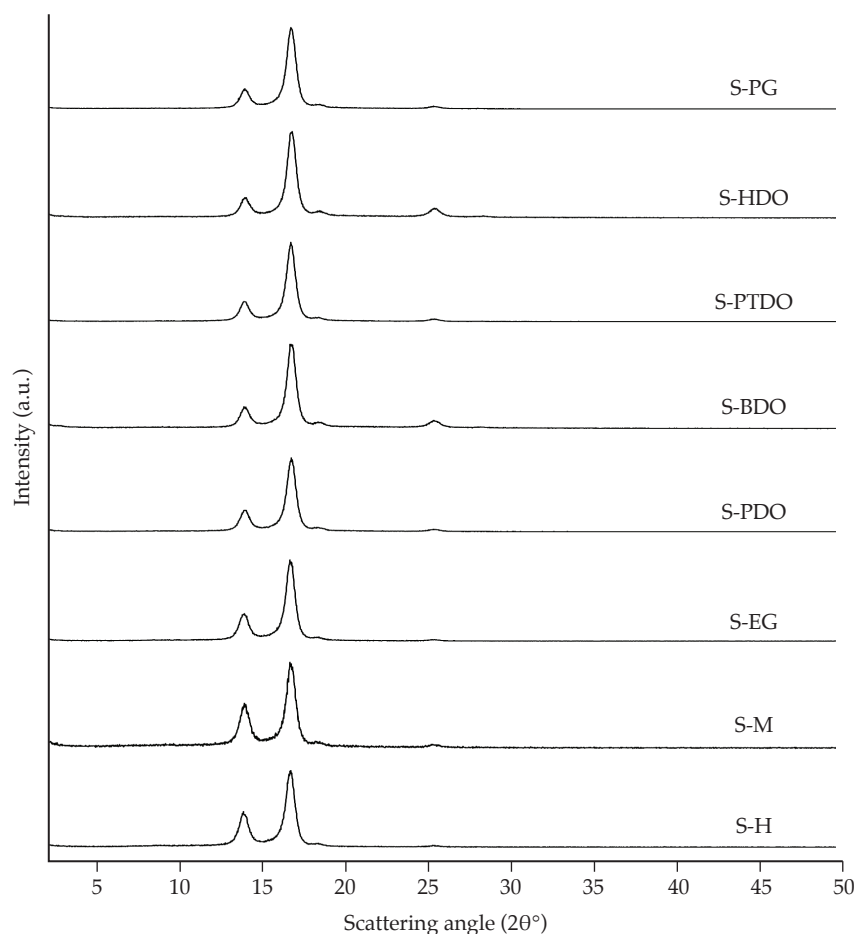


Figure 5. Wide angle X-ray diffraction (WAXD) diffractograms of SPU made from palm olein-based polyols, measured at 25°C.

TABLE 4. SCATTERING ANGLE, *d*-SPACING, RELATIVE INTENSITY, FWHM, CRYSTALLITE SIZE AND CRYSTALLINITY OF SPU MADE FROM PALM OLEIN-BASED POLYOLS (two main peaks)

Designation		S-H	S-M	S-EG	S-PDO	S-BDO	S-PTDO	S-HDO	S-PG
Peak 1	Scattering angle, 2θ (°)	13.84	13.89	13.85	13.90	13.91	13.89	13.92	13.91
	<i>d</i> -spacing (Å)	6.39	6.37	6.39	6.36	6.36	6.37	6.35	6.36
	Relative intensity (%)	43	48	32	28	23	25	21	23
	FWHM, 2θ (°)	0.6889	0.7189	0.7071	0.6975	0.7004	0.6778	0.6941	0.7046
	Crystallite size (nm)	12.14	11.63	11.83	11.99	11.94	12.34	12.05	11.87
Peak 2	Scattering angle, 2θ (°)	16.63	16.68	16.64	16.70	16.71	16.67	16.72	16.69
	<i>d</i> -spacing (Å)	5.32	5.31	5.32	5.30	5.30	5.31	5.30	5.31
	Relative intensity (%)	100	100	100	100	100	100	100	100
	FWHM, 2θ (°)	0.6716	0.6828	0.6682	0.6743	0.6610	0.6623	0.6630	0.6889
	Crystallite size (nm)	12.49	12.29	12.55	12.44	12.69	12.67	12.65	12.18
Degree of crystallinity (%)		92	90	93	95	94	95	95	94

Note: FWHM - full width at half maximum.

Solvent Resistance of SPUs

The resistance of SPUs towards solvents was evaluated in water, 0.1 N hydrochloric acid (HCl), 0.1 N sodium hydroxide (NaOH), toluene, mineral

oil and MEK. Percentages of weight changes of SPUs after day 1 and day 7 were recorded. In general, all SPUs samples had good resistance toward water, acid (0.1 N HCl), base (0.1 N NaOH) and mineral oil, indicated by the low percentages of weight changes

(0.04% to 0.69%), except S-H and S-M, which had slightly higher percentage of weight changes in mineral oil, between 1.18% and 8.07% (Table 5). All the SPU samples were swollen in toluene and MEK, indicated by the higher percentages of weight changes (41.88% to 101.17% in toluene and 33.89% to 92.65% in MEK), which showed that all the SPUs were less resistant to non-polar organic solvents. In both toluene and MEK, the S-M exhibited the highest weight change due to the lower degree of crosslinking or hydrogen bonding presence in this SPU (Ionescu, 2005). This observation correlated well with POoP M structure, in which the polyol had the lowest hydroxyl number and functionality with almost 100% secondary hydroxyl and one methoxy group hanging in the triglyceride molecule (Tuan Noor Maznee *et al.*, 2018a).

In the case of S-H, even though it also has 100% secondary hydroxyl, the SPU displayed relatively low weight change in both toluene and MEK. This might be due to higher degree of crosslinking present in the S-H, contributed by high hydroxyl number and functionality of POoP H (Tuan Noor Maznee *et al.*, 2018a), which help in increasing the crosslinking density of SPU (Ionescu, 2005).

CONCLUSION

POoPs have been used to prepare SPUs by reacting the polyols with MDI and chain extended with BDO. The prepared SPUs were evaluated in terms of mechanical, dynamic and morphological properties, thermal behaviour and solvent resistance. HS concentration and crosslink density seems to greatly influence the mechanical properties of the SPUs as witnessed in S-H and S-M. HS concentration is dependent on the hydroxyl number of polyol, in which polyol with higher hydroxyl number will generate higher concentration of HS and physical crosslinking. All SPUs exhibited phase-mixed morphologies between the HS and SS as indicated by DMA analysis. All SPUs were of highly crystalline polymer as shown by WAXD analysis. The solvent resistant test indicated that SPUs made from POoPs were dimensionally stable in most solvents tested except in toluene and MEK. Overall, properties of SPUs were not significantly affected by number of carbon atoms in nucleophiles used to make POoPs, however, they were significantly affected by HS concentration and crosslink density of SPU. Therefore, from commercial point of view, SPUs made from POoP H (water as the nucleophile) and POoP EG (1,2-ethanediol as the nucleophile) could serve as the most economical POoPs to be produced whereby SPU that requires high hardness could be produced using POoP H and SPU which requires high elongation and tear strength could be produced using POoP EG.

TABLE 5. SOLVENT RESISTANCE TEST OF SOLID POLYURETHANES (SPU) (% WEIGHT CHANGE) MEASURED AT 28°C

Designation	Water		0.1 N HCl		0.1 N NaOH		Toluene		Mineral oil		MEK	
	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7
S-H	0.34 ± 0.08	0.71 ± 0.09	0.18 ± 0.05	0.65 ± 0.21	0.32 ± 0.12	0.48 ± 0.07	42.22 ± 0.26	41.88 ± 0.72	1.18 ± 0.05	2.18 ± 0.36	48.68 ± 2.53	47.69 ± 1.97
S-M	0.23 ± 0.11	0.36 ± 0.18	0.30 ± 0.08	0.30 ± 0.06	0.44 ± 0.17	0.49 ± 0.11	101.17 ± 1.71	100.79 ± 1.44	4.99 ± 0.68	8.07 ± 0.85	92.65 ± 6.98	87.11 ± 6.66
S-EG	0.26 ± 0.08	0.26 ± 0.09	0.14 ± 0.05	0.32 ± 0.08	0.16 ± 0.08	0.30 ± 0.03	40.19 ± 0.53	43.79 ± 1.81	0.40 ± 0.05	0.60 ± 0.09	52.86 ± 0.39	33.89 ± 0.59
S-PDO	0.20 ± 0.16	0.23 ± 0.16	0.11 ± 0.01	0.14 ± 0.03	0.11 ± 0.07	0.20 ± 0.11	59.68 ± 0.69	60.78 ± 0.50	0.26 ± 0.09	0.38 ± 0.04	51.98 ± 0.60	50.33 ± 0.33
S-BDO	0.13 ± 0.01	0.27 ± 0.07	0.13 ± 0.03	0.36 ± 0.07	0.13 ± 0.02	0.27 ± 0.04	55.72 ± 0.42	56.66 ± 2.03	-1.44 ± 3.02	-1.12 ± 3.07	56.79 ± 0.72	48.56 ± 3.85
S-PTDO	0.10 ± 0.03	0.17 ± 0.06	0.05 ± 0.03	0.14 ± 0.02	0.04 ± 0.02	0.16 ± 0.01	55.14 ± 0.52	56.49 ± 0.43	0.37 ± 0.12	0.69 ± 0.23	48.68 ± 0.38	48.77 ± 0.69
S-HDO	0.11 ± 0.04	0.38 ± 0.07	0.16 ± 0.04	0.30 ± 0.09	0.20 ± 0.03	0.30 ± 0.02	49.58 ± 1.15	54.37 ± 0.51	0.30 ± 0.05	0.62 ± 0.05	53.67 ± 2.26	54.55 ± 1.88
S-PG	0.10 ± 0.01	0.31 ± 0.04	0.14 ± 0.05	0.32 ± 0.05	0.29 ± 0.06	0.42 ± 0.11	51.41 ± 0.57	54.34 ± 1.21	0.34 ± 0.09	0.54 ± 0.11	52.59 ± 1.24	54.16 ± 1.94

Note: ± - standard deviation.

ACKNOWLEDGEMENT

The authors would like to thank the Director-General of MPOB for permission to publish this article. The authors would also like to acknowledge Norhidayah Ahmad Yusoff, Muhammad Ihsan Ismail, Mohamed Ahir Musa, Asura Abd Rahman, Mohd Taib Samsudin and Ahmad Syafiq Ahmad Hazmi of the Advanced Oleochemical Technology Division, MPOB for their technical assistance.

REFERENCES

- Armylisas, A H N; Siti Hazirah, M F; Yeong, S K and Hazimah, A H (2017). Modification of olefinic double bonds of unsaturated fatty acids and other vegetable oil derivatives via epoxidation: A review. *Grasas y Aceites*, 68: e174.
- Brokenbrow, B E; Sims, D and Wright, J (1971). Relationships between polyurethane elastomer structure and ageing properties Part 2: Effect of backbone polyol. Technical Report No. 53, Ministry of Defence, Explosives Research and Development Establishment, 1971. <http://www.dtic.mil/dtic/tr/fulltext/u2/745982.pdf>, accessed on 20 February 2017.
- Caillol, S; Desroches, M; Boutevin, G; Loubat, C; Auvergne, R and Boutevin, B (2012). Synthesis of new polyester polyols from epoxidised vegetable oils and biobased acids. *Eur. J. Lipid Sci. Technol.*, 114: 1447-1459.
- Calliauw, G; Gibon, V; De Greyt, W; Plees, L; Foubert, I and Dewettinck (2007). Phase composition during palm olein fractionation and its effect on soft PMF and superolein quality. *J. Am. Oil Chem. Soc.*, 84: 885-891.
- Chen, R; Zhang, C and Kessler, M R (2014). Polyols and polyurethanes prepared from epoxidised soybean oil ring-opened by polyhydroxy fatty acids with varying OH numbers. *J. Appl. Polym. Sci.*, 132: 41213.
- Costa, V; Nohales, A; Felix, P; Guillem, C; Gutiérrez, D and Gómez, C M (2014). Structure-property relationships of polycarbonate diol-based polyurethanes as a function of soft segment content and molar mass. *J. Appl. Polym. Sci.*, 132: 41704.
- Cruz, S M and Viana, J C (2015). Structure-property relationships in thermoplastic polyurethane elastomer nanocomposites: Interactions between polymer phases and nanofillers. *Macromol. Mater. Eng.*, 300: 1153-1162.
- Datta, J and Kasprzyk, P (2018). Thermoplastic polyurethanes derived from petrochemical or renewable resources: A comprehensive review. *Polym. Eng. Sci.*, 58: E14-E35.
- Del Rio, E M; Lligadas, G; Ronda, J C; Galià, M and Cádiz, V (2010). Bio-based polyurethanes from polyether polyols obtained by ionic-coordinative polymerisation of epoxidised methyl oleate. *J. Polym. Sci. A Polym. Chem.*, 48: 5009-5017.
- Drobný, J G (2007). *Handbook of Thermoplastic Elastomers*. First edition. William Andrew Inc, New York. 215 pp.
- Hepburn, C (1992). Trends in polyurethane elastomer technology. *Iranian J. Polym. Sci. Technol.*, 1(2): 84-110.
- Hojabari, L; Kong, X and Narine, S S (2010). Functional thermoplastics from linear diols and diisocyanates produced entirely from renewable lipid sources. *Biomacromolecules*, 11: 911-918.
- Ionescu, M (2005). *Chemistry and Technology of Polyols for Polyurethanes*. Rapra Technology Limited, Shawbury, Shropshire, United Kingdom. p. 32-41.
- Lligadas, G; Ronda, J C; Galià, M and Cadiz, V (2010). Plant oils as platform chemicals for polyurethane synthesis: Current state-of-the-art. *Biomacromolecules*, 11: 2825-2835.
- Mohd Norhisham, S; Sendijarevic, I; Tuan Noor Maznee, T I; Nurul 'Ain, H; Kosheela Devi, P P; Srihanum, A; Norhayati, M N; Yeong, S K; Hazimah, A H; Schiffman, C M; Sendijarevic, A and Sendijarevic, V (2017). Soft polyurethane elastomers with adhesion properties based on palm olein and palm oil fatty acid methyl ester polyols. *Int. J. Adhes. Adhes.*, 73: 38-44.
- Omonov, T S; Kharraz, E and Curtis, J M (2016). The epoxidation of canola oil and its derivatives. *RSC Adv.*, 6: 92874-92886.
- Parveez, G K A; Azmil Haizam, A T; Shamala, S; Loh, S K; Meilina, O-A; Kosheela Devi, P P; Kamalrudin, M S; Sheilyza, M I and Zainab, I (2021). Oil palm economic performance in Malaysia and R&D progress in 2020. *J. Oil Palm Res.*, 33(2): 181-214.
- Petrović, Z S (2008). Polyurethanes from vegetable oils. *Polym. Rev.*, 48(1): 109-155.
- Shetranjiwalla, S; Li, S; Bouzidi, L and Narine, S S (2017). Impact of polymerisation protocol on

structure-property relationships of entirely lipid-derived poly(ester urethane)s. *J. Renew. Mater.*, 5(5): 333-344.

Sonnenschein, M F; Guillaudeu, S J; Landes, B G and Wendt, B (2010). Comparison of adipate and succinate polyesters in thermoplastic polyurethanes. *Polymer*, 51: 3685-3692.

Srihanum, A; Sendijarevic, I; Tuan Noor Maznee, T I; Nurul 'Ain, H; Kosheela Devi, P P; Mohd Norhisham, S; Yeong, S K; Zainab, I; Campara, I; Schiffman, C M; Pietrzyk, K and Sendijarevic, V (2017). Impact of the hard-segment concentration on highly resilient polyurethane foams based on palm olein polyol. *J. Appl. Polym. Sci.*, 134(45): 45440.

Tanaka, R; Hirose, S and Hatakeyama, H (2008). Preparation and characterisation of polyurethane foams using a palm oil-based polyol. *Bioresour. Technol.*, 99: 3810-3816.

Tuan Noor Maznee, T I; Ibrahim, N A; Sendijarevic, V; Sendijarevic, I; Schiffman, C M; Hoong, S S; Mohd Noor, M A; Kosheela Devi, P P; Yeong, S K; Zainab, I; Emilia, A M; Norhazlin, Z and Sendijarevic, A (2019). Thermal and mechanical properties of thermoplastic urethanes made from crystalline and amorphous azelate polyols. *J. Appl. Polym. Sci.*, 136: 47890.

Tuan Noor Maznee, T I; Ibrahim, N A; Mohd Noor, M A; Hoong, S S; Kosheela Devi, P P; Yeong, S K;

Zainab, I; Schiffman, C M; Sendijarevic, I; Emilia, A M; Norhazlin, Z and Sendijarevic, V (2018a). Oligomeric composition of palm olein-based polyols: The effect of nucleophiles. *Eur. J. Lipid Sci. Technol.*, 120(4): 1700354.

Tuan Noor Maznee, T I; Ibrahim, N A; Mohd Noor, M A; Hoong, S S; Kosheela Devi, P P; Yeong, S K; Zainab, I; Schiffman, C M; Sendijarevic, I; Emilia, A M; Norhazlin, Z and Sendijarevic, V (2018b). Oligomeric composition of polyols from fatty acid methyl ester: The effect of ring-opening reactants of epoxide groups. *J. Am. Oil Chem. Soc.*, 95(4): 509-523.

Tuan Noor Maznee, T I; Norin, Z K S; Ooi, T L; Salmiah, A and Gan, L H (2001). Effect of additives on palm-based polyurethane foams. *J. Oil Palm Res.*, 13: 7-15.

Xie, R; Bhattacharjee, D and Argyropoulos, J (2009). Polyurethane elastomers based on 1,3 and 1,4-bis(isocyanatomethyl)cyclohexane. *J. Appl. Polym. Sci.*, 113: 839-848.

Xu, W; Zhou, L; Sun, W; Zhang, J and Tu, W (2015). Effect of difunctional acids on the physicochemical, thermal and mechanical properties of polyester polyol-based polyurethane coatings. *J. Appl. Polym. Sci.*, 132: 41246.

Zlatanić, A; Lava, C; Zhang, W and Petrović, Z S (2004). Effect of structure on properties of polyols and polyurethanes based on different vegetable oils. *J. Polym. Sci. B Polym. Phys.*, 42: 809-819.

METHOD FOR THE DETERMINATION OF TOTAL CHLORIDE CONTENT IN EDIBLE OILS

ABDUL NIEFAIZAL ABDUL HAMMID¹; AZMIL HAIZAM AHMAD TARMIZI¹; MUHAMAD RODDY RAMLI^{1*};
AINIE KUNTOM¹ and LEE HOCK CHIN²

ABSTRACT

Edible oils are important component of food products and have to meet with food safety requirements. However, a group of compounds called chloropropanols has been detected in edible oils which have compromised its safety. The precursor for these compounds is chloride. The chlorinated compounds can be in the form of organic or inorganic contaminants. The growing importance of chemical measurements for this entity has greatly pressured the method development to improve the quality of analytical results and to guarantee quality to the end users. In this work, a method for the determination of total chloride (TC) in edible oils was validated. The analysis of samples was performed by a combination of combustion and titration process using a Total Chloride Analyser (TCA). The results showed good linearity in the range of 0.5 to 20.0 $\mu\text{g mL}^{-1}$, with the correlation coefficient (R^2) of more than 0.999. The average recoveries of TC evaluated at three spike levels were 80% to 105% with relative standard deviations (RSD) of less than 10%. The limit of detection (LOD) and limit of quantification (LOQ) were 0.03 and 0.10 $\mu\text{g mL}^{-1}$, respectively. The results indicated that this method could be used for routine analysis of TC in edible oils.

Keywords: chloropropanols, combustion, edible oils, titration, total chloride.

Received: 14 October 2021; **Accepted:** 31 January 2022; **Published online:** 9 March 2022.

INTRODUCTION

Edible oils are important ingredients in most food products. However, the presence of chloropropanols has been a major food safety issues in the processed edible oils. The chloropropanols detected are 3-monochloropropane-1,2-diol esters (3-MCPDE) and 2-monochloropropane-1,3-diol esters (2-MCPDE). They are a group of food processed contaminants formed from acylglycerols and chlorides during the refining process of edible oils. Glycidyl esters (GE) are mainly formed during the

deodourisation step in the refining process of edible oils and therefore occur in almost all refined edible oils (Smidrkal *et al.*, 2016).

Studies have shown that 3-MCPDE is formed in oils during deodourisation, which is performed at temperatures up to 270°C. Chemically, 3-monochloropropane-1,2-diol (3-MCPD) is a glycerol chlorohydrin formed when one hydroxyl group in a glycerol molecule is replaced by a chlorine atom. The single positional isomer, 2-chloropropane-1,3-diol (2-MCPD) and the two enantiomers of 3-MCPD are formed when -OH is replaced by -Cl at the *sn*-1 or *sn*-3 positions on the glycerol backbone (Figure 1) (Hamlet and Sadd, 2002).

The presence of 2-MCPDE, 3-MCPDE and GE in the diet is a potential health concern since these esters are hydrolysed by enzymes in the gastrointestinal tract and transform into their free forms, which have been considered toxic (Hamlet *et al.*, 2002). The free forms of 2-MCPDE, 3-MCPDE

¹ Malaysian Palm Oil Board,
6 Persiaran Institusi, Bandar Baru Bangi,
43000 Kajang, Selangor, Malaysia.

² QS Instrument Sdn. Bhd.,
No. 9 Jalan Juru Ukur U1/119,
40150 Shah Alam, Selangor, Malaysia.

* Corresponding author e-mail: roddy@mpob.gov.my

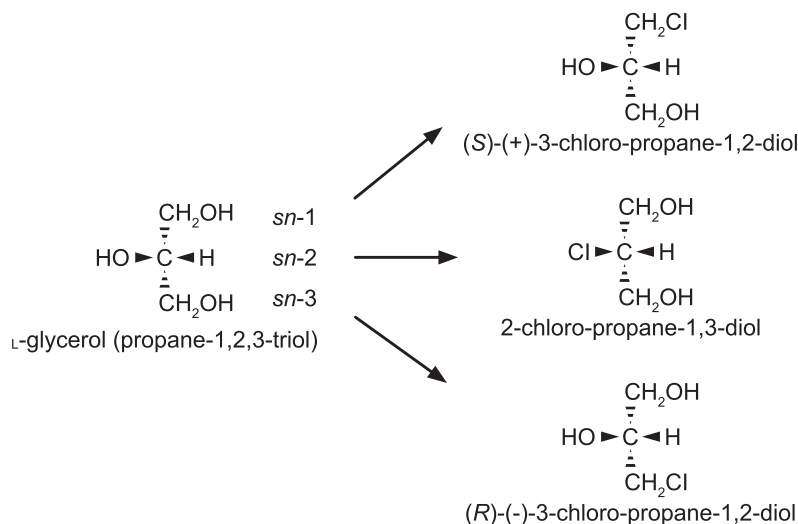


Figure 1. Formation of 2-MCPD and 3-MCPD with the presence of chloride atom.

and GE are free 2-MCPD, free 3-MCPD and glycidol, respectively. According to the International Agency for Research on Cancer (IARC), free 3-MCPD is classified as a possible human carcinogen (Group 2B) and glycidol as a probable human carcinogen (Group 2A) (Abraham *et al.*, 2013; IARC, 2012). For the free 2-MCPD and its esters, there is no risk assessment published in the literature to date, due to the lack of data on this contaminant (IARC, 2012).

The presence of a chloride 'donour' in crude oil is critical in the formation of 3-MCPDE and 2-MCPDE. This may be due to high levels of chlorinated substances generated by the plant's endogenous metabolism, the use of chloride salts in fertilisers, and the use of hydrochloric acid activated clays during the bleaching step of the refining process (Craft and Destailats, 2014; EFSA, 2016).

One of the strategies to minimise the formation of these contaminants in refined edible oils is the reduction of their precursors before deodourisation. Washing palm oil before deodourisation may be a viable option since it removes some of the precursors from the oil, mostly chloride 'donour' compounds, preventing the formation of 2-MCPDE and 3-MCPDE (Ramli *et al.*, 2020). In the literature, it was reported that washing crude palm oil (CPO) and fruit pulps reduced 3-MCPDE levels in refined oil by up to 90% (Ramli *et al.*, 2015; Silva *et al.*, 2014; Stadler, 2015).

Determination of chloride content is often conducted through titrimetric method due to its inherent reproducibility, accuracy and low costing absolute method of analysis. Nevertheless, detection of chloride in palm oil is not straight forward due to the complexity of the sample, comprising both polar and non-polar chlorides, as well as the possibility of analyte loss during the analytical procedures before the analysis.

There are numerous publications on the determination of chloride in soil, water and plant tissues. Analysis of chloride in these matrices are usually conducted by decomposition of the samples or salt extraction and followed by analysis using ion chromatography (IC), precipitation titration, potentiometric titration, and inductively coupled plasma mass spectrometry (ICP-MS) apparatus with special equipment (Krzyzaniak *et al.*, 2016). Chloride content in soil is determined in a soil-water extract. For plant tissue, it is either washed with lime or extracted with diluted nitric acid to determine chloride (Mesko *et al.*, 2016). Ion chromatography is well established as a routine technique for the determination of chloride in environmental samples, such as natural water, tap water, drinking water and soil. In fact, one of the earliest applications of IC is determination of chloride in soils (Mishra *et al.*, 2018).

The methods of precipitation and potentiometric titration use silver nitrate standard solution to determine the concentration of chloride by calculating the consumption of silver nitrate in the standard solution. The distinction between the two methods is the end point of titration. In potentiometric titration method, the end point is determined by the indicator electrode that records the change of the potential as a function of the amount (usually the volume) of the added titrant of exactly known concentration. In the precipitation titration method, the end point is determined by the formation of orange colour precipitate. Potentiometric titration is shown to be more sensitive than precipitation titration (Dianne, 2021).

The oldest method to determine chloride is the classical gravimetric method whereby chloride is measured as silver chloride. The Mohr and Volhard methods, which were established in the late 1800s, are classical volumetric chloride methods that have

been extensively used for soil and plant analysis. The Association of Official Analytical Chemists (AOAC) has official methods for the gravimetric and potentiometric determination of chloride in plant tissue, and for the volumetric determination of chloride in mineral water (Tian *et al.*, 2020). All of these methods are accurate. Nevertheless, any methods involving final weighing or manual titration is time consuming. Rapid measurements of chloride content have been reported for plant materials using an automatic chloride titrator with accuracy and precision comparable to manual potentiometric titration (Liao and Chou, 2006). A sensitive chloride colorimetric method developed by Zall *et al.* (1956) for determining chloride in water provides the basis for the development of an automated chloride colorimetric method.

A number of procedures for determination of chloride in oil matrix have been published and recognised as official methods for the American Society for Testing and Materials (ASTM) [ASTM D3230, ASTM D6470-6 and ASTM D4929-7], US Environmental Protection Agency (EPA) [Method; EPA 9075.8] and the Universal Oil Products (UOP) [Method UOP 779]. The ASTM D3230 and ASTM D6470 Methods are indirect quantification of chloride through the measurement of salt content. The ASTM D4929 and UOP 779 describe the quantification of organic chlorides while EPA 9075 defines the total chloride (TC) in petroleum products.

It is important to note that all the described official methods are solely for the measurement of chloride content in mineral oils or petroleum derivatives. Currently, there are still no methods available to detect the presence of chloride in edible oils, particularly CPO. Realising the importance to determine the level of chloride content in edible oils, an analytical method needs to be established by taking into account the distinction of chemical compositions and physical properties between mineral and edible oils. The availability of method to measure chloride content in edible oils would benefit the palm oil industry in gauging the CPO feedstock and thus, mitigates the presence of 3-MCPDE in the refined oils.

Therefore, in this article, a modified ASTM D4929 analytical procedure is proposed to determine the TC content in edible oils using a Total Chloride Analyser (TCA).

MATERIALS AND METHODS

Sample Collection

A total of 40 samples of various edible oils were collected. Twenty samples were palm oil products including CPO and refined, bleached and

deodourised palm oil (RBDPO) and were collected from local mill and refinery, respectively. The remaining 20 samples of commercial cooking oils consisted of soybean oil, canola oil, sunflower oil, corn oil, rice bran oil, macadamia oil, walnut oil, black seed oil, grape seed oil and olive oil and were purchased from local supermarket in Malaysia.

Chemicals and Reagents

Milli-Q system (Millipore Corp. Bedford, USA) ultrapure water was used to prepare all reference solutions and reagents. The resistivity of ultrapure water used has to be maintained at 18.2 MΩ cm or higher. The 2,4-nitrochlorobenzene stock standard solution was purchased from Merck (Darmstadt, Germany), while toluene, acetic acid, sodium acetate, potassium nitrate, potassium chloride and hydrochloric acid were obtained from Sigma-Aldrich Chemical, USA. All chemicals and organic solvents were of analytical grade. Carrier gases *i.e.*, argon (99.98% pure) and oxygen (99.70% pure) were purchased from MOX-Linde, Malaysia. All the plastic and glass wares were cleaned by soaking in a combination of diluted nitric acid and distilled water, in the ratio of 1:9 and were rinsed with ultrapure water before use, to avoid any possible contamination.

Chloride Stock Solution

Stock standard solutions of 5000 µg mL⁻¹ chloride was prepared by adding of 2.857 g (X) of 2,4-dinitrochlorobenzene in 100 mL of toluene (Y). The chloride concentration of this stock solution is obtained according to the following Equation (1) (Mitsubishi Chemical Analytech, 2014):

$$\text{Chloride concentration} \left(\mu\text{g mL}^{-1} \right) = \frac{X \text{ (g)}}{Y \text{ (mL)}} \times \frac{MW_{\text{Cl}}}{MW_{\text{Cl Std}}} \times 10^6 \quad (1)$$

where

MW_{Cl} is molecular weight of chloride (35.45)

$MW_{\text{Cl Std}}$ is molecular weight of chloride standard (202.55)

X is weight of chloride standard

Y is volume of toluene

Chloride Working Standard Solution

Working standard solution of 100 µg mL⁻¹ chloride was prepared daily by diluting 5000 µg mL⁻¹ chloride stock standard solution with toluene.

Preparation of Standard Calibration Curve

The calibration curve was established using five different chloride concentrations ranging from

0.5 to 20.0 $\mu\text{g mL}^{-1}$. Standard solutions of 0.5, 2.0, 5.0, 10.0 and 20.0 $\mu\text{g mL}^{-1}$ were prepared by diluting 100 $\mu\text{g mL}^{-1}$ working standard solution with appropriate volumes of toluene. The calibration ranges were selected according to the expected concentrations of the chloride in the edible oils.

Analytical Instruments and Devices

Quantitative analyses of the chloride were carried out using a TCA (NSX2100H, Nittoseiko Analytech Co. Ltd., Japan) equipped with an auto boat controller (ABC-210, Nittoseiko Analytech Co. Ltd., Japan), horizontal furnace (HF-210, Nittoseiko Analytech Co. Ltd., Japan) and coulometer detector (MCD-210, Nittoseiko Analytech Co. Ltd., Japan). Table 1 shows the typical operating conditions of the TCA for the TC analysis.

Sample Preparation

The oil aliquot was first heated in a water bath at temperature of approximately 70°C for 20 min, to ensure homogeneity prior to sampling. About 50 mg of sample was then weighed and placed in a ceramic boat for combustion and titration process in the TCA. The combustion and titration conditions for the TCA are as shown in Table 1.

TABLE 1. OPERATING CONDITIONS OF TCA FOR COMBUSTION PROCEDURE

Parameter	Condition
Reactant gas flow, oxygen	200 mL min ⁻¹
Carrier gas flow, argon	200 mL min ⁻¹
Furnace temperature inlet	1 000°C
Furnace temperature outlet	1 100°C
Sample amount	40-60 mg
Detection mode	Coulometric titration

Validation and Recoveries

The analytical method was validated in-house for the determination of each TC in all edible oils in terms of linearity, accuracy, repeatability, reproducibility, limit of detection (LOD) and limit of quantification (LOQ).

Linearity

Linearity or calibration of instruments was estimated by injecting 2,4-dinitrochlorobenzene/toluene (chloride) standard solution at five different concentrations (0.5, 1.0, 5.0, 10.0, 20.0 $\mu\text{g mL}^{-1}$) in triplicate. Linearity acceptance criteria were at a correlation coefficient (R^2) >0.999.

Recovery, Repeatability and Reproducibility

Recovery studies were carried out by spiking 2,4-dinitrochlorobenzene in four selected oil samples, namely CPO, RBDPO, sunflower oil and canola oil at concentrations of 0.5, 1.0 and 5.0 $\mu\text{g mL}^{-1}$ in six replications. The percentage recovery of the analyte was calculated to evaluate the accuracy of the analytical procedure. Recovery (R) was then calculated as Equation (2):

$$\% R = \frac{\text{Concentration in spiked sample} - \text{Concentration in unspiked sample}}{\text{Actual spike concentration}} \times 100 \quad (2)$$

Repeatability was measured in terms of precision in order to determine the variability of independent test results obtained with the same method by the same operator using the same equipment in a series of analyses for similar samples (Nagy *et al.*, 2011). The repeatability (RSD_r) of the method was carried out by conducting recovery experiments using 2,4-dinitrochlorobenzene standard solutions at three different concentration levels (0.5, 1.0, 5.0 $\mu\text{g mL}^{-1}$) in six replicates of CPO, RBDPO, sunflower oil and canola oil which was then analysed using the established method. To evaluate within-laboratory reproducibility (RSD_R), two different analysts performed the recovery experiment with six replicates, for three consecutive days, using the same concentration, samples and method. Repeatability and reproducibility were determined according to the ISO5725-2 Guidelines and expressed by Relative Standard Deviation (RSD).

LOD and LOQ

The reagent blanks (toluene) were prepared following the same procedures for the quantification of chloride in the samples, in order to determine LOD and LOQ of the analytical procedure. LOD is the minimum concentration of analyte that can be detected but not necessarily quantified with an acceptable uncertainty. LOQ is the lowest concentration of an analyte in a sample which can be quantitatively determined with acceptable uncertainty. First, the standard deviation (SD) must be evaluated before the calculation of LOD and LOQ. The Equation (3) for determining the LOD and LOQ is as follows:

$$\text{Standard Deviation (SD)} = \frac{A \times B}{100} \quad (3)$$

where

A is an average count value of chloride in reagent blank (μg)

B is RSD of chloride in reagent blank

Next, the Equation (4) for the calculation of LOD and LOQ is as follows:

$$LOD/LOQ = \frac{C \times SD \text{ (derived from above calculation)}}{D \times E} \quad (4)$$

where

C is sigma (σ) (use 3 for LOD calculation and use 10 for LOQ calculation)

D is slope (B) of calibration curve

E is injection volume used for calibration (mL)

Harmonisation of Sample Preparation Procedure

To assess the homogeneity of chloride, the CPO samples were heated at 50°C, 60°C and 70°C and at each temperature, the samples were melted at two different periods; 10 and 20 min. Seven sets of samples of each CPO were placed in the oven at the indicated temperature and time. Once melted, the samples were vortexed for 1 min to ensure the homogeneity of chloride in the samples. Then, the samples were analysed on TC using the above validated method.

Statistical Method

The descriptive statistics (denoted as mean, SD and range) and analysis using the Students't-test were employed using SPSS software version 13.0 (IBM Corporation, New York).

RESULTS AND DISCUSSION

The development of the proposed methodology was performed in two steps: (1) validation and optimisation of the instrument conditions, and (2) application to real samples (edible oils).

Method Performance

Evaluation of linearity, recovery percentage (repeatability and reproducibility), LOD and LOQ are essential to assess the method performance (Zanella *et al.*, 2000). The calibration curve was obtained by analysing five different solutions of known concentrations of chloride between 0.5 and 20.0 $\mu\text{g mL}^{-1}$ for three times.

The data for the calibration curve of chloride standard is shown in Table 2. The data illustrate excellent range of correlation coefficient (R^2) values of more than 0.999. This shows good linearity of the analytical method which reflects feasibility of the method. Values of the RSD are less than 10% for all concentrations (0.5, 1.0, 5.0, 10.0, 20.0 $\mu\text{g mL}^{-1}$) which are within the acceptable range according to the Association of Official Analytical Chemists (AOCS, 2016).

The LOD and LOQ for the method are shown in Table 3. The LOD and LOQ were found to be 0.03 and 0.10 $\mu\text{g mL}^{-1}$, respectively. These results demonstrate that the validated method is very sensible for detecting and quantifying chloride.

TABLE 2. CALIBRATION CURVE DATA OF CHLORIDE STANDARD

Concentration of chloride standard ($\mu\text{g mL}^{-1}$)	Injection volume (μL)	Mean value ($\mu\text{g mL}^{-1}$)	SD	RSD (%)
0.5	40	0.49	0.02	3.10
1.0	40	1.01	0.07	6.57
5.0	40	4.93	0.15	3.10
10.0	40	9.90	0.20	2.02
20.0	40	19.93	0.15	0.77

Note: SD - standard deviation; RSD - relative standard deviation.

TABLE 3. LINEAR EQUATION, LOD AND LOQ OBTAINED FOR QUANTIFICATION OF TC

Parameter	Data
LOD ($\mu\text{g mL}^{-1}$)	0.03
LOQ ($\mu\text{g mL}^{-1}$)	0.10
Calibration curve	$y = 0.9035x + 0.006$
R^2	0.999

Note: LOD - limit of detection; LOQ - limit of quantification; R^2 - correlation coefficient.

The accuracy and precision of the results, estimated in percent average of standard addition recoveries, was higher than 80% at all spiked levels (Table 4). Recoveries were found to be 84% to 95%, 82% to 86%, 81% to 92% and 87% to 91% for CPO, RBDPO, sunflower oil and canola oil, respectively. Average recoveries for all samples were in the range of 81% and 95%, which demonstrated the conformity to the guidelines for the recovery analysis from 70% and 110%. This coincides with the requirement of the Association of Official Analytical Chemists (AOCS, 2016). It is not possible to compare the experimental mean recoveries with reference values since there is no Reference Material (RM) for chloride in edible oil for these matrices.

TABLE 4. RECOVERIES OF CHLORIDE IN SPIKED SAMPLES

Sample	Concentration of spiked chloride ($\mu\text{g mL}^{-1}$)	Mean recovery $\pm$ SD	Recovery (%)
CPO	0.5	0.42 ± 0.01	84
	1.0	0.86 ± 0.02	86
	5.0	4.77 ± 0.24	95
RBDPO	0.5	0.41 ± 0.01	82
	1.0	0.85 ± 0.01	85
	5.0	4.32 ± 0.31	86
Sunflower oil	0.5	0.44 ± 0.01	88
	1.0	0.81 ± 0.01	81
	5.0	4.63 ± 0.09	92
Canola oil	0.5	0.45 ± 0.02	90
	1.0	0.87 ± 0.01	87
	5.0	4.55 ± 0.17	91

Note: CPO - crude palm oil; RBDPO - refined, bleached and deodourised palm oil; SD - standard deviation.

The precision of the methods was assessed by repeatability and reproducibility, as described in Materials and Methods. RSD were calculated under RSD repeatability (RSD_r) and RSD reproducibility (RSD_R) conditions, to evaluate the precision of the method. The RSD for repeatability of chloride was found to be 0.8% to 2.4% in unspiked CPO, 0.4% to 3.6% in CPO, 1.8% to 2.7% in unspiked RBDPO, 0.9% to 2.6% in RBDPO, 2.3% to 3.2% in unspiked sunflower oil, 1.5% to 3.5% in sunflower oil, 0.9% to 2.1% in unspiked canola oil and 1.5% to 3.8% in canola oil. The RSD_R was found to be between 1.2% to 5.1% (Table 5). However, the developed method was within the acceptable range of RSDs of less than 20%, with the performance criteria requirement of the Association of Official Analytical Chemists (AOCS, 2016) and less than 15%, in agreement with the Food and Drug Administration guidelines (FDA, 2012). Hence, this indicates that the results are satisfactory and the method can be successfully applied for accurate and routine analysis of chloride in edible oils.

The study also demonstrated that the TC recovery increased with the function of combustion temperature and oxygen flow rate. A preliminary study revealed that the TC percentage recovery from CPO increased in concomitant with the increase of the combustion temperature and oxygen flow rate (Table 6). The combustion temperature of 950°C and 200 mL min⁻¹ of oxygen flow rate is the optimal condition for the chloride analysis and this observation is in line with the findings by Cavallo *et al.* (2010). This combustion temperature of more than 950°C with the oxygen flow rate at 200 mL min⁻¹ ensures enough energy to break the chemical

TABLE 5. REPEATABILITY (RSD_r) AND REPRODUCIBILITY (RSD_R) FOR CHLORIDE DETERMINATION IN OPTIMAL INSTRUMENT CONDITIONS

Sample	Spike ($\mu\text{g mL}^{-1}$)	Repeatability (RSD_r) %			Reproducibility (RSD_R) %
		Day 1	Day 2	Day 3	
Unspiked CPO	-	1.5	2.4	0.8	2.6
CPO	0.5	2.8	1.3	3.6	2.3
	1.0	0.4	2.8	2.8	2.1
	5.0	3.2	1.8	1.7	2.6
Unspiked RBDPO	-	2.1	2.7	1.8	3.2
RBDPO	0.5	1.2	2.6	1.5	2.1
	1.0	0.9	1.5	1.4	1.2
	5.0	1.6	1.3	2.1	1.5
Unspiked sunflower oil	-	3.2	2.5	2.3	2.7
Sunflower oil	0.5	2.0	3.3	3.1	2.7
	1.0	1.7	2.7	3.5	4.2
	5.0	1.5	3.1	2.3	5.1
Unspiked canola oil	-	0.9	2.1	1.5	3.1
Canola oil	0.5	3.8	1.8	2.6	2.8
	1.0	1.8	3.2	2.6	1.7
	5.0	3.6	1.5	1.8	5.1

Note: CPO - crude palm oil; RBDPO - refined, bleached and deodourised palm oil.

bond between organic and inorganic chloride in order to reach the maximum chloride recovery. From this study, the developed method can accurately determine the TC content which comprises of organochloride and inorganic chloride in edible oil samples. However, a comprehensive study needs to be conducted to support this theory and to gain more valuable data.

TABLE 6. CHLORIDE RECOVERY OF DIFFERENT COMBUSTION TEMPERATURES AND OXYGEN FLOW RATES

Combustion temperature (°C)	Oxygen flow rate (mL min ⁻¹)	% Recovery		
		0.5 µg mL ⁻¹	1.0 µg mL ⁻¹	5.0 µg mL ⁻¹
750	150	66	58	70
850		71	73	69
950		81	75	82
750	200	73	79	69
850		77	74	82
950		89	86	92

Harmonisation of Sample Preparation of Crude Palm Oil

Research by the Analytical Methods Committee (AMC) showed that sample preparation is the most common cause of errors in trace analysis and that contamination is always a major concern during sample preparation (Paul, 2013). Appropriate sample preparation techniques are always required in analytical analysis to enrich target analytes, or transform the sample into a suitable form for subsequent instrumental analysis (Hu and He, 2012).

Before the analysis of TC, the preparation of the CPO needs to be optimised to obtain homogeneous and representative test portions to avoid incorrect data interpretation. Data on TC obtained in different samples of pre-treated CPO is shown in Table 7.

From the study, it was discovered that the suitable temperature and duration melting time for sample pre-treatment was at 70°C and 20 min. Data obtained from this parameter showed that the TC level was consistent with low SD value and RSD percentage. On the contrary, the inconsistency of data obtained from other parameters may be due to samples being not completely melted and thus, affecting the homogeneity of chloride in the samples. This inconsistency has caused a wide range of SD and RSD percentages.

Based on statistical terms, the smaller the SD and RSD percentages, the closer the numbers are to the mean (In *et al.*, 2015). In addition, the percentage RSD also provides general information about the performance of a method, where 10% or less generally indicates a good method performance. It is proposed that the melting temperature of 70°C and duration for melting procedure at 20 min as suitable to ensure proper homogeneity and consistency in obtaining chloride in a sample.

Analysis of TC Content in Commercial Cooking Oils

The developed method was applied to determine the TC present in palm oil. The average concentrations of TC in each of the palm oil samples used in the study are shown in Table 8. The results show a wide variation in TC concentrations among the samples analysed and the chloride content of CPO is higher than RBDPO.

The high chloride level in the CPO may be due to uptake of chloride to the palm fruit from soil, water and fertiliser (Ibrahim *et al.*, 2015). During the processing of the palm fruit, the steam used for sterilisation may affect the chloride level in CPO (Craft *et al.*, 2012). Among the many potential sources of inorganic chloride in the oil palm supply chain, potassium chloride (KCl) and ammonium chloride

TABLE 7. CONCENTRATION OF TC IN CPO AT DIFFERENT PRETREATED PARAMETERS

No.	Pre-treated parameter					
	50°C/10 min	50°C/20 min	60°C/10 min	60°C/20 min	70°C/10 min	70°C/20 min
1	2.6	3.5	2.4	3.8	3.3	3.6
2	3.4	1.9	2.8	3.2	3.1	3.2
3	2.7	2.5	1.6	2.4	2.8	3.4
4	2.8	2.8	3.1	2.6	2.6	3.0
5	1.9	2.4	3.5	2.9	3.4	2.9
6	3.5	1.7	2.6	3.3	3.9	3.7
7	3.9	3.7	2.7	1.8	2.4	3.2
Mean	3.0	2.6	2.7	2.9	3.1	3.3
SD	0.7	0.8	0.6	0.7	0.5	0.3
RSD (%)	22.6	28.5	22.2	23.0	16.8	9.0

Note: TC - total chloride; CPO - crude palm oil; SD - standard deviation; RSD - relative standard deviation.

(NH_4Cl) are regularly used as fertilisers to support the growth of oil palms and increase bunch yield (Freudenstein *et al.*, 2013). Other inorganic chlorides used as fertiliser such as calcium chloride (CaCl_2), magnesium chloride (MgCl_2), ferrous chloride (FeCl_2) and ferric chloride (FeCl_3), are also commonly used in oil palm plantation (Abdollah, 2010). These inorganic sources of chloride are suggested to accumulate in the palm fruits during oil palm growth. The muriate of potash fertiliser used in oil palm plantations as well as the coagulant used for water treatment in palm oil mills were also believed to be the source of chloride in CPO (Andrew, 2018). Ferric chloride (FeCl_3) is a well-known coagulant used for water treatment in Malaysia (Chua *et al.*, 2020) and therefore acts as a potential source of chloride-containing compounds in the water within the oil palm environment.

TABLE 8. CONCENTRATIONS OF TC IN 20 PALM OIL SAMPLES ANALYSED USING TCA

No.	Matrix	TC ($\mu\text{g mL}^{-1}$) (mean $\pm$ SD)
1	CPO	6.86 $\pm$ 0.49
2	CPO	4.51 $\pm$ 0.51
3	CPO	5.67 $\pm$ 1.08
4	CPO	3.91 $\pm$ 0.32
5	CPO	2.26 $\pm$ 0.29
6	CPO	4.91 $\pm$ 1.01
7	CPO	2.88 $\pm$ 0.58
8	CPO	3.21 $\pm$ 0.52
9	CPO	4.73 $\pm$ 1.51
10	CPO	8.16 $\pm$ 0.93
11	CPO	3.97 $\pm$ 1.18
12	CPO	3.53 $\pm$ 0.62
13	CPO	1.92 $\pm$ 0.33
14	CPO	4.85 $\pm$ 0.81
15	CPO	2.31 $\pm$ 0.48
16	CPO	2.91 $\pm$ 0.32
17	RBDPO	0.41 $\pm$ 0.12
18	RBDPO	1.01 $\pm$ 0.15
19	RBDPO	0.75 $\pm$ 0.12
20	RBDPO	0.66 $\pm$ 0.09

Note: TC - total chloride; TCA - total chloride analyser; CPO - crude palm oil; RBDPO - refined, bleached and deodourised palm oil; SD - standard deviation.

The study also showed that 93.75% of the CPO samples contained TC concentrations varying from 1.92 to 8.16 $\mu\text{g mL}^{-1}$. Empty fruit bunches (EFB) and palm oil mill sludge (POMS) contain a small amount of residual oil that is worth recovered. Hence, some

mills recover and blend these residual oils into CPO to increase oil extraction rate (OER). This practice has affected the quality parameters of CPO such as free fatty acids (FFA), peroxide value (PV), phosphorus level and also increases the level of chloride in CPO (Nizam and Mahmud, 2021).

CPO normally undergoes the refining process through degumming, bleaching and deodourisation. During bleaching process some of the chlorinated compounds are adsorbed onto the bleaching earth and hence reduced the chloride content in RBDPO (Ramli *et al.*, 2020).

The analysis of chloride in other commercial edible oils also showed a wide variation in TC level. Table 9 shows that in almost all edible oils of soft oil cluster, the chloride level was below 1 $\mu\text{g mL}^{-1}$. However, for the nut oil cluster, only walnut oil and peanut oil showed chloride levels of more than 1 $\mu\text{g mL}^{-1}$. The differences in the levels of chloride in each of the edible oil may be due to the oil processing during extraction and refining processes. For fruit oils, some vegetative parts of the fruits may contribute to the chloride content, as seen in CPO and if the vegetative parts are removed during processing, the chloride content is expected to be low, as in the extra virgin olive oil. However, further pressing of the olive pressed mass showed an increase in the chloride content as in the olive oil and pomace oil.

Most of the seed oils are extracted using solvent and followed by chemical refining. The alkaline and bleaching earth in the refining process have the capacity to remove the chloride in the oils. Hence, soft oils contain low chloride as shown in Table 9. Nut oils as in peanuts contain high levels of chloride because of the exposure and direct contact with the soil. Analysis of blended oils shown in Table 9 indicates that blending of soft oils with palm olein had slightly increased the chloride content, while blending between soft oils does not affect the chloride content.

The TC content in various refined oils as illustrated in Table 9, reflects the 3-MCPDE content in the oils. Mixture of palm olein with soft oils had exhibited higher TC content as compared to other blended soft oils. It was reported by Abd. Razak *et al.* (2012) that blended palm olein, sesame and peanut showed the highest 3-MCPDE content compared to other soft oils including canola, corn, sunflower, rice bran and soybean oils. The TC content in these soft oils is among the lowest. It was also found that palm olein and olive pomace oil contained the second and third highest 3-MCPDE content, respectively (Abd. Razak *et al.*, 2012), as reflected on the higher TC content in these types of oils. Hence, TC content in edible oils as tabulated in Table 9 is very useful in predicting the amount of 3-MCPDE content in the oils.

TABLE 9. CONCENTRATIONS OF TC IN 20 COMMERCIAL REFINED OIL SAMPLES ANALYSED USING TCA

No.	Sample	TC ($\mu\text{g mL}^{-1}$) (mean $\pm$ SD)
Soft oils		
1	Canola oil	0.670 ± 0.062
2	Sunflower oil	0.577 ± 0.024
3	Rice bran oil	0.523 ± 0.006
4	Roasted sesame oil	0.715 ± 0.006
5	High oleic sunflower oil	0.317 ± 0.021
6	Grapeseed oil	0.269 ± 0.030
7	Black seed oil	0.249 ± 0.042
Fruit oils		
8	Extra virgin olive oil	ND
9	Olive oil	0.563 ± 0.075
10	Olive pomace oil	0.990 ± 0.002
11	Palm olein	1.400 ± 0.073
Lauric oils		
12	Coconut oil	0.287 ± 0.042
13	Cold pressed virgin coconut oil	0.448 ± 0.045
14	Palm kernel oil	ND
Nut oils		
15	Walnut oil	2.053 ± 0.144
16	Peanut oil	1.104 ± 0.134
Blended oils		
17	Mixture palm olein and sunflower oil	0.853 ± 0.037
18	Mixture canola oil and corn oil	0.203 ± 0.018
19	Mixture canola oil and sunflower oil	0.442 ± 0.063
20	Mixture canola oil and soybean oil	0.130 ± 0.015

Note: TC - total chloride; TCA - total chloride analyser; ND - not detected; SD - standard deviation.

CONCLUSION

The validated method for chloride determination in edible oils by the TCA presented satisfactory values of linearity, LOD and LOQ. In the same way, the acceptance criteria for validating the methodology was achieved, with a percentage RSD lower than 10% in the precision assays and a recovery of above 80%. This method can potentially be applied for the determination of chloride in edible oils. The validated methods were successfully applied for the determination of TC in commercial cooking oils purchased in Malaysia. The monitoring and elimination of chloride in crude edible oil should be implemented by the industry in order to ensure that edible oils produced are safe for human consumption and free from process developed contaminants, in this case 3-MCPDE.

ACKNOWLEDGEMENT

The authors wish to thank the Director-General of MPOB for permission to publish this article. Appreciation also goes to Mohd Noor Petrus, Norazilah Pardi and those who have directly or indirectly contributed their technical assistance in ensuring the success of this work.

REFERENCES

- Abdollah, N A B (2010). *Some problem in chemical and physical treatment of water supply*. Ph.D thesis, Universiti Teknologi Malaysia.
- Abd. Razak, R A; Kuntom, A; Siew, W L; Ibrahim, N A; Ramli, M R; Hussein, R and Nesaretnam, K (2012). Detection and monitoring of 3-monochloropropane-1, 2-diol (3-MCPD) esters in cooking oils. *Food Control*, 25: 355-360.
- Abraham, K; Appel, K E; Berger-Preiss, E; Apel, E; Gerling, S; Mielke, H; Creutzenberg, O and Lampen, A (2013). Relative oral bioavailability of 3-MCPD from 3-MCPD fatty acid esters in rats. *Arch. Toxicol.*, 87: 649-659.
- Andrew, Y K C (2018). Crude palm oil de-chlorination. *Palm Oil Engineering Bulletin*, 128: 51-54.
- AOCS (2016). Guidelines for Standard Method Performance Requirements. Appendix F. 29th edition. Vol. 2. Association of Analytical Chemists (AOCS).
- Cavallo, G; Metrangolo, P; Pilati, T; Resnati, G; Sansotera, M and Terraneoab, G (2010). Halogen bonding: A general route in anion recognition and coordination. *Chem. Soc. Rev.*, 39: 3772-3783.
- Chua, S C; Chong, F K; Malek, M A; Mustafa, M R; Ismail, N; Sujarwo, W; Lim, J W and Ho, Y C (2020). Optimised use of ferric chloride and sesbania seed gum (SSG) as sustainable coagulant aid for turbidity reduction in drinking water treatment. *Sustainability*, 12(6): 2273.
- Craft, B D and Destailats, F (2014). Formation mechanisms. *Process Contaminants in Edible Oils* (MacMahon ed.). AOCS Press, Urbana, Illinois. p. 7-21.
- Craft, B D; Nagy, C K; Sandoz, L and Destailats, L (2012). Factors impacting the formation of monochloropropanediol (MCPD) fatty acid diesters during palm (*Elaeis guineensis*) oil production. *Food Addit. Contam. A*, 29(3): 354-361.

- Dianne, J (2021). The advantages of potentiometric Titration. <https://sciencing.com/advantages-potentiometric-titration-6532902.html>, accessed on 30 August 2021.
- EFSA (2016). Risks for human health related to the presence of 3-and 2-monochloropropanediol (MCPD), and their fatty acid esters, and glycidyl fatty acid esters in food. *EFSA Journal*, 14(5): 4426.
- European Commission Regulation (2006). No. 1881/2006 of 19 December 2006 on setting maximum levels for certain contaminants in foodstuffs. *Official Journal of the European Union*, L364: 5-24.
- FDA (2012). FDA foods program guidelines for chemical methods, Version 1.0 2/28/2012.
- Freudenstein, A; Weking, J and Matthäus, B (2013). Influence of precursors on the formation of 3-MCPD and glycidyl esters in a model oil under simulated deodorization conditions. *Eur. J. Lipid Sci. Technol.*, 115: 286-294.
- Hamlet, C and Sadd, P (2002). Kinetics of 3-chloropropane-1,2-diol (3-MCPD) degradation in high temperature model systems. *Eur. Food Res. Technol.*, 215: 46-50.
- Hamlet, C G; Sadd, P A; Crews, C; Velisek, J and Baxter, D E (2002). Occurrence of 3-chloropropane-1,2-diol (3-MCPD) and related compounds in foods: A review. *Food Addit. Contam.*, 19: 619-631.
- Hu, B and He, M (2012). Pre-concentration and sample treatment techniques for trace element analysis. *Reference Module in Chemistry, Molecular Sciences and Chemical Engineering*, 3: 365-394.
- Ibrahim, N A; Ramli, M R and Lin, S W (2015). Possible factors that cause the formation of 3-monochloropropane-1,2-diol (3-MCPD) esters. *Palm Oil Developments*, 57: 1-6.
- In, J H; Kim, C K and Jeong, S (2015). Analysis of relative standard deviation of spectral line intensity and intensity ratio in laser-induced breakdown spectroscopy using $\text{Cu}_{1-x}\text{Ga}_x\text{Se}_2$ thin film samples. *J. Anal. At. Spectrom.*, 30(10): 2107-2119.
- International Agency for Research on Cancer (IARC) (2012). 3-Monochloro-1,2-propanediol. IARC monographs on the evaluation of carcinogenic risks to humans.
- Krzyzaniak, S R; Santos, R F; Dalla Nora, F M; Cruz, S M; Flores, E M M and Mello, P A (2016). Determination of halogens and sulphur in high-purity polyimide by IC after digestion by MIC. *Talanta*, 158: 193-197.
- Liao, W Y and Chou, T C (2006). Fabrication of a planar-form screen-printed solid electrolyte modified Ag/AgCl reference electrode for application in a potentiometric biosensor. *Anal. Chem.*, 78: 4219-4223.
- Mesko, M F; Costa, V C; Picoloto, R S; Bizzi, C A and Mello, P A (2016). Halogen determination in food and biological materials using plasma-based techniques: Challenges and trends of sample preparation. *J. Anal. At. Spectrom.*, 31: 1243-1261.
- Mishra, V G; Das, M K; Shah, D J; Jeyakumar, S; Tomar, B S and Ramakumar, K L (2018). Simultaneous determination of borate, chloride and molybdate in pyrohydrolysis distillates of plant and soil samples by ion chromatography. *J. Chromatogr. A*, 1532: 144-149.
- Mitsubishi Chemical Analytech (2014). Operational manual (horizontal furnace) for coulometer Model MCD-210.
- Nagy, K; Sandoz, L; Craft, B D and Destailats, F (2011). Mass defect filtering of isotope signatures reveals the source of chlorinated palm oil contaminants. *Food Addit. Contam. A*, 28: 1492-1500.
- Nizam, A F A and Mahmud, M S (2021). Food quality assurance of crude palm oil: A review on toxic ester feedstock. *OCL*, 28(6): 23.
- Paul, W (2013). Minimizing contamination during sample preparation for trace analysis. <https://www.elgalabwater.com/blog/minimizing-contamination-during-sample-preparation-trace-analysis>, accessed on 19 August 2021.
- Ramli, M R; Siew, W L; Ibrahim, N A; Kuntom, A and Abd Razak, R A (2015). Other factors to consider in the formation of chloropropandiol fatty esters in oil processes. *Food Addit. Contam. A*, 32: 817-824.
- Ramli, M R; Tarmizi, A H A; Hammid, A N A; Razak, R A A; Kuntom, A; Lin, S W and Radzian, R (2020). Preliminary large scale mitigation of 3-monochloropropane-1,2-diol (3-MCPD) esters and glycidyl esters in palm oil. *J. Oleo Sci.*, 69: 815-824.
- Silva, S M; Sampaio, K A; Ceriani, R; Verhé, R; Stevens, C; De Greyt, W and Meirelles, A J A (2014). Effect of type of bleaching earth on the final colour of refined palm oil. *LWT - Food Sci. Technol.*, 59: 1258-1264.

Smidrkal, J; Tesarova, M; Hradkova, I; Bercikova, M; Adamcikova, A and Filip, V (2016). Mechanism of formation of 3-chloropropan-1,2-diol (3-MCPD) esters under conditions of the vegetable oil refining. *Food Chem.*, 211: 124-129.

Stadler, R H (2015). Monochloropropane-1,2-diol esters (MCPDEs) and glycidyl esters (GEs): An update. *Curr. Opin. Food Sci.*, 6: 12-18.

Tian, Y; Zhang, P; Zhao, K; Du, Z and Zhao, T (2020). Application of Ag/AgCl sensor for chloride

monitoring of mortar under dry-wet cycles. *Sensors*, 20(5): 1394.

Zall, D M; Fisher, D and Garner, M Q (1956). Photometric determination of chlorides in water. *Anal. Chem.*, 28: 1665-1668.

Zanella, R; Primel, E G; Goncalves, F F and Martins, A F (2000). Development and validation of a high performance liquid chromatographic method for the determination of clomazone residues in surface water. *J. Chromatogr. A*, 904: 257-262.

EFFECTS OF PALM OIL-BASED SURFACTANT USED IN PESTICIDE FORMULATION ON TERRESTRIAL PLANTS

ASMA LIYANA SHAARI^{1*}; NOORAZAH ZOLKARNAIN¹; ISMAIL AB. RAMAN¹ and
RAZMAH GHAZALI^{1*}

ABSTRACT

Polyoxyethylene (20) sorbitan monooleate, or POE 20S, is one of palm-based surfactants commonly used as adjuvant in pesticide formulation. Toxicity study of POE 20S toward plants is still lacking since public is usually misinformed that adjuvant is non-toxic. Therefore, a study was conducted to investigate the effect of POE 20S on terrestrial plants, i.e., tomato and corn. At every one-week interval, aqueous solutions of POE 20S at 1%, 3%, 5%, 7% and 10% w/v were sprayed onto planted seeds. Germination time, number of seedlings survived, shoot length, shoot weight and visual symptoms of injury were determined and compared with control. The results showed that the first spray of all POE 20S concentrations did not inhibit the germination of both tomato and corn seeds. All seeds germinated and emerged. However, the next 10% spray treatment of POE 20S showed significantly lower survival in both plants due to necrosis and severe shrivelling of their leaves. In terms of germination time, shoot length and shoot weight, only tomato plants showed no significant differences between all concentrations tested and the control. We concluded that POE 20S is safe and not harmful to non-target terrestrial plants if appropriate dilution is used for pesticide formulation.

Keywords: ecotoxicity, palm oil-based surfactant, pesticide, polyoxyethylene (20) sorbitan monooleate, seed germination.

Received: 1 September 2021; **Accepted:** 16 February 2022; **Published online:** 16 March 2022.

INTRODUCTION

Tremendous benefits have been derived from the use of pesticides, especially in agriculture sector, where pesticides help to increase world food production by reducing losses from weeds, diseases and insect pests (Alavanja, 2009). In 2019 alone, approximately 2 million tonnes of pesticides were utilised worldwide, where China is the major user, followed by the United States of America (USA) and Argentina. By the year 2020, the global pesticide usage has been estimated to increase up to

3.5 million tonnes (Sharma *et al.*, 2019). Consumptions of pesticide also increase rapidly in developing countries in Southeast Asia, *e.g.*, Malaysia, Cambodia, Laos and Vietnam (Schreinemachers *et al.*, 2015), where Malaysia recorded 67 300 t pesticide consumption in 2017 (Müller, 2020). The use of pesticides was predicted to increase yearly due to agricultural activities (FAO, 2017).

The pesticide formulation is a mixture of active and other ingredients. Active ingredients function as the toxicant, which are capable of preventing, destroying, repelling or mitigating pests. Meanwhile, other ingredients include the solvent, carrier and adjuvant (Herzfeld and Sargent, 2011), which are inactive and with no toxic effect, but are added to pesticide formulations to improve the pesticide's ability to control pests (Abhilash and Singh, 2009). Surfactants are the major type of

¹ Malaysian Palm Oil Board,
6 Persiaran Institusi, Bandar Baru Bangi,
43000 Kajang, Selangor, Malaysia.

* Corresponding author e-mail: asmaliyana@mpob.gov.my;
razmah@mpob.gov.my

adjuvant (Krogh *et al.*, 2003), which helps to improve the emulsifying, dispersing, spreading, sticking or penetrating properties of pesticides (Miller and Westra, 1998) and enhances the effectiveness of pesticide application on foliage (Aumatell, 1996). On soils, surfactants act as agents responsible for increasing the pesticide mobility (Huggenberger *et al.*, 1973). Non-ionic surfactants are the most important type of surfactant that are employed in pesticide formulations (Seaman, 1990). This is because, non-ionic surfactants are compatible with most active ingredients and appropriate to use with systemic pesticides.

Recent oil palm downstream research and development (R&D) efforts have focused more on the development of products utilising various oleochemical derivatives, including palm-based surfactant (Parveez *et al.*, 2020). For example, one of the studies conducted by the Malaysian Palm Oil Board (MPOB) indicated that polyoxyethylene (20) sorbitan monooleate or POE 20S has the potential to be used as adjuvant in pesticide formulation (Ismail *et al.*, 1998; 2010; 2014) since it was observed that mixed surfactants of POE 20S and C₁₂-C₁₄ fatty alcohol ethoxylate exhibited superior emulsification efficiency and stability (Sumaiyah *et al.*, 2019). This surfactant is an oily liquid derived from ethoxylated sorbitan (a derivative of sorbitol) and esterified with fatty acids (Herzfeld and Sargent, 2011). At present, POE 20S is among the non-ionic surfactants that are commonly used in pesticide formulation (Ismail *et al.*, 1998; 2010; Sumaiyah *et al.*, 2019).

According to Norris (1982) and Ismail *et al.* (2014), most surfactants used in pesticide formulation, including POE 20S, are generally considered to be inert and non-phytotoxic. In some cases, surfactant may cause mild phytotoxicity but with no effect to overall growth, and in other cases, surfactant may inhibit plant growth when the concentrations increased (Bhat *et al.*, 1992). However, according to US EPA (1997), many consumers have a misleading impression of the term 'inert ingredient', and believe it is harmless. Numerous studies have found that despite the widespread assumption that adjuvants or inert ingredients are physically, chemically and biologically inactive, substantial evidence suggested that inert ingredients by themselves harm all non-target organisms studied (Mullin *et al.*, 2016).

As described by Fishel (2014), some plants are affected by surfactant because certain surfactants may be capable of causing phytotoxicity or injury in terrestrial plants. However, the phytotoxicity data on terrestrial plants are quite scattered and they are mainly measured for linear alkylbenzene sulphonate (LAS), a synthetic surfactant derived from mineral oil (Endo *et al.*, 1969; Luxmoore *et al.*, 1974; Ying, 2006) and no toxicity data of POE 20S towards terrestrial plants are reported anywhere.

Since POE 20S has been widely used as adjuvant in pesticides, it is essential to assess its toxicity in order to provide an estimate of its potential to cause harm on terrestrial plants. This is because, theoretically, active ingredient used should kill the target pest or weed, but there is also possibility that the surfactant used as adjuvant may cause toxicity to surrounding plants. Hence, the surfactant assessment toward terrestrial plants is relevant and necessary in order to obtain more toxicity data on available surfactant. This data can contribute in finding the tolerance limit of certain surfactants to terrestrial plants and thus, a comprehensive database of surfactant toxicity can be developed.

According to Asma Liyana *et al.* (2020), no toxicity study of surfactant toward Malaysian native terrestrial plants has been reported. Since MPOB has developed palm-based surfactants and formulated pesticides, it is the opportunity for MPOB to establish a facility for toxicity testing of these products toward terrestrial plants, especially to cater for the local research. The data generated will indirectly provide important information for reference by current and future generation. For that reason, the present study was performed to evaluate the toxicity effect of POE 20S, a palm oil-based surfactant on the germination and emergence of terrestrial plants' seeds, *i.e.*, corn and tomato under laboratory conditions.

MATERIALS AND METHODS

Plant Species

Seeds from two plant species, *Zea mays* or corn (represents monocotyledonous plant) and *Solanum lycopersicum* or tomato (represents dicotyledonous plant), purchased from a local supplier, were used in this study. These species were chosen because they are two of the test species recommended in the test guideline Organization for Economic Co-operation and Development (OECD) 208 - Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test (OECD 208, 2006). Besides, they are economically important, widely distributed, abundance and commonly used in research. Seeds used in this study were not treated with fungicides, insecticides or repellents prior to study initiation.

Soil Substrate

The soil used in the study was commercial sandy loam potting soil produced by local supplier. The soil was sent for elemental analysis (determination of nitrogen, carbon and sulphur content) before use in the experiment in order to ensure the required quality of soil used is consistent throughout the study. The properties of the soil were as follows: total nitrogen 1.34%, total carbon 44.00%, organic

carbon 41.17%, total sulphur 1.52% and pH 5.78. The most important criteria of the commercial potting soil used is that it must contain not less than 1.50% organic carbon.

Experimental Design and Test Conditions

The experimental design for this study followed a standard method by OECD, *i.e.*, test guideline OECD 208 - Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test (OECD 208, 2006), which consisted of a control and treatment groups. A control group, which received no test substance, was maintained to assure that effects observed are associated with exposure to the test substance and this group was identical in every aspect to the treatment groups except for exposure to the test substances. With the help of marked stick, seeds were sown at uniform depth of 2 cm and 4 cm for respective tomato and corn in a temperature-controlled laboratory. One and 2-3 seeds were planted per pot for tomato and corn, respectively. Each treatment of both species consisted of 40 replicated pots.

Tomato and corn required different environmental (light and temperature) conditions to grow. Therefore, a preliminary study was performed to obtain the suitable environmental conditions for both plants. For tomato seeds, growth tests were performed under laboratory conditions for 21 days with additional aquarium lamp mounted on the culture rack to obtain light intensity of 1500 ± 500 lux. The photoperiod was set for 16 hr light and 8 hr dark using timer, while growth temperature was set at $22^\circ\text{C} \pm 2^\circ\text{C}$. Corn seeds were planted in the same laboratory conditions as tomato. However, germinated corn seeds need warmer condition with average temperature of $25^\circ\text{C} \pm 5^\circ\text{C}$ and young corn plants with leaves need higher light intensity from natural sunlight (approximately 4000 ± 1000 lux) for 12 hr per day. During the growth period, tomato plants were watered using sub-irrigated planter system, where a cup filled with reverse osmosis water was placed under each test pot. Meanwhile, corn plants were watered by spraying manually two times a day with reverse osmosis water.

Application of Test Substance

Polyoxyethylene (20) sorbitan monooleate (POE 20S, Sigma-Aldrich) was used as a test substance in this study. POE 20S, also known as Tween 80, is generally used as a non-ionic surfactant and emulsifier in pesticide and cosmetic formulation. A purity of 98% and hydrophilic-lipophilic balance (HLB) of 10 is provided in the product specification. After seeds sowing, the solutions of different POE 20S concentrations were sprayed immediately onto the planted seeds in each pot using a manual hand

sprayer. POE 20S doses were applied again onto both plant species at a one-week interval. A sufficient dosage of surfactant solutions (approximately 3-5 mL per spray per pot) were sprayed into the pots to uniformly wet the planted area or emerged shoots and leaves. Concentrations tested were 0 (control), 1%, 3%, 5%, 7% and 10% w/v in aqueous solution. Selection of these concentrations was based on water solubility properties of the POE 20S, where 10% w/v was the highest concentration used since this is the maximum solubility level for POE 20S to be totally soluble in water (Chemical Book, 2016).

Observation and Measurement

The germination of tomato seeds was completed within 10 days of planting, while corn seeds germination was completed within shorter time, *i.e.*, seven days. The number of seedlings germinated were counted in each pot to determine the germination percentage (GP) (Mahmood and Usman, 2014) as in Equation (1):

$$\text{Germination percentage (GP)} = \frac{\text{Total number of seedlings germinated}}{\text{Total number of seeds planted}} \times 100 \quad (1)$$

Experiments were continued for another 11 days and seven days for tomato and corn, respectively, to observe differences in young plants' growth and toxicity symptoms. Besides, percentage of survivals of emerged plants was also calculated at the end of experiment, *i.e.*, on day 21 and 14 for tomato and corn, respectively as in Equation (2):

$$\text{Survival} = \frac{\text{Total number of live plants at the end of experiment}}{\text{Total number of emerged plants}} \times 100 \quad (2)$$

The endpoints seed germination, shoot length, and fresh and dry biomass were assessed for each species after 21 days and 14 days for tomato and corn plants, respectively. The test was considered valid only if more than 70% of seeds in the control had a successful germination. At harvest, shoots were cut at the shoot-root junction and the shoot length was measured. The fresh shoots were dried in an oven at $60^\circ\text{C} \pm 1^\circ\text{C}$ for 24 hr to determine the dry biomass.

Phytotoxicity Test

Germination and early growth of tomato and corn plants treated with POE 20S aqueous solution may show phytotoxicity in some treatments.

Therefore, all treatments were assessed for phytotoxicity potential using test guideline OECD 208 - Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test (OECD 208, 2006). Visual symptoms of treatments and control groups were observed and recorded. At harvest, visual phytotoxicity symptoms (*e.g.*, chlorosis, necrosis, wilting, *etc.*) were determined based on leaf injury using a modified morphological score for phytotoxicity (Vanhala *et al.*, 2004) as in *Table 1*.

TABLE 1. MODIFIED MORPHOLOGICAL SCORE FOR PHYTOTOXICITY

Score	Effects/ symptoms
0	No symptom/ damage/ injury
10	Negligible symptom
20	Chlorosis or yellowing of the leaf tips
30	Chlorosis up to the whole lamina
40	Twisting leaves and stunting, but no burning
50	Wilting leaves and stunting, but no burning
60	Necrosis or browning/ burning of the leaf tips
70	Moderate necrosis up to the whole lamina
80	Severe necrosis of the whole leaves and stem
90	All leaves dry, shriveled and necrotic
100	Dead plant

Source: Vanhala *et al.* (2004).

Phytotoxicity symptoms were rated based on severity of leaf injury or plant damage by visual observation on plant morphology using phytotoxicity rating scale of 0 to 10 (Ambarish *et al.*, 2017) as in *Table 2*.

TABLE 2. PHYTOTOXICITY RATING SCALE

Rating	% Phytotoxicity (leaf injury/ damage)
0	No phytotoxicity
1	1-10
2	11-20
3	21-30
4	31-40
5	41-50
6	51-60
7	61-70
8	71-80
9	81-90
10	91-100

Source: Ambarish *et al.* (2017).

Based on phytotoxicity rating scale, percent phytotoxicity index (PPI) was computed using the Equation (3):

$$\text{PPI} = \frac{\text{Sum of all numerical ratings}}{\text{Total number of plants observed} \times \text{Maximum phytotoxicity rating}} \times 100 \quad (3)$$

Based on PPI, which measures the mean percent of leaf injury or plant damage, phytotoxicity levels of surfactants were determined as low, medium or high as in *Table 3*.

TABLE 3. DETERMINATION OF PHYTOTOXICITY LEVEL

PPI	Phytotoxicity level
0	Nil
1-5	Low
5-10	Medium
>10	High

Source: Ambarish *et al.* (2017).

Statistical Analysis

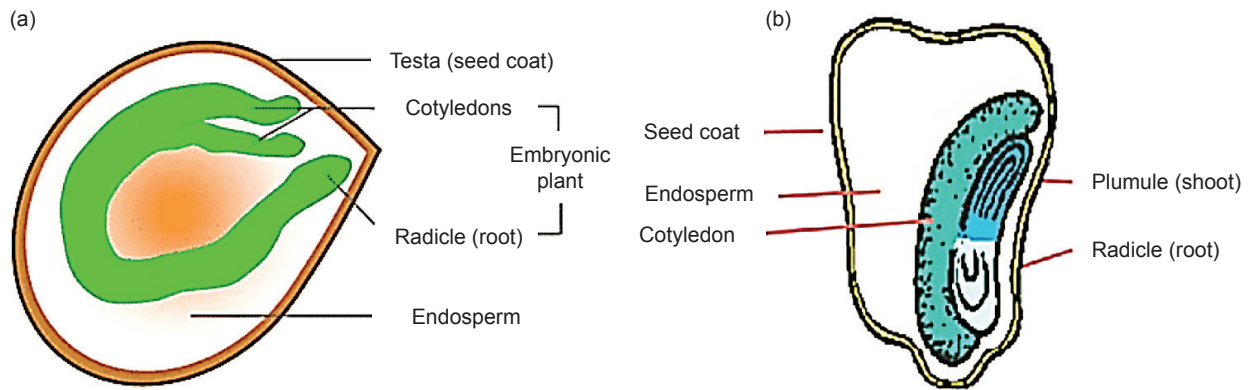
Data of seed germination, plants survival, germination time, shoot length and shoot weight were analysed using one-way analysis of variance (ANOVA) on SPSS 23 software (IBM Corporation, Armonk, New York, USA). The results are presented as mean $\pm$ standard deviation and data from the different treatment and control were compared by Tukey's honestly significant difference (HSD) range test. Differences were considered significant when $p < 0.05$.

RESULTS AND DISCUSSION

Seed Germination

Seed germination is a resumption of embryo growth resulting in seed coat rupture and emergence of the young plant (Kozłowski and Pallardy, 1997). *Figure 1* provides a simple diagram of tomato and corn seeds before germination. Percentage of seed germination is an important parameter for analysing the growth of seed under laboratory conditions. In this study, the seedling germination of tomato and corn fulfilled the validity criteria described by the standard guideline, where more than 70% of the seeds in the control group successfully germinated.

After the first spray treatment of 1%, 3%, 5%, 7% and 10% w/v POE 20S aqueous solution, *i.e.*, immediately after seeds have been planted, all tomato and corn seeds germinated and there were



Source: e-Limu (2015); Lichtenthaler (1996).

Figure 1. (a) Tomato, and (b) corn seeds before germination.

no significant adverse effects observed. Thus, the differences of GP between control and respective treatments were not statistically significant with GP value around 100%. The results indicated that the spraying of POE 20S solution immediately after seed planting did not inhibit the seed germination of both plants. In general, GP remained at a relatively high level for both plants at all five POE 20S concentrations tested. Even at the highest concentration tested, *i.e.*, 10%, there was no inhibition in the seed germination. This may be due to the fact that natural coating around the seeds act as a barrier between the embryo and its immediate environment. Therefore, the spraying of POE 20S solution immediately after seed planting may lead the solution to be absorbed by the seed coats and the seed coats simultaneously protected the embryo, thus, did not affect the germination (Di Salvatore *et al.*, 2008). Besides, Wong *et al.* (2001) also reported that seed germination is less sensitive to chemicals or toxicants than seed growth, in which the effect of exposure to the toxicant is higher only after the radical has emerged.

Survival and Growth Measurement

POE 20S solution was applied into the pot at one-week intervals throughout the experiment, regardless of whether or not germination occurred. Not all germinated seeds grew healthily until the end of the test period. In 5% w/v and 7% w/v treatments, only 85.0% and 62.5% of tomato, respectively, survived until end of the test period, while seeds treated with 10.0% w/v POE 20S exhibited the lowest survival of young tomato plants, *i.e.*, 30.0%. As for corn, all young plants in all treatments survived except plants treated with 10.0% w/v PEO 20S solution. At this concentration, only 42.5% of the corn plants survived, and the

rest were found dead at the end of the test. A trend was observed where an increase in concentration of tested surfactant decreased the percentage of seed germination and seedlings survival (Galvez *et al.*, 2018). In this study, as seen in both tomato and corn, the increasing concentrations of POE 20S solution also reduced the survival of the emerged plants. Significant differences ($p < 0.5$) in percentage of survived plants were found among the concentrations studied, *i.e.*, control, 1% and 3% > 5% and 7% > 10% in tomato; and control, 1%, 3%, 5% and 7% > 10% in corn. The survival percentages of emerged tomato and corn until end of the test period are shown in Figure 2.

As expected, the increase in surfactant concentrations resulted in a reduction of survival in emerged plants. Poor survival of emerged plants of both species in concentrated treatments (5%, 7% and 10% w/v) suggested that phytotoxicity symptoms may appear only at high POE 20S concentrations, *i.e.*, 5%, 7% and 10% w/v. When comparing the survival trend of both plants, it was clearly observed that corn survived better in POE 20S as compared to tomato. Ilic *et al.* (2015) explained that in the monocotyledonous seed, the embryo is enclosed by a thicker endosperm. It is possible that the thicker endosperm of corn seed protects the embryo from the effect of surfactant, particularly at the higher surfactant concentration.

Different indices, *i.e.*, germination time, shoot length and shoot weight of survived tomato and corn were measured and calculated after 21 and 14 days, respectively. The results are presented in Tables 4 and 5. As compared to control, the seedlings of tomato and corn showed delay of one to five days in their germination time with the increasing concentrations of POE 20S solution. According to Kimball *et al.* (2011), a delay in germination has direct influences on seedling

growth and physiology. As observed in this study, increasing POE 20S solution's concentration caused delay in seed germination and significantly affected both shoot length and shoot weight as

well. The observed data allow formulators to select appropriate concentration of POE 20S solution to be used as adjuvant in their products that will not pose bad effect on other non-target plants.

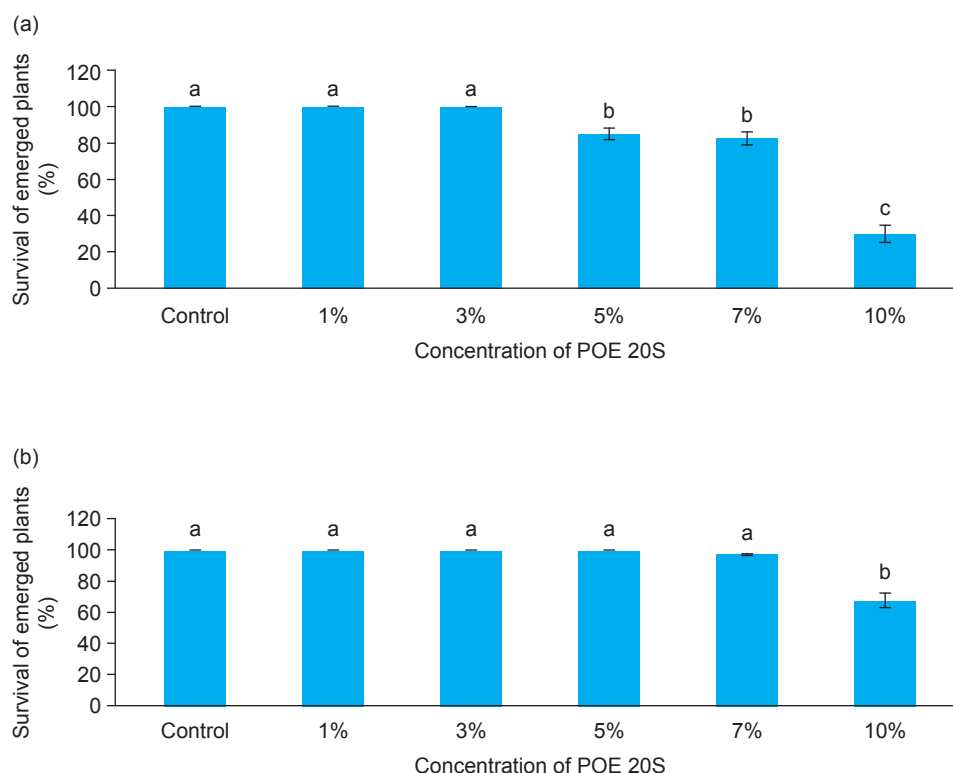


Figure 2. Percentage of survived emerged (a) tomato and (b) corn at the end of test, i.e., day 21 and 14, respectively. Error bars represent standard deviation. Columns with the same letter above them are not significantly different.

TABLE 4. MEAN $\pm$ STANDARD DEVIATION OF GERMINATION TIME, SHOOT LENGTH AND SHOOT DRY WEIGHT OF TOMATO PLANTS AT THE END OF TEST (Day 21)

POE 20S concentration (%)	Germination time (days)	Shoot length (cm)	Shoot dry weight (g)
0 (control)	5 $\pm$ 1	6.0 $\pm$ 0.3a	0.0048 $\pm$ 0.0009a
1	5 $\pm$ 1	5.4 $\pm$ 0.8b	0.0042 $\pm$ 0.0009b
3	6 $\pm$ 1	4.2 $\pm$ 0.7c	0.0030 $\pm$ 0.0005c
5	7 $\pm$ 1	3.8 $\pm$ 0.7cd	0.0027 $\pm$ 0.0005c
7	7 $\pm$ 1	3.4 $\pm$ 0.8d	0.0024 $\pm$ 0.0005cd
10	7 $\pm$ 1	3.3 $\pm$ 0.3d	0.0021 $\pm$ 0.0006d

Note: Means in the same column with the same letter are not significantly different.

TABLE 5. MEAN $\pm$ STANDARD DEVIATION OF GERMINATION TIME, SHOOT LENGTH AND SHOOT DRY WEIGHT OF CORN PLANTS AT THE END OF TEST (Day 14)

POE 20S concentration (%)	Germination time (days)	Shoot length (cm)	Shoot dry weight (g)
0 (control)	4 $\pm$ 1	17.5 $\pm$ 1.1a	0.0289 $\pm$ 0.0034a
1	4 $\pm$ 1	17.3 $\pm$ 1.0a	0.0277 $\pm$ 0.0028ab
3	4 $\pm$ 1	17.1 $\pm$ 0.7ab	0.0261 $\pm$ 0.0033b
5	5 $\pm$ 1	16.6 $\pm$ 1.0b	0.0229 $\pm$ 0.0035c
7	6 $\pm$ 1	14.6 $\pm$ 0.9c	0.0176 $\pm$ 0.0033d
10	7 $\pm$ 1	14.2 $\pm$ 1.0c	0.0166 $\pm$ 0.0012d

Note: Means in the same column with the same letter are not significantly different.

Phytotoxicity Test

Several differences in visible symptoms of toxicity were observed between control and treated groups at the end of experiments. In control, no detrimental symptoms were detected for both plants and so were the 1% and 3% w/v POE 20S for tomato. However, 5% and 7% w/v POE 20S resulted in moderate necrosis, where clear burning symptoms could be seen on tips of the leaves, while 10% w/v POE 20S contributed to severe drying of tomato plants towards the end of test (Figure 3).

Corn treated with 1% w/v POE 20S exhibited negligible symptoms, but most of the corn seedlings treated with 3% and 5% w/v POE 20S exhibited yellowish leaves, either on the tips or over the whole lamina. In 7% w/v POE 20S, mild necrosis could be seen on the tip of leaves and over the edge of leaves' lamina, while severe wrinkling and shrivelling of leaves could be found in the corn plants treated with 10% w/v POE 20S (Figure 4). Thus, this proves that symptoms of toxicity toward emerged seedlings are proportionate with the POE 20S concentrations tested.

Among the three parameters evaluated (seed germination, survival of germinated seeds and shoot growth), the clearest examples of toxicity effects were observed for shoot growth. These observations have been supported by Chang *et al.* (2015) who found that, since the shoot are the first target tissue to be exposed to the surfactants, the toxic symptoms appear to be greater for them compared to other parts of plant.

The toxicity symptoms observed were rated based on severity of leaves damage and were used to calculate PPI as in Table 6. From the results obtained, increased concentration of POE 20S solution resulted in an increased PPI level and toxicity effect in both monocotyledonous and dicotyledonous plants. As such, POE 20S solution can be declared as safe at lower concentrations, *i.e.*, 1% and 3% w/v POE 20S since there are no inhibitory effects on both species in the treatments and the toxicity level was also low. Similar results were found by Galvez *et al.* (2018), who revealed that POE 20S solution was among the least phytotoxic compound when tested with onion (monocotyledonous) and lettuce (dicotyledonous), where the germination index was between 80%-85% and with absence of toxicity symptom.

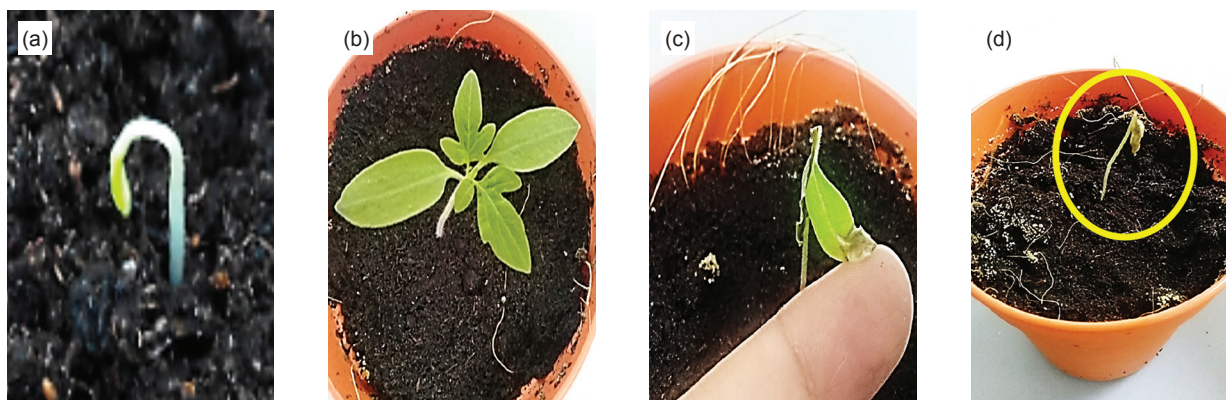


Figure 3. From left, (a) germinated tomato seed in control group on day 6, (b) tomato plant in control group on day 21, (c) moderate necrosis on tomato leaf treated with 7% POE 20S on day 21, and (d) severe drying of tomato plant treated with 10% POE 20S on day 21.

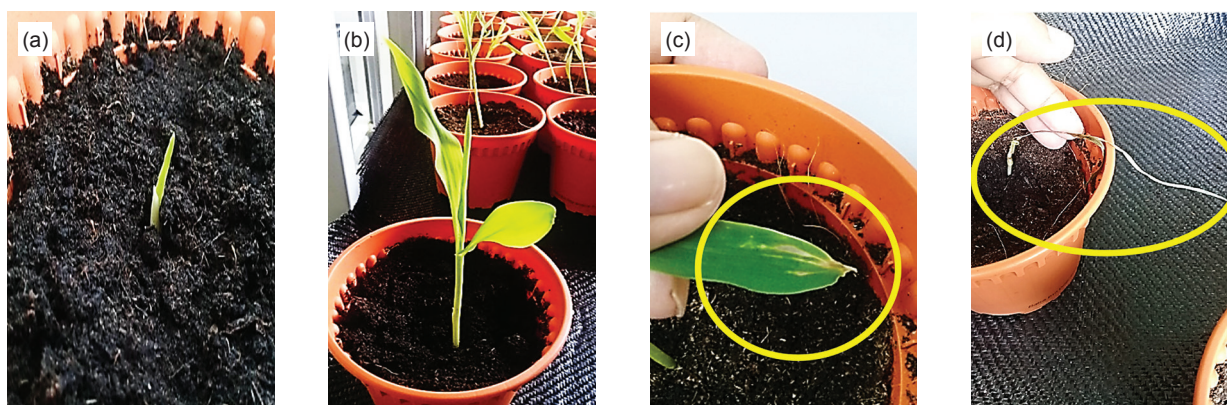


Figure 4. From left, (a) germinated corn seed in control group on day 5, (b) corn plant in control group on day 14, (c) mild necrosis on the tip of corn leaf treated with 7% POE 20S on day 14 and (d) severe shrivelling of corn plant treated with 10% POE 20S on day 14.

CONCLUSION

The results showed that higher concentration of POE 20S solution, i.e., 5%, 7% and 10% w/v resulted in reduced survival of emerged plants of both species and increased level of phytotoxicity effect towards both dicotyledonous and monocotyledonous plants. With phytotoxicity level rated as low to medium for 1% to 10% w/v POE 20S solution, respectively, it is confirmed that, with appropriate dilution of POE 20S in pesticide formulation will not bring harm to non-target terrestrial plants. Data obtained from this study can be used as a reference to encourage manufacturers to select efficient vegetable-based surfactants for their pesticide formulation that will not bring harm to terrestrial environment, especially terrestrial plants.

ACKNOWLEDGEMENT

The authors would like to thank the Director-General of MPOB for permission to publish this article. We appreciate the technical assistance by Muhamad Fierdaus Mohd Jasmi in laboratory experiments. Thanks, are also extended to members of the Ecotoxicology Laboratories, Advanced Oleochemical Technology Division (AOTD), MPOB for their assistance and support in this study and Oleo Product Development Unit, AOTD for supplying POE 20S sample.

REFERENCES

- Abhilash, P C and Singh, N (2009). Pesticides use and application: An Indian scenario. *J. Hazard. Mater.*, 165: 1-12. DOI: 10.1016/j.jhazmat.2008.10.061.
- Alavanja, M C R (2009). Pesticides use and exposure extensive worldwide. *Rev. Environ. Health*, 24(4): 303-309. DOI: 10.1515/rev.2009.24.4.303.
- Ambarish, S; Biradar, A P; Jagginavar, S B and Karbhantanal, S S (2017). Effect of phytotoxicity of pesticides on grain yield of Rabi sorghum [*Sorghum bicolor* (L.) Moench]. *J. Pharmacogn. Phytochem.*, 6(5): 1850-1853.
- Asma Liyana, S; Noorazah, Z and Razmah, G (2020). Ecotoxicity of surfactants toward terrestrial plants: An overview. *Palm Oil Developments*, 72: 8-14.
- Aumatell, A (1996). Agrochemical formulations. *Chemistry in Australia*, 63(11): 497-499.
- Bhat, N R; Prince, T L; Tayama, H K and Carver, S A (1992). Rooted cutting establishment in media containing wetting agent. *Hort. Sci.*, 27(1): 78.
- Chang, G; Zhang, Q; Zhang, L; Lu, Y and Gao, T (2015). Effect of sodium dodecyl sulphate on wheat (*Triticum aestivum* L.) seedlings. *Environ. Prog. Sustainable Energy*, 34: 1142-1147. DOI: 10.1002/ep.12107.
- Chemical Book (2016). Product catalog. www.chemicalbook.com/ProductCatalog_EN/ 18.html, accessed on 14 June 2019.
- Di Salvatore, M; Carafa, A M and Carratù, G (2008). Assessment of heavy metals phytotoxicity using seed germination and root elongation tests: A comparison of two growth substrates. *Chemosphere*, 73(9): 1461-1464. DOI: 10.1016/j.chemosphere.2008.07.061.
- E-Limu E-Learning Company Limited (2015). Plants. www.learn.e-limu.org, accessed on 14 March 2020.
- Endo, R M; Letey, J; Valoras, N and Osborn, J F (1969). Effects of non-ionic surfactants on monocots. *Agron. J.*, 61: 850-854. DOI: 10.2134/agronj1969.00021962006100060007x.
- Food and Agriculture Organization of the United Nations (FAO) (2017). Pesticide residues in food. <http://www.fao.org/documents/card/en/c/I8258EN/>, accessed on 14 January 2020.
- Fishel, F M (2014). Pesticide effect on non-target organisms. <http://edis.ifas.ufl.edu>, accessed on 20 December 2016.
- Galvez, A; Lopez-Galindo, A and Pena, A (2018). Effect of different surfactants on germination and root elongation of two horticulture crops: Implications for seed coating. *N. Z. J. Crop. Hortic. Sci.*, 47(2): 83-98. DOI: 10.1080/01140671.2018.1538051.
- Herzfeld, D and Sargent, K (2011). *Private Pesticide Applicator Safety Education Manual*. 19th edition. University of Minnesota Extension. p. 85-108.
- Huggenberger, F; Letey, J and Farmer, W J (1973). Effect of two non-ionic surfactants on adsorption and mobility of selected pesticides in a soil system. *Soil Sci. Soc. Am. J.*, 37: 215-219. DOI: 10.2136/sssaj1973.03615995003700020018x.
- Ilic, S Z; Mirecki, N; Trajkovic, R; Kapoulas, N and Milenkovic, L (2015). Effect of Pb on germination of different seed and his translocation in bean seed during sprouting. *Fresenius Environ. Bull.*, 24(2a): 670-675.
- Ismail, A R; Dzolkifli, O; Ooi, T L and Salmiah, A (1998). The effect of palm-based surfactants as spray adjuvants in glyphosate isopropylamine (IPA). *J. Oil Palm Res.*, 10(2): 45-56.

- Ismail, A R; Meor Badli Shah, A R; Sim, K C; Rosnah, I; Hazimah, A H; Shamsudin, B and Tan, A S H (2010). Performance of palm oil-based adjuvant in glyphosate herbicide formulations for weed control. *MPOB Information Series*, 467 and 468.
- Ismail, A R; Nursakinah, I and Hazimah, A H (2014). Palm-based emulsion-in-water (EW) termiticide. *MPOB Information Series*, 555.
- Kimball, S; Angert, A L; Huxman, T E and Venable, D L (2011). Differences in the timing of germination and reproduction relate to growth physiology and population dynamics of Sonoran Desert winter annuals. *Am. J. Bot.*, 98: 1773-1781. DOI: 10.3732/ajb.1100034.
- Kozlowski, T T and Pallardy, S G (1997). Seed germination and seedling growth. *Growth Control in Woody Plants*. Academic Press, USA. p. 14-72.
- Krogh, K A; Halling-Sørensen, B; Mogensen, B B and Vejrup, K V (2003). Environmental properties and effects of nonionic surfactant adjuvants in pesticides: A review. *Chemosphere*, 50(7): 871-901. DOI: 10.1016/s0045-6535(02)00648-3.
- Lichtenthaler, H K (1996). Vegetation stress: An introduction to the stress concept in plants. *J. Plant Physiol.*, 148(1-2): 4-14. DOI: 10.1016/S0176-1617(96)80287-2.
- Luxmoore, R J; Valoras, N and Letey, J (1974). Non-ionic surfactant effects on growth and porosity of barley roots. *Agron. J.*, 66: 673-675. DOI: 10.2134/agronj1974.0002196200660050020x.
- Mahmood, S and Usman, M (2014). Consequences of magnetized water application on maize seed emergence in sand culture. *J. Agric. Sci. Technol.*, 16: 47-55.
- Miller, P and Westra, P (1998). How surfactants work, no. 0.564. Colorado State University Cooperative Extension. Crop Fact Sheet. http://thanyagroup.com/research/download/25530111_2.pdf, accessed on 6 August 2019.
- Müller, J (2020). Volume of pesticides used in Malaysia 2009-2017. <https://www.statista.com/statistics/1101033/malaysia-pesticide-use-volume/>, accessed on 11 September 2020.
- Mullin, C A; Fine, J D; Reynolds, R D and Frazier, M T (2016). Toxicological risks of agrochemical spray adjuvants: Organosilicone surfactants may not be safe. *Front. Public Health*, 4: 92. DOI: 10.3389/fpubh.2016.00092.
- Norris, R F (1982). Action and fate of adjuvants in plants. *Adjuvants for Herbicides* (Hodgson, R H ed.). Weed Science Society of America, Champaign, IL. p. 68-83.
- OECD 208 (2006). Guidelines for testing chemicals: Terrestrial plant test - seedling emergence and seedling growth test. Organization for Economic Co-operation and Development. Paris, Italy.
- Parveez, G K A; Elina, H; Loh, S K; Meilina, O A; Kamalrudin, M S; Mohd Noor Izuddin, Z A; Shamala, S; Zafarizal, A A H and Zainab, I (2020). Oil palm economic performance in Malaysia and R&D progress in 2019. *J. Oil Palm Res.*, 32(2): 159-190. DOI: 10.21894/jopr.2020.0032.
- Schreinemachers, P; Afari-Sefa, V; Heng, C H; Dung, P T M; Praneetvatakul, S and Srinivasan, R (2015). Safe and sustainable crop protection in Southeast Asia: Status, challenges and policy options. *Environ. Sci. Policy*, 54: 357-366.
- Seaman, D (1990). Trends in the formulation of pesticides - An overview. *Pestic. Sci.*, 29: 437-449. DOI: 10.1002/ps.2780290408.
- Sharma, A; Kumar, V; Shahzad, B; Tanveer, M; Sidhu, G P S; Handa, N; Kohli, S K; Yadav, P; Bali, A S; Parihar, R D; Dar, O I; Singh, K; Jasrotia, S; Bakshi, P; Ramakrishnan, M; Kumar, S; Bhardwaj, R and Thukral, A K (2019). Worldwide pesticide usage and its impact on ecosystem. *SN Applied Science*. DOI: 10.1007/s42452-019-1485-1.
- Sumaiyah, M N M; Ismail, A R; Zafarizal, A A H and Zainab, I (2019). Influence of surfactant structure in physical stability and physiochemical properties of insecticide emulsions. *J. Oil Palm Res.*, 31(4): 670-680. DOI: 10.21894/jopr.2019.0025.
- US Environmental Protection Agency (US EPA) (1997). Pesticide regulation notice 97-6. Use of term 'inert' in the label ingredients statement. http://www.epa.gov/opppmsd1/PR_Notices/pr97-6.html, accessed on 25 October 2016.
- Vanhala, P; Kurstjens, D; Ascard, J; Bertram, A; Cloutier, D C; Mead, A; Raffaelli, M and Rasmussen, J (2004). Guidelines for physical weed control research: flame weeding, weed harrowing and intra-row cultivation. *6th Workshop on physical and cultural weed control*. European Weed Research Society. Lillehammer, Norway. 8-10 March 2004. p. 208-239.

Wong, J W C; Li, K; Fang, M and Su, D C (2001). Toxicity evaluation of sewage sludge in Hong Kong. *Environ. Int.*, 27: 373-380. DOI: 10.1016/S0160-4120(01)00088-5.

Ying, G G (2006). Fate, behaviour and effects of surfactants and their degradation products in the environment. *Environ. Int.*, 32: 417-431. DOI: 10.1016/j.envint.2005.07.004.

ASSOCIATION OF FATTY ACIDS AND POLAR COMPOUND FRACTIONS WITH ACRYLAMIDE FORMATION DURING INTERMITTENT FRYING

SU LEE KUEK¹; AZMIL HAIZAM AHMAD TARMIZI^{2*}; RAZNIM ARNI ABD RAZAK²; SELAMAT JINAP^{1,3}; SAPARIN NORLIZA⁴ and MAIMUNAH SANNY^{1,3*}

ABSTRACT

This study aimed to investigate the relationship of fatty acids composition (FAC) and total polar compounds (TPC) on acrylamide formation during intermittent frying of French fries. Four vegetable oils namely palm olein, red palm olein, soybean and sunflower oil were subjected to 80 frying cycles over five consecutive days. The FAC was analysed in the fresh oil (before frying) and 80th frying cycles while TPC was quantified for every 16th frying cycle. The saturated fatty acids (SFA) significantly increased over 80 frying cycles; however, polyunsaturated fatty acids (PUFA) demonstrated a significant reduction. A strong positive correlation ($r=0.864$, $p\leq 0.05$) was observed between acrylamide content and PUFA whereas a weak positive but significant correlation for SFA ($r=0.284$, $p\leq 0.05$) and monounsaturated fatty acids (MUFA) ($r=0.358$, $p\leq 0.05$). Polymerised triacylglycerols (PTAG), oxidised triacylglycerols (OxTAG), and diacylglycerols (DAG) were significantly increased over frying cycles. A weak but significant negative correlation ($r=-0.269$, $p\leq 0.05$) was observed between PTAG in oils and acrylamide in French fries. FAC and PTAG significantly contributed to the formation of acrylamide in French fries. This indicates that lipid oxidation, particularly tertiary oxidation, exhibits a significant contribution to acrylamide development during frying.

Keywords: acrylamide, fatty acids composition, intermittent frying, total polar compounds, vegetable oils.

Received: 11 May 2021; **Accepted:** 16 February 2022; **Published online:** 18 March 2022.

INTRODUCTION

Frying is one of the food preparation methods involving immersion of food in edible oil above the boiling point of water that cause changes in

the characteristics of fried products such as colour, texture and flavour of the fried products (Hubbard and Farkas, 2007). The dehydration process at excessive temperatures is reflected by a rapid heat and mass transfer between oil and food (Ahmad Tarmizi and Niranjana, 2013). Albeit considerable oil content in fried food, frying is still widely employed in domestic industrial sectors (Ahmad Tarmizi *et al.*, 2016). Intermittent frying refers to cooking at discrete batches when the oil is heated and kept after use for subsequent frying operation (Seppanen and Csallany, 2006). In the presence of oxygen, moisture, trace elements and free radicals, frying leads to oil degradation through myriad chemical reactions such as thermal oxidation, hydrolysis, and polymerisation (Andrikopoulos *et al.*, 2002).

As fried food absorbs oil, deterioration of oil used for frying not only yields undesired sensory characteristics of the fried product (Gertz and Behmer, 2014) but also generate new compounds that

¹ Department of Food Science, Faculty of Food Science and Technology, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.

² Malaysian Palm Oil Board, 6 Persiaran Institusi, Bandar Baru Bangi, 43000 Kajang, Selangor, Malaysia.

³ Laboratory of Food Safety and Food Integrity, Institute of Tropical Agriculture and Food Security, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.

⁴ Sime Darby Research Sdn. Bhd., Lot 2664, Jalan Pulau Carey, 42960 Pulau Carey, Selangor, Malaysia.

* Corresponding author e-mail: azmil_haizam@mpob.gov.my; s_maimunah@upm.edu.my

could be harmful to human health (Kushairi *et al.*, 2019). The presence of acrylamide is one of the major concerns associated with fried products. Acrylamide is classified by the International Agency for Research on Cancer (IARC) as a probably carcinogenic (group 2A) to humans (IARC, 1994). Potato-based products such as French fries are reported to have notable levels of acrylamide (Luning and Sanny, 2016; Sanny *et al.*, 2012). The European Commission has set a threshold for French fries at 500 $\mu\text{g kg}^{-1}$ (European-Commission, 2017). It is well-established that the Maillard reaction is the major pathway for the formation of acrylamide (Stadler *et al.*, 2002). Meanwhile, lipid oxidation is reported as the minor pathway for acrylamide manifestation when its precursor, which is acrylic acid, is developed via the degraded product namely acrolein (Daniali *et al.*, 2016; Gertz and Klostermann, 2002; Yasuhara *et al.*, 2003).

Fatty acid composition (FAC) is often used to determine the authenticity and stability of oils for frying (Ahmad Tarmizi *et al.*, 2016). Their thermal resistance is associated with the degree of saturation. Oils with higher saturated fatty acids are more stable than conventional liquids oils such as canola and sunflower oils (Ahmad Tarmizi *et al.*, 2016). The amount of double bonds in fatty acids determines the severity of oil oxidation. A higher degree of oil unsaturation is more prone to thermal oxidation (Ahmad Tarmizi *et al.*, 2016). Several publications have correlated the degree of unsaturation and thermal oxidation with acrylamide formation in the fried products (Lim *et al.*, 2014; Wang *et al.*, 2019). Factors such as frying conditions (Abdul Hamid *et al.*, 2019; Kamarudin *et al.*, 2018; Sanny *et al.*, 2010) and frying media (Ahmad *et al.*, 2021; Daniali *et al.*, 2018; Lim *et al.*, 2014; Zhang *et al.*, 2015) have shown to onset the formation of acrylamide in fried products.

The thermal oxidation mechanism mainly involves initiation, propagation, and termination (Choe and Min, 2009). Peroxide value (PV), which measures hydroperoxides in the oil, is used to evaluate the initial stages of oxidation. The *p*-anisidine value (AnV), which is denoted as secondary oxidation, quantifies the level of non-volatile aldehydes and ketones in oil during frying (Ahmad Tarmizi *et al.*, 2016). The advancement of secondary oxidation or known as tertiary oxidation is mainly responsible for the formation of compounds with relatively higher molecular weight. The measurement of total polar compounds (TPC) is perceived as one of the most objective methods to determine the cumulative oil degradation from primary, secondary, and tertiary oxidation. TPC can be further cascade down to several fractions namely polymerised triacylglycerols (PTAG), diacylglycerols (DAG), monoacylglycerols (MAG), oxidised triacylglycerols (OxTAG) and free fatty

acids (FFA) (Santos *et al.*, 2013). Many European countries have set the TPC maximum limit between 25% and 30% in used oils (Berger, 2005). A considerable amount of TPC in the oil as a result of high OxTAG and PTAG build-up could possibly affect human health (Petersen *et al.*, 2013). Animal studies have shown that PTAG and OxTAG are potent for higher risk of skin diseases (Cohn, 2002) and gastrointestinal irritants (Saguy and Dana, 2003). Realising the amount of frying oil imparts the fried products is significant, oil characteristics are highly concern in ensuring oil deterioration is minimal (Ahmad Tarmizi *et al.*, 2016).

Most authors reported the insignificant association between PV and acrylamide content in fried food (Abd Razak *et al.*, 2021; Ahmad *et al.*, 2021; Kuek *et al.*, 2020; Williams, 2005; Zhang *et al.*, 2015). Despite our current study did not observe any significant correlation between AnV and acrylamide content in fried beef nuggets, the relationship was reported to be significant in French fries (Abd Razak *et al.*, 2021; Kuek *et al.*, 2020). In fact, Abd Razak *et al.* (2021) has recently observed a significant association between TPC and acrylamide content in French fries. As most publications highlighted a significant correlation between the FAC and acrylamide content (Lim *et al.*, 2014; Wang *et al.*, 2019), limited studies have been carried out to explore the relationship between TPC and acrylamide. Thus, our study aimed to establish a relationship between TPC and FAC of different vegetable oils on acrylamide formation during intermittent frying of French fries. The acrylamide data from our published article (Kuek *et al.*, 2020) was used in the present study to determine the strength of the relationship between FAC and TPC with acrylamide concentration.

MATERIALS AND METHODS

Raw Materials

Palm olein, soybean oil and sunflower oil of the same batch were purchased from local supermarkets. The red palm olein (industrial grade) was procured from a palm oil refinery that is located in Selangor, Malaysia. Shoestring pre-fried French fries ($0.5 \times 0.4 \text{ cm}^2$ in cross-section and 6 to 7 cm long) of the same batch were obtained from a local frozen food distributor. The potato product was stored at -20°C upon purchase and thawed at 4°C overnight before the frying experiment.

Frying Experiment

The frying setup was executed with reference to Ahmad Tarmizi *et al.* (2019a) with some slight modification. Approximately 5 kg of oil was firstly

weighed, placed into two split pots of fryer DFT-6000 (MSM, Malaysia) and heated to 180°C. A batch of 150 g of par-fried potato strips was fried for every cycle for 3.5 min with the interval of 30 min for 8 hr daily for five consecutive days. The frying protocol was designed to simulate conditions in the food service sector (Ismail, 2005). Upon completion of each frying cycle, the finished product was collected and stored in a zip-lock bag at -20°C. Approximately 450 g of used oil was sampled in a dark amber bottle and cooled to room temperature before flushing with nitrogen and stored at -20°C for subsequent analyses. Fresh oil was added to the fryer on the next day of operation to replenish oil sampling. Each type of oil underwent frying experiments in duplicate.

Chemicals and Reagents

The n-hexane, tetrahydrophuran, sodium sulphate, and silica gel 60 (0.063 to 0.200 mm, 70 to 230 mesh ASTM) were purchased from Merck (Darmstadt, Germany). Petroleum ether (PE), diethyl ether (DE), and sea sand (acid wash) were purchased from System (Selangor, Malaysia).

Analysis of Oil

Fatty acid composition (FAC). The quantification of FAC was carried out following AOCS Official Method Ce 1i-07 (AOCS, 2011) using Hewlett-Packard 6890 Series gas chromatography (Agilent Technologies, Palo Alto, CA, USA). The GC was equipped with a BPX70 capillary column (60 m × 0.25 mm, i.d. 0.25 µm film thickness), flame ionisation detector (FID), electronic integrator, and data processor (J&W Scientific, Folsom, USA). The sample injection volume was set at 1 µL while helium gas was used as the carrier gas with the flow rate of 0.8 mL min⁻¹ and a pre-column split ratio of 100:1. The temperature for the injection port and FID detector was set at 240°C while the column temperature remained at 185°C. Fatty acid methyl esters (FAME) were quantified by comparing their retention times and peak areas. Reference mix comprising of RM-5 and RM-6 methyl ester standards (Supelco, Dorset, United Kingdom) was used considering the variation of fatty acids in the studied oils. The percentage of mass fractions is used to express the level of each fatty acid.

Total polar compound (TPC) fractions. The TPC was performed according to IUPAC 2.507 with minor modification (Dobarganes *et al.*, 2000). About 2.5 g of the sample was measured in a 50 mL volumetric flask. PE and DE solution (87:13, respectively) was then added up to the mark. The empty round bottom flask dried in an oven at 103°C overnight and allowed to cool in a desiccator until

constant weight. A 45 cm glass column was packed with 25 g of silica gel, suspended in PE-DE mixture, and layered with 4 g of sea sand.

The nonpolar fraction was eluted using a 150 mL PE-DE mixture for approximately 60 to 65 min and collected in a round bottom flask. A similar procedure was repeated for polar fractions using DE. The round bottom flask was then dried using a rotary evaporator (Büchi Labortechnik AG, Flawil, Switzerland) at 60°C, rotation at 130 rpm for 15 min, and vacuum level at 380 mbar, and rotation at 130 rpm for 15 min. After drying, nitrogen gas was then flushed slowly into the flask for another 15 min and weighed. About 0.5 g of residue in the round bottom flask was taken out and re-dissolved using 10 mL of tetrahydrophuran. The solution was then filtered using a 0.45 µm nylon membrane filter and collected in a vial before injection into high performance liquid chromatography (HPLC).

Partitioning of polar compound fractions. Polar compound fractions namely OxTAG, PTAG, DAG, MAG, and FFA were quantified using HPLC equipped with an evaporative light scattering detector (ELSD) (Agilent 1260 Infinity, Santa Clara, USA) and three PLgel 500 Å columns consist of 5 µm particle size (7.5 mm × 300 mm) in series (Agilent 1260 Infinity, Santa Clara, USA). Tetrahydrophuran was used as a mobile phase used at the flow rate of 1 mL min⁻¹. Temperature columns were set at 40°C while the evaporator and nebuliser of the ELSD were fixed at 40°C and 30°C, respectively. The nitrogen flow rate of the ELSD was established at 1.7 standard L min⁻¹ (SLM). Analysis time for each sample required 30 min to complete. The principle of quantifying the polar compounds fractions was based on the molecule size. Fractions with larger molecule size will appear as the earliest peak on the chromatogram: PTAG > OxTAG > DAG > MAG > FFA. It is important to note that partitioning of polar fractions is made from the basis of polarity reflecting specific routes of a chemical reaction during frying.

Statistical Analysis

General linear model analysis of variance (ANOVA) was performed using Minitab Statistical Software 17 (Minitab Inc., Pa, USA). The means of analysis were evaluated following Tukey's multiple comparison test. Pearson's correlation was used to determine the strength of the relationship between quality parameters in the oil and acrylamide content in the fried product. The correlation was confirmed for possible violations from the model assumptions during the analysis. *p*-value, which was less or equal to 0.05, indicates the significance of the relationship between variables.

RESULTS AND DISCUSSION

Table 1 shows the significant variation ($p \leq 0.05$) occurring in FAC in all oils across 80 frying cycles. Alteration of fatty acids is associated with the levels of unsaturated fatty acids, heat treatment, and number of frying cycles (Alireza *et al.*, 2010). It is also noted that myristic acid (C14:0), stearic acid (C18:0) and oleic acid (C18:1) were significantly increased over 80 frying cycles regardless of oil types. This observation was in agreement with the findings by Ahmad Tarmizi *et al.* (2019a) and Li *et al.* (2017). An increase in SFA was mainly contributed by the conversion of unsaturated fatty acids into saturated form as a result of oxidation (Alireza *et al.*, 2010). Indeed, the elevation of palmitic acid (C16:0), C18:0 and C18:1 was likely of oxidation of linoleic acid (C18:2) and linolenic acid (C18:3) during frying (Li *et al.*, 2017). The C18:1 significantly increased in sunflower and soybean oils as a function of frying

cycles (Table 1). In the case of sunflower oil, an increase of C18:1 showed in this study contradicted with Romero *et al.* (1998) but consistent with the observation made by Ahmad Tarmizi *et al.* (2019a). However, C18:1 was significantly lowered in palm olein, and this coincides with Li *et al.* (2017). Interestingly, C18:1 of red palm olein did not show any significant changes even after 80 frying cycles compared to palm olein (Table 1). The C18:2 and C18:3 demonstrated a significant reduction across frying cycles in all oils (Table 1), which was found similar with Li *et al.* (2017). In the light of soybean oil, Sebedio *et al.* (1990) observed an enhancement of C18:1, C18:2 and C18:3 in soybean oil due to degradation of C18:2 and C18:3. Reduction of C18:2 and C18:3 can be explained by higher tendency of the oil towards oxidation (Alireza *et al.*, 2010). Oxidation of unsaturated fatty acids lowered the unsaturated fatty acid content (Li *et al.*, 2017).

TABLE 1. FATTY ACID COMPOSITION OF VEGETABLE OILS ACROSS FRYING CYCLES

Fatty acid (%)	Frying cycle	Palm olein	Sunflower oil	Red palm olein	Soybean oil
C12:0	Fresh	0.41 ± 0.00 ^{ab}	0.00 ± 0.00 ^{aA}	0.31 ± 0.01 ^{bb}	0.00 ± 0.00 ^{cB}
	80 th	0.44 ± 0.01 ^{aA}	0.00 ± 0.00 ^{dA}	0.35 ± 0.02 ^{bA}	0.05 ± 0.00 ^{cA}
C14:0	Fresh	1.04 ± 0.00 ^{ab}	0.08 ± 0.01 ^{dB}	0.65 ± 0.07 ^{ba}	0.09 ± 0.00 ^{cB}
	80 th	1.16 ± 0.03 ^{aA}	0.25 ± 0.06 ^{cA}	0.57 ± 0.07 ^{bb}	0.22 ± 0.01 ^{cA}
C16:0	Fresh	37.96 ± 0.00 ^{ab}	6.17 ± 0.10 ^{dB}	35.08 ± 0.38 ^{bb}	10.73 ± 0.02 ^{cB}
	80 th	43.46 ± 0.20 ^{aA}	11.71 ± 0.04 ^{dA}	40.16 ± 0.31 ^{ba}	16.50 ± 0.02 ^{cA}
C16:1	Fresh	0.26 ± 0.02 ^{aA}	0.10 ± 0.00 ^{cB}	0.20 ± 0.00 ^{ba}	0.08 ± 0.00 ^{dB}
	80 th	0.20 ± 0.01 ^{aB}	0.13 ± 0.01 ^{ba}	0.21 ± 0.01 ^{aA}	0.10 ± 0.00 ^{cA}
C18:0	Fresh	3.97 ± 0.00 ^{bb}	3.28 ± 0.02 ^{dB}	3.78 ± 0.05 ^{cB}	4.36 ± 0.01 ^{aB}
	80 th	4.57 ± 0.10 ^{ba}	3.96 ± 0.08 ^{cA}	4.44 ± 0.22 ^{ba}	5.01 ± 0.01 ^{aA}
C18:1	Fresh	43.05 ± 0.62 ^{ba}	31.05 ± 0.05 ^{cB}	46.38 ± 0.47 ^{aA}	23.28 ± 0.01 ^{dB}
	80 th	42.58 ± 0.38 ^{bb}	35.40 ± 0.08 ^{cA}	44.97 ± 0.53 ^{bb}	28.05 ± 0.02 ^{dA}
C18:2	Fresh	11.74 ± 0.01 ^{dA}	57.49 ± 0.07 ^{aA}	12.10 ± 0.12 ^{cA}	52.83 ± 0.03 ^{ba}
	80 th	6.90 ± 0.12 ^{dB}	46.75 ± 0.06 ^{aB}	8.11 ± 0.04 ^{cB}	43.75 ± 0.02 ^{bb}
C18:3	Fresh	0.34 ± 0.01 ^{ba}	0.32 ± 0.05 ^{cA}	0.32 ± 0.01 ^{ba}	7.47 ± 0.00 ^{aA}
	80 th	0.13 ± 0.02 ^{cB}	0.24 ± 0.04 ^{bb}	0.12 ± 0.01 ^{cB}	4.57 ± 0.00 ^{aB}
C20:0	Fresh	0.38 ± 0.00 ^{ab}	0.23 ± 0.01 ^{ba}	0.38 ± 0.0 ^{aB}	0.38 ± 0.01 ^{aB}
	80 th	0.50 ± 0.06 ^{aA}	0.27 ± 0.01 ^{cA}	0.45 ± 0.02 ^{abA}	0.42 ± 0.01 ^{ba}
C20:1	Fresh	0.00 ± 0.00 ^{cA}	0.21 ± 0.04 ^{abA}	0.16 ± 0.00 ^{ba}	0.20 ± 0.00 ^{aA}
	80 th	0.00 ± 0.00 ^{cA}	0.16 ± 0.02 ^{ba}	0.17 ± 0.01 ^{ba}	0.19 ± 0.00 ^{aB}
C22:0	Fresh	0.00 ± 0.00 ^{cA}	0.71 ± 0.01 ^{aA}	0.00 ± 0.00 ^{cB}	0.42 ± 0.01 ^{bb}
	80 th	0.00 ± 0.00 ^{dA}	0.70 ± 0.03 ^{aA}	0.05 ± 0.00 ^{cA}	0.54 ± 0.01 ^{ba}
C24:0	Fresh	0.00 ± 0.00 ^{aA}	0.00 ± 0.00 ^{aA}	0.00 ± 0.00 ^{aA}	0.00 ± 0.00 ^{aA}
	80 th	0.00 ± 0.00 ^{aA}	0.00 ± 0.00 ^{aA}	0.00 ± 0.00 ^{aA}	0.00 ± 0.00 ^{aA}
Others	Fresh	0.85 ± 0.00 ^{aA}	0.07 ± 0.01 ^{dB}	0.35 ± 0.01 ^{bb}	0.15 ± 0.00 ^{cB}
	80 th	0.26 ± 0.05 ^{bb}	0.44 ± 0.01 ^{aA}	0.45 ± 0.01 ^{aA}	0.21 ± 0.00 ^{ba}
SFA	Fresh	43.76 ± 0.00 ^{ab}	10.47 ± 0.10 ^{dB}	40.38 ± 0.29 ^{bb}	15.98 ± 0.03 ^{cB}
	80 th	50.14 ± 0.22 ^{aA}	16.88 ± 0.08 ^{dA}	46.31 ± 0.32 ^{ba}	23.04 ± 0.03 ^{cA}
MUFA	Fresh	43.31 ± 0.63 ^{ba}	31.25 ± 0.15 ^{cB}	46.64 ± 0.48 ^{aA}	23.43 ± 0.11 ^{dB}
	80 th	42.78 ± 0.38 ^{bb}	35.58 ± 0.11 ^{cA}	45.43 ± 0.54 ^{bb}	28.21 ± 0.11 ^{dA}
PUFA	Fresh	12.08 ± 0.01 ^{dA}	57.81 ± 0.08 ^{ba}	12.42 ± 0.12 ^{cA}	60.30 ± 0.03 ^{aA}
	80 th	7.04 ± 0.13 ^{dB}	46.99 ± 0.09 ^{bb}	8.23 ± 0.04 ^{cB}	48.31 ± 0.03 ^{aB}
C18:2/C16:0	Fresh	0.31	9.32	0.34	4.93
	80 th	0.16	3.99	0.20	2.65

Note: ^{a-d} values within the same row with different lowercase letters are significantly different ($p \leq 0.05$).

^{A-B} values within the same fatty acid chain column with different lowercase letters are significantly different ($p \leq 0.05$).

Value are the means ± SD of duplicate experiments with triplicate determinations.

An increase in SFA for all oils was found significant while polyunsaturated fatty acids (PUFA) exhibited a significant decline over frying cycles. A higher degree of unsaturation reflects the tendency of oil towards oxidation (Ahmad Tarmizi *et al.*, 2016). Oxidation of C18:3 causes an increase in SFA but reduces the PUFA content (Li *et al.*, 2017). Unlike PUFA and SFA, the trend for monounsaturated fatty acids (MUFA) as depicted in Table 1 is very much dependent on the oil types. For sunflower and soybean oils, MUFA increased significantly over 80 frying cycles. However, palm olein displayed a significant drop in MUFA with the increase of frying cycles. In the case of red palm olein, MUFA did not show any significant changes over frying cycles due to the absence of alteration in C18:1. The ratio of C18:2/C16:0 is calculated to distinguish the degree of oxidative deterioration in used oils (Alireza *et al.*, 2010). Table 1 also shows the reduction of C18:2/C16:0 for all vegetable oils throughout the frying cycles. Such observation was somewhat similar to Zribi *et al.* (2014), who emphasised on the C18:2/C18:1 decrement over frying time indicates the occurrence of lipid deterioration under excessive heating. Sunflower oil exhibited the highest C18:2/C16:0 as its relatively higher C18:2 content (Alireza *et al.*, 2010). Interestingly, palm olein demonstrated the lowest ratio of C18:2/C16:0 when compared to other oils which reflect its stability and resistance towards heat even after five days of frying. This study also revealed that C18:2/C16:0 for red palm olein and palm olein was apparently identical (Table 1). This further demonstrated that both palm olein and red palm olein were found stable and thermally resistant against high temperature under long frying operation.

Table 2 shows the Pearson's correlation between the degree of saturation and acrylamide content upon extended frying. The results showed a strong positive correlation ($r=0.864$, $p\leq 0.05$) between acrylamide with PUFA while a weak positive but significant correlation was pronounced in SFA ($r=0.284$, $p\leq 0.05$) and MUFA ($r=0.358$, $p\leq 0.05$). Comparison of results with published literature could not be made due to the absence of research works that associate FAC with acrylamide content fried products. Nevertheless, Ehling and Shibamoto (2005) reported on the heating of asparagine with C16:0 could generate a relatively high acrylamide content. The presence of SFA could onset the acrylamide formation albeit the correlation was comparatively weak. A similar study by Ehling and Shibamoto (2005) also concluded that heating asparagine in lipid with higher unsaturated fatty acids induces acrylamide formation of which the C18:1 and C18:2 generated 10 folds higher in acrylamide in comparison to C16:0 when heating was undertaken at 180°C. Table 2 also concluded that the correlation between PUFA and acrylamide

was stronger than MUFA and SFA considering PUFA is highly prone to oxidation. L alas (2009) reported that C18:2 is more susceptible to oxidation when compared to C18:1 considering the presence of conjugated diene. The results obtained agree with the outcome from Capuano *et al.* (2010) where an increase in unsaturation is highly prone to lipid oxidation and thus, promotes acrylamide formation. Lipid oxidation generates more carbonyl groups such as aldehydes and ketones that can further react with asparagine to form acrylamide even in the absence of reducing sugar (Ehling and Shibamoto, 2005). Yasuhara *et al.* (2003) also opined that acrylamide could be developed through the formation of acrolein. Acrolein, which is one of the oil breakdowns, can further oxidise to acrylic acid and subsequently reacts with ammonia to form acrylamide.

TABLE 2. PEARSON'S CORRELATION BETWEEN ACRYLAMIDE CONCENTRATION AND SFA, MUFA AND PUFA OF VEGETABLE OILS, RESPECTIVELY

Parameters	Pearson's correlation	
	<i>r</i>	<i>p</i> -value
SFA	0.284	$p\leq 0.05$
MUFA	0.358	$p\leq 0.05$
PUFA	0.864	$p\leq 0.05$

A significant increase ($p\leq 0.05$) in TPC of palm olein, red palm olein, sunflower oil and soybean oil was also evidenced as a function of frying cycle (Table 3) as frying oil was continuously exposed to elevated temperature, oxidation of oil increased and thus, the TPC (Aniolowska and Kita, 2016). The TPC development was likely contributed to the increase of OxTAG and hydrolytic breakdown products such as FFA and DAG. The rate of TPC development over frying time is much dependent on the oil composition (Ahmad Tarmizi *et al.*, 2016). Many European countries tolerate an upper TPC limit of 24% to 27% due to health concerns of used oil (Li *et al.*, 2016). By taking 24% as a benchmark, frying in palm olein and soybean oil surpassed the limit after the 48th frying cycle, whereas sunflower oil gave a shorter frying time (32nd cycle). The results in Table 3 clearly indicate that red palm olein was the most stable as its TPC content was still acceptable even after the 60th frying cycle.

With respect to polar compound fractions, PTAG, OTAG, and DAG of all vegetable oils were significantly increased ($p\leq 0.05$) over frying cycles (Table 3). PTAG is developed when oils undergo tertiary oxidation and thermal alteration (Ahmad Tarmizi *et al.*, 2019b). The PTAG of sunflower and soybean oils were found to be higher than other oils. This result can be explained by the higher C18:2 content in oils. Oils rich in C18:2 are more easily polymerised during frying than those oils containing

higher C18:1 (Tompkins and Perkins, 2000). It is also reported that the formation rate of cyclic monomers and polymers was higher when the oils contain considerable level of C18:3 (Tompkins and Perkins, 2000).

Results in Table 3 showed that the OxTAG for all oils significantly increased ($p \leq 0.05$) across frying cycles. The trends of OxTAG in palm olein and soybean oil exhibited a rapid increase until the 48th cycle and did not show any notable increase thereafter. The results were consistent with the observation made by Ahmad Tarmizi *et al.* (2019b). For sunflower oil, OxTAG rapidly increased until the 32nd frying cycle and stabilised throughout the remaining frying cycle. Such occurrence is due to lipid oxidation where aldehydes and ketones were converted into compounds with high-molecular-

weight (Tsaknis *et al.*, 2002). However, a similar profile was not observed in red palm olein as OxTAG continued to build up consistently throughout 80 frying cycles. The increase in OxTAG was possibly caused by higher concentration of beta-carotenes that could be responsible as pro-oxidant during frying (Palozza *et al.*, 2003). Sunflower and soybean oils gave higher OxTAG when compared to palm olein and red palm olein even before frying. The result was similar to those observed by Li *et al.* (2017), who concluded that the presence of OxTAG was prominent in sunflower oil. However, after 80 frying cycles, all oils yielded comparable OxTAG contents due to prolonged oxidation during frying. According to Li *et al.* (2017), OxTAG swiftly increases at the beginning of frying and reaches a plateau as the frying progresses.

TABLE 3. POLAR COMPOUND FRACTIONS OF VEGETABLE OILS ACROSS FRYING CYCLES

Parameters (%)	Frying cycle	Palm olein	Sunflower oil	Red palm olein	Soybean oil
PTAG	0 th	0.02 ± 0.00 ^{cE}	0.40 ± 0.03 ^{aF}	0.01 ± 0.00 ^{dF}	0.12 ± 0.03 ^{bE}
	16 th	2.68 ± 0.16 ^{cD}	8.12 ± 0.30 ^{aE}	2.51 ± 0.12 ^{cE}	7.16 ± 0.24 ^{bD}
	32 nd	6.12 ± 0.22 ^{cC}	14.04 ± 0.21 ^{aD}	6.36 ± 0.19 ^{cD}	11.87 ± 0.41 ^{bC}
	48 th	8.97 ± 0.17 ^{cB}	18.40 ± 0.27 ^{aC}	8.25 ± 0.54 ^{cC}	16.15 ± 1.02 ^{bB}
	60 th	10.08 ± 0.83 ^{cA}	21.26 ± 0.21 ^{aB}	10.55 ± 0.56 ^{cB}	16.55 ± 1.58 ^{bB}
	80 th	10.69 ± 2.05 ^{dA}	22.95 ± 0.32 ^{aA}	13.06 ± 0.36 ^{cA}	19.06 ± 0.39 ^{bA}
OxTAG	0 th	0.53 ± 0.17 ^{bD}	1.84 ± 0.02 ^{aE}	0.42 ± 0.11 ^{bF}	1.81 ± 0.42 ^{aD}
	16 th	4.73 ± 0.13 ^{cC}	6.34 ± 0.11 ^{bD}	3.56 ± 0.47 ^{dE}	7.07 ± 0.27 ^{aC}
	32 nd	8.42 ± 0.59 ^{aB}	8.75 ± 0.13 ^{aC}	8.33 ± 0.31 ^{aD}	8.67 ± 0.55 ^{aB}
	48 th	10.54 ± 0.43 ^{aA}	9.91 ± 0.43 ^{aB}	9.75 ± 0.71 ^{aC}	10.00 ± 0.76 ^{aA}
	60 th	11.15 ± 0.52 ^{aA}	11.37 ± 0.40 ^{aA}	11.00 ± 0.47 ^{aB}	10.86 ± 0.61 ^{aA}
	80 th	11.96 ± 1.54 ^{abA}	11.61 ± 0.28 ^{ba}	13.20 ± 0.84 ^{aA}	11.76 ± 1.54 ^{abA}
DAG	0 th	6.67 ± 0.03 ^{aB}	1.55 ± 0.05 ^{cD}	3.47 ± 1.03 ^{bD}	1.00 ± 0.11 ^{dD}
	16 th	8.05 ± 0.24 ^{aA}	1.25 ± 0.02 ^{dF}	3.83 ± 0.24 ^{bD}	1.47 ± 0.04 ^{cC}
	32 nd	7.72 ± 0.07 ^{aA}	1.38 ± 0.05 ^{dE}	5.07 ± 0.13 ^{bC}	1.60 ± 0.13 ^{cC}
	48 th	7.72 ± 0.17 ^{aA}	1.65 ± 0.04 ^{dC}	5.38 ± 0.34 ^{bC}	1.91 ± 0.16 ^{cB}
	60 th	7.78 ± 0.40 ^{aA}	1.97 ± 0.05 ^{dB}	6.28 ± 0.36 ^{bB}	2.37 ± 0.60 ^{cAB}
	80 th	8.19 ± 0.89 ^{aA}	2.37 ± 0.05 ^{dA}	7.54 ± 0.30 ^{ba}	2.64 ± 0.08 ^{cA}
MAG	0 th	0.00 ± 0.00 ^{bA}	0.00 ± 0.00 ^{bA}	0.01 ± 0.00 ^{aB}	0.00 ± 0.00 ^{bA}
	16 th	0.00 ± 0.00 ^{bA}	0.00 ± 0.00 ^{bA}	0.01 ± 0.00 ^{aB}	0.00 ± 0.00 ^{bA}
	32 nd	0.00 ± 0.00 ^{aA}	0.00 ± 0.00 ^{aA}	0.00 ± 0.00 ^{aC}	0.00 ± 0.00 ^{aA}
	48 th	0.00 ± 0.00 ^{aA}	0.00 ± 0.00 ^{aA}	0.00 ± 0.00 ^{aC}	0.00 ± 0.00 ^{aA}
	60 th	0.00 ± 0.00 ^{bA}	0.00 ± 0.00 ^{bA}	0.01 ± 0.00 ^{aB}	0.00 ± 0.00 ^{bA}
	80 th	0.00 ± 0.00 ^{bA}	0.00 ± 0.00 ^{bA}	0.03 ± 0.00 ^{aA}	0.00 ± 0.00 ^{bA}
FFA	0 th	0.02 ± 0.00 ^{dB}	0.26 ± 0.03 ^{aA}	0.05 ± 0.01 ^{cBC}	0.13 ± 0.00 ^{bB}
	16 th	0.03 ± 0.01 ^{bA}	0.19 ± 0.02 ^{aB}	0.04 ± 0.01 ^{bC}	0.17 ± 0.03 ^{aA}
	32 nd	0.03 ± 0.02 ^{cA}	0.24 ± 0.01 ^{aA}	0.07 ± 0.02 ^{cB}	0.20 ± 0.02 ^{bA}
	48 th	0.05 ± 0.01 ^{bA}	0.29 ± 0.04 ^{aA}	0.09 ± 0.03 ^{bB}	0.29 ± 0.09 ^{aA}
	60 th	0.06 ± 0.02 ^{cA}	0.30 ± 0.07 ^{aA}	0.11 ± 0.02 ^{bAB}	0.21 ± 0.02 ^{aA}
	80 th	0.06 ± 0.03 ^{cA}	0.33 ± 0.08 ^{aA}	0.16 ± 0.03 ^{ba}	0.24 ± 0.04 ^{aA}
TPC	0 th	7.24 ± 0.24 ^{aD}	4.05 ± 0.11 ^{bF}	3.97 ± 1.42 ^{bF}	3.09 ± 0.59 ^{cE}
	16 th	15.50 ± 0.53 ^{aC}	15.90 ± 0.37 ^{aE}	9.94 ± 0.79 ^{bE}	15.88 ± 0.12 ^{aD}
	32 nd	22.38 ± 0.55 ^{bB}	24.40 ± 0.37 ^{aD}	19.90 ± 0.06 ^{cD}	22.37 ± 0.96 ^{bC}
	48 th	27.28 ± 0.23 ^{bA}	30.24 ± 0.66 ^{aC}	23.48 ± 1.29 ^{cC}	28.35 ± 1.95 ^{bB}
	60 th	28.96 ± 1.68 ^{cA}	35.00 ± 0.49 ^{aB}	27.95 ± 1.43 ^{cB}	30.90 ± 1.41 ^{bB}
	80 th	30.90 ± 4.82 ^{cA}	37.26 ± 0.28 ^{aA}	34.00 ± 0.98 ^{baA}	33.70 ± 0.04 ^{baA}

Note: ^{a-d} values within the same row with different letters are significantly different ($p < 0.05$).

^{A-F} values within the same column with different letters are significantly different ($p < 0.05$).

Value are the means ± SD of duplicate experiments with triplicate determinations.

Irrespective of oil types, the DAG showed a significant increase ($p \leq 0.05$) as frying progressed (Table 3). Elevation in DAG is due to the hydrolytic reaction when moisture released from food interacts with oil during frying (Li *et al.*, 2017). In palm olein, DAG built-up coincides with frying cycles where short-chain fatty acids attached to TAG molecules are more susceptible to hydrolysis (Li *et al.*, 2017). It is a known fact that palm olein and red palm olein have naturally higher DAG content than other soft oils (Berger, 2005). For such circumstances, the measurement of TPC to solely distinguish the degree of oil deterioration seems unfair to palm oil products as the inherently higher DAG content could lead to misleading information on oil quality and stability (Ahmad Tarmizi *et al.*, 2019b). On that note, the focus should emphasise more on PTAG and OxTAG in judging the degree of oil deterioration.

The Pearson's correlation between polar compound fractions and acrylamide content upon extended frying is summarised in Table 4. A weak negative correlation ($r = -0.269$, $p \leq 0.05$) was observed between PTAG in oils and acrylamide content in the fried product. However, there was no correlation ($p > 0.05$) found between TPC, OxTAG, PTAG + OxTAG, and DAG on the presence of acrylamide in the fried product irrespective to frying media. Comparison of results with published literature was not possible due to the unavailability of such studies. Gertz *et al.* (2003) reported that TPC development enables to motivate heat conductivity of the oil over frying time. Enhancement of heat transfer would further allow more interaction between acrylamide precursor and hot oil, which in turn, increases the incidence of acrylamide formation. Our study, however, showed that the formation of acrylamide in fried products ceased with the PTAG built-up over frying time as evidenced by a weak negative correlation (Table 4). PTAG evolution would compete with glycerol molecules to form either acrylamide or skewed towards polymerisation to form PTAG.

TABLE 4. PEARSON'S CORRELATION BETWEEN ACRYLAMIDE CONCENTRATION AND PTAG, OTAG AND DAG OF VEGETABLE OILS, RESPECTIVELY

Parameters	Pearson's correlation	
	<i>r</i>	<i>p</i> -value
TPC	-0.152	0.181
PTAG	-0.269	$p \leq 0.05$
OxTAG	-0.023	0.838
PTAG + OxTAG	-0.209	0.065
DAG	0.192	0.091

This study showed that the alteration of FAC for all vegetable oils was significant across 80 frying cycles. The SFA gave a significant increase while PUFA exhibited a significant opposite trend

as a result of prolonged frying. A significant positive correlation was perceived among SFA, MUFA, and PUFA in vegetable oils and acrylamide content in the fried product. The results denoted that a higher degree of oil unsaturation could fasten lipid oxidation and thus, increase the manifestation of acrylamide formation. The amount of TPC developed in vegetable oils also portrayed a significant increase over frying cycles. Considerable elevation of PTAG, OxTAG, and DAG upon the increment of the frying cycle is due to the occurrence of oxidation aside from hydrolysis and polymerisation during frying. A significant negative correlation was observed between PTAG in vegetable oils and acrylamide content in French fries.

CONCLUSION

This study showed that the alteration of FAC for all vegetable oils was significant across 80 frying cycles. The SFA gave a significant increase while PUFA exhibited a significant opposite trend as a result of prolonged frying. A significant positive correlation was perceived among SFA, MUFA, and PUFA in vegetable oils and acrylamide content in the fried product. The results denoted that a higher degree of oil unsaturation could fasten lipid oxidation and thus, increase the manifestation of acrylamide formation. The amount of TPC developed in vegetable oils also portrayed a significant increase over frying cycles. Considerable elevation of PTAG, OxTAG, and DAG upon the increment of the frying cycle is due to the occurrence of oxidation aside from hydrolysis and polymerisation during frying. A significant negative correlation was observed between PTAG in vegetable oils and acrylamide content in French fries.

ACKNOWLEDGEMENT

We acknowledge the financial support from the Ministry of Education of Malaysia (Project No. FRGS/1/2020/STG04/UPM/02/16 and UPM/800-4/11/MRUN/2019/5539123).

REFERENCES

Abd Razak, R A; Ahmad Tarmizi, A H; Kuntom, A; Sanny, M and Ismail, I S (2021). Intermittent frying effect on French fries in palm olein, sunflower, soybean and canola oils on quality indices, 3-monochloropropane-1,2-diol esters (3-MCPDE), glycidyl esters (GE) and acrylamide contents. *Food Control*, 124: 107887.

- Abdul Hamid, N; Omar, S and Sanny, M (2019). Effect of thawing conditions and corresponding frying temperature profiles on the formation of acrylamide in French fries. *J. Saudi Soc. Agric. Sci.*, 18: 396-400.
- Ahmad, S N S; Tarmizi, A H A; Razak, R A A; Jinap, S; Norliza, S; Sulaiman, R and Sanny, M (2021). Selection of vegetable oils and frying cycles influencing acrylamide formation in the intermittently fried beef nuggets. *Foods*, 10: 257.
- Ahmad Tarmizi, A H; Abd Razak, R A; Abdul Hamid, A N and Kuntom, A (2019a). Effect of anti-clouding agent on the fate of 3-monochloropropane-1,2-diol esters and glycidyl esters in palm olein during repeated frying. *Molecules (Basel, Switzerland)*, 24: 2332.
- Ahmad Tarmizi, A H; Hishamuddin, E and Abd Razak, R A (2019b). Impartial assessment of oil degradation through partitioning of polar compounds in vegetable oils under simulated frying practice of fast food restaurants. *Food Control*, 96: 445-455.
- Ahmad Tarmizi, A H; Ismail, R and Kuntom, A (2016). Effect of frying on the palm oil quality attributes - A review. *J. Oil Palm Res.*, 28(2): 143-153.
- Ahmad Tarmizi, A H and Niranjana, K (2013). Combination of moderate vacuum frying with high vacuum drainage-relationship between process conditions and oil uptake. *Food Bioprocess Technol.*, 6: 2600-2608.
- Alireza, S; Tan, C P; Hamed, M and Che Man, Y B (2010). Effect of frying process on fatty acid composition and iodine value of selected vegetable oils and their blends. *Int. Food Res. J.*, 17: 295-302.
- Andrikopoulos, N K; Kalogeropoulos, N; Faliro, A and Barbogianni, M N (2002). Performance of virgin olive oil and vegetable shortening during domestic deep-frying and pan-frying of potatoes. *Int. J. Food Sci. Technol.*, 37: 177-190.
- Aniolowska, M and Kita, A (2016). The effect of frying on glycidyl esters content in palm oil. *Food Chem.*, 203: 95-103.
- AOCS (2011). Official Method Ce 1i-07. Official Methods and Recommended Practices of the AOCS. Champaign, IL, USA, AOCS.
- Berger, K G (2005). The use of palm oil in frying. Malaysian Palm Oil Council. Kelana Jaya, Selangor.
- Capuano, E; Oliviero, T; Acar, O C; Gokmen, V and Fogliano, V (2010). Lipid oxidation promotes acrylamide formation in fat-rich model systems. *Food Res. Int.*, 43: 1021-1026.
- Choe, E and Min, D B (2009). Mechanisms of antioxidants in the oxidation of foods. *Compr. Rev. Food Sci. Food Saf.*, 8: 345-358.
- Cohn, J (2002). Oxidized fat in the diet, postprandial lipaemia and cardiovascular disease. *Curr. Opin. Lipidol.*, 13: 19-24.
- Daniali, G; Jinap, S; Hajeb, P; Sanny, M and Tan, C P (2016). Acrylamide formation in vegetable oils and animal fats during heat treatment. *Food Chem.*, 212: 244-249.
- Daniali, G; Jinap, S; Sanny, M and Tan, C P (2018). Effect of amino acids and frequency of reuse frying oils at different temperature on acrylamide formation in palm olein and soybean oils via modeling system. *Food Chem.*, 245: 1-6.
- Dobarganes, M C; Velasco, J and Dieffenbacher, A (2000). Determination of polar compounds, polymerized and oxidized triacylglycerols, and diacylglycerols in oils and fats: Results of collaborative studies and the standardized method (Technical report). *Pure Appl. Chem.*, 72: 1563-1575.
- Ehling, S and Shibamoto, T (2005). Correlation of acrylamide generation in thermally processed model systems of asparagine and glucose with color formation, amounts of pyrazines formed and antioxidative properties of extracts. *J. Agric. Food Chem.*, 53: 4813-4819.
- European-Commission (2017). ANNEXES to the Commission Regulation (EU) establishing mitigation measures and benchmark levels for the reduction of the presence of acrylamide in food. https://ec.europa.eu/info/law/better-regulation/initiatives/ares-2017-2895100_en, accessed on 30 November 2016.
- Gertz, C and Behmer, D (2014). Application of FT-NIR spectroscopy in assessment of used frying fats and oils. *Eur. J. Lipid Sci. Technol.*, 116: 756-762.
- Gertz, C and Klostermann, S (2002). Analysis of acrylamide and mechanisms of its formation in deep-fried products. *Eur. J. Lipid Sci. Technol.*, 104: 762-771.
- Gertz, C; Klostermann, S and Kochhar, S P (2003). Deep frying: The role of water from food being fried and acrylamide formation. *Oleagineux Corps Gras Lipides*, 10: 297-303.

- Hubbard, L J and Farkas, B (2007). A method for determining the convective heat transfer coefficient during immersion frying. *J. Food Process Eng.*, 22: 201-214.
- IARC (1994). Some industrial chemicals. Lyon. <https://monographs.iarc.fr/wp-content/uploads/2018/06/mono60.pdf>, accessed on 12 October 2018.
- Ismail, R (2005). Palm oil and palm olein frying applications. *Asia Pac. J. Clin. Nutr.*, 14: 414-419.
- Kamarudin, S A; Jinap, S; Sukor, R; Foo, S P and Sanny, M (2018). Effect of fat-soluble anti-oxidants in vegetable oils on acrylamide concentrations during deep-fat frying of French fries. *Malays. J. Med. Sci.*, 25: 128-139.
- Kuek, S L; Ahmad Tarmizi, A H; Abd Razak, R A; Jinap, S; Norliza, S and Sanny, M (2020). Contribution of lipid towards acrylamide formation during intermittent frying of French fries. *Food Control*, 118: 107430.
- Kushairi, A; Ong-Abdullah, M; Nambiappan, B; Hishamuddin, E; Bidin, M N I Z; Ghazali, R; Subramaniam, V; Sundram, S and Parveez, G K A (2019). Oil palm economic performance in Malaysia and R&D progress in 2018. *J. Oil Palm Res.*, 31: 165-194.
- Lalas, S (2009). Quality of frying oil. *Advances in Deep-Fat Frying of Foods* (Sahin, S and Sumnu, S G eds.). CRC Press, Taylor and Francis Group, Florida, USA. p. 57-80.
- Li, J; Li, X; Cai, W and Liu, Y (2016). Comparison of different polar compounds-induced cytotoxicity in human hepatocellular carcinoma HepG2 cells. *Lipids Health Dis.*, 15: 30.
- Li, X; Li, J; Wang, Y; Cao, P and Liu, Y (2017). Effects of frying oils' fatty acids profile on the formation of polar lipids components and their retention in French fries over deep-frying process. *Food Chem.*, 237: 98-105.
- Lim, P K; Jinap, S; Sanny, M; Tan, C P and Khatib, A (2014). The influence of deep frying using various vegetable oils on acrylamide formation in sweet potato (*Ipomoea batatas* L. Lam) chips. *J. Food Sci.*, 79: T115-T121.
- Luning, P A and Sanny, M (2016). Acrylamide in fried potato products. *Acrylamide in Food: Analysis, Content and Potential Health Effects* (Gokmen, V ed.). Elsevier Inc., Waltham, USA. p. 159-179.
- Palozza, P; Serini, S; Di Nicuolo, F; Piccioni, E and Calviello, G (2003). Prooxidant effects of beta-carotene in cultured cells. *Mol. Aspects Med.*, 24: 353-362.
- Petersen, K D; Jahreis, G; Busch-Stockfisch, M and Fritsche, J (2013). Chemical and sensory assessment of deep-frying oil alternatives for the processing of French fries. *Eur. J. Lipid Sci. Technol.*, 115: 935-945.
- Romero, A; Cuesta, C and Sanchez-Muniz, F J (1998). Effect of oil replenishment during deep-fat frying of frozen foods in sunflower oil and high-oleic acid sunflower oil. *J. Am. Oil Chem. Soc.*, 75: 161-167.
- Saguy, I S and Dana, D (2003). Integrated approach to deep fat frying: Engineering, nutrition, health and consumer aspects. *J. Food Eng.*, 56: 143-152.
- Sanny, M; Jinap, S; Bakker, E J; Van Boekel, M A J S and Luning, P A (2012). Possible causes of variation in acrylamide concentration in French fries prepared in food service establishments: An observational study. *Food Chem.*, 132: 134-143.
- Sanny, M; Luning, P A; Marcelis, W J; Jinap, S and van Boekel, M A J S (2010). Impact of control behaviour on unacceptable variation in acrylamide in French fries. *Trends Food Sci. Technol.*, 21: 256-267.
- Santos, C S P; Cruz, R; Cunha, S C and Casal, S (2013). Effect of cooking on olive oil quality attributes. *Food Res. Int.*, 54: 2016-2024.
- Sebedio, J L; Bonpunt, A; Grandgirard, A and Prevost, J (1990). Deep fat frying of frozen prefried french fries: Influence of the amount of linolenic acid in the frying medium. *J. Agric. Food Chem.*, 38: 1862-1867.
- Seppanen, M C and Csallany, A (2006). The effect of intermittent and continuous heating of soybean oil at frying temperature on the formation of 4-hydroxy-2- trans-nonenal and other alpha, beta-unsaturated hydroxylaldehydes. *J. Am. Oil Chem. Soc.*, 83: 121-127.
- Stadler, R H; Blank, I; Varga, N; Robert, F; Hau, J; Guy, P A; Robert, M C and Riediker, S (2002). Acrylamide from Maillard reaction products. *Nature*, 419: 449-450.
- Tompkins, C and Perkins, E C (2000). Frying performance of low-linolenic acid soybean oil. *J. Am. Oil Chem. Soc.*, 77: 223-229.
- Tsaknis, J; Lalas, S and Protopapa, E (2002). Effectiveness of the antioxidants BHA and BHT in

selected vegetable oils during intermittent heating. *Grasas y Aceites*, 53: 199-205.

Wang, Y; Hu, H; McClements, D J; Nie, S; Shen, M; Li, C; Huang, Y; Zhong, Y; Chen, J; Zeng, M and Xie, M (2019). pH and lipid unsaturation impact the formation of acrylamide and 5-hydroxymethylfurfural in model system at frying temperature. *Food Res. Int.*, 123: 403-413.

Williams, J S E (2005). Influence of variety and processing conditions on acrylamide levels in fried potato crisps. *Food Chem.*, 90: 875-881.

Yasuhara, A; Tanaka, Y; Hengel, M and Shibamoto, T (2003). Gas chromatographic investigation of

acrylamide formation in browning model systems. *J. Agric. Food Chem.*, 51: 3999-4003.

Zhang, H; Cheng, L; Wang, L and Qian, H (2015). Influence of deep-frying using various commercial oils on acrylamide formation in French fries. *Food Addit. Contam. - Part A Chem. Anal. Control Expo. Risk Assess.*, 32: 1083-1088.

Zribi, A; Jabeur, H; Aladedunye, F; Rebai, A; Matthaus, B and Bouaziz, M (2014). Monitoring of quality and stability characteristics and fatty acid compositions of refined olive and seed oils during repeated pan - and deep-frying using GC, FT-NIRS, and chemometrics. *J. Agric. Food Chem.*, 62: 10357-10367.

ASSESSMENT OF FATTY ACID AND VITAMIN E PROFILES IN EDIBLE OILS IN MALAYSIA

RADHIKA LOGANATHAN¹ and TENG KIM-TIU^{1*}

ABSTRACT

Cooking oil plays a pivotal role in our daily diet. The present investigation was designed to characterise the fatty acid composition and eight homologs of vitamin E in a total of 52 locally available vegetable oils [types: pure oils (n=10) and blended oils (n=6)] in Putrajaya, Malaysia. The analyses were performed by gas chromatography for fatty acid composition, the acetic acid-isooctane method for peroxide value (PV), and high-performance liquid chromatography (HPLC) for vitamin E profiling. Following multivariate discriminant analysis, the oils were categorised into two groups. Most studies and oil standards have reported only part of eight vitamin E homologs, therefore this current study is novel as it provides the complete vitamin E profiling of the tested oils. There was no major discrepancy found between the assayed values and packaging labels on the fatty acid composition, trans-fat, and vitamin E content. The PV and trans-fats were within the recommended range, indicating good quality compliance. Fatty acid and vitamin E content were within standard oil specifications. These observations implied that the oil manufacturers and retailers in Malaysia had good acquiescence to international standards and local regulations. This study provides consumers with technical insights in oil selection, based on the functionality needed.

Keywords: blended oils, edible oils, fatty acid composition, peroxide value, vitamin E.

Received: 12 November 2021; **Accepted:** 22 February 2022; **Published online:** 14 April 2022.

INTRODUCTION

The total world consumption for oils and fats in the year 2020 was 234.8 million tonnes (Oil World Annual, 2020). Palm oil (32.2%) was the most widely consumed amongst the 17 listed major oils and fats (Oil World Annual, 2020). This is followed by soybean oil (24.1%), rapeseed oil (10.5%), sunflower oil (9.0%), tallow (4.2%), butter (3.7%), palm kernel oil (3.4%), lard (3.3%), cotton oil (2.0%), corn oil (1.8%), groundnut oil (1.7%), olive oil (1.5%), coconut oil (1.2%), fish oil (0.4%), sesame oil (0.4%), linseed oil (0.4%) and lastly castor oil (0.3%) (Oil World Annual, 2020).

Cooking oil is an essential part of our daily diet preparation. Fats and oils are made up of triglycerides which consist of a glycerol backbone

esterified with three fatty acids (Figure 1). Fats and oils play a pivotal role in maintaining good health and they provide more calories per gram (9 kcal g⁻¹) than any other nutrients (Chowdhury *et al.*, 2007). Fatty acids are grouped into three broad categories namely saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) depending on the number of double bonds (Chowdhury *et al.*, 2007). The World Health Organization (WHO) recommends 20.0%-35.0% energy (E) total fat from total energy intake for adults (WHO, 2008). Intake of SFA and trans-fats are recommended to be <10.0% and <1.0% respectively of total energy intake. The acceptable range for total PUFA (n-6 and n-3 fatty acids) consumption is 6.0%-11.0% E from total energy intake of which n-3 fatty acid intake ranges between 0.5%-2.0% energy [$>0.5\%$ E alpha-linolenic acid (ALA) plus eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (0.25-2.00 g/day)], whereas n-6 fatty acids (linoleic acid, LA) intake ranges between 2.5%-9.0% E (Elmadfa

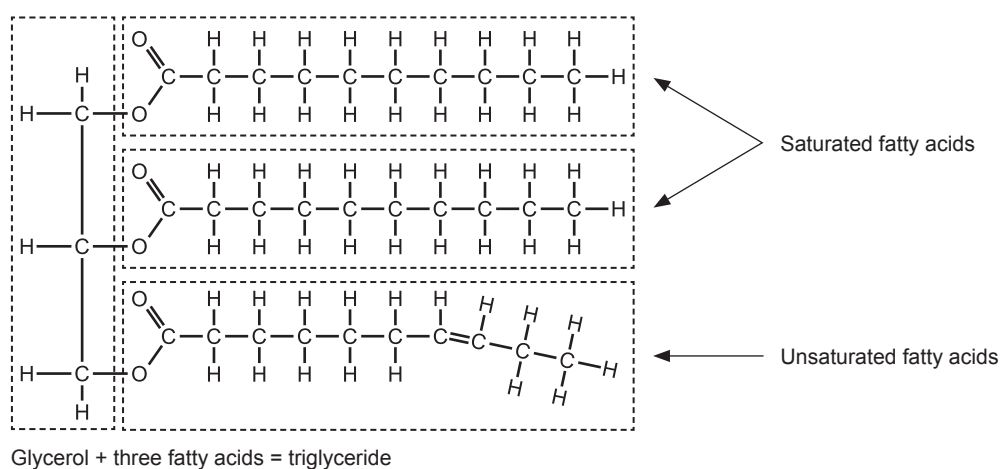
¹ Malaysian Palm Oil Board,
6 Persiaran Institusi, Bandar Baru Bangi,
43000 Kajang, Selangor, Malaysia.

* Corresponding author e-mail: kimtiu@mpob.gov.my

and Kornsteiner, 2009; WHO, 2008). Wallingford *et al.* (2004) reported that the fatty acid profile of edible oils in China is often incompletely and incorrectly labelled. There are some literature on the characterisation of fatty acids in commercial oils in China (Haiyan *et al.*, 2007; Wallingford *et al.*, 2004), India (Dorni *et al.*, 2018), the Spanish market (Rueda *et al.*, 2014), Iran (Piravi-Vanak *et al.*, 2009) and Bangladesh (Chowdhury *et al.*, 2007). However, none were found in the Malaysian setting.

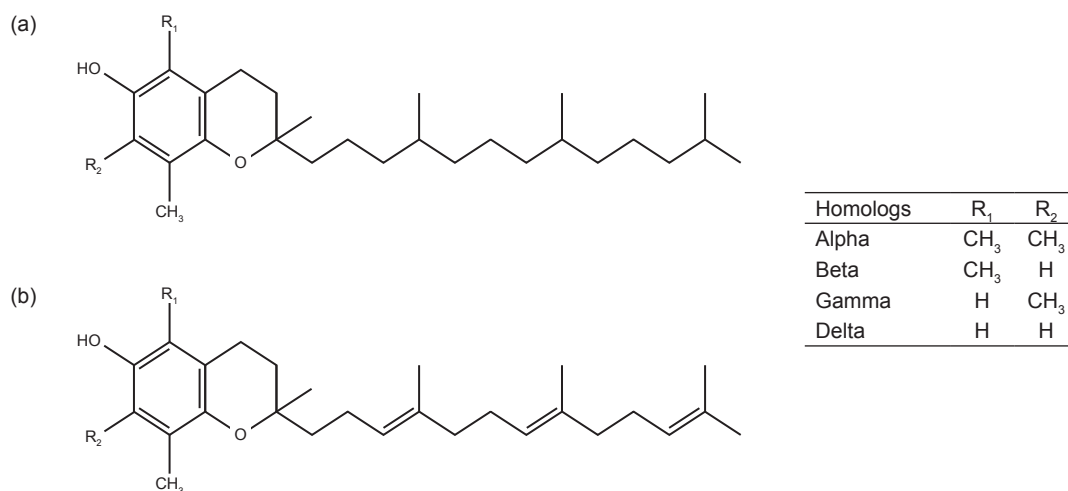
Vitamin E or tocols is a fat-soluble vitamin that is found abundantly in oilseeds and nuts (Aksoz *et al.*, 2020). Vitamin E is a family of eight similar homologs that exists naturally as two families namely tocopherols and tocotrienols (Figure 2). Each

vitamin E family exists naturally in four isoforms namely alpha, beta, delta and gamma (Loganathan *et al.*, 2021). Vitamin E is a potent antioxidant with lipoperoxyl radical scavenging activities which could protect the oils against oxidative deterioration (Loganathan *et al.*, 2020a; 2020b). Besides, the content of vitamin E in oil reflects the oil quality (Wen *et al.*, 2020). Most studies (Aksoz *et al.*, 2020; Gliszczynska-Swiglo *et al.*, 2007; Matthaus *et al.*, 2016; Petersen *et al.*, 2012) and standards (Codex Alimentarius, 1999; 2001; 2019) have reported only part of eight vitamin E homologs, and more attention has been given to tocopherols as compared to tocotrienols. Therefore, there is a gap of knowledge in understanding the complete vitamin E profiling.



Source: Lichtenstein (2013).

Figure 1. Molecular structure of a triglyceride.



Source: Fu *et al.* (2014).

Figure 2. Molecular structure of (a) tocopherols and (b) tocotrienols with homologs determined based on the presence and position of methyl group(s) as side chains at R1 and R2 on the chromanol ring.

Fats and oils emanate a great deal of variation and uniqueness by nature in their fatty acid compositions, phytonutrient contents, and physiological properties. The aims of this work were to (1) characterise the fatty acid present and vitamin E content of locally available vegetable oils; (2) study the quality of oils on the shelf in terms of peroxide value (PV); (3) determine the accuracy of the packaging labels to the assayed values.

MATERIALS AND METHODS

Edible Oil Samples

Edible oil samples of 11 different varieties: Palm oil (five samples), canola oil (five samples), sunflower oil (nine samples), corn oil (seven samples), soybean oil (four samples), olive oil (11 samples), rice bran oil (one sample), avocado oil (one sample), grapeseed oil (one sample), rapeseed oil (one sample), and blended oils (13 samples) were purchased from the local supermarket around Putrajaya, Malaysia area between October to November 2015. Oils were transferred into six dark amber bottles, blanketed with nitrogen, and stored at -20°C for subsequent analyses. All labels on oil samples were tabulated and analysed alongside.

Chemicals and Reagents

Tocopherol and tocotrienol homologs (alpha, beta, gamma and delta) were purchased from Davos Life Sciences Pte. Ltd. (Singapore); 2,2,5,7,8-pentamethyl-6-hydroxychromane (PMC) from Wako (Osaka, Japan); AOCS oil reference mixture RM-6 from Supelco (Bellefonte, USA); High-Performance Liquid Chromatography (HPLC) gradient grade methanol, 1-propanol, *n*-hexane, toluene and dioxane from Merck (Darmstadt, Germany); potassium hydrogen carbonate, glacial acetic acid, potassium iodide, sodium chloride isooctane, starch, sodium thiosulphate from System ChemAR (Shah Alam, Malaysia).

Preparation of Fatty Acid Methyl Ester (FAME)

The fatty acid composition of the test fats was determined by converting fatty acids of triglyceride to FAME according to AOCS Official Method Ch 1-91 (The American Oil Chemists' Society, 2017c).

Fatty Acid Composition Analysis by Gas Chromatography (GC)

Analysis of fatty acid composition was carried out according to AOCS Official Method Ce 1a-13 (The American Oil Chemists' Society, 2017b) on GC

Perkin Elmer Autosystem Model, USA. The GC was fitted with the SGE Capillary BPX70 column from SGE Analytical Science Pty. Ltd. (Milton Keynes, United Kingdom). The identification of fatty acids was based on AOCS oil reference mixture RM-6. The results were expressed as relative percentages of wet weight.

Vitamin E Analysis by HPLC

Vitamin E content was analysed by normal phase HPLC Agilent model 1100 series, the US with fluorescence detector. The mean recovery of internal standard, namely 2,2,5,7,8-pentamethyl-6-hydroxy-Chromane (PMC) was 93.07% $\pm$ 0.72%. The chromatographic analysis of the compounds was conducted on Phenomenex® Luna 5 μ M Silica analytical column (250 mm x 4.6 mm i.d) and a mobile phase of hexane/dioxane/isopropyl alcohol (970:25:5) at a flow rate of 1.0 mL min⁻¹. Identification and quantification of the components were done by comparison of the peak areas with those pure standards. Values are presented as mg kg⁻¹ of wet weight.

Peroxide Value (PV)

Analysis of PV was performed with reference to the AOCS Official Method Cd 8b-90 (The American Oil Chemists' Society, 2017a). Briefly, an oil sample weighing 5.0 g was placed in the conical flask and 50.0 mL of acetic acid/iso-octane solution (3:2) was added. The sample was stirred until it has been well dissolved before the addition of 0.5 mL of saturated potassium iodide. The solution was shaken thoroughly and thereafter, immediately, with 30.0 mL of distilled water and 0.5 mL of starch solution. The sample was titrated with 0.1 M sodium thiosulphate until the blue colour disappears. PV of the oil samples was measured in milliequivalent of peroxide per kg of oil sample (wet weight).

Data Presentation

All analytical determinations were performed at minimum in duplicates and presented as the mean values $\pm$ standard deviation. One-way analysis of variance ANOVA followed by Tukey's *post hoc* test was applied to assess the differences between oils ($p < 0.05$). Cluster analyses were performed by multivariate statistical classification methods. Following hierarchical cluster analysis, discriminant analysis was applied to analyse differences among the oil groups. Statistical analysis was conducted using GraphPad Prism version 9.3.1 and SPSS version 11.5.

RESULTS AND DISCUSSION

All assayed samples had fatty acid profiles that fall within standard database values (Codex Alimentarius, 1999; 2001; 2019) and corresponded closely with the total fatty acid content of the packaging label. The fatty acid composition of the oils is shown in Table 1 and Figure 3. Heat map visualisation in Figure 4 provides a direct intuitive visualisation of the different contents of total SFA, total MUFA, and total PUFA in assayed edible oils by category.

Following cluster analysis depicted in Figure 5, the oils can be classified into two main groups namely high MUFA: high oleic sunflower (85.00%) > olive > avocado > rapeseed > canola > red palm olein and canola blend/canola and sunflower blend > palm olein/palm olein, peanut and sesame blend > soybean, palm olein and canola blend (38.00%); and high PUFA [grapeseed (71.95%) > sunflower > soybean > olive and sunflower blend > corn oil > corn, soybean, and canola blend (50.47%)] oils.

Within the MUFA group, the oils were further classified into five sub-classes (Figure 5). The first subclass had an equal composition of MUFA (45.11%-45.41%) and SFA (42.54%-43.42%). Palm olein is a member of the first subclass. Palm olein is derived from the mesocarp of the fruit of the oil palm, *Elaeis guineensis* (Kushairi *et al.*, 2019). Olein fractions are widely used as cooking oils and suitable as a heavy-duty frying oil. There are two main grades of palm olein, namely single-fractionated palm olein and double-fractionated palm olein (Ahmad Tarmizi and Siew, 2008). All five tested palm olein were found to be of double fractionated grade. The tertiary blend of palm olein, peanut, and sesame oil (n=3) had an almost similar fatty acid composition to that of palm olein. The second subclass had MUFA concentrations ranging from 51.91%-64.06%. Low erucic acid canola and rapeseed oils classified in this group were derived from a variety of rapeseed cultivars of the plant family *Brassicaceae* (Codex Alimentarius, 1999; Matthaus *et al.*, 2016). Similar to Matthaus *et al.* (2016), we too find these oils consisted mainly of oleic (C18:1) (51.80%-63.80%), linoleic (C18:2 N6C) (18.60%-25.30%), and n-3 fatty acid (C18:3N3, 8.00%-11.20%). The binary blend of high MUFA canola oil with high PUFA sunflower oil (n=4) as well as the blend of MUFA rich oils namely red palm olein and canola oil (n=1) also belongs to this group. These unique blends resulted in low SFA content (9.23%-18.18%) but high MUFA (51.91%-54.14%) and PUFA (27.68%-38.87%) profiles. High oleic sunflower oil is the only member of subclass three with distinctly high MUFA content. High oleic acid sunflower oil is produced from high oleic acid oil-bearing seeds of the same plant (*Helianthus annuus* L.) (Codex Alimentarius, 1999). Following previous

bibliographic data (Chowdhury *et al.*, 2007; Petersen *et al.*, 2012), the fractions corresponded almost entirely to C18:1N9C (84.53%). Olive and avocado oils were classified under sub-class four. Olive oil is the oil obtained from the fruit of the long-lived olive tree (*Olea europaea* L.) (Codex Alimentarius, 2001). We have evaluated three types of olive oils, namely extra virgin (n=3), extra light (n=2) and refined/pure (n=5). All three types of olive oil had similar fatty acid compositions and this was in agreement with data from the literature (Piravi-Vanak *et al.*, 2009) and standards (Codex Alimentarius, 2001; International Olive Council, 2006). Another member of this subclass namely avocado oil is an edible oil extracted from the fruit of *Persea Americana* (Haytowitz *et al.*, 2011). Similar to the current study, Haiyan *et al.* (2007) also reported avocado oil had an almost similar fatty acid composition to that of canola/rapeseed oil, with predominantly MUFA (C18:1N9C). Avocado oil can be distinguished from other tested oil with a notable presence of C16:1 (7.80%), of which a similar observation was also reported in the literature (Rueda *et al.*, 2014). A tertiary blend of soybean, palm olein, and canola oils was classified as subclass five. This blend had SFA (30.44%): MUFA (37.82%): PUFA (31.74%) content at a 1:1:1 ratio.

PUFA group, on the other hand, can be further divided into two big clusters. Sunflower, soybean, corn, binary blended oil (olive and sunflower), and tertiary blended oil (corn, soybean, and canola) were classified as one group (sub-class six). These oils have almost similar fatty acid composition (PUFA: 50.47%-62.49%, MUFA: 23.93%-37.06%, and SFA: 10.65%-15.71%). Sunflower (*Helianthus annuus* L.) is an annual crop that belongs to the family Asteraceae or Compositae (Codex Alimentarius, 1999). Sunflower oil is extracted from the sunflower seeds. Corn oil is a by-product from the wet or dry milling of maize germ (the embryos of *Zea mays*) (Jovanović *et al.*, 2005). The primary product from this milling is starch (Jovanović *et al.*, 2005). Soybean oil is derived from soybeans (*Glycine max* (L.) Merr.) that belong to the nitrogen-fixing leguminous plant (Codex Alimentarius, 1999). Soybean oil can be distinguished from the other members of the subclass as it had the highest levels of C18:3N3 (5.50%-7.10%) (Chowdhury *et al.*, 2007; Haiyan *et al.*, 2007). Grapeseed oil was classified as a separate subclass (No. 7) under the PUFA group. Grapeseed oil is derived from the seeds of the grapes (*Vitis vinifera* L.) (Codex Alimentarius, 1999). Grapeseed oil is a winery industry by-product (Martin *et al.*, 2020) and was found to have the highest PUFA content (72.00%) in the form of C18:2N6C as compared to other tested oils.

Most oil groups had no *trans*-fatty acid (C18:2N6T- linolelaidic acid) except for sunflower, corn, soybean, binary blends of canola and

sunflower oil, and tertiary blends of soybean, palm olein, and canola oils (Table 1). A slight variation was found between the assayed samples values as compared to packaging labels for canola oil (samples 6-9), sunflower oil (samples 11-17), and soybean oil (sample 27).

The original American Heart Association (AHA) Step I Diet fat recommended 30% E from dietary fat and fatty acid balance, SFA: MUFA: PUFA at 1:1:1 (Krauss *et al.*, 2000), corresponding to soybean, palm olein, and canola blended oil. Currently, the AHA recommends slightly less SFA (<10% of calories), up to 10% from PUFA, and as much as 15% from MUFA (Krauss *et al.*, 2000). Raising the dietary PUFA/SFA ratio has been recommended to reduce cardiovascular disease event risk and improve glycaemic control (Kang *et al.*, 2005). WHO recommended PUFA/SFA ratio above 0.4 (Coskuntuna *et al.*, 2015). Nevertheless, a high dietary PUFA/SFA ratio increases oxidative stress since PUFA are highly prone to lipid peroxidation (Kang *et al.*, 2005). Only olive oil, avocado oil, and tertiary blended oil (soybean, palm olein, and canola oil) had a desirable PUFA/SFA ratio of 0.4-1.0. Palm olein had a lower than ideal PUFA/SFA ratio (>0.4). Whereas sunflower oil (5.90), grapeseed oil (8.83), and binary blended olive and sunflower oil (5.59) had a very high ratio of PUFA/SFA content. Edible oils are usually blended to contain a better proportion of SFAs, MUFAs, PUFAs, and essential fatty acids. The downside of blended oils was that the ratio of blending was not written on any of the labels. It was interesting to note that the same type of blended oil carried similar fatty acid composition, implying a similar ratio of blending, although the manufacturers were different.

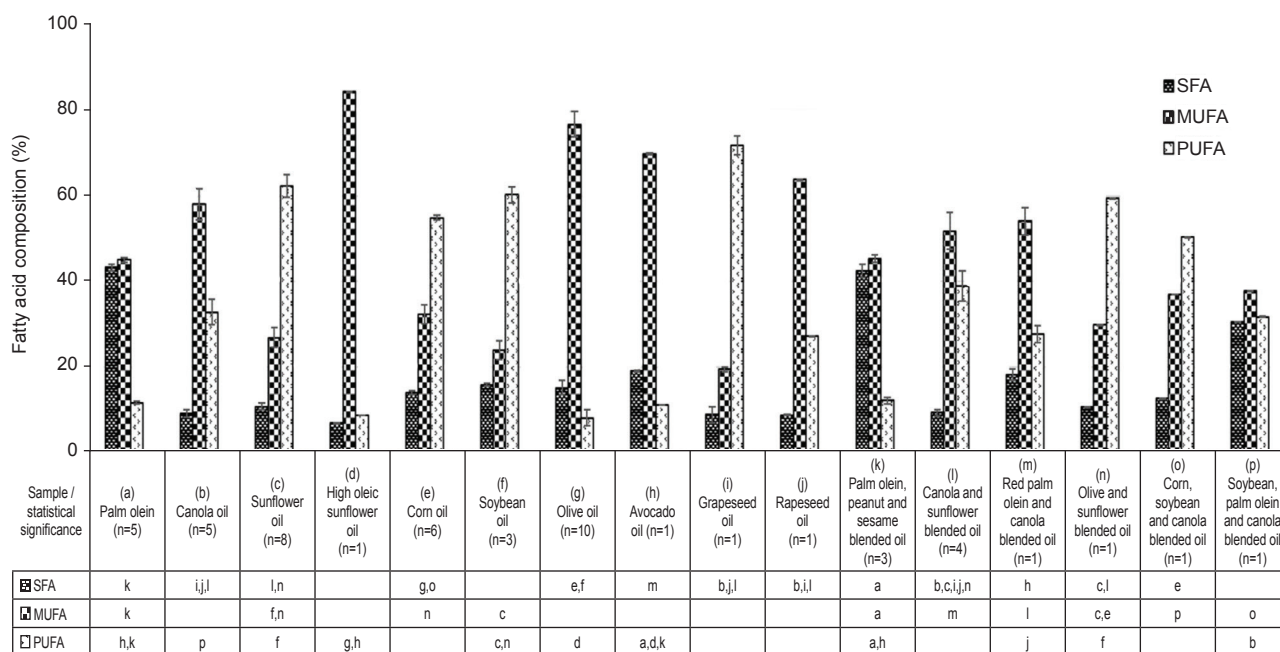
The primary oxidation products are usually measured with a PV test. Peroxides are intermediate oxidation products of oil, that lead to the formation of a complex mixture of volatile compounds *i.e.* aldehydes, ketones, hydrocarbons, alcohols, and esters (Loganathan *et al.*, 2020a; 2020b). Peroxides form in oils, especially at undesirable storage conditions such as elevated temperatures as well as exposure to oxygen and/or light (Loganathan *et al.*, 2020a; 2020b). The acceptable range of PV in refined palm oil is less than 10 meq O₂ kg⁻¹. PVs of all the oil samples were within the recommended range (<10 meq O₂ kg⁻¹) except for sample No. 6 (Table 1).

All assayed samples had almost similar total vitamin E and its homologs content (Table 2 and Figure 6) as compared to standard database values (Codex Alimentarius, 1999; 2001; 2019). Additional to Codex Alimentarius standards' data, we also report beta-tocotrienol content in the assayed oils. Only 30 companies reported on vitamin E content in their packaging labels, whereby 50% of these labels (n=15) had vitamin E content within the assayed range (with $\pm$ 30% deviation), 14 were under-

TABLE 1. MAIN FATTY ACIDS (%) AND PV, WET WEIGHT BASIS IN ASSAYED EDIBLE OILS

Sample	C16:0 (Palmitic)	C18:0 (Stearic)	C18:1n9C (Oleic)	C18:2n6C (Linoleic)	C18:2n6T (Linoleic)	C18:3n3 (Alpha linolenic)	PV (meq O ₂ kg ⁻¹)
(a) Palm olein (n=5)	37.93 $\pm$ 0.77 ^k	3.9 $\pm$ 0.09 ^{cknp}	44.93 $\pm$ 0.58 ^k	11.03 $\pm$ 0.38 ^{bk}	ND ^{bcd}	0.54 $\pm$ 0.05 ^{cdeghikn}	1.57 $\pm$ 0.85 ^{klm}
(b) Canola oil (n=5)	5.1 $\pm$ 0.57 ^{ijl}	2.15 $\pm$ 0.18 ^{del}	56.87 $\pm$ 3.65 ^m	22.57 $\pm$ 2.27	ND ^{acdeghijklmno}	9.2 $\pm$ 0.98 ^j	5.2 $\pm$ 2.42 ^{dfhlop}
(c) Sunflower oil (n=8)	6.22 $\pm$ 0.22 ^{lin}	3.59 $\pm$ 0.19 ^{anp}	26.02 $\pm$ 1.75 ^f	61.42 $\pm$ 2.79	0.05 $\pm$ 0.14 ^{abdeghijklmno}	0.38 $\pm$ 0.34 ^{adghikn}	10.67 $\pm$ 0.73 ⁿ
(d) High oleic sunflower oil (n=1)	3.87 $\pm$ 0 ⁱ	2.37 $\pm$ 0.01 ^{begilmno}	84.53 $\pm$ 0.01	7.79 $\pm$ 0.02 ^g	ND ^{abceghijklmno}	ND ^{acghikn}	3.67 $\pm$ 0.22 ^{behtijklmop}
(e) Corn oil (n=6)	11.19 $\pm$ 0.18 ^{fg}	2.05 $\pm$ 0.1 ^{bdjl}	30.94 $\pm$ 0.48 ⁿ	53.54 $\pm$ 0.47 ^f	0.06 $\pm$ 0.07 ^{abdeghijklmno}	1.25 $\pm$ 0.4 ^{ag}	3.07 $\pm$ 0.81 ^{adhtijklmop}
(f) Soybean oil (n=3)	10.52 $\pm$ 0.28 ^e	4.43 $\pm$ 0.27 ^k	23.66 $\pm$ 2.1 ^c	52.39 $\pm$ 1.1 ^e	0.29 $\pm$ 0.44 ^p	6.15 $\pm$ 0.74 ^l	5.49 $\pm$ 2.09 ^{bcdhlop}
(g) Olive oil (n=10)	11.69 $\pm$ 1.45 ^e	2.85 $\pm$ 0.52 ^{dilmno}	75.66 $\pm$ 3.12	7.24 $\pm$ 1.85 ^d	ND ^{abceghijklmno}	0.6 $\pm$ 0.18 ^{acdehikn}	7.96 $\pm$ 2.48 ⁿ
(h) Avocado oil (n=1)	18.15 $\pm$ 0.01	0.77 $\pm$ 0.19	62.07 $\pm$ 0.23 ^j	10.5 $\pm$ 0.01 ^{ak}	ND ^{abceghijklmno}	0.18 $\pm$ 0.28 ^{acdeghikn}	3.77 $\pm$ 0.36
(i) Grapeseed oil (n=1)	5.87 $\pm$ 0.89 ^{bcdln}	2.78 $\pm$ 1.12 ^{dglmno}	19.16 $\pm$ 0.34	71.85 $\pm$ 2.39	ND ^{abceghijklmno}	0.1 $\pm$ 0.09 ^{acdeghikn}	3.17 $\pm$ 0.08 ^{bcdhtijklmop}
(j) Rapeseed oil (n=1)	4.39 $\pm$ 0 ^{bdl}	1.61 $\pm$ 0 ^e	63.84 $\pm$ 0.04 ^h	18.24 $\pm$ 0.02 ^m	ND ^{abceghijklmno}	8.68 $\pm$ 0.03 ^b	4.33 $\pm$ 0.29 ^{bcdhtlop}
(k) Palm olein, peanut and sesame blended oil (n=3)	36.89 $\pm$ 1.41 ^a	3.96 $\pm$ 0.14 ^{acfnp}	45.16 $\pm$ 0.73 ^a	11.46 $\pm$ 0.79 ^{ah}	ND ^{abceghijklmno}	0.54 $\pm$ 0.11 ^{acdeghikn}	2.11 $\pm$ 1.59 ^{adehlm}
(l) Canola and sunflower blended oil (n=4)	5.29 $\pm$ 0.39 ^{bcdj}	2.46 $\pm$ 0.21 ^{bdeghlmno}	51.58 $\pm$ 4.35 ^m	31.06 $\pm$ 3.82	0.07 $\pm$ 0.07 ^{abdeghijklmno}	6.69 $\pm$ 0.92 ^{fm}	4.47 $\pm$ 1.1 ^{bcdhtlop}
(m) Red palm olein and canola blended oil (n=1)	13.99 $\pm$ 0.99	2.81 $\pm$ 0.19 ^{dglmno}	53.95 $\pm$ 3.32 ^{bl}	19.31 $\pm$ 1.37 ⁱ	ND ^{abceghijklmno}	7.37 $\pm$ 0.5 ^l	1.93 $\pm$ 0.22 ^{adehik}
(n) Olive and sunflower blended oil (n=1)	6.87 $\pm$ 0.01 ^{ci}	3.66 $\pm$ 0.01 ^{ackp}	29.76 $\pm$ 0.01 ^e	58.66 $\pm$ 0.02	ND ^{abceghijklmno}	0.33 $\pm$ 0.02 ^{acdeghik}	8.8 $\pm$ 0.39 ^{cg}
(o) Corn, soybean and canola blended oil (n=1)	8.84 $\pm$ 0	2.85 $\pm$ 0 ^{dglm}	36.36 $\pm$ 0 ^p	44.53 $\pm$ 0.05	ND ^{abceghijklmno}	5.07 $\pm$ 0	5.03 $\pm$ 0.36 ^{bcdhtlop}
(p) Soybean, palm olein and canola blended oil (n=1)	25.7 $\pm$ 0.03	3.79 $\pm$ 0.03 ^{ackn}	37.69 $\pm$ 0.02 ^o	28.06 $\pm$ 0	0.32 $\pm$ 0.01 ^f	2.85 $\pm$ 0.01	4.4 $\pm$ 0.14 ^{bcdhtlop}

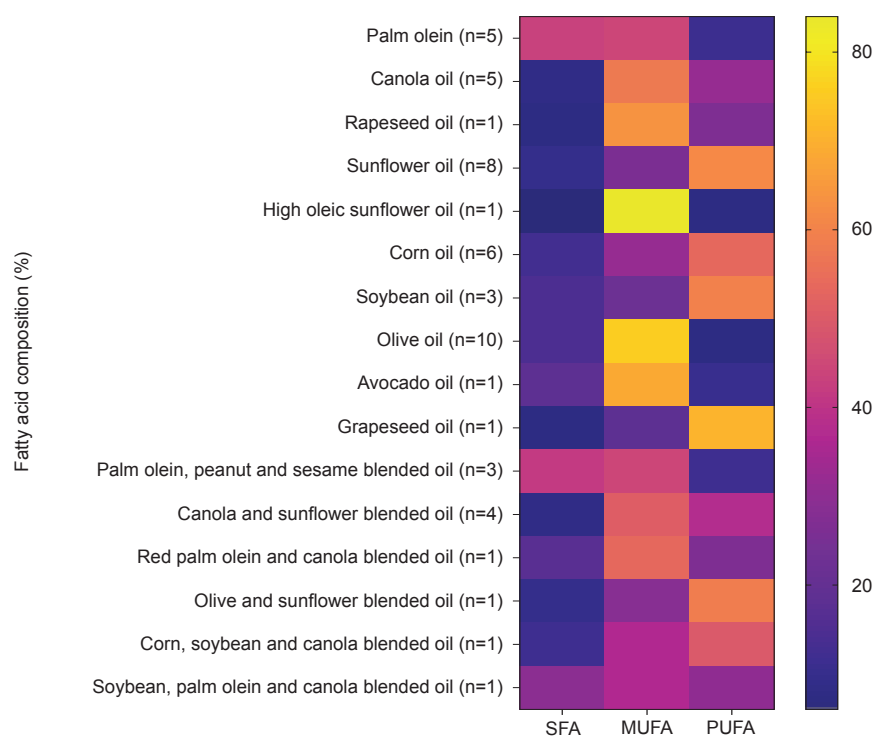
Note: Values are mean value $\pm$ SD. Analyses were done in replicates for fatty acid composition (%) and triplicates for PVs. Data was measured by one-way analysis of variance (ANOVA) with Tukey *post hoc* test. Statistical differences ($p < 0.05$) were found between test oils except those indicated by the alphabet to represent the sample of each row. ND - not detected.



Note: Values are mean value $\pm$ SD. Analyses were done in replicates for fatty acid composition (%). Data was measured by one-way analysis of variance (ANOVA) with Tukey *post hoc* test. Statistical differences ($p < 0.05$) were found between test oils except those indicated by the alphabet to represent the sample of each column.

SFA - saturated fatty acids; MUFA - monounsaturated fatty acids; and PUFA - polyunsaturated fatty acids.

Figure 3. Total SFA, total MUFA, and total PUFA wet weight basis in assayed edible oils by category.



Note: Yellow (■) indicates fatty acids distributed at high concentration and blue (■) indicates fatty acids distributed at a low concentration). SFA (saturated fatty acids), MUFA (monounsaturated fatty acids), and PUFA (polyunsaturated fatty acids).

Figure 4. Heatmap explains the different contents of total SFA, total MUFA, and total PUFA wet weight basis in assayed edible oils by category.

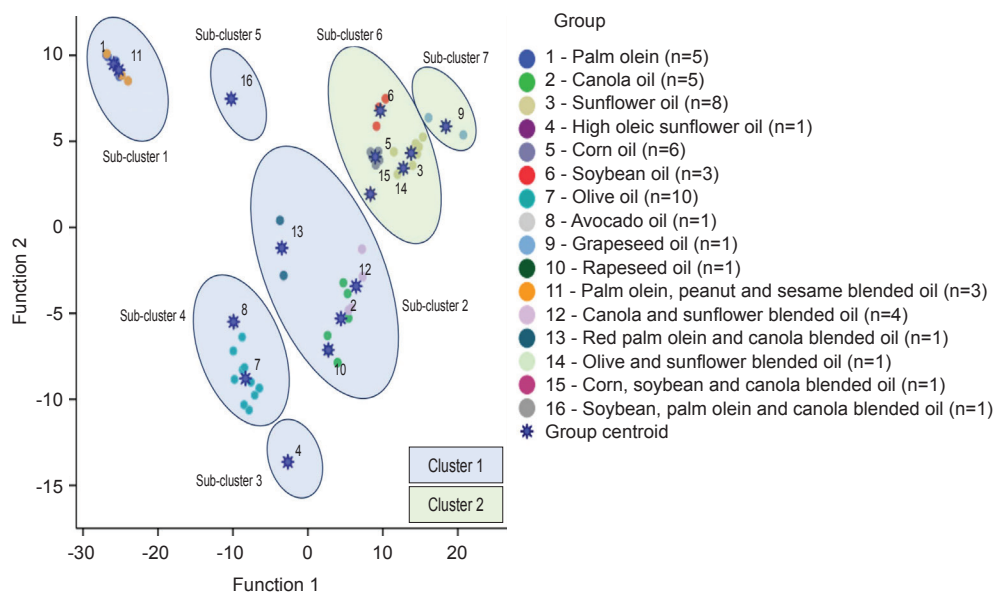


Figure 5. Classification of assayed edible oils with canonical discriminant analysis.

reported, and one over-reported. The present study shows that total vitamin E, total tocopherols, total tocotrienols, and vitamin E homologs of the assayed samples varied a wide range in assayed edible oils (Table 2 and Figure 6).

As depicted in Figure 6 and Table 2, the total vitamin E in the assayed oils were compared and listed in decreasing order: palm olein and peanut blended oil (780 mg kg^{-1})/palm olein (769 mg kg^{-1})/soybean oil (736 mg kg^{-1}) > corn oil (642 mg kg^{-1})/soybean, palm olein and canola blended oil (673 mg kg^{-1})/corn, soybean, and canola blended oil (671 mg kg^{-1})/red palm olein and canola blended oil (581 mg kg^{-1}) > sunflower oil (511 mg kg^{-1})/rapeseed oil (486 mg kg^{-1})/canola and sunflower blended oil (481 mg kg^{-1})/grapeseed oil (448 mg kg^{-1}) > olive and sunflower oil (375 mg kg^{-1}) > olive oil (186 mg kg^{-1}) > avocado oil (89 mg kg^{-1}).

The heat map in Figure 6 provides a clearer visual representation of the hierarchical concentrations of vitamin E homologs, total tocopherols, total tocotrienols, and total vitamin E in assayed edible oils by category. In accordance with previous findings, alpha-tocopherol was found to be the most abundant form of vitamin E in the studied vegetable oils and blends (Aksoz *et al.*, 2020; Gliszczynska-Swiglo *et al.*, 2007; Wen *et al.*, 2020). The richest alpha-tocopherol source in the current pool of samples was sunflower oil (429 mg kg^{-1}). Whereas, beta-tocopherol was found in low quantities in vegetable oils (Gliszczynska-Swiglo *et al.*, 2007). Palm olein, grapeseed, and blended oils with palm olein (palm olein, peanut, and sesame blended oil; soybean, palm olein and canola blended

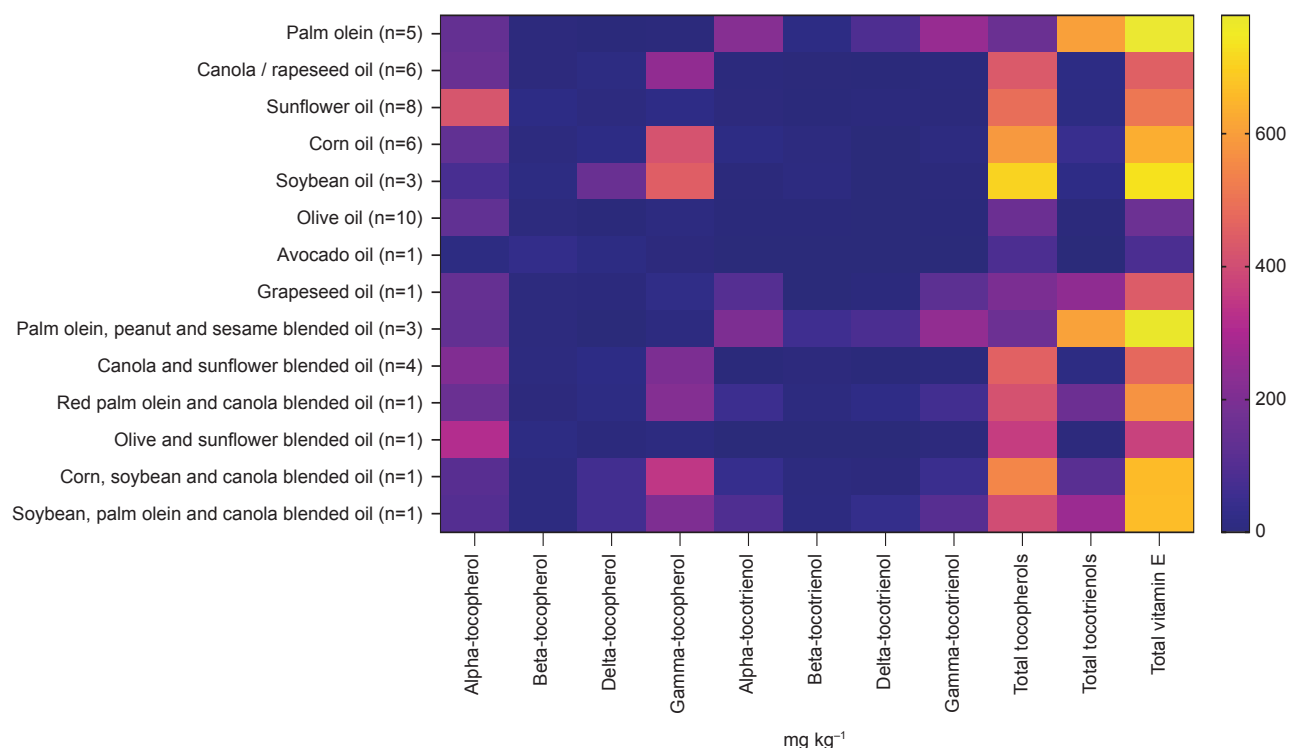
oil; red palm olein and canola blended oil; and corn, soybean, and canola blended oil) were unique as they were the only oils bearing tocotrienols mainly in the forms of alpha, delta and gamma. Inferior to the current study, most studies have reported (delta- and gamma-) tocopherols together due to lack of separation (Aksoz *et al.*, 2020; Gliszczynska-Swiglo *et al.*, 2007). Soybean oil was found to be another rich source of vitamin E ($549.2\text{--}862.3 \text{ mg kg}^{-1}$), mainly in the form of tocopherols: gamma (61.8%), delta (21.6%), and alpha (10.9%). Research conducted by Wen *et al.* (2020) reported higher concentrations of vitamin E in soybean oil 1053 mg kg^{-1} with almost similar concentrations of tocopherols: gamma 64.0%, delta 25.0%, and alpha 11.0% (Wen *et al.*, 2020). Grapeseed oil had a balanced composition of tocopherols (45.2%) and tocotrienols (55.8%). Sunflower oil, olive oil, and their blend (sunflower and olive blended oil) resulted in more than 96.0% of tocopherols mainly in the form of alpha-tocopherol (>81.0%). Similar to Matthaus *et al.* (2016), we too report that corn oil, canola oil, and rapeseed oil can be distinguished from other analysed oils as they consist of only alpha- and gamma-tocopherols. Similar to the current study, Petersen *et al.* (2012) also observed that high oleic sunflower oil and sunflower oil possess comparable vitamin E content and composition. Corn, soybean, and canola blended oil display fairly high vitamin E content, mostly in the form of tocopherols: gamma (52.6%), alpha (17.7%), delta (10.2%), and trace amounts of gamma (7.7%) and alpha (6.8%) tocotrienols. Vitamin E content was lowest in avocado oil (88.8 mg kg^{-1}) as compared to other assayed samples.

TABLE 2. HOMOLOGS AND TOTAL TOCOPHEROLS AND TOTOTRIENOLS WET WEIGHT BASIS IN ASSAYED EDIBLE OILS BY CATEGORY

Tocopherols (mg kg ⁻¹)		Alpha	Beta	Delta	Gamma	Total
(a)	Palm olein (n=5)	144.19 ± 6.08 ^{b,d,f,h,j,l,n}	9.81 ± 0.28 ^{b,f,h,i,j,l}	1.36 ± 2.15 ^{c,f,h,j,m}	5.08 ± 2.7 ^{c,f,g,h,j,m}	160.45 ± 9.92 ^{th,j}
(b)	Canola oil (n=5)	155.54 ± 11.08 ^{a,d,f,h,i,j,l}	9.83 ± 0.27 ^{a,f,h,i,j,l}	18.65 ± 3.5 ^{c,d,g,i,k,l,m}	249.48 ± 20.59 ^{i,k,l,o}	433.5 ± 26.36 ^{c,i,k,l,o}
(c)	Sunflower oil (n=10)	429.51 ± 49.67	23.45 ± 1.98	11.3 ± 5.79 ^{a,b,g,h,i,l,m}	27.33 ± 16.59 ^{a,f,g,h,j,m}	491.58 ± 66.45 ^{b,i,k,n}
(d)	Corn oil (n=6)	136.34 ± 12.61 ^{a,b,f,h,j,l,n}	12.84 ± 0.34 ^{k,o}	23.97 ± 2 ^{b,g,k,l}	419.81 ± 81.87 ^e	592.95 ± 91.01 ⁿ
(e)	Soybean oil (n=3)	80.04 ± 13.3	17.61 ± 1.12	158.25 ± 23.63	453.97 ± 51.48 ^d	709.87 ± 78.44
(f)	Olive oil (n=10)	136.32 ± 20.36 ^{a,b,d,h,j,l}	10.47 ± 0.51 ^{a,b,h,j,l}	0.34 ± 1.06 ^{a,h,j,m}	15.57 ± 3.05 ^{a,c,g,h,j,m}	162.69 ± 21.6 ^{a,h,j}
(g)	Avocado oil (n=1)	18.77 ± 0.23	39.72 ± 0.11	20.54 ± 0.03 ^{b,c,d,i,k,l}	9.75 ± 0.04 ^{a,c,f,h,j,m}	88.77 ± 0.41
(h)	Grapeseed oil (n=1)	149.26 ± 1.39 ^{a,b,d,f,i,j,k,l}	10.94 ± 0.01 ^{a,b,f,j}	7.67 ± 0.08 ^{a,c,f,i,j,m}	32.58 ± 0.19 ^{a,c,f,g,j,m}	200.44 ± 1.66 ^{a,f,j}
(i)	Rapeseed oil (n=1)	170.46 ± 0.94 ^{b,h,l}	9.58 ± 0.01 ^{a,b,f,j,l}	12.57 ± 0.01 ^{b,c,g,h,l,m}	266.77 ± 1.3 ^{b,l}	459.38 ± 2.26 ^{b,c,k,l,o}
(j)	Palm olein, peanut and sesame blended oil (n=3)	143.2 ± 5.61 ^{a,b,d,f,h,j,l,n}	10.57 ± 1.06 ^{a,b,f,h,i,l}	0 ± 0 ^{a,f,h,m}	13.26 ± 2.8 ^{a,c,f,g,h,m}	167.02 ± 6.18 ^{a,f,h}
(k)	Canola and sunflower blended oil (n=4)	218.28 ± 18.48	12.85 ± 0.72 ^{d,n,o}	24.39 ± 7.27 ^{b,d,g,l}	204.34 ± 52.15 ^{b,l,o}	459.86 ± 40.48 ^{b,c,i,l,o}
(l)	Red palm olein and canola blended oil (n=1)	161.47 ± 0.71 ^{a,b,d,f,h,i,j}	9.71 ± 0.03 ^{a,b,f,i,j}	20.07 ± 0.17 ^{b,c,d,g,i,k}	224.24 ± 0.16 ^{b,i,k,o}	415.49 ± 0.4 ^{b,i,k,m,o}
(m)	Olive and sunflower blended oil (n=1)	320.51 ± 1.19	21.21 ± 0.01	8.26 ± 0.01 ^{a,b,c,f,h,j}	15.53 ± 0.05 ^{a,c,f,g,h,j}	365.52 ± 1.16 ^{lo}
(n)	Corn, soybean and canola blended oil (n=1)	118.91 ± 0.62 ^{a,d,f,j,o}	13.95 ± 0.04 ^{d,k,o}	68.58 ± 0.09 ^o	350.69 ± 2.03	552.13 ± 2.6 ^{c,d}
(o)	Soybean, palm olein and canola blended oil (n=1)	109.88 ± 0.86 ^o	13.85 ± 0.07 ^{d,k,o}	68.11 ± 0.43 ⁿ	209.37 ± 2.8 ^{b,k,l}	401.22 ± 1.44 ^{b,i,k,l,m}

Tocotrienols (mg kg ⁻¹)		Alpha	Beta	Delta	Gamma	Total
(a)	Palm olein (n=5)	226.88 ± 8.47	22.74 ± 1.22 ^o	95.32 ± 6.36	263.77 ± 12.22 ^j	608.72 ± 24.57 ^j
(b)	Canola oil (n=5)	6.98 ± 3.57 ^{c,e,f,g,i,k,m}	4.91 ± 3.44 ^{c,d,f,g,h,k,l,m}	1.46 ± 2.31 ^{b,d,e,f,g,k,m}	8 ± 1.46 ^{c,d,e,f,g,i,k,m}	21.35 ± 6.96 ^{c,e,f,i,k,m}
(c)	Sunflower oil (n=10)	9.57 ± 5.5 ^{b,e,f,i,k}	1.87 ± 3.7 ^{b,f,g,h,i,m}	0.78 ± 2.34 ^{b,c,d,e,f,g,k,m}	6.88 ± 3.91 ^{b,d,e,f,g,i,k,m}	19.09 ± 9.72 ^{b,e,f,i,k,m}
(d)	Corn oil (n=6)	22.36 ± 1.36	11.3 ± 1.12 ^{b,e,k,l,n,o}	0 ± 0 ^{b,c,e,f,g,k,m}	15.78 ± 1.81 ^{b,c,i,k,m}	49.44 ± 3.99
(e)	Soybean oil (n=3)	5.44 ± 0.22 ^{b,c,f,g,i,k,m}	14.58 ± 1.68 ^{d,k,l,n,o}	0 ± 0 ^{b,c,d,f,g,k,m}	6.21 ± 2.69 ^{b,c,f,g,i,k,m}	26.23 ± 4.41 ^{b,c,i,k,m}
(f)	Olive oil (n=10)	3 ± 4.2 ^{b,c,e,g,i,k,m}	1.27 ± 2.87 ^{b,c,g,h,i,m}	0 ± 0 ^{b,c,d,e,g,k,m}	1.28 ± 2.89 ^{b,c,e,g,i,k,m}	5.54 ± 7.37 ^{b,c,g,k,m}
(g)	Avocado oil (n=1)	0 ± 0 ^{b,e,f,k,m}	0 ± 0 ^{b,c,f,g,h,m}	0 ± 0 ^{b,c,d,e,f,k,m}	0 ± 0 ^{b,c,e,f,i,k,m}	0 ± 0 ^{f,m}
(h)	Grapeseed oil (n=1)	110.97 ± 1.04	0 ± 0 ^{b,c,f,g,i,m}	8.9 ± 0.02 ^{l,n}	127.7 ± 1.04	247.58 ± 2.06
(i)	Rapeseed oil (n=1)	10.25 ± 0.18 ^{b,c,e,f,k}	0 ± 0 ^{b,c,f,g,h,m}	7.45 ± 0.02 ^{h,n}	8.49 ± 0.06 ^{b,c,d,e,f,g,l,m}	26.19 ± 0.26 ^{b,c,e,k,m}
(j)	Palm olein, peanut and sesame blended oil (n=3)	207.09 ± 18.04	64.39 ± 16.03	87.25 ± 7.05	254.68 ± 18.06 ^a	613.41 ± 29.19 ^a
(k)	Canola and sunflower blended oil (n=4)	1.25 ± 2.17 ^{b,c,e,f,g,i,m}	9.41 ± 0.64 ^{b,d,e,l,n,o}	1.81 ± 3.13 ^{b,c,d,e,f,g,m}	8.71 ± 0.36 ^{b,c,d,g,e,f,i,m}	21.17 ± 5.25 ^{b,c,e,f,i,m}
(l)	Red palm olein and canola blended oil (n=1)	56.95 ± 1.26	11.49 ± 0.65 ^{b,d,e,k,n,o}	28.23 ± 0.17	69.07 ± 0.25	165.74 ± 0.68
(m)	Olive and sunflower blended oil (n=1)	0 ± 0 ^{b,e,f,g,k}	0 ± 0 ^{b,c,f,g,h,i}	0 ± 0 ^{b,c,d,e,f,g,k}	9.21 ± 0.07 ^{b,c,d,e,f,g,i,k}	9.21 ± 0.07 ^{b,c,e,f,g,i,k}
(n)	Corn, soybean and canola blended oil (n=1)	45.6 ± 0.04	12.56 ± 0.92 ^{d,e,k,l,o}	9.34 ± 0 ^{h,i}	51.24 ± 0.17	118.75 ± 1.13
(o)	Soybean, palm olein and canola blended oil (n=1)	98.54 ± 0.72	15.97 ± 0.02 ^{a,d,e,k,l,n}	40.56 ± 1.69	116.24 ± 0.83	271.31 ± 3.22

Note: Values are mean value ± SD. Analyses were done in replicates for vitamin E analysis. Data was measured by one-way analysis of variance (ANOVA) with Tukey *post hoc* test. Statistical differences (*p*<0.05) were found between test oils except those indicated by the alphabet to represent the sample of each column.



Note: Yellow (■) indicates fatty acids distributed at high concentration and blue (■) indicates fatty acids distributed at a low concentration). SFA (saturated fatty acids), MUFA (monounsaturated fatty acids), and PUFA (polyunsaturated fatty acids).

Figure 6. Heatmap explains the different contents of vitamin E homologs, total tocopherols, total tocotrienols and total vitamin E (tocols) wet weight basis in assayed edible oils by category.

Antioxidants are intentionally added at times to improve the oxidative stability of oils (Loganathan *et al.*, 2020b). All the oils used in the current study were in their natural form and none were fortified. The recommended dietary allowance (RDA) for vitamin E by the Institute of Medicine National Academies (Russell *et al.*, 2001) is 15 mg day⁻¹. All the oils except for olive and avocado oils could fulfil the required daily intake of vitamin E. In general, vitamin E deficiency is very rare in healthy people as vitamin E is found in a variety of foods (Aksoz *et al.*, 2020).

Limitations of the current study were erucic acid (C22:1n9 cis), elaidic acid (C18:1n9t) and stereospecific analysis of fatty acids were not analysed. There could be a little variation in fatty acid composition and vitamin E content within the same type/cultivar of oil. This could be due to variations in oilseed/plant, the degree of fruit ripeness/plant maturity, the geographical location of plantations, climatic conditions, oil extraction and processing method, storage conditions, and analytical method (Aksoz *et al.*, 2020; Loganathan *et al.*, 2017; Matthauss *et al.*, 2016; Wen *et al.*, 2020).

It's important to have a healthy balance of the fatty acids in our diets. Each oil has a different fatty acid profile and unique uses in meal preparations. This can be attained by choosing the

right commercially available blended oil. Another option is using a combination of edible oils. For example, in a meal, sesame oil can be used to season rice porridge, sunflower oil to sauté green leaves, palm olein to deep fry chicken fillets, and olive oil to prepare the salad dressing. Regardless of how healthy or how balanced it is, whatever is consumed in excess is deleterious to health. Hence, appropriate calorie intake and a balanced diet are important.

CONCLUSION

By analysing the pattern of fatty acid and vitamin E profiles, the current study provides novel evidence that edible oils in the Malaysian market are of good quality and appropriately labelled. We did not find any discrepancy in the fatty acid composition labelling in any of the samples assayed. Although vitamin E content was underreported on a few labels of the assayed samples, however, all assayed samples had almost similar fatty acid and vitamin E profiles as compared to standard oil specifications. The PVs were also within the recommended range, indicating good quality compliance. These observations implied that the oil manufacturers had good acquiescence to international standards and local regulations. In

addition to that, consumers in Malaysia can be assured that the edible oils on the market shelves are well maintained during processing, delivery, and storage. Current reporting on complete vitamin E profiling is important as the functional property of each homolog varies. This study offers a clear understanding of the characterisation of fatty acid and vitamin E profiles which could serve as a benchmark for the oil industry and dietary nutrition. Besides, this study will be helpful for consumers to select suitable edible oils for various cooking methods.

ACKNOWLEDGEMENT

The authors would like to thank the Director-General of MPOB for the permission to publish the study. Sincere acknowledgement to research assistants (Fatmawati, Noraqmar and Ghazali) and intern student (Aida Farzana-UiTM) for the assistance in laboratory analysis.

REFERENCES

- Ahmad Tarmizi, A H and Siew, W L (2008). Quality assessment of palm products upon prolonged heat treatment. *J. Oleo Sci.*, 57(12): 639-48.
- Aksoz, E; Korkut, O; Aksit, D and Gokbulut, C (2020). Vitamin E (α -, β + γ - and δ -tocopherol) levels in plant oils. *Flavour Fragr. J.*, 35(5): 504-510.
- Chowdhury, K; Banu, L A; Khan, S and Latif, A (2007). Studies on the fatty acid composition of edible oil. *Bangladesh J. Sci. Ind. Res.*, 42(3): 311-316.
- Codex Alimentarius (1999). *Codex standard for named vegetable oils*. Food and Agriculture Organization of the United Nations. World Health Organization.
- Codex Alimentarius (2001). *Codex standard for olive oil, virgin and refined, and for refined olive-pomace oil*. Food and Agriculture Organization of the United Nations. World Health Organization.
- Codex Alimentarius (2019). *Proposed Draft Version to the Standard for Named Vegetable Oils (CXS 210-1999): Inclusion of Walnut oil, Almond oil, Hazelnut oil, Pistachio oil, Flaxseed oil and Avocado oil*. Food and Agriculture Organization of the United Nations. World Health Organization.
- Coskuntuna, L; Gecgel, U; Yilmaz, I; Gecgel, U and Dulger, G C (2015). Investigating fatty acid composition of samples were homogenized various meat and offal products from Turkey. *J. Am. Oil Chem. Soc.*, 92(5): 659-665.
- Dorni, C; Sharma, P; Saikia, G and Longvah, T (2018). Fatty acid profile of edible oils and fats consumed in India. *Food Chem.*, 238: 9-15.
- Elmadfa, I and Kornsteiner, M (2009). Fats and fatty acid requirements for adults. *Ann. Nutr. Metab.*, 55(1/3): 56-75.
- Fu, J Y; Che, H L; Tan, D M Y and Teng, K T (2014). Bioavailability of tocotrienols: Evidence in human studies. *Nutr. Metab. (Lond)*, 11(1): 1-10.
- Gliszczynska-Swiglo, A; Sikorska, E; Khmelinskii, I and Sikorski, M (2007). Tocopherol content in edible plant oils. *Pol. J. Food Nutr. Sci.*, 57(4 [A]): 157-161.
- Haiyan, Z; Bedgood Jr, D R; Bishop, A G; Prenzler, P D and Robards, K (2007). Endogenous biophenol, fatty acid and volatile profiles of selected oils. *Food Chem.*, 100(4): 1544-1551.
- Haytowitz, D; Lemar, L; Pehrsson, P; Exler, J; Patterson, K; Thomas, R; Nickle, M; Williams, J; Showell, B and Khan, M (2011). USDA national nutrient database for standard reference, release 24. US Department of Agriculture, Washington DC, USA.
- International Olive Council (2006). *Global Method for the Detection of Extraneous Oils in Olive Oils*. International Olive Council (IOC), Madrid, Spain.
- Jovanović, P L; Dokić, L P and Marić, B J (2005). Fatty acid composition of maize germ oil from high-oil hybrids wet-milling processing. *Acta Period. Technol.*, 36: 43-50.
- Kang, M J; Shin, M S; Park, J N and Lee, S S (2005). The effects of polyunsaturated: Saturated fatty acids ratios and peroxidisability index values of dietary fats on serum lipid profiles and hepatic enzyme activities in rats. *Bri. J. Nutr.*, 94(4): 526-532.
- Krauss, R M; Eckel, R H; Howard, B; Appel, L J; Daniels, S R; Deckelbaum, R J; Erdman Jr, J W; Kris-Etherton, P; Goldberg, I J and Kotchen, T A (2000). AHA dietary guidelines: Revision 2000: A statement for healthcare professionals from the Nutrition Committee of the American Heart Association. *Circulation*, 102(18): 2284-2299.
- Kushairi, A; Ong-Abdullah, M; Nambiappan, B; Hishamuddin, E; Bidin, M; Ghazali, R; Subramaniam, V; Sundram, S and Parveez, G K A (2019). Oil palm economic performance in

Malaysia and R&D progress in 2018. *J. Oil Palm Res.*, 31(2): 165-194.

Lichtenstein, A (2013). Fats and oils. *Reference Module in Biomedical Sciences Encyclopedia of Human Nutrition*. 3rd edition. Academic Press, Elsevier Ltd., Waltham, MA, USA. p. 201-208.

Loganathan, R; Selvaduray, K R; Nesaretnam, K and Radhakrishnan, A (2021). Palm tocotrienols cause cleavage of poly-(ADP)-ribose polymerase enzyme and down-regulation of cyclooxygenase-2 protein level in human breast cancer cells. *J. Oil Palm Res.*, 33(1): 119-128.

Loganathan, R; Subramaniam, K M; Radhakrishnan, A K; Choo, Y-M and Teng, K-T (2017). Health-promoting effects of red palm oil: Evidence from animal and human studies. *Nutr. Rev.*, 75(2): 98-113.

Loganathan, R; Tarmizi, A H A; Vethakkan, S R and Teng, K T (2020a). Retention of carotenes and vitamin E, and physico-chemical changes occurring upon heating red palm olein using deep-fat fryer, microwave oven and conventional oven. *J. Oleo. Sci.*, 69(3): 167-183.

Loganathan, R; Tarmizi, A H A; Vethakkan, S R and Teng, K T (2020b). Storage stability assessment of red palm olein in comparison to palm olein. *J. Oleo. Sci.*, 69(10): 1163-1179.

Martin, M E; Grao-Cruces, E; Millan-Linares, M C and Montserrat-De La Paz, S (2020). Grape (*Vitis vinifera* L.) seed oil: A functional food from the winemaking industry. *Foods*, 9(10): 1360.

Matthaus, B; Özcan, M M and Al Juhaimi, F (2016). Some rape/canola seed oils: Fatty acid composition and tocopherols. *Z. Naturforsch., C, J. Biosci.*, 71(3-4): 73-77.

Oil World Annual (2020). *ISTA Mielke GmbH*. Hamburg, Germany.

Petersen, K D; Kleeberg, K K; Jahreis, G and Fritsche, J (2012). Assessment of the oxidative stability of conventional and high-oleic sunflower oil by means of solid-phase microextraction-gas chromatography. *Int. J. Food Sci. Nutr.*, 63(2): 160-169.

Piravi-Vanak, Z; Ghavami, M; Ezzatpanah, H; Arab, J; Safafar, H and Ghasemi, J B (2009). Evaluation of authenticity of Iranian olive oil by fatty acid and triacylglycerol profiles. *J Am. Oil Chem. Soc.*, 86(9): 827-833.

Rueda, A; Seiquer, I; Olalla, M; Giménez, R; Lara, L and Cabrera-Vique, C (2014). Characterization of fatty acid profile of argan oil and other edible vegetable oils by gas chromatography and discriminant analysis. *J. Chem.*, 2014. DOI: 10.1155/2014/843908.

Russell, R; Beard, J L; Cousins, R J; Dunn, J T; Ferland, G; Hambidge, K; Lynch, S; Penland, J; Ross, A and Stoecker, B (2001). Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. A Report of the Panel on Micronutrients, Subcommittees on Upper Reference Levels of Nutrients and of Interpretation and Uses of Dietary Reference Intakes, and the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes Food and Nutrition Board Institute of Medicine.

The American Oil Chemists' Society (2017a). AOCS Official Method Cd 8b-90: Peroxide Value Acetic Acid-Isooctane Method, Official Methods and Recommended Practices of the AOCS. 7th edition. American Oil Chemists Society Press, Champaign. AOCS Press.

The American Oil Chemists' Society (2017b). AOCS Official Method Ce 1a-13: Determination of Fatty acids in Edible Oils and Fats by Capillary GLC, Official Methods and Recommended Practices of the AOCS. 7th edition. American Oil Chemists Society Press, Champaign. AOCS Press.

The American Oil Chemists' Society (2017c). AOCS Official Method Ch 1-91: Preparation of Methyl Esters of Long-Chain Fatty Acids, Official Methods and Recommended Practices of the AOCS. 7th edition. American Oil Chemists Society Press, Champaign. AOCS Press.

Wallingford, J C; Yuhas, R; Du, S; Zhai, F and Popkin, B M (2004). Fatty acids in Chinese edible oils: value of direct analysis as a basis for labeling. *Food Nutr. Bull.*, 25(4): 330-336.

Wen, Y; Xu, L; Xue, C; Jiang, X and Wei, Z (2020). Assessing the impact of oil types and grades on tocopherol and tocotrienol contents in vegetable oils with chemometric methods. *Molecules*, 25(21): 5076.

WHO (2008). *Interim summary of conclusions and dietary recommendations on total fat and fatty acids*. Joint FAO/WHO expert consultation on fats and fatty acids in human nutrition.

PROPERTIES OF BREAD MADE WITH PALM-BASED FLUID SHORTENING

NUR HAQIM ISMAIL^{1*}; NORAZURA AILA MOHD HASSIM¹; SAW MEI HUEY¹; NOOR LIDA HABI MAT DIAN¹ and SIVARUBY KANAGARATNAM¹

ABSTRACT

Fluid shortening is frequently used as an alternative to solid shortening in the food industry. Fluid shortening is preferred over solid shortening because it is more convenient and easier to handle. The aim of this study was to examine the effects of palm-based fluid and solid shortenings and palm olein (POo) on the physical, firmness and sensory properties of bread. Fluid shortening produced the highest weight of bread and had similar volume, specific volume and density to bread made with solid shortening, while bread made with POo had the lowest volume, specific volume and density. The colour was similar in terms of lightness (L) and blueness-yellowness (b*) of the crusts of all the bread. The crumbs of all the bread had similar L* and a* (greenness-redness) values. Crumbs of the bread made with fluid shortening were the softest of all. In terms of sensory properties, the bread made with fluid and solid shortenings were preferred, and both received comparable sensory scores for taste, colour, aroma and overall acceptability. On the contrary, bread produced from POo were of inferior physical, textural, and sensory quality. These results indicate that fluid shortening produced bread has comparable properties to those made with solid shortening.*

Keywords: bread, palm-based fluid shortening, texture.

Received: 19 September 2021; **Accepted:** 2 March 2022; **Published online:** 20 April 2022.

INTRODUCTION

Bread is classified as a fermented product made from flour, shortening, yeast, water, sugar and salt. The bread-making process includes mixing flour with water, yeast, sugar, salt and fat to form a dough. The dough is kneaded until the gluten structure is developed through the hydration of the proteins in the flour (Cauvain, 2012). Fat is an important ingredient in baking products and it plays a variety of roles in providing desirable textural properties to baked goods. The presence of fat, commonly in the form of shortening, is essential in bread-making. The amount of shortening added is approximately 2%-5% of the flour weight (Pareyt *et al.*, 2011).

Shortening is made of 100% of oils and fats. Plant oils and fats are the most common lipid used for shortening. However, animal fat can also be used to produce shortening (Aguilar *et al.*, 2015). In the making of shortening, the fat blend is texturised to obtain a homogenous and plastic texture. Shortening can be in a solid or liquid/fluid form, depending on the application (Ghotra *et al.*, 2002; Yılmaz and Ögütçü, 2015). It can lubricate, lessen, or shorten the products in which it is used. Furthermore, the addition of shortening contributes to tenderness and texture, incorporates air, acts as a medium for heat transfer, and improves the shelf-life of the food products (Ghotra *et al.*, 2002; Huang *et al.*, 2019).

In bread making, shortening has a vital impact on the incorporation of air during the mixing process which contributes to the volume of the final loaf (Pareyt *et al.*, 2011). Shortening contributes to the development of thin and expandable gluten films that assist in the incorporation of air and for

¹ Malaysian Palm Oil Board,
6 Persiaran Institusi, Bandar Baru Bangi,
43000 Kajang, Selangor, Malaysia.

* Corresponding author e-mail: nurhaqim.ismail@mpob.gov.my

stabilisation during the mixing process (Watanabe *et al.*, 2003). Shortening with sufficient solid fat is necessary to strengthen the dough, and hence to enhance gas retention during proofing (Chin *et al.*, 2010). According to Ghotra *et al.* (2002), shortenings used in bread-making should have a broad range of plasticity at ambient temperature. The plasticity is determined by the solid-to-liquid ratio of the fat used in the shortening formulation. An extremely high solid fat content (SFC) will negatively affect aeration, while excessive liquid oil will affect the shortening's air-holding ability through the mixing process, (Rios *et al.*, 2014). Othman *et al.* (2015) reported that bakery shortenings used in bread-making should have at least 20% SFC at 25°C and 5% SFC at 40°C. A study by Smith and Johansson (2004) concluded that more preferable bread was obtained when a shortening with a higher SFC was used in the shortening formulation, which resulted in a bread with more volume compared to a bread made without solid fat. Dough made with liquid oil or without any lipid have a more porous structure, very thin cell walls and bigger gas cells (Osuna *et al.*, 2018). According to Stauffer (1996), the inclusion of 5% shortening increased the loaf volume up to 15%-25%. However, excessive fat/shortening can prevent the dough from rising during proving, hence reducing the bread's volume (Dunford, 2017).

Fluid shortening is widely used in the food industry as an alternative to solid shortening. Fluid shortening is preferred over solid shortening as the former is more convenient and easier to handle as it can minimise fat loss due to the residues or fats which remain on the packing materials. It is formulated in such a way so that it can be poured or pumped out at an ambient temperature and remains stable at temperatures ranging from 16°C-32°C (Ghotra *et al.*, 2002; O'Brien, 2020). Typically, fluid shortening is used as a frying fat. However, it can also be used in bakery products such as bread, rolls, buns, pie crusts and cakes (Dunford, 2017). The roles of fluid shortening in bakery products are similar to those of solid shortening. Nur Haqim *et al.* (2018) reported that the performance of fluid shortening made with palm oil (PO) in biscuits is comparable to that of solid shortening. Zhou *et al.* (2011) reported that fluid shortening can produce cakes of good characteristics and firmness.

Application of solid shortening in bakery products was broadly studied (Aguilar *et al.*, 2015; Artan *et al.*, 2010; Chin *et al.*, 2010; Dilek and Bilgiçli, 2021; Manaf *et al.*, 2019; Saghafi *et al.*, 2019). Solid shortening is widely available and used in Malaysia. However, a thorough evaluation of the effects of using fluid shortening on the physical, texture, and sensory qualities of bread has yet to be conducted. Thus, this study was aimed to

investigate the performance of fluid shortening made with PO, in comparison with palm-based solid shortening and shortening made with palm olein (POo) which is liquid at ambient temperature. The effects of PO fluid shortening on the physical, textural and sensory properties of bread were also investigated.

MATERIALS AND METHODS

Materials

Refined, bleached and deodourised (RBD) PO (iodine value, IV 52) and palm stearin (POs) (IV 33) were obtained from Sawit Raya Sdn. Bhd., Malaysia. Palm olein (IV 58) (POo) (Delima Oil Products Sdn. Bhd., Malaysia), high protein wheat flour (12.8% protein) (Dr. Oetker Nona, Malaysia), sugar (Central Sugars Berhad, Malaysia), yeast (AB Mauri, Malaysia) and salt (Adabi Consumer Industries Sdn. Bhd., Malaysia) were purchased from the local market.

Production of shortening. Fluid shortening was produced solely from PO according to the method described by Che Man *et al.* (2010). The sample was melted at 70°C for 30 min to eliminate the crystal memory before being placed in a water-jacketed reactor vessel linked to a water bath with flowing hot and cold water. The product was cooled to 20°C and held for 80 min before being raised to 30°C for 40 min and cooled again to 20°C for 40 min while being agitated at a constant speed. Solid shortening containing a mixture of 80% PO and 20% POs was produced in a pilot plant (Gerstenberg and Agger, Copenhagen, Denmark) using the method described by Kanagaratnam *et al.* (2013). The blend was heated to 65°C for an hour to achieve complete melting. The product was cooled to 45°C while passing through the first chilling unit and cooled again to 25°C while passing through the second chilling unit. The blend was subsequently homogenised by passing through a pin worker with a shaft rotation speed of 150 rpm. The shortening was collected in a container and stored at 25°C.

Slip melting point (SMP). SMP of the fats was measured according to Malaysian Palm Oil Board (MPOB) Test Method p4.2: 2004 (MPOB, 2005). A 10 mm fat column was loaded into three capillary tubes. The fat column was chilled by pressing the ends of the tubes containing the samples against a piece of ice and rolling them until the oil column solidified. The tubes were placed in a test tube and kept in a beaker of water that had been calibrated at $10 \pm 1^\circ\text{C}$ in a thermostat water bath. The beaker was transferred to the water bath and maintained at a temperature of $10 \pm 1^\circ\text{C}$ for 16 hr.

Then, the capillary tubes were removed from the test tube and secured with a rubber band to a thermometer, with the lower ends of the tubes aligned with the bottom of the thermometer's mercury bulb. The thermometer was immersed in 400 mL boiling distilled water, with the lower end submerged to a depth of 30 mm in the water. The starting temperature of the bath was adjusted to 8°C-10°C below the expected SMP of the sample. A magnetic stirrer was used to stir the water, and the heat was applied at a rate of 1°C/min, steadily decreasing to 0.5°C/min once achieving the slip point. The heating was continued until the fat column was raised. The SMP was recorded as the temperature at which the fat in the column rise.

Solid fat content (SFC). The SFC of the fats was evaluated using pulsed nuclear magnetic resonance (NMR) (NMS Minispec from Bruker, Germany) in accordance with the American Oil Chemists Society (AOCS) Official Method Cd 16b-93 (AOCS, 2012). Samples were melted at 80°C for 1 hr and filled into NMR tubes of 0.8 cm (diameter) and 3.0 cm (height). Samples were then chilled for 90 min at 0°C, followed by tempering for 40 hr at 26°C, 90 min at 0°C and finally kept for 60 min at desired test temperatures of 5°C, 10°C, 15°C, 20°C, 25°C, 30°C, 35°C, 37°C, 40°C, 45°C, 50°C, 55°C and 60°C prior to each measurement.

Bread preparation. The bread was produced using 1 kg high protein flour, 600 g of water, 40 g of fluid shortening or solid shortening or POo, 40 g of sugar, 20 g of yeast and 10 g of salt. All the dry ingredients (high protein flour, sugar, yeast and salt) were incorporated using an electric mixer (Hobart, US) for 1 min at speed No. 1. Water was then slowly added to the dry ingredients and the mixture was blended for another 1.5 min at the same speed. Subsequently, fluid or solid shortening, or POo were added, and the dough was kneaded at speed No. 2 for 14 min. The dough was then allowed to rest for 10 min at ambient temperature under a damp cloth. After resting, the dough was divided into 250 g portions and proofed for 1 hr before being baked at 200°C (Salva Modular Deck Oven, Industrial S.L.U, Spain) for 16 min. The bread was cooled at ambient temperature for 1 hr before packing and storage.

Weight. The weight of the bread was determined using a digital balance (Mettler, Switzerland).

Volume, specific volume and density. The volume was measured using the rapeseed displacement method according to Approved Method 10-05

(AACC, 2000). The specific volume (mL/g) and density (g/mL) were calculated using the following Equations (1) and (2):

$$\text{Specific volume of bread (mL/g)} = \frac{\text{Volume of bread}}{\text{Weight of bread}} \quad (1)$$

$$\text{Density of bread (g/mL)} = \frac{\text{Weight of bread}}{\text{Volume of bread}} \quad (2)$$

Crust and crumb colours. The colours of the crust and crumb were determined using a Chroma Meter CR-400 (Konica Minolta Sensing, Japan), as described by Makinde and Akinoso (2014). The bread was sliced into cubes of $2 \times 2 \times 2 \text{ cm}^3$ and placed in the colourimeter. The parameters determined were Commission on Illumination (CIE) colour values L^* [$L^*=0$ (black) and $L^*=100$ (white)], a^* ($-a^*$ =greenness and $+a^*$ =redness) and b^* ($-b^*$ =blueness and $+b^*$ =yellowness). The total colour difference (δE^*) was calculated using the Scofield Equation (3) below:

$$\delta E^* = \sqrt{(\delta L^*)^2 + (\delta a^*)^2 + (\delta b^*)^2} \quad (3)$$

where $\delta L^* = L^* - L^*_{\text{reference}}$, $\delta a^* = a^* - a^*_{\text{reference}}$, and $\delta b^* = b^* - b^*_{\text{reference}}$ and $L^*_{\text{reference}} = 100$, $a^*_{\text{reference}} = 0$, and $b^*_{\text{reference}} = 0$ (Chin *et al.*, 2010).

The colour attributes were recorded using Spectramagic software version 2.11 (1998). All measurements were conducted in three replicates.

Firmness. Firmness was done using a texture analyser (model TA.XT plus, Stable Micro System, England) with a 50 mm probe (P/50) diameter cylindrical probe. Bread crumb samples were cut into cubes of $4 \times 4 \times 4 \text{ cm}^3$. The conditions for the analysis were: a pre-test speed of 2.0 mm s^{-1} , a test speed of 5.0 mm s^{-1} , a post-test speed of 5.0 mm s^{-1} , a distance of 20.0 mm, trigger type of auto-20 g, and time of 5 s (Akyüz and Mazı, 2020). Measurements were conducted in three replicates.

Sensory evaluation. Sensory evaluation of crumbs of the fresh bread was conducted by 30 untrained panellists consisting of MPOB staff and students. The sensory evaluation was performed in a laboratory using a 9-point hedonic scale under fluorescent light and at ambient temperature. The sample of bread was cut in $4 \times 4 \text{ cm}^2$ and coded with random three digits. Panellists were asked to determine the sensory attributes of the bread samples based on their degree of like (scale of 1-9) where 1=dislike extremely, 2=dislike very much, 3=dislike moderately, 4=dislike slightly, 5=neither

like nor dislike, 6=like slightly, 7=like moderately, 8=like very much and 9=like extremely. The attributes evaluated were appearance, texture, taste, colour, aroma and overall acceptability as suggested by Feili *et al.* (2013).

Statistical analysis. The data were statistically analysed using Minitab (Version 16, Minitab Inc., USA) statistical software package by which the data were subjected to analysis of variance (ANOVA). Means were subjected to Tukey's test and a p -value <0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Slip Melting Point (SMP)

SMP is the temperature at which the fat softens. The SMP of fluid shortening, solid shortening and POo, and the phase of the products at an ambient temperature of 25°C are as shown in Table 1. The SMP of all the samples were significantly different ($p<0.05$) as the products ranged from POo which was liquid with SMP of 16.66°C, fluid shortening was semi solid with SMP of 38.23°C and solid shortening with SMP of showed significantly highest ($p<0.05$) SMP (46.53°C). Solid shortening is commonly used in bread making. Othman *et al.* (2015) suggested that the applicable SMP for bakery shortening ranged from 40.40°C to 45.25°C.

TABLE 1. SMP OF FLUID SHORTENING, SOLID SHORTENING AND PALM OLEIN

Sample	Phase at an ambient temperature of 25°C	Slip melting point (°C)
Fluid shortening	Partially solid	38.23 ^b ± 0.30
Solid shortening	Solid	46.53 ^a ± 0.25
RBD POo	Liquid	16.66 ^c ± 0.28

Note: *Means within each column bearing different superscript letters differ significantly ($p<0.05$) from one another.

Solid Fat Content (SFC)

SFC is defined as the proportion of solid present at a particular temperature. The values describe the physical properties of a product at a specific temperature (Ramli *et al.*, 2008). The SFC of fluid shortening, solid shortening and POo are as shown in Figure 1. In this study, the bread was made at 25°C (ambient temperature), hence the percentage of solid fat present at this temperature was crucial. Fluid shortening had a low percentage of solid fat of 15.53% SFC at 25°C. The lower level of solid fat in the fluid shortening enables the product to be flowable and pourable. Solid shortening had 25.29% SFC at 25°C, hence, this product was firm and soft, making

it an acceptable fat for bread making. POo was fully liquid at 25°C. Saghafi *et al.* (2019) explained that the SFC and melting point of fats are interrelated, an increase in SFC, which is an increase in the percentage of solid fat will increase the melting point of the fat.

The presence of solid fats and liquid oils in a shortening (semisolid or partial solids state) are crucial in ensuring the required quality of bread (Nor Aini and Che Maimon, 1996). The liquid oil in shortening provides a lubrication effect on bread, allowing the lubrication of gluten particles by breaking the protein and starch structure (Tamstorf *et al.*, 1988). The liquid oil portion softens and tenderises the crumb, hence, the moisture mouthfeel during chewing (Stauffer, 2002). During storage, the liquid component keeps the bread crumb moist by preventing moisture migration from the core of the bread to the dry surface region, which might result in a consistent leathery texture. This effect allows the bread to have a longer shelf life in terms of firmness. The solid fats in a shortening are essential to help the development of the dough's structure, influencing important qualities including volume, grain, and texture of the final product. Brooker (1996) stated that the aeration is aided by fat crystals, which form membranes to capture microscopic air bubbles. Hence, the specific concentration of solid fats is necessary to support the required performance of shortening. Shortening is made from 100% oils and fats, thus, it has two distinct properties that are essential for bread making, which is being insoluble in water and cannot be hydrated by it (Figoni, 2004). Therefore, solid fats encapsulate the structure of gluten protein and starch granules, prohibiting them from absorbing water during the mixing process to form a firm dough structure. Solid fats also play an important role in the proofing stage. The solid fats, which coat the gluten matrix during mixing, further strengthen the gluten matrix, allowing for more gas retention during proofing. On the other hand, the use of liquid oil had a deleterious impact on the gluten network, making it weaker and less extensible than dough made with shortening containing saturated fats. The liquid oil is absorbed into the gluten structure, causing it to clump together. Bread made with liquid oil exhibited the porous structure of the gluten network with large gas cells (Osuna *et al.*, 2018; Watanabe *et al.*, 2003).

Weight of the Bread

The weight of bread made with fluid shortening was not significantly different ($p>0.05$) from that of bread made with POo (Table 2). The bread made with solid shortening was one time lighter ($p<0.05$) than the other two breads. These results indicate that the SFC of the fats affects the weight of the bread. The same behaviour was reported by Smith

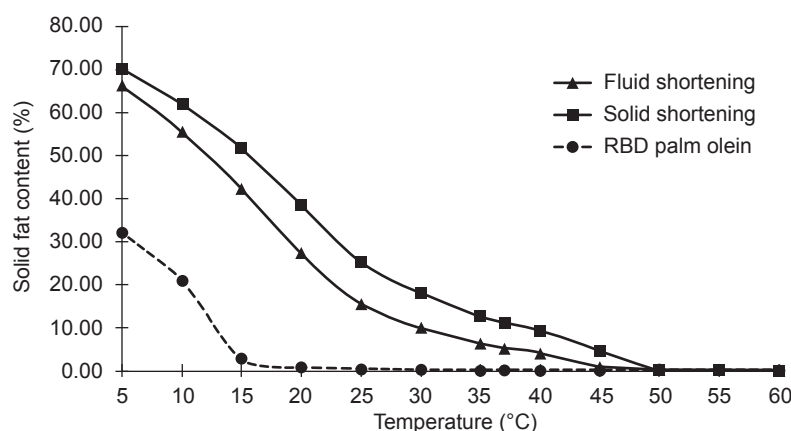


Figure 1. SFC as a function of temperature for fluid shortening, solid shortening and palm olein.

TABLE 2. WEIGHT, COLOUR AND FIRMNESS OF BREAD MADE WITH FLUID SHORTENING, SOLID SHORTENING AND PALM OLEIN AT 25°C

Parameter	Sample		
	Fluid shortening	Solid shortening	POo
Weight (g)	223.67 ^a ± 1.60	220.47 ^b ± 1.22	226.13 ^a ± 0.64
Colour of crust	L*	53.57 ^a ± 2.06	57.13 ^a ± 1.71
	a*	12.52 ^a ± 0.25	11.84 ^{ab} ± 0.53
	b*	19.84 ^a ± 1.44	23.12 ^a ± 1.56
	ΔE*	52.05 ^a ± 1.29	50.15 ^a ± 0.87
Colour of crumbs	L*	73.75 ^a ± 2.81	72.24 ^a ± 0.88
	a*	-0.85 ^a ± 0.16	-0.87 ^a ± 0.08
	b*	9.89 ^b ± 0.11	9.53 ^b ± 0.08
	ΔE*	28.08 ^a ± 2.58	29.36 ^a ± 0.82
Firmness (g)	183.80 ^c ± 11.95	232.39 ^b ± 3.59	377.11 ^a ± 4.79

Note: *Means within each row bearing different superscript letters differ significantly ($p < 0.05$) from one another.

L* - lightness-darkness; a* - greenness-redness; b* - blueness-yellowness.

and Johansson (2004), who reported that the bread made with shortening that contains 60% SFC at ambient temperature was lighter compared to those made with shortening that contains 20% of SFC. Chin *et al.* (2010) claimed that the weight of bread made with higher melting point shortening produced lighter bread. Fluid shortening which had a lower SFC than solid shortening at 25°C was not able to provide the lightness provided by solid shortening.

Volume and Specific Volume of the Bread

Cross-sections of the bread showing differences in volume are depicted in Figure 2. Bread made with fluid and solid shortenings had comparable values for volume (Figure 3) and specific volume (Figure 4). The volume of the bread samples made with solid and liquid shortenings was significantly higher ($p < 0.05$) than that of the bread samples

made with POo, and this explained the higher SFC level in the fluid and solid shortenings. During the bread-making process, solid fat shortening acts as a gas-stabilising agent, influencing the incorporation of air into the dough (Alvarez-Jubete *et al.*, 2010; Scheuer *et al.*, 2014), which impacts loaf volume (Pareyt *et al.*, 2011; Scheuer *et al.*, 2014). Smith and Johansson (2004) explained that during baking, the loaf volume of bread made with shortening of high solid fat increased, due to an increase in the surface area, thus, facilitating more evaporation of water. This results in drier bread with a lower weight. The findings suggest that the SFC of the shortening influenced bread volume. A higher SFC resulted in a higher loaf volume. These findings are in line with the findings of Smith and Johansson (2004) who reported that the loaf volume of bread was higher when made with shortening with higher solid fat than those made with liquid oil.

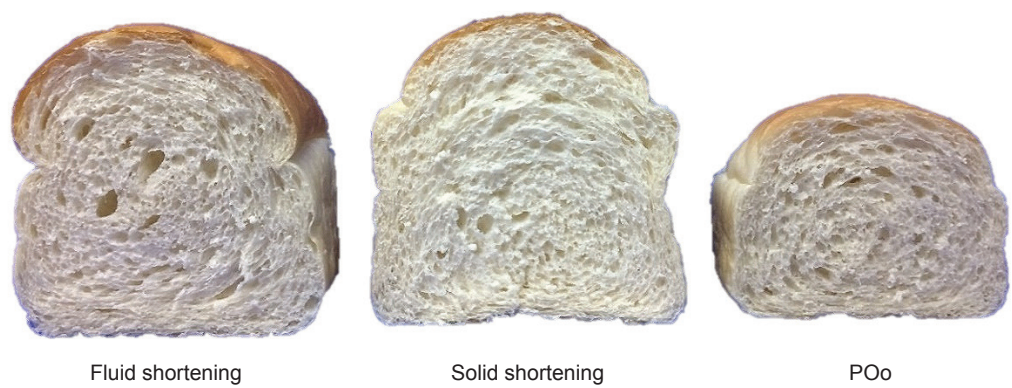


Figure 2. Cross-sections of bread showing the differences of volume as affected by the type of shortening.

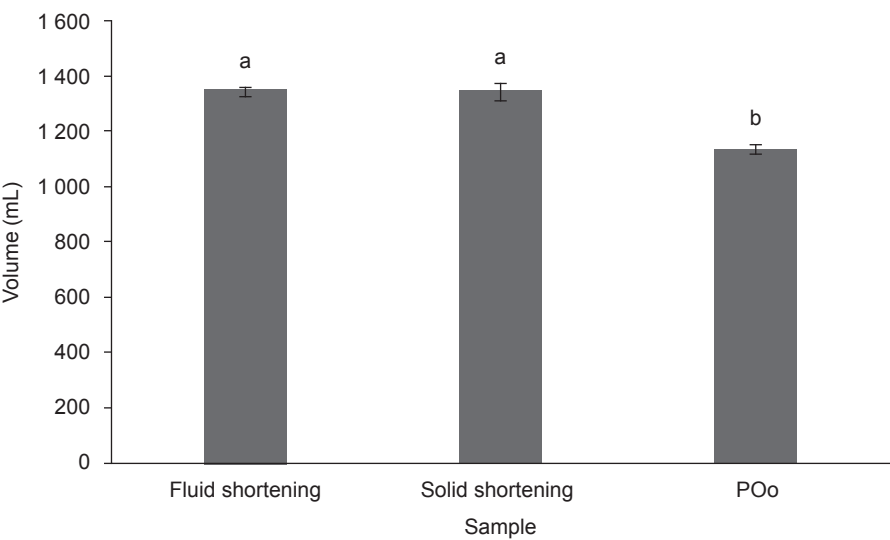


Figure 3. The volume of bread made with fluid shortening, solid shortening and palm olein at 25°C.

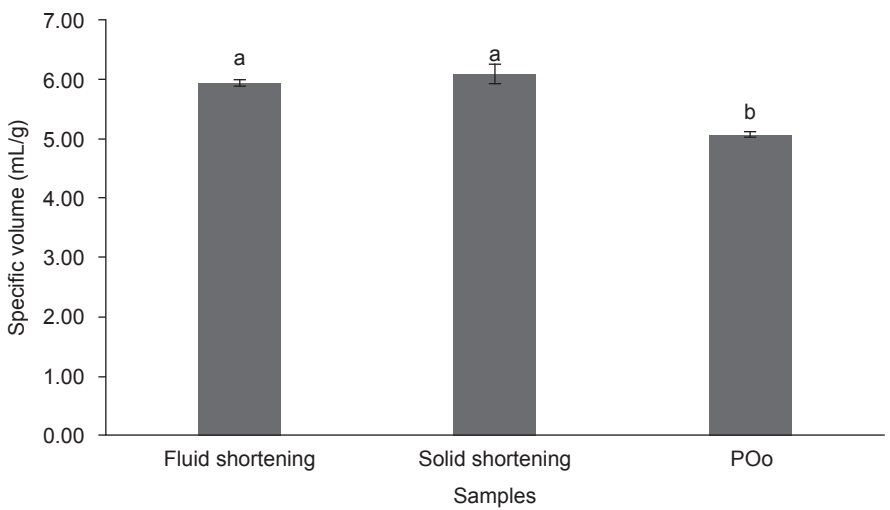


Figure 4. Specific volume of bread made with fluid shortening, solid shortening and palm olein at 25°C.

Artan *et al.* (2010) reported that the volume of bread made with shortening containing POs (hard fraction of PO) increased with an increase in the POs content in the shortening formulation. Osuna *et al.* (2018) described that the addition of canola oil into the bread produced a negative impact on the bread volume compared to the bread made with bovine fat which had higher SFC. The fluid shortening used in this study had an SFC of 15% at 25°C and 4% at 40°C. However, the solid shortening had SFC of 25.53% at 25°C and 9.31% at 40°C, which are within the specifications for an optimum bakery shortening. Ghotra *et al.* (2002) and Smith and Johansson (2004) reported that adequate SFC in the shortening can prevent air cells (produced by yeast from the dough) from being released by enclosing them within protein-lipid films. This process is crucial at the beginning of baking to make the dough rise. A higher amount of SFC in shortening produced a higher loaf volume due to this process (Osuna *et al.*, 2018). Notwithstanding this, the shortening itself has also a good leavening ability as it can mix homogeneously in the flour, providing a sufficiently aerated structure. Calligaris *et al.* (2013) explained that liquid oil did not homogeneously disperse throughout the flour, which prohibits the development of a sufficiently aerated structure. This showed that the SFC of fluid and solid shortenings has a good leavening ability.

Colour

The colour attributes of the breadcrumbs and crusts made with fluid shortening, solid shortening and POo are tabulated in Table 2. The a^* value of bread crust made with fluid shortening was higher ($p < 0.05$) compared to other samples, showing that the crust is redder than the other samples and this might be due to the difference in the colour of the PO and POo. Almeida *et al.* (2019) reported variation of colour in RBD palm oil and POo upon storage at various temperatures. The colour of all the bread crusts was similar ($p > 0.05$) in terms of L^* , b^* and δE values. These findings are similar to what was reported by Aguilar *et al.* (2015), of which the shortening levels did not affect the lightness of the crust although the levels of shortening were different in their studies. The type of oils and fats used in the bread also had no significant effect ($p > 0.05$) on the colour of the breadcrumbs. The crumbs from all bread had similar whitish colour, with L^* , a^* and δE values ranging from 72.24 to 73.75, -0.87 to -0.82 and 28.08 to 29.36, respectively. However, the b^* of the crumb showed variation between fluid and solid shortenings with those made with POo. The crumb of bread made POo was yellower than the other samples.

Firmness

Firmness is the important criterion that contributes to the perception of bread with good quality (Majeed *et al.*, 2017). Firmness is described as the force required to bite into the bread, with a low force indicating a soft bread crumb texture (Ulziijargal *et al.*, 2013). The firmness of the bread made with fluid shortening, solid shortening and POo at 25°C are presented in Table 2.

The bread made with fluid shortening had the softest crumbs, followed by the bread made with solid shortening. The bread made with POo had the firmest crumbs. The crumbs of the bread made with POo was 1.6 and 1.2 firmer than the crumbs of bread made with fluid shortening and solid shortening, respectively, which was due to the absence of any solid fat in the bread formulation. A previous report by Chin *et al.* (2010) stated that bread made with shortening had a softer crumb. This has been related to the lubricating and tenderising effect of fat, as well as better aeration and breaking up the gluten network. Shortening also helps to reduce moisture migration from the core of the loaf to the drier outside crust region, which would otherwise result in the loaf losing its crispness later in the baking process.

A certain amount of solid fat is needed in bread as the high melting crystals contribute to the higher gas development of the air cells during baking (Pehlivanoglu *et al.*, 2018). The findings also align with those of Farmani *et al.* (2016) who discovered that bread with a higher addition of oil was firmer. In addition, the incorporation of shortening containing solid fats in a bread formulation formed a thin and expandable gluten film in the dough, while liquid oil caused gluten aggregation (Scheuer *et al.*, 2014; Watanabe *et al.*, 2003). Gluten in the bread dough made with liquid oil contained a large number of holes that cause a fragile and easily ruptured structure. Smith and Johansson (2004) clarified that liquid oil cannot attach to the air cells, and therefore, specific concentrations of solid fats are necessary for a good shortening. Bread with lower firmness values showed lower chewiness values, indicating that a lower effort is required to break down the bread to the state ready for swallowing. Although solid shortening in the present study had recommended levels of SFC for a good bakery shortening, fluid shortening produced a much softer crumb texture than solid shortening.

Sensory Analyses

The mean scores attributed to appearance, texture, taste, colour, aroma and overall acceptability of the bread are shown in Table 3. The bread made with solid and fluid shortenings received mean scores above 6.0 for all the attributes,

TABLE 3. MEAN SCORES FOR SENSORY ATTRIBUTES OF THE BREAD MADE WITH FLUID SHORTENING, SOLID SHORTENING AND PALM OLEIN

Attribute	Sample		
	Fluid shortening	Solid shortening	POo
Appearance	6.63 ^a ± 1.28	6.59 ^a ± 1.73	5.25 ^b ± 1.45
Texture	6.19 ^b ± 1.17	7.09 ^a ± 1.35	4.78 ^c ± 1.45
Taste	6.50 ^a ± 1.24	6.84 ^a ± 1.24	5.47 ^b ± 1.50
Colour	6.78 ^a ± 1.18	6.84 ^a ± 1.24	5.47 ^b ± 1.50
Aroma	6.59 ^a ± 1.07	6.84 ^a ± 1.19	5.31 ^b ± 1.28
Overall acceptability	6.98 ^a ± 1.11	6.84 ^a ± 1.19	5.47 ^b ± 1.19

Note: *Means within each row bearing different superscript letters differ significantly ($p < 0.05$) from one another.

indicating that the bread were well accepted by the panellists. The bread made with solid shortening received the highest scores for all the attributes except for appearance and overall acceptability, but these were not significantly different ($p > 0.05$) from the scores for the bread made with fluid shortening, except for texture. As a result, it can be suggested that customers are unable to identify any differences in appearance, taste and aroma between the bread made with fluid shortening and solid shortening. Bread made with fluid shortening, despite its outstanding softness was less preferred in terms of texture compared to the bread made with solid shortening. However, bread made with fluid shortening received the highest score for overall acceptability. The texture of bread made with POo received the lowest score indicating that the panellists slightly dislike the texture of the bread as the bread was the firmest. The bread made with POo was the least accepted by the panellists, as shown by its lower scores, with mean scores below 5.56 for all the sensory attributes. Lowest volume and hard texture as well as appearance scores appear to be the factors that contribute to panellists rating POo bread as the least preferred. The highest b^* value (yellowness) of the bread made with POo might affect the perception of the panellists resulting lower score of appearance of this sample.

CONCLUSION

In conclusion, bread made with fluid shortening had comparable qualities in terms of volume, specific volume and density with those made of solid shortening, while bread made with POo had significantly ($p < 0.05$) lower volume, specific volume and density. Crumbs of the bread made with fluid shortening were the softest while bread made with POo had the firmest crumb. Different types of fats used in the bread had no significant effects ($p > 0.05$) on the L^* and b^* of the crusts and L^* and a^* of the crumbs of all the breads. The bread made with fluid and solid shortening received

comparable sensory scores for taste, colour, aroma and overall acceptability, except for texture. It was discovered in this study that the softest texture does not always imply the most preferred in terms of texture. The results indicate that bread made with fluid shortening had comparable physical, textural and sensory properties to bread made with solid shortening. This evaluation does not recommend POo for bread making as the use of POo resulted in bread with inferior physical, textural and sensory qualities. The findings of this study may provide researchers and the bakery industry with vital information on shortenings. Future work on this research could include baking tests with different sorts of bakery products using fluid shortenings.

ACKNOWLEDGEMENT

The authors would like to thank the management of MPOB for permission to execute and publish this work. The authors would also like to thank the staff of the Food Technology Group for their contribution in ensuring the success of this study.

REFERENCES

- AACC (2000). Measurement of volume by rapeseed displacement: Methods 22-10.01. Approved Methods of the American Association of Cereal Chemistry. American Association Cereal Chemistry. Inc., St. Paul, Minnesota.
- Aguilar, N; Albanell, E; Miñarro, B and Capellas, M (2015). Chickpea and tiger nut flours as alternatives to emulsifier and shortening in gluten-free bread. *LWT - Food Sci. Technol.*, 62(1): 225-232.
- Akyüz, G and Mazi, B G (2020). Physicochemical and sensory characterization of bread produced from different dough formulations by *Kluyveromyces lactis*. *J. Food Process. Preserv.*, 44(6): e14498.

- Almeida, D T d; Viana, T V; Costa, M M; Silva, C d S and Feitosa, S (2019). Effects of different storage conditions on the oxidative stability of crude and refined palm oil, olein and stearin (*Elaeis guineensis*). *Food Sci. Technol.*, 39(1): 211-217.
- Alvarez-Jubete, L; Auty, M; Arendt, E K and Gallagher, E (2010). Baking properties and microstructure of pseudocereal flours in gluten free bread formulations. *Eur. Food Res. Technol.*, 230(3): 437-445.
- AOCS (2012). Official methods and recommended practices of the AOCS. 6th edition. American Oil Chemists' Society (AOCS).
- Artan, M Y; Karim, R and Boo, H C (2010). The influence of different formulations of palm oil/ palm stearin-based shortenings on the quality of the white bread. *Middle-East J. Sci. Res.*, 5(6): 469-476.
- Brooker, B E (1996). The role of fat in the stabilisation of gas cells in bread dough. *J. Cereal Sci.*, 24: 187-198.
- Calligaris, S; Manzocco, L; Valoppi, F and Nicoli, M C (2013). Effect of palm oil replacement with monoglyceride organogel and hydrogel on sweet bread properties. *Food Res. Int.*, 51(2): 596-602.
- Cauvain, S P (2012). *Breadmaking: An Overview. Breadmaking: Improving Quality* (Cauvain, S P ed.). Elsevier Science, Woodhead Publishing Series in Food Science, Technology and Nutrition, United Kingdom. p. 9-11.
- Che Man, Y B; Miskandar, M S; Abdul Rahman, R; Nor Aini, I and Yusoff, M S A (2010). Monitoring crystal development in palm oil-based fluid shortening production by FT-IR spectroscopy. *J. Food Process. Preserv.*, 34(1): 68-82.
- Chin, N L; Rahman, R A; Hashim, D M and Kwong, S Y (2010). Palm oil shortening effects on baking performance of white bread. *J. Food Process Eng.*, 33(3): 413-433.
- Dilek, N M and Bilgiçli, N (2021). Effect of taro (*Colocasia esculenta* (L.) Schott) flour and different shortening ratios on physical and chemical properties of gluten-free cookie. *J. Food Process. Preserv.*, 45(11): e15894.
- Dunford, N (2017). Shortenings. Food technology fact sheet. *FAPC*, 212: 1-4.
- Farmani, J; Miarkiani, F and Maghsoudlou, Y (2016). Dough characteristics, baking performance, and staling of taftoon bread as affected by supplementation with sesame oil. *J. Culin. Sci. Technol.*, 14(4): 318-331.
- Feili, R; Zaman, W; Wan Abdullah, W N and Yang, T A (2013). Physical and sensory analysis of high fiber bread incorporated with jackfruit rind flour. *Food Sci. Technol.*, 1(2): 30-36.
- Figoni, P (2004). *How Baking Works*. John Wiley and Sons, Inc., Hoboken, New Jersey, USA. p. 30-39, 64-67, 176-193.
- Ghotra, B S; Dyal, S D and Narine, S S (2002). Lipid shortenings: A review. *Food Res. Int.*, 35(10): 1015-1048.
- Huang, Z; Stipkovits, L; Zheng, H; Serventi, Lu and Brennan, C (2019). Bovine milk fats and their replacers in baked goods: A review. *Foods*, 8(9): 383.
- Kanagaratnam, S; Hoque, M E; Miskandar, M S and Spowage, A (2013). Investigating the effect of deforming temperature on the oil-binding capacity of palm oil based shortening. *J. Food Eng.*, 118: 90-99.
- Majeed, M; Khan, M U; Owaid, M N; Khan, M R; Shariati, M A; Igor, P and Ntsefong, G N (2017). Development of oyster mushroom powder and its effects on physicochemical and rheological properties of bakery products. *J. Microbiol. Biotech. Food Sci.*, 6(5): 1221-1227.
- Makinde, F M and Akinoso, R (2014). Physical, nutritional and sensory qualities of bread samples made with wheat and black sesame (*Sesamum indicum* Linn.) flours. *Int. Food Res. J.*, 21(4): 1635-1640.
- Manaf, Y N; Marikkar, J M N; Mustafa, S; Van Bockstaele, F and Nusantara, B P (2019). Effect of three plant-based shortenings and lard on cookie dough properties and cookies quality. *Int. Food Res. J.*, 26(6): 1795-1802.
- MPOB (2005). *MPOB Test Methods*. MPOB, Bangi. 336 pp.
- Nor Aini, I and Che Maimon, C H (1996). Characteristics of white pan bread as affected by tempering of the fat ingredient. *Cereal Chem.*, 73: 462-465.
- Nur Haqim, I; Miskandar, M S and Rafidah, A H (2018). Influence of palm-based fluid shortening on the physical and textural properties of biscuits. *J. Oil Palm Res.*, 30(2): 299-305.

O'Brien, R D; Oswell, N J and Pegg, R B (2020). Shortenings: Types and formulations. *Bailey's Industrial Oil and Fat Products* (Shahidi, F ed.). John Wiley and Sons, Ltd. p. 1-25.

Osuna, M; Romero, A M; Avallone, C M; Judis, M A and Bertola, N C (2018). Animal fat replacement by vegetable oils in formulations of breads with flour mixes. *J. Food Sci. Technol.*, 55: 1-10.

Othman, N H; Md Noor, A and Yusoff, M S A (2015). Physicochemical properties, baking performance and sensory evaluation of bakery shortening enriched with diacylglycerol. *J. Food Sci. Eng.*, 5: 58-66.

Pareyt, B; Finnie, S M; Putseys, J A and Delcour, J A (2011). Lipids in bread making: Sources, interactions, and impact on bread quality. *J. Cereal Sci.*, 54(3): 266-279.

Pehlivanoglu, H; Ozulku, G; Yildirim, R. M; Demirci, M; Toker, O S and Sagdic, O (2018). Investigating the usage of unsaturated fatty acid-rich and low-calorie oleogels as a shortening mimetic in cake. *J. Food Process. Preserv.*, 42(6): e13621.

Ramli, M R; Siew, W L; Cheah, K Y; Idris, N A and Mat Sahri, M (2008). Physicochemical properties and performance of high oleic and palm-based shortenings. *J. Oleo Sci.*, 57(11): 605-612.

Rios, R V; Pessanha, M D F; Almeida, P F D; Viana, C L and Lannes, S C D S (2014). Application of fats in some food products. *Food Sci. Technol.*, 34(1): 3-15.

Saghafi, Z; Naeli, M H; Tabibiazar, M and Zargaraan, A (2019). Zero-trans cake shortening: Formulation and characterization of physicochemical, rheological, and textural properties. *J. Am. Oil Chem. Soc.*, 95(2): 171-183.

Scheuer, P M; Bruna Mattioni, B; Barreto, P L M; Montenegro, F M; Ruffi, C R G; Biondi, S; Kilpp, M and Francisco, A D (2014). Effects of fat replacement on properties of whole wheat bread. *Braz. J. Pharm. Sci.*, 50(4): 703-712.

Smith, P R and Johansson, J (2004). Influences of the proportion of solid fat in a shortening on loaf volume and staling of bread. *J. Food Process. Preserv.*, 28(5): 359-367.

Stauffer, C E (1996). Bakery product applications. Fats and Oils (Practical Guides for the Food Industry). American Association of Cereal Chemists, St Paul, Minnesota, USA. p. 61-69.

Stauffer, C D (2002). *Soy protein in baking*. Technical Foods Consultants Cincinnati, Ohio, USA.

Tamstorf, S; Jonnson, T and Krog, N (1988). The role of fats and emulsifiers in baked products. *Chemistry and Physics of Baking* (Blanshard, J M V; Frazier, P J and Galliard, T eds.). The Royal Society of Chemistry, London. p. 75-88.

Ulziijargal, E; Yang, J H; Lin, L Y; Chen, C P and Mau, J L (2013). Quality of bread supplemented with mushroom mycelia. *Food Chem.*, 138: 70-76.

Watanabe, A; Yokomizo, K and Eliasson, A C (2003). Effect of physical states of nonpolar lipids on rheology, ultracentrifugation, and microstructure of wheat flour dough. *Cereal Chem.*, 80(3): 281-284.

Yilmaz, E and Ögütçü, M (2015). The texture, sensory properties and stability of cookies prepared with wax oleogels. *Food Function*, 6(4): 1194-1204.

Zhou, J; Faubion, J M and Chuck, E and Walker, C E (2011). Evaluation of different types of fats for use in high-ratio layer cakes. *LWT - Food Sci. Technol.*, 44(8): 1802-1808.

OPTIMISATION OF ETHYLENE TREATMENT CONDITIONS FOR OIL PALM FRUIT DETACHMENT USING RESPONSE SURFACE METHODOLOGY

MOHD IBNUR SYAWAL ZAKARIA^{1*}; ARUTCHELVAM BALAKRISHNAN¹; BEE AIK TAN¹; JAIME YOKE SUM LOW¹; SHWU FUN KUA¹; CHIN MING LIM¹ and DAVID ROSS APPLETON¹

ABSTRACT

Ethylene gas can be applied to oil palm fresh fruit bunches (FFB) to enhance fruit detachment and produce fresh loose fruits (LF) that could be processed separately to reduce oil loss due to empty fruit bunch (EFB) absorption during the milling process. Response surface methodology (RSM) with central composite design (CCD) was employed to achieve optimum fruit detachment in oil palm FFB as the main response with two experimental variables, which were ethylene concentration (500 to 2000 ppm) and incubation period (18 to 24 hr). The proposed model showed an R^2 value of 0.85 where the experimental variables were significant to the result. From the model, 26% of the targeted fruit detachment was attained with ethylene concentration of 1102.7 ppm and an incubation period of 21.5 hr. The application of optimum ethylene gas and incubation time from the study can be then tested on a pilot scale to reduce optimisation time.

Keywords: ethylene, fresh fruit bunch, fruit detachment, loose fruits, RSM.

Received: 24 November 2021; **Accepted:** 24 May 2022; **Published online:** 21 July 2022.

INTRODUCTION

Oil palm is the main commodity crop in Malaysia and has contributed significantly to the country's economic development. In 2020, the Department of Statistics Malaysia reported that palm oil accounted for about 38% of Malaysia's agricultural output value and contributed to 3% of its gross domestic product in 2019 (Department of Statistics, 2020).

One of the most important attributes in determining the crude palm oil (CPO) quality is the harvested fresh fruit bunches (FFB). Typically, FFB quality is classified based on colour, shape, and ripeness (Basiron, 2007). Harvesting FFB at the right ripeness will potentially maximise oil yield as individual fruit can further accumulate oil and achieve the highest oil content before detachment from the bunch (Mohanaraj and Donough, 2016). Harvested FFB will then be delivered to an oil mill for a series of milling processes to produce CPO. The mass ratio between extracted CPO and

processed FFB is normally used to estimate oil extraction rate (OER) (Corley and Tinker, 2003) and serves as an important key performance indicator for palm oil mills (Zulkefli *et al.*, 2017). Aside from FFB quality, many other factors could impact OER such as oil content in oil palm fruitlets, palm age, soil conditions, climate, harvesting practices and oil extraction efficiency in mills (Chew *et al.*, 2021).

Nadzim *et al.* (2020) reported the OER in our country has fluctuated for the last 20 years. Low OER implies low oil productivity, low revenue, and some oil loss during a particular processing stage (Chang *et al.*, 2003; Chew *et al.*, 2021; Zulkefli *et al.*, 2017). There are several sources of oil loss in the conventional palm oil milling process including empty fruit bunches (EFB) and unstripped bunches (USB) after threshing, press cake fibre from screw press, steriliser condensate and sludge from the separator. The approximate oil loss encountered in EFB ranges from 0.3% to 0.5% with a ratio to FFB (Nadzim *et al.*, 2020; Ng, 1994). To minimise oil loss caused by EFB absorption during sterilisation, we explored fruit detachment induced by ethylene gas in FFB so that only high-quality loose fruits will be processed in mills. Our previous study by Balakrishnan *et al.* (2021) showed that fruit

¹ Sime Darby Plantation Technology Centre Sdn. Bhd., 43400 Serdang, Selangor, Malaysia.

* Corresponding author e-mail: ibnur.syawal.zakaria@sime-darbyplantation.com

detachment achieved was $29.4 \pm 1.9\%$ incubated with 750 ppm ethylene gas for 24 hr. The ethylene gas and incubation period were only tested at 500 ppm to 1250 ppm for 24 hr. A similar study by Nualwijit and Lerslerwong (2014) used higher ethylene concentration with the same incubation period applied on the unripe FFB stimulated fruit abscission. There is no prior published research on the optimum conditions of these two parameters: ethylene concentration and incubation period on FFB fruit detachment other than from our previous study (Balakrishnan *et al.*, 2021; Chew *et al.*, 2021). From our findings, both ethylene concentration and incubation duration have more influence on fruit detachment. Therefore, we decided to deploy the Response Surface Methodology (RSM) to further investigate the combined effect of these two parameters at the lab and pre-pilot scale.

RSM is a collection of statistical techniques for designing experiments, building models, evaluating the effect of variables, and searching for optimum conditions of variables for desirable responses (Montgomery, 2017). RSM explores the relationships between several experimental variables and one or more responses could be employed (Bhagwat *et al.*, 2021). Consequently, the objective of this article was to determine the processing parameters and functional relationship of exogenous ethylene concentration and incubation period to achieve optimum fruit detachment with the RSM tool.

MATERIALS AND METHODS

Plant Material

Oil palm FFBs of the *Tenera* variety were harvested from the commercial fields in Banting and Carey Island, Malaysia. Only ripe bunches that had undergone a colour change (from deep violet to yellowish-orange), according to the ripeness standard established by MPOB (2003) (Table 1) and weighing between 14 to 20 kg were selected for this study. The bunches were transported to the research facility within 2 hr of harvesting.

Response Surface Methodology Experimental Design

Central composite design (CCD) of RSM was used to determine the number of experiments to be evaluated for optimisation of variables and response using State-Ease software (Design Expert v11.1.2.0). This CCD consisted of two independent variables, which were ethylene concentration and incubation period to investigate the optimum condition that gives a better percentage of fruit detachment, known as the response in this experiment (Table 2). The minimum, intermediate and maximum

TABLE 1. OIL PALM FRUITS RIPENESS STANDARD

Fruit ripeness	Description
Unripe	No fruitlets detached from a fruit bunch
Under-ripe	1 to 10 fruitlets detached from a fruit bunch
Ripe	>10 fruitlets detached and 50% of fruitlets are still intact in a fruit bunch
Over-ripe	> 50% of the fruitlets detached from a fruit bunch

Source: MPOB (2003).

TABLE 2. LEVELS AND VALUES OF THE INDEPENDENT VARIABLES FOR THE CENTRAL COMPOSITE DESIGN OF RESPONSE SURFACE METHODOLOGY

Experiment variables	Coded factors	Levels of coded factor		
		-1	0	1
Ethylene concentration, x_1 (ppm)	A	500	1 250	2 000
Incubation period, x_2 (hr)	B	18	21	24

values of each variable were coded as -1, 0 and +1, respectively. The experimental design consisted of 11 experimental runs, including three centre points, four factorial and four axial points to cover the possible combinations of variables and their effect on the investigated response. Random experimental orders were performed in triplicate to minimise the unexpected variability effects on the observed response due to systematic error.

Experiment Set-up

Ethylene treatment was performed on individual ripe bunches and each treatment consisted of five replicates ($n=5$). The bunches were individually placed inside a covered incubation box ($V=150$ L). Ethylene gas (Gaslink, Malaysia) was transferred into the box by a fixed pressure at 0.3 bar, with a flowrate of 0.1 L min^{-1} at the designed concentration and incubation period as presented in Table 2. The gas concentration was monitored using a portable gas detector (PG610, Henan Inte Electrical Equipment Co., Ltd).

For the validation study, optimum points determined by the RSM model including ethylene concentration of 1102.73 ppm at 21.55 hr were used at the lab and pre-pilot trial. Approximately 150 kg of ripe FFB were collected and placed inside a larger covered incubation bin ($V=1100$ L) for validation study during the pre-pilot trial. Five replicates ($n=5$) of experimental runs were performed for both control and treatment. Post ethylene treatment, the fruitlets on FFB were hand stripped manually and all the detached fruits were weighed.

Fruit detachment was determined according to the Equation (1):

$$\text{Fruit detachment (\%)} = \frac{\text{(Total weight of detached fruits / total weight of bunches)} \times 100\% \quad (1)$$

Statistical Analysis

Design Expert v11.1.2.0 was used for statistical and graphical analyses of the experimental data. Statistical significance was evaluated using Fisher's F-test. The quality of the predictive model was statistically evaluated by using the coefficient of determination. All experiments were carried out in triplicate and the average values were reported.

RESULTS AND DISCUSSION

Experimental Design Matrix and Results Analysis

The design matrix to optimise fruit detachment is summarised in Table 3. The correlation between two variables and response was developed by CCD. The fruit detachment obtained from experiment data ranged from 11.1% to 30.8%. Three replication of the central point (standard order 9 to 11) were used to predict the error that represents the accuracy of the experiment. The obtained standard error from the actual experiment was 1.5, which was higher than the optimum range of lesser than 1.0 (Feng *et al.*, 2017). The high standard error may be due to varied weight and ripeness conditions of different biological replicates of FFB randomly selected from the commercial field for this experiment.

Based on the results, fruit detachment increased with incubation time at a fixed ethylene concentration. At 500 ppm of ethylene concentration, detachment achieved was only at 11.1% but increased to 23.7% and 27.8% after 21 hr and 24 hr of incubation. The observation can be due to the autocatalytic reaction of ethylene response in the FFB that could have started only after 18 hr with exposure of lower exogenous ethylene initially (500 ppm) as displayed by other climacteric fruits such as tomato, papaya, and banana (Fabi and do Prado, 2019; Liu *et al.*, 1999; Yokotani *et al.*, 2009). Autocatalysis of ethylene production is a characteristic feature of the ripening of climacteric fruits in which a massive increase in ethylene production is triggered by exposure to ethylene at a concentration above a threshold level (Liu *et al.*, 2015). In this study, endogenous ethylene produced by FFB was insufficient, so when exogenous ethylene was applied, it induced an autocatalytic ethylene production system in FFB.

The results showed that with similar exposure time at higher exogenous ethylene of 1250 ppm and 2000 ppm, there was a drastic increase in fruit detachment, which indicated that a large amount of ethylene was sufficient to induce an autocatalytic reaction from basal level (system 1, S1) to ripening and senescence-associated system 2 (S2) (Hewitt and Dhingra, 2020; Yokotani *et al.*, 2009). Under the S1 mode in non-climacteric fruits such as sweet cherry, kiwi, and pineapple, ethylene biosynthesis terminates upon reaching the basal level and is not affected by the ripening process (Bapat *et al.*, 2010; Hewitt *et al.*, 2021). In contrast, the ethylene biosynthesis in climacteric fruits such as oil palm, apple, banana, and mango increase during the transition from preclimacteric

TABLE 3. VARIABLES, LEVELS, AND THE RESPONSE OF FRUIT DETACHMENT BASED ON ETHYLENE CONCENTRATION AND INCUBATION PERIOD

Std. order	Variables levels ^a		Response		Residual
	Ethylene concentration, A (ppm)	Incubation period, B (hr)	Fruit detachment (%)	Predicted value (%)	
1	500 (-1)	18 (-1)	11.10	12.96	-1.86
2	2 000 (1)	18 (-1)	22.50	22.98	-0.48
3	500 (-1)	24 (1)	27.80	28.78	-0.98
4	2 000 (1)	24 (1)	25.70	25.29	0.41
5	500 (-1)	21 (0)	23.70	20.87	2.83
6	2 000 (1)	21 (0)	24.20	24.13	0.07
7	1 250 (0)	18 (-1)	23.50	20.95	2.55
8	1 250 (0)	24 (1)	30.80	30.01	0.79
9	1 250 (0)	21 (0)	22.40	25.28	-3.08
10	1 250 (0)	21 (0)	27.40	25.48	1.92
11	1 250 (0)	21 (0)	23.30	25.48	-2.18

Note: ^a Coded level is with brackets.

(S1) mode to climacteric (S2) mode (Chen *et al.*, 2018) upon induction by exogenous ethylene (Gwanpua *et al.*, 2018). Therefore, with higher concentrations of ethylene at 1250 ppm and 2000 ppm, it was observed that 23.5% and 22.5% of detachment were achieved at 18 hr of incubation, which indicated that autocatalytic reaction of ethylene can be activated much earlier by increasing ethylene concentration. The incubation time variable was not set lower than 18 hr taking into consideration the duration from post-harvest FFB and typical daily mill operation.

Central Composite Design Model Development and Analysis

The sequential model sum of squares for this study was shown in Table 4. The highest-order polynomial was used to select the appropriate model. Two-factor interaction (2FI) model was proposed with *p*-value of 0.0578 (*p*<0.1) and high *F*-value of 5.13.

To assess the fitness and reliability of the proposed model, three main tests were used, namely, significant of terms, lack-of-fit and regression model. An examination of the significance and fitness of the 2FI model based on the *p*-value and *F*-test was carried out to ensure the sufficiency of the suggested model (Table 5). Based on the ANOVA model, the *p*-value was 0.0128, indicating that the model was statistically significant.

The model is reliable and reproducible based on the regression coefficient ($R^2=0.85$), coefficient of variation (C.V.%=10.53%) and precision residual sum of square (PRESS=140.62), respectively. In this study, the PRESS is greater than 4, indicating there is a good correlation between experimental data and the predicted model. The incubation period was found to be the most influential factor. The non-significant lack of fit (*p*-value=0.6093) confirms the significance and adequacy of the model.

RSM Model Validation

Based on the obtained results, the model built for fruit detachment using ethylene concentration was in Equation (2). This equation was generated

from the coded levels, as shown in Table 2. The coded equation is useful for identifying the relative impact of the variables by comparing them with the factor coefficients:

$$\text{Fruit detachment (\%)} = 25.48 + 1.63A + 4.53B - 3.38AB - 2.98A^2 \quad (2)$$

The equation in terms of the actual factors:

$$\text{Fruit detachment (\%)} = -56.63 + 0.05x_1 + 3.39x_2 - 0.002x_1x_2 - 5.30 \times 10^{-6}x_1^2 \quad (3)$$

A graphical representation of the model's quality was illustrated in Figure 1a. Predicted data by ANOVA *vs.* actual values for fruit detachment showed that the model almost formed a straight line. The residuals plot of the actual value (Figure 1b) showed that all the points were located within the acceptable range and were close to the centre line within the threshold cut-off at ± 4.8819 . This indicated that the experimental data was adequately fitted to the model and can be employed to assess, evaluate, and predict the interactions between the significant variables in obtaining high fruit detachment efficiency.

Parameter Optimisation Based on RSM

The three-dimensional (3D) response surface plot (Figure 2) was facilitated to study the effects of variables and their interaction on FFB fruit detachment based on the developed model (Equation 2).

The 3D plot illustrated the fruit detachment increased with increasing ethylene concentration and incubation period. It was found that the optimum ethylene concentration and incubation period were 1102.73 ppm and 21.55 hr, to achieve targeted fruit detachment set at 26%. Based on our previous work, the FFB outer layer consists of 25%-28% of the total bunch weight where the fruits can be easily detached compared to the middle and inner layers (Balakrishnan *et al.*, 2021). This was confirmed when 26% of fruit detachment was achieved from our preliminary trials.

TABLE 4. SEQUENTIAL MODEL SUM OF SQUARES

Source	Sum of squares	Degree of freedom	Mean square	F-value	<i>p</i> -value (prob>F)	Remark
Mean <i>vs.</i> total	6 259.43	1	6 259.43			
Linear <i>vs.</i> mean	139.31	2	69.66	5.18	0.0361	
2FI <i>vs.</i> linear	45.56	1	45.56	5.13	0.0578	Suggested
Quadratic <i>vs.</i> 2FI	24.30	2	12.15	1.61	0.2892	
Cubic <i>vs.</i> quadratic	8.08	2	4.04	0.4077	0.6972	Aliased
Residual	29.73	3	9.91			
Total	6 506.42	11	591.49			

TABLE 5. ANOVA RESULTS FOR RESPONSE SURFACE MODEL

Source	Sum of square	DF	Mean square	F-value	p-value (prob>F)	Remark
Model	209.10	4	52.27	8.28	0.0128	Significant
A, ethylene concentration	16.01	1	16.01	2.53	0.1625	Not significant
B, incubation period	123.31	1	123.31	19.52	0.0045	Significant
AB	45.56	1	45.56	7.21	0.0362	Significant
A ²	24.22	1	24.22	3.83	0.0979	Significant
Residual	37.89	6	6.32			
Lack of fit	23.69	4	5.92	0.8336	0.6093	Not significant
Pure error	14.21	2	7.10			
Corrected total	246.99	10				
Standard deviation	2.51		R ²	0.8466		
Mean	23.85		Adjusted R ²	0.7443		
C.V.%	10.53		Predicted R ²	0.4307		
Press	140.62		Adeq. precision	10.0662		

Note: Significant at a significance level of 0.1.

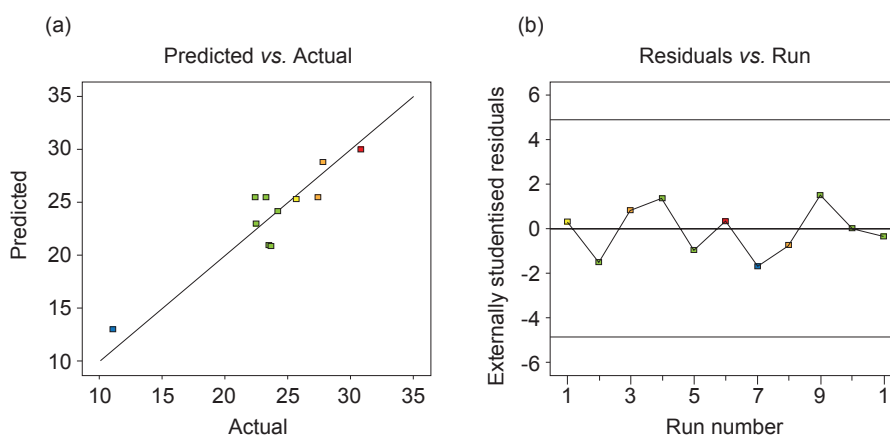
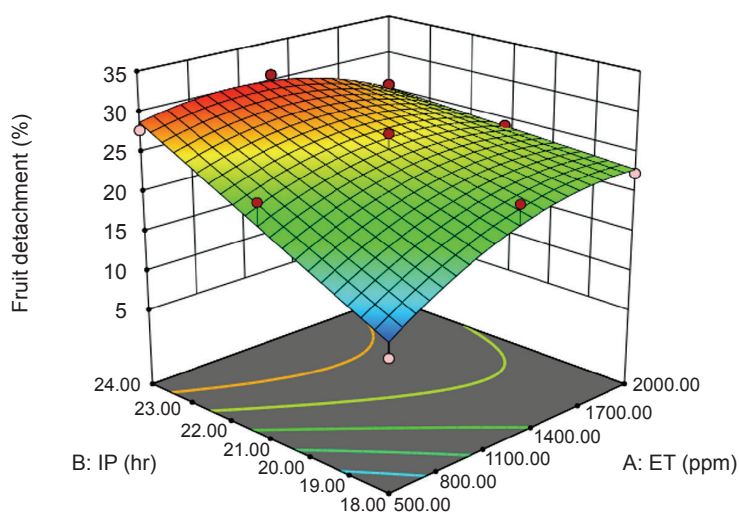


Figure 1. Diagnostic plot for developed model adequacy. (a) Actual response vs. predicted response for fruit detachment, (b) Studentised residuals vs. run number, the threshold cut-off at ± 4.8819 .



Note: ET - ethylene concentration; IP - incubation period.

Figure 2. Three-dimensional response surface design of the fruit detachment vs. ethylene concentration and incubation period.

TABLE 6. COMPARISON BETWEEN ACTUAL AND PREDICTED VALUE OF FRUIT DETACHMENT AT THE OPTIMUM POINTS

Validation	Optimum point		Predicted fruit detachment (%)	Actual fruit detachment (%)
	Ethylene concentration (ppm)	Incubation period (hr)		
Lab	1 102.73	21.55	26	27.94 ± 1.38
Pre-pilot	1 102.73	21.55	26	27.00 ± 1.12

Model Validation

Model validation was performed using the optimum point of variables determined in this study (Table 6) for both lab and pre-pilot trials. Based on the model, fruit detachment was set at 26%, while the lab and pre-pilot give the average actual fruit detachment of $27.94 \pm 1.38\%$ and $27.00 \pm 1.12\%$ with insignificant difference ($p=0.117$ and $p=0.197$), respectively. Therefore, the results revealed that predicted responses from RSM were similar to experimental responses which were displayed in experimental trials. This demonstrated that the RSM is a good tool in ethylene concentration and incubation period optimisation for fruit detachment.

CONCLUSION

The fruit detachment study with multiple variables was successfully deployed using RSM through designing, analysing and discovering the optimum point, as well as evaluating the effects of variables. A 2FI model with R^2 value of 0.85, was effectively applied to explain and predict the response of fruit detachment. Numerical optimisation for targeted fruit detachment of 26%, was predicted to be achieved at ethylene concentration of 1102.73 ppm and an incubation period of 21.55 hr. The model was validated and found to have similar responses between predicted and actual results in both lab and pre-pilot trials. The application of ethylene is a realistic and novel solution for the palm oil industry that may revamp the oil palm mill process to reduce the oil losses through separate processing of high-quality loose fruits. However, the application of this technology in a commercial setting must be tested using the model's optimised variables as a proof of concept.

ACKNOWLEDGEMENT

This work was fully funded by Sime Darby Plantation Berhad and conducted by researchers affiliated with Sime Darby Plantation Technology Centre Sdn. Bhd. We would like to thank our commercial fields in Banting and Carey Island for providing oil palm FFB used in this study. This

study was conducted in Sime Darby Plantation R&D Centre, which is fully supported by Sime Darby Plantation, Malaysia.

REFERENCES

- Balakrishnan, A; Zakaria, M I S; Tan, B A; Low, J Y S; Kua, S F; Lim, C M and Appleton, D R (2021). Postharvest fruit detachment in oil palm bunches with ethephon and ethylene gas application. *Agric.*, 11(11): 1030.
- Bapat, V A; Trivedi, P K; Ghosh, A; Sane, V A; Ganapathi, T R and Nath, P (2010). Ripening of fleshy fruit: Molecular insight and the role of ethylene. *Biotechnol. Adv.*, 28(1): 94-107.
- Basiron, Y (2007). Palm oil production through sustainable plantations. *Eur. J. Lipid Sci. Technol.*, 109(4): 289-295.
- Bhagwat, K; Choutmal, S and Digraskar, R (2021). Process parameters optimisation on CNC engraving machine by using response methodology (RSM). *Int. J. Res. Sci. Eng.*, 9(7): 28-34.
- Chang, L C; Sani, A R A and Basran, Z (2003). An economic perspective of oil extraction rate in the oil palm industry of Malaysia. *Oil Palm Industry Economic J.*, 3(1): 25-31.
- Chen, Y; Grimplet, J; David, K; Castellarin, S D; Terol, J; Wong, C J; Luo, Z; Schaffer, R; Celton, J M; Talon, M; Gambetta, G A and Chervin, C (2018). Ethylene receptors and related proteins in climacteric and non-climacteric fruits. *Plant Sci.*, 276: 63-72.
- Chew, C L; Lim, C M; Kua, S F; Low, J Y S; Tan, B A; Balakrishnan, A; Zakaria, M I S; Noor Haizar, A H; Syed Hilmi, S M H; Mohd Hakimi, N I N; Mustaner, M and Mat Hassan, N S (2021). Process for producing crude palm fruit oil and virgin palm fruit oil. Malaysia patent application PI2019007397.
- Chew, C L; Ng, C Y; Hong, W O; Wu, T Y; Lee, Y Y; Low, L E; Kong, P S and Chan, E S (2021). Improving sustainability of palm oil production by increasing oil extraction rate: A review. *Food Bioproc. Technol.*, 14(4): 573-586.

- Corley, R H V and Tinker, P B (2003). *The Oil Palm*. 4th edition. Blackwell Science, Oxford. p. 459.
- Department of Statistics (2020). Selected agricultural indicators, Malaysia, 2020. <https://www.dosm.gov.my>, accessed on 17 September 2021.
- Fabi, J P and Do Prado, S B R (2019). Fast and furious: Ethylene-triggered changes in the metabolism of papaya fruit during ripening. *Front. Plant Sci.*, 10: 535-535.
- Feng, J; Zhang, J; Zhang, J; He, Y; Zhang, R; Chen, C and Liu, G (2017). Enhanced methane production of vinegar residue by response surface methodology (RSM). *AMB Expr.*, 7(1): 1-8.
- Gwanpua, S G; Qian, Z and East, A R (2018). Modelling ethylene regulated changes in 'Hass' avocado quality. *Postharvest Biol. Technol.*, 136: 12-22.
- Hewitt, S and Dhingra, A (2020). Beyond ethylene: New insights regarding the role of alternative oxidase in the respiratory climacteric. *Front. Plant Sci.*, 11: 1578.
- Hewitt, S; Kilian, B; Koepke, T; Abarca, J; Whiting, M and Dhingra, A (2021). Transcriptome analysis reveals potential mechanisms for ethylene-inducible pedicel-fruit abscission zone activation in non-climacteric sweet cherry (*Prunus avium* L.). *Hortic.*, 7(9): 270.
- Liu, X; Shiomi, S; Nakatsuka, A; Kubo, Y; Nakamura, R and Inaba, A (1999). Characterisation of ethylene biosynthesis associated with ripening in banana fruit. *Plant Physiol.*, 121(4): 1257-1266.
- Liu, M; Pirrello, J; Chervin, C; Roustan, J P and Bouzayen, M (2015). Ethylene control of fruit ripening: Revisiting the complex network of transcriptional regulation. *Plant Physiol.*, 169(4): 2380-2390.
- Mohanaraj, S and Donough, C (2016). Harvesting practices for maximum yield in oil palm: Results from a re-assessment at IJM Plantations, Sabah. *Oil Palm Bulletin*. p. 32-37.
- Montgomery, D C (2017). *Design and Analysis of Experiments*. 9th edition. John Wiley and Sons, New Jersey. p. 490.
- MPOB (2003). *Oil Palm Fruit Grading Manual*. 2nd edition. MPOB, Bangi. p. 4.
- Nadzim, U K H M; Yunus, R; Omar, R and Lim, B Y (2020). Factors contributing to oil losses in crude palm oil production process in Malaysia: A review. *Int. J. Biomass Renew.*, 9(1): 10-24.
- Ng, S B (1994). Measurement of oil extraction ratio (OER) and milling losses. Paper presented at the National Seminar on Palm Oil Extraction Rate: Problems and Issues. PORIM, Kuala Lumpur. 21-22 December 1993.
- Nualwijit, N and Lerslerwong, L (2014). Post harvest ripening of oil palm fruit is accelerated by application of exogenous ethylene. *Songklanakarin J. Sci. Technol.*, 36(3): 255-259.
- Yokotani, N; Nakano, R; Imanishi, S; Nagata, M; Inaba, A and Kubo, Y (2009). Ripening-associated ethylene biosynthesis in tomato fruit is autocatalytically and developmentally regulated. *J. Exp. Bot.*, 60(12): 3433-3442.
- Zulkefli, F; Othman, N; Syahlan, S; Zaini, M R and Bakar, M A (2017). Fresh fruit bunch quality and oil losses in milling processes as factors that affect the extraction rate of palm oil. *Int. J. Agric. Forest Plant.*, 5: 99-103.

JOURNAL OF OIL PALM RESEARCH

GUIDE FOR AUTHORS

(for more details, kindly surf <http://jopr.mpob.gov.my>)

Type of Articles

1. Regular Article

Full-length original empirical investigations, consisting of introduction, materials and methods, results and discussion, conclusions. Original work must provide references and an explanation on research findings that contain new and significant findings. Conclusion should be brief and focus on the research output, should not be in point form. These papers should not exceed 6000 words of text (including tables, figures and references) and generally not more than a total of 10 figures and tables. After peer-review, the article word count limit can be extended to a maximum of 8000 words to better address the reviewers' and editors' comments. Any additional figures or tables can be included in the supplementary data. Please note that papers submitted to JOPR will be sent back to authors because of poor figure resolution or exceeding the number of figures permitted.

2. Short Communication

Significant new information to readers of the Journal in a short but complete form. Preferably not exceeding 3000 words (including tables, figures and references), and is intended for rapid publication. They are not intended for publishing preliminary results or to be a reduced version of regular article.

3. Review Article

Critical evaluation of materials about current research that have already been published by organising, integrating, and evaluating previously published materials. Re-analyses as meta-analysis and systemic reviews are encouraged. Review articles provide systemic overview, evaluation and interpretation of research in a given field. They should not exceed 12 000 words (excluding references only) and should contain no more than a total of 20 figures and tables. Any additional figures or tables can be included in the supplementary data. Please note that papers submitted to JOPR will be sent back to authors because of poor figure resolution or exceeding the number of figures permitted. The same information should not be repeated in a figure and a table.

Language

Please write your text in good English (only British English is accepted). We do not accept American English or a mixture of these.

JOPR's Template

JOPR's template, which is a standard format that facilitates the manuscript writing and copyediting process. This template is created to provide a detail and clear house style of JOPR. The template is drafted according to JOPR's house style, but in standard word version format. When writing a paper, authors need to format their papers to fit into the journal's house style. To make this easier, Word templates are available for many of other established journals, ready for them to download and apply to their research paper format. It is crucial for author to write a research paper while considering formatting. Each journal has its own guidelines for formatting; hence, the template defines how an article will look when it is published online or in print.

JOPR's Aims & Scope

This is established to provide a detail and clear aims and scope for author reference. Authors should declare in the cover letter how the research fits the aims and scope of JOPR.

JOPR's House Style

A detail listing of JOPR's house style for authors and a checklist to facilitate the copyediting process and standardise the copyediting process. The JOPR's house style remains the same and is drafted into a detail version for author's reference.

Manuscript Submission

- Manuscripts should be submitted via: <https://mc04.manuscriptcentral.com/jopres>
- JOPR does not permit dual submission, publication and/or any archive platform (preprint) in violation of journal ethical practices.

For more details and to download the JOPR's House Style and Template, kindly surf <http://jopr.mpob.gov.my>

CALL FOR PAPERS

JOURNAL OF OIL PALM RESEARCH

The JOPR is the flagship journal of Malaysian Palm Oil Board (MPOB)

- Quartile: Q3
- Internationally refereed
- No processing fee
- Open access
- Publishes four volumes annually

Impact Factor (2021) 1.594

CiteScore (2021) 3.3

Send your manuscript at <http://jopr.mpob.gov.my> or scan the QR Code. Contact us jopr.admin@mpob.gov.my

Contents of the Coming Issue

Journal of Oil Palm Research

Vol. 35 (1) March 2023*

- Impact of *Elaeidobius kamerunicus* (Faust) Introduction on Oil Palm Fruit Formation in Malaysia and Factors Affecting its Pollination Efficiency: A Review
Saharul Abillah Mohamad; Mohamed Mazmira Mohd Masri; Norman Kamarudin; Mohamad Rosman Sulaiman; Arnaud Costa; Meilina Ong-Abdullah; Hasnol Othman; Siti Nurulhidayah Ahmad; Muhammad Nurul Yaqin Syarif; Zam Abdul Karim; Idris Abdul Ghani; Samsudin Amit; Azlina Zakaria; Su Chong Ming; Siaw Ting Chuan; Johari Jalinias; Yap Yau Koong; Ahmad Anuar Sairi; Syed Mohd Faizal Syed Ali; Mahbob Abdullah; Sivapragasam Annamalai; King Jie Hung; Muhamad Azmi Mohammed; Idris Abu Seman; Ramle Moslim and Ghulam Kadir Ahmad Parveez
- Spatial Distribution of *Rattus* Species (Rodentia: Muridae) in Oil Palm Plantations of Peninsular Malaysia with Species Verification using Cytochrome Oxidase I (COI) Gene
Mohamad Harris Nasir; Muhamad Shakirin Mispan; Subha Bhassu; Khoo Jing Jing; Sazaly Abubakar; Siti Nurul Izzah Mohd Azami; Siti Nabilah Ishak; Farah Shafawati Mohd-Taib and Hasmahzaiti Omar
- A Comparative Study of Oil Palm Fronds Torrefaction Under Flue Gas and Nitrogen Atmospheres
Premchand; Masaharu Komiyama; Aqsha and Yoshimitsu Uemura
- Counting of Oil Palm Fresh Fruit Bunches Using Computer Vision
Minarni Shiddiq; Dodi Sofyan Arief; Defrianto; Vicky Vernando Dasta; Deborah Margaretha Panjaitan and Doni Saputra
- Improving the Growth Performance of Oil Palm Seedlings by Mixtures of Organic and Chemical Fertilisers
Mohd Fahmi Keni; Nurhafizhoh Zainuddin and Mohamed Mazmira Mohd Masri
- Transpiration of Oil Palm (*Elaeis guineensis* Jacq.) Based on Sap Flow Measurement: The Relation to Soil and Climate Variables
Iput Pradiko; Suroso Rahutomo; Rana Farrasati; Eko Noviandi Ginting; Fandi Hidayat and Muhdan Syarovy

Note: * Subject to change.