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EXTRACTION AND PURIFICATION OF PHYTOSTEROLS MIXTURE FROM PALM FATTY ACID DISTILLATE (PFAD) USING MULTISTAGE EXTRACTION PROCESSES

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ABSTRACT

Phytosterols are among the bioactive compounds naturally present in vegetable oils and their by-products or derivatives. A phytosterol resource (PSR), solid by-product from the extraction of vitamin E in palm fatty acid distillate (PFAD), contains 2%-4% (w/w) total sterols. Therefore, the extraction of phytosterols from the PSR in a mini-pilot scale involving multistage extraction processes was developed to recover the valuable minor component. The multistage extraction and purification processes comprised of solid-liquid extraction (SLE) with hexane at 35°C in 1 hr, saponification reaction at the reflux temperature of 80°C for 1 hr, liquid-liquid extraction (LLE) with hexane and water, and crystallisation and vacuum filtration at -5°C for 20 hr. On average, gas chromatographic (GC) analysis showed the phytosterols recovered from the extraction and purification process had more than 80% purity. The recovery of total sterols from the PSR was 84% composed of β-sitosterol (21%-22%), campesterol (13%-20%) and stigmasterol (59%-64%). This extraction process is technically feasible to extract and produce crude phytosterols from a PFAD by-product.

Keywords: palm fatty acid distillate (PFAD), phytosterols, multistage extraction, palm oil by-product.

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INTRODUCTION

Phytosterols (plant sterols) are one of the bioactive compounds that occur naturally in vegetable oils and their by-products or derivatives. Phytosterols can be found in olive oil, sunflower oil and pistachios. Several types of phytosterols have been identified and reported but only β-sitosterol, campesterol and stigmasterol exist in significant amount in these resources (Moreau *et al*., 2018; Massimo *et al*., 2019). Other types of phytosterols such as brassicasterol, Δ^5-avenasterol, Δ^7-avenasterol, sitostanol, campstanol and Δ^7-stigmasterol can also be found in small quantities (Fernandes and Cabral, 2007). Furthermore, Moreau *et al*. (2018) have determined other types of phytosterols, which are stanols and their conjugates in foods.

Consumption of phytosterols has been reported to be able to reduce cholesterol absorption and lowering total serum and low density lipoprotein (LDL) cholesterol levels in animals and humans (Jones *et al*., 1999; Tasan *et al*., 2006). Phytosterols also showed anti-cancer property toward breast, colon and prostate cancer cell lines (Awad and Fink,

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2000). These important beneficial characteristics rendered phytosterols to have wide applications in nutraceuticals and functional food industries. Therefore, phytosterols can be incorporated into various types of food for enrichment to provide enough phytosterols for daily intake of 2 g per day (Devaraj and Jialal, 2006). Examples of functional food products containing phytosterols are margarine, butter, cereals, milk and spreads products, which are enriched with plant-derived sterols and their esters (Kowalski, 2017).

Commercial phytosterols are extracted from soyabean oil, corn oil, rapeseed oil, sunflower oil as well as tall oil. In palm oil, sterols can be found as minor component; together with tocotrienols, tocopherols, carotene, coenzyme Q10 and squalene. The sterols content in crude palm oil (CPO) ranges from 250 to 730 ppm (Chandrasekaram, 2009). Phytosterols are also present in the by-products of palm oil mill and refinery such as palm pressed fibre oil (PPFO) and palm fatty acid distillate (PFAD) in various concentrations depending on the processes (Ab Gapor, 2010; Lau *et al*., 2008). In Malaysia, 53 palm oil refineries are in operation with a total annual refining capacity of 27.33 million tonnes and thus about 765 000 t of PFAD are also being generated from the refining process (Kushairi *et al*., 2018). On average, PFAD contains about 0.4% phytosterols (Ab Gapor, 2010) and thus, it is estimated that about 3000 t of phytosterols are available to be extracted from the PFAD.

Extraction of phytosterols can be conducted using several methods and extraction technologies depending on the source of raw materials (Fernandes and Cabral, 2007). The common recovery processes of phytosterols from oil are multistage processes of esterification, saponification, molecular distillation, crystallisation and filtration (Choo *et al*., 2005). Sterols in raw materials are partially found in ester form. As such, pre-treatments involving chemical modification such as saponification and hydrolysis are required to convert a component in the substance into different properties to allow for easier separation. These processes will convert sterol esters into unsaponifiable free sterols and also convert fatty acids and their esters into soap matrix (Fernandes and Cabral, 2007). Selective adsorption and desorption method using styrene-diviynlbenzene type adsorbent with various type of solvents type such as methanol, isopropanol and hexane is also applied to enrich the phytonutrient content in CPO and PPFO (Phoon *et al*., 2018). A greener technology such as supercritical fluid extraction (SFE) was introduced to extract oil enriched with phytonutrient from PPFO, olive oil deodouriser and PFAD (Akgun, 2011; Lau *et al*., 2008; Norhidayah *et al*., 2012; Sugihara *et al*., 2010). The SFE is able to simultaneously extract carotene, vitamin E, squalene and phytosterols from the oil, which are then collected as fractional products from SFE based on the temperature and pressure of the supercritical carbon dioxide set during the operation. Ng and Choo (2013) developed a method using flash chromatography to recover phytonutrients such as carotenes from palm oil. Aqueous enzymatic method has also been found to be able to increase phytonutrients content in PPFO (Noorshamsiana *et al*., 2017). All of these green systems are able to recover phytonutrients from the feedstock. However, phytosterols concentration recovered were low, below 2%. Therefore, additional processes are required in order to obtain high purity of individual phytonutrients. Moreover, as compared to conventional processes, these new technologies incur high capital cost and need skilled manpower.

Previous studies mainly focused on phytosterols extraction directly from CPO and deodourised distillates. Hence, few oil palm by-products from palm oil mills and refinery have been collected and analysed for their phytosterols content. This includes a solid by-product of the commercial vitamin E extraction from PFAD that has huge amount of unrecovered phytosterols. Suitable extraction and purification processes have yet to be properly developed to recover the phytosterols from this particular resource. Therefore, the aim of this study is to extract and purify the phytosterols from the solid by-product in a mini-pilot scale multistage extraction process. The recovery of this valuable component from the by-product of oil palm processing will help the oil palm industry to generate additional income and subsequently sustain the palm oil industry in Malaysia.

MATERIALS AND METHOD

Materials Preparation

CPO, PPFO, sludge palm oil (SPO) and oil palm empty fruit bunch (OPEFB) residual oil are collected from various sampling points at several palm oil mills in Peninsular Malaysia. All samples were stored at 4°C prior to analysis. PFAD was purchased from an oil refinery company (MOI Foods Malaysia Sdn Bhd, Selangor, Malaysia) and stored in a stainless steel drum before the extraction processes.

PFAD was then subjected to several processes, consisting of esterification, transesterification, distillation, crystallisation and ion exchange adsorption as depicted in *Figure 1*, adapted from vitamin E extraction method by Ab Gapor *et al*. (1993). The solid by-product after the crystallisation process known as phytosterol resources (PSR) was collected and analysed for its phytonutrients content.

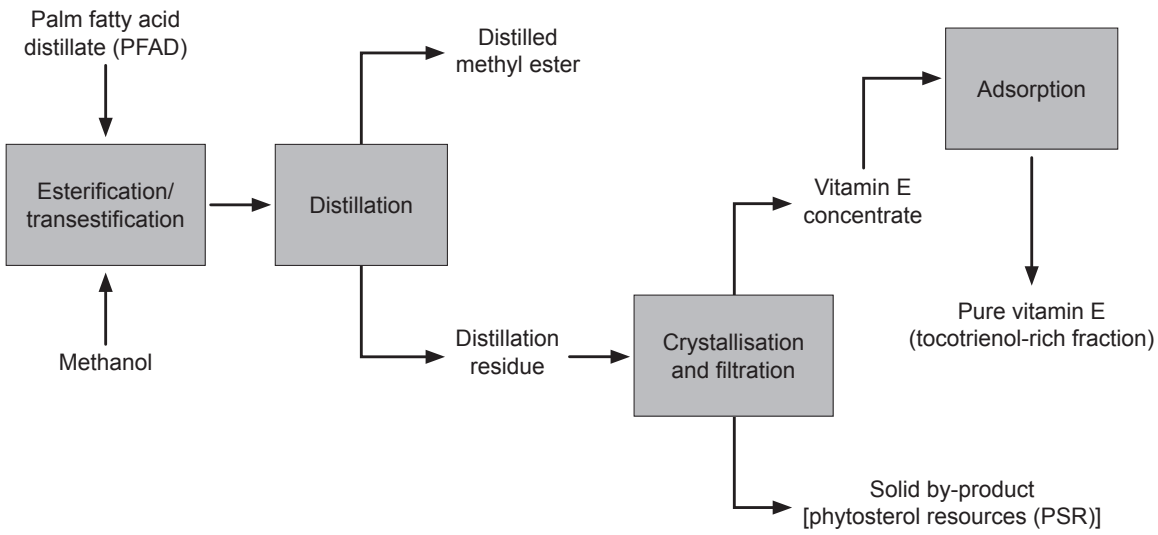

Figure 1. Extraction of vitamin E from palm fatty acid distillate (PFAD).

Extraction of Phytosterols in Laboratory Scale

A sample of 5 g PSR was mixed with 50 ml of ethanol and 2.5 g of potassium hydroxide (KOH), and then subjected to saponification reaction in 250 ml round bottom flask equipped with reflux condenser. The reaction was conducted at the ethanol reflux temperature for 1 hr. The reacted mixture was then extracted five times with hexane until a colourless organic layer was obtained. The extracted organic layer was then washed with distilled water until the neutral pH was obtained. Excess solvent was evaporated and the sample was then mixed with various solvents namely hexane, ethanol, acetone and methanol before being subjected to purification stage. A 1 g of extracted sample phytosterols-rich fraction (PSRF) added with 10 ml of solvent are heated to 60°C and then crystallised in deep freezer at temperature of -5°C for 20 hr. The formed crystal was filtered and dried in oven. The final product was expected in a form of pure phytosterols mixture.

Extraction of Phytosterols in Mini-pilot Scale Multistage Extraction Processes

Extraction of phytosterols mixture. All the PSR samples were subjected to solid-liquid extraction (SLE) process using an overflow method with two different temperatures, 35°C and 50°C. The SLE unit (SOLTEQ®) was equipped with a 20-litre solvent pot with reboiler, distillation column, distillate condenser, extraction vessel and solvent collection vessel.

About 200 g of extracted product was subjected to saponification reaction for the recovery of unsaponifiable material (USM). The extract was then mixed with 100 g KOH and ethanol (10 x sample weight), refluxed at 80°C to 90°C for 1 hr to 4 hr. The reaction was conducted in a 10-litre multipurpose glass reactor equipped with a condenser, which was purchased from Buchiglasuster, Switzerland.

The reaction mixture was then subjected to liquid-liquid extraction (LLE) by mixing the reaction mixture with hexane and water at the ratio of 10:10:1 (hexane:water:sample) in order to extract the USM. The USM extracts or known as PSRF was collected in the light phase together with hexane, leaving the water-soluble compound in the heavy phase. The process was repeated for five cycles. The LLE process was conducted in a DN50 1500 mm borosilicate column (25 litres capacity), manufactured by SOLTEQ®, Malaysia.

Purification of phytosterols mixture. The PSRF extract was subsequently subjected to crystallisation process in a 5 litre crystallisation vessel equipped with vacuum filtration (Buchiglasuster, Switzerland) in order to further purify the phytosterols present in PSR samples. Different types of solvents (hexane, ethanol, methanol and acetone) were mixed with the PSRF with solvent to PSRF ratio (volume to weight) of 10:1. Then, the mixture was heated at 60°C for 1 hr and cooled down to -5°C for 19 hr. Finally, the mixture was filtered under vacuum condition, while the temperature was maintained at -5°C during filtration. The solid and filtrate were analysed for total sterols content and quantified for product yield and recovery. The multistage extraction processes flow is illustrated in *Figure 2*.

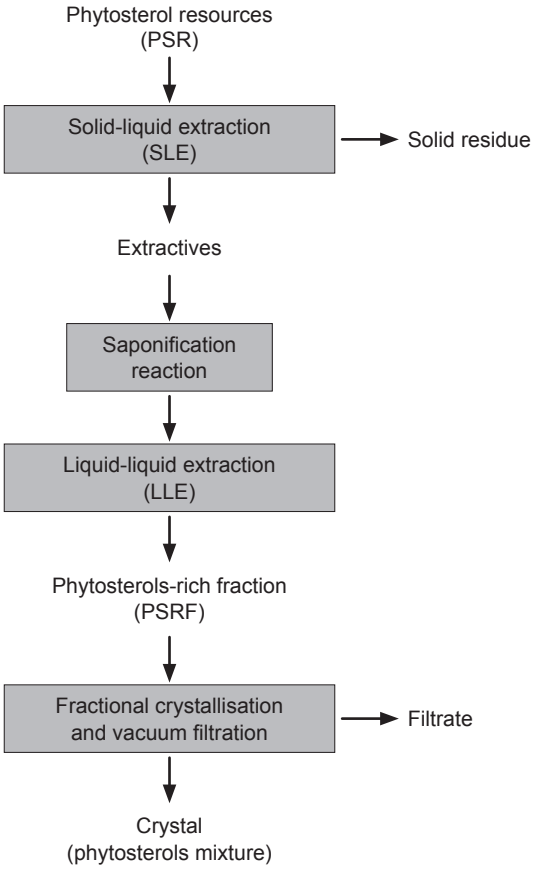

Figure 2. Multistage extraction processes of phytosterols mixture from the phytosterols resources (PSR).

Analysis of Phytonutrients

Samples preparation. All samples (except for PSRF and phytosterols mixture) were pre-treated via saponification prior to the analysis of sterols based on MPOB Test Method (MPOB, 2004). Samples weighing 5.0 g were mixed with 2.5 g 10% (w/v) KOH in an ethanolic solution and then refluxed for 1 hr at temperature between 70°C to 80°C. Then, hexane was used to extract the USM from the saponified mixture and the extracts were washed using distilled water until the neutral pH was obtained. The remaining moisture was removed using sodium sulphate ($NaSO_4$) and hexane was then removed using rotary evaporator. The USM was weighed to quantify the recovery yield as in Equation (1).

$$\text{Yield of USM recovery} = \frac{\text{Weight of PSR - Weight of dried USM}}{\text{Weight of PSR}} \times 100 \qquad \text{Equation (1)}$$

Phytosterols, squalene and vitamin E determination using gas chromatography (GC) analyser. The samples were dissolved and diluted in 100% n-hexane to an appropriate concentration. High purity samples such as PSRF and phytosterols mixture are directly dissolved in warm n-hexane without undergoing any pre-treatment. All the diluted sample were analysed using GC Autosystem XL, Perkin Elmer as a platform for the sterols separation equipped with flame ionisation detector (FID) for peak detection. The samples were separated in the capillary column purchased from Supelco SAC™-5 (Sigma) with the length of 30 m and internal diameter of 0.25 mm, bonded with a 0.25 µm film of 5% phenyl/95% dimethylpolysiloxane in the presence of helium gas as mobile phase. The initial oven temperature, maximum oven temperature and detector temperature were set at 270°C, 320°C and 270°C, respectively. Identification of compounds was achieved by comparing their retention times in GC spectra with those of standards (*Table 1*). For quantitative analysis, calibration curves were prepared by analysing different concentrations of squalene, vitamin E and sterol standards and by representing peak area versus concentration.

TABLE 1. RETENTION TIME FOR SQUALENE, VITAMIN E AND STEROLS DURING GAS CHROMATOGRAPHY-FLAME IONISATION DETECTOR (GC-FID) ANALYSIS

Components	Retention time (min)
Squalene	5.20
Vitamin E	
α-tocopherol	7.60
α-tocotrienol	8.60
γ-tocotrienol	9.30
δ-tocotrienol	10.92
Sterols	
Cholesterol	8.80
Campestrols	10.80
Stigmasterols	11.40
β-sitosterols	12.70

Fourier transform infrared (FTIR) analysis. The infrared spectrum of sterols samples was recorded using Perkin-Elmer Spectrum One FTIR Spectrometer with wavelength in the range of 4000-650 cm^{-1}. The spectrums were compared with the individual sterols standard.

Nuclear magnetic resonance (NMR) analysis. Samples were dissolved in 200 µl deuterated chloroform, shaken and placed in a 5 mm NMR capillary tube. The ^{1}H and ^{13}C experiments were performed using 600 MHz JEOL Spectrometer for compound identification [adapted from Teh *et al.* (2017)].

RESULTS AND DISCUSSIONS

Phytosterols Composition in Palm Oil and its By-products

Various oil palm by-products were analysed for their phytosterols content, The composition of extracted USM and total phytosterols content

in palm oil and its by-products are as tabulated in *Table 2*. Results showed that OPEFB residual oil and CPO have low USM content of 0.82%-0.83%. These two samples composed of only 5.48%-6.19% of phytosterols in USM, with only 450-500 ppm of total phytosterols composition in these PSR. Another component of USM might be carotene, which makes the oil reddish in colour (Rusnani *et al*., 2012; Md Yunos *et al*., 2015). Other than these two samples, the phytosterols content in SPO was recorded at 800-1100 ppm, followed by PPFO with 1700-4100 ppm phytosterols. USM recovery percentage of both samples were less than 2%. It was observed that the phytosterols composition in this SPO (800-1000 ppm) was higher than that in the slurry obtained from the heavy phase of CPO clarification tank (508.7 ppm). This might be due to the dilution of the sludge with water from CPO clarification process (Teh *et al*., 2017). Meanwhile, the SPO composed of only oil recovered and concentrated from that particular sludge. Besides, it was reported that most of USM in POME composed from carotene, squalene and vitamin E (Sangkharak *et al*., 2016; Teh *et al*., 2017). As expected, the highest phytosterols content of 20 300-141 000 ppm was found in solid by-product from vitamin E extraction process. This PSR sample contained 3.82%-15.15% USM. The phytosterols content in this PSR sample is relatively higher than palm phytonutrient concentrate produced from palm methyl ester as reported by Chandrasekaram (2009). The higher value of phytosterols in this sample was due to the fact that most of the esters and glycerides were primarily removed during the distillation process for vitamin E extraction. The remaining esters, squalene and vitamin E were also extracted in liquid fraction during the crystallisation and filtration process. This sample also had the highest total phytosterols content in USM of more than 50%, which allowed simpler purification step to obtain phytosterols as compared to other PSR samples. Besides high total phytosterols content, relatively low fatty acids content reduces the requirement of the equipment size towards the downstream stages of the extraction processes (Fernandes and Cabral, 2007).

Extraction of Phytosterols Mixture from PSR

Process for extraction of vitamin E from PFAD produced 1.50% (w/w) solid by-product. This solid by-product PSR composed of up to 15.15% USM that mostly consisted of phytosterols. This contributed to high content of phytosterols in the PSR samples amounting to 14.10%. The extraction of phytosterols from this PSR was conducted through SLE using 100% n-hexane at 35°C and 50°C. Temperature of 35°C was selected to represent the extraction at constant room temperature adapted from the maceration method (Azwanida, 2015). Warm solvent at 50°C enhanced the extraction of phytosterols. However, the temperature for extraction must be below hexane boiling point in order to avoid solvent evaporation. Solvent extraction at 35°C yielded 47% extractives (*Table 3*). The yield was increased to more than 90% when extraction temperature was set at 50°C. The extractive yield was lower but the extract contained higher USM, which consequently increased the total sterols content in the extract. Solubility of fatty acids and methyl ester in hexane is lesser at lower temperature (Calvo *et al*., 2009). Therefore, solvent temperature at 35°C is preferred for extraction of phytosterols in order to minimise the carry over of fatty acids and methyl ester in the extract. Different solvent temperatures will give different products and recovery yields due to the differences in component solubility in hexane.

After the SLE process, saponification was carried out to convert all sterol esters into free sterol and all glycerides into water-soluble fatty acid soap. The reaction was conducted in laboratory scale by varying the duration of reaction time between 1 hr to 4 hr in order to determine the highest USM recovery. It was found that as the reaction time increased, the recovery of USM reduced from 43.70% at 1 hr to 23.60% at 4 hr (*Figure 3*). The longer reaction time has continuously exposed samples to heat and it may have destroyed some phytonutrients in USM such as sterols and squalene (Lau *et al*., 2005).

TABLE 2. PHYTOSTEROLS CONTENT IN VARIOUS PALM OIL AND ITS BY-PRODUCTS

Phytosterols resources	Unsaponifiable matter recovery (%)	Phytosterols composition in unsaponifiable matter form (%)	Phytosterols composition (ppm)
Crude palm oil*	0.83	6.19	500
Palm fatty acid distillate	1.86 - 2.87	2.39 - 15.33	600 - 4 200
Palm pressed fibre oil	0.96 - 1.58	17.99 - 25.93	1 700 - 4 100
Oil palm empty fruit bunch residual oil*	0.82	5.48	450
Sludge palm oil	1.15 - 1.17	7.16 - 9.04	800 - 1 100
Solid by-product of vitamin E extraction process	3.82 - 15.15	50.35 - 93.12	20 300 -141 000

Note: *Analysis of one sample.

TABLE 3. TOTAL STEROLS AND ITS COMPOSITION DURING SOLID-LIQUID EXTRACTION

Solvent temperatures	35°C			50°C		
Extracted components	**PSR**	**Extractives**	**Solid residue**	**PSR**	**Extractives**	**Solid residue**
USM (%)	19.45	41.27	0.07	16.10	16.97	6.82
Total sterols in samples (%)	13.75	25.35	0.01	8.55	8.61	5.82
Sterols composition in USM (%)*						
Cholesterol	1.39	4.12	4.12	1.42	1.58	1.43
Campesterol	22.66	23.53	20.29	22.72	23.26	23.73
Stigmasterol	14.96	16.60	21.32	15.55	15.67	19.33
β-sitosterol	60.99	55.75	54.26	60.32	59.49	55.51
Yield (%)	-	47	53	-	91.5	8.5
Sterols recovery (%)	-	99.95	-	-	94.09	-

Note: PSR - phytosterol resources; USM - unsaponifiable matter.
*Normalised to 100%.

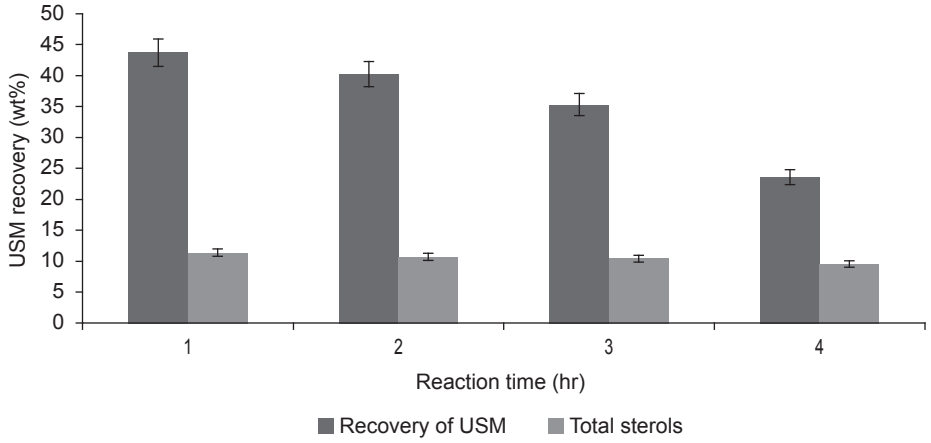

Figure 3. Effect of reaction time on the recovery of unsaponifiable matter (USM) from solid residue of vitamin E extraction.

As such, the mini-pilot test was conducted using optimised saponification reaction time of 1 hr. The amount of solvent used in mini-pilot liquid-liquid extraction was fixed at a ratio of 10:1 in order to maximise the extraction of phytosterols. The USM recovery in mini-pilot test was in the range of 14.32%-19.95% after five runs. The average sterols content was 47.64%±4.78%. Apart from phytosterols, the USM also contained squalene and traces of unreacted glycerides. Sterols and squalene have great differences in melting point and hence, solvent-assisted crystallisation process was used to isolate the phytosterols from the squalene and other USM mixtures. The separation of free phytosterols and tocopherols is usually performed through fractional crystallisation as free phytosterols tend to precipitate at a low temperature (Moreira and Baltanás, 2004).

Purification of Phytosterols Mixture

Phytosterols mixture extract was further purified using crystallisation. Crystallisation is one of the best and cheapest methods available for the purification of solids from impure solutions. There are several types of solvents and solvent mixtures suitable for the crystallisation procedure. However, a simple purification process should use only one solvent instead of mixed solvents for recovery and recycling purposes. In this study, four types of solvents have been tested in laboratory scale crystallisation; they were ethanol, methanol, acetone and hexane (*Table 4*). From this experiment, crystallisation of USM with hexane gave the highest crystal yield of 62% with pure phytosterols (100% purity), followed by ethanol and then acetone. The lowest crystal yield obtained was when methanol was used as solvent, producing 25% crystal yield with phytosterols content of 48.25%. Other solvents such as benzene, toluene and cyclohexane may give higher sterols purity, but with lower yield and highly toxic (Yan *et al*., 2011). Thus, these solvents are not desirable for crystallisation of phytosterols.

The crystallisation process was further tested at mini-plant scale, using hexane and ethanol as solvents. The yield of crystal and filtrate are tabulated in *Table 5*. The crystal yield and sterol content for mini-pilot scale were lower than that

of laboratory scale crystallisation. This was due to higher phytosterols loss in the filtrate. The purity of phytosterols obtained was also lower than that of laboratory scale extraction. However, the crude phytosterols purity was maintained at more than 80%. This was due to the presence of other impurities such as unreacted glycerides that have close melting point as sterols.

Characterisation of Phytosterols Mixture

Phytosterols mixture after the purification process was a waxy solid and white in colour. *Table 6* shows the individual sterols composition in PFAD, phytosterols mixture (phytosterols-mix) extracted in laboratory and mini-pilot scale in comparison with commercial vegetable oil sterols. It was found that β-sitosterol content in palm-based sterols is higher than commercial sterols, while stigmasterols content is lower. The β-sitosterol has advantages to treat men's health problems such as increased urinary flow rates, decreasing the amount of urine left in the bladder after urinating and improving the overall quality of life (Wilt *et al*., 1999).

Apart from GC analysis, the extracted phytosterols were further analysed using FTIR and NMR to confirm their characteristics. FTIR spectrum (*Figure 4*) showed the absorption bands at 3345.68 cm^{-1} (OH), 2920.02 cm^{-1} (CH_2) and 2850.54 cm^{-1} (CH). The absorption at 1641.6 cm^{-1} for the olefinic bond in stigmasterol was also sighted. However, the band was weakly absorbed due to the C=C stretching. A bending frequency of cyclic (CH_2) and $-CH_2(CH_3)\gamma$ were observed at 1461.81 cm^{-1} and 1377.95 cm^{-1} respectively. The absorption frequency at 1051.89 cm^{-1} and 958.79 cm^{-1} were that of trisubstituted olefin, which were usually referred to as β-sitosterol and stigmasterol (Jamaluddin *et al*., 1994).

The ^{13}C NMR spectrum (*Figure 5*) shows that the presence of the compound in phytosterols mixture is in a form of steroid skeleton due to similar chemical shift obtained as compared in the literature (Jain and Bari, 2010; Jamaluddin *et al*., 1994). The main difference between the three main sterols is the presence of double bond at C22=C23 in stigmasterols with a chemical shift at 138.404 and 129.247 ppm, while β-sitosterols and campesterol both have a chemical shift at 34.020 and 26.150 ppm corresponding to C22-C23 single bond. The ^{13}C NMR spectrum for campesterol and β-sitosterols were identical, thus, the presence of those two compounds were confirmed.

Based on mini-pilot plant trial, it was estimated that one tonne of PFAD will produce 15 kg by-product of PSR during the vitamin E extraction process. After multistage extraction process, 1.08 kg phytosterols mixture with average purity of 87.23% was obtained with overall sterols recovery from the PSR of about 84%. Overall mass balance on the multistage production of phytosterols mixture from PFAD is given in *Figure 6*.

TABLE 4. PURIFICATION OF PHYTOSTEROLS-RICH FRACTION (PSRF) THROUGH CRYSTALLISATION WITH DIFFERENT TYPES OF SOLVENTS IN LABORATORY SCALE

Solvent type	Samples	Sterols composition (%)				Total sterols (%)	Yield (%)
		Cholesterol	Campesterol	Stigmasterol	β-Sitoterol		
Ethanol	Crystal	7.02	18.58	16.37	58.03	93.92	43
	Filtrate	0.00	24.32	0.00	75.68	22.24	
Methanol	Crystal	10.20	3.42	28.81	57.57	48.25	25
	Filtrate	12.57	23.29	0.00	64.14	11.41	
Acetone	Crystal	5.39	17.32	18.31	58.98	100.00	32
	Filtrate	11.12	29.06	19.62	40.20	22.19	
Hexane	Crystal	4.87	19.18	17.16	58.78	100.00	62
	Filtrate	8.47	20.43	0.00	71.11	32.97	

TABLE 5. PURIFICATION OF PHYTOSTEROLS-RICH FRACTION (PSRF) THROUGH CRYSTALLISATION IN MINI-PILOT SCALE

Solvent type	Samples	Phytonutrient composition (%)				Yield of phytosterols mixture (%)
		Sterol	Squalene	Vitamin E	Others	
Ethanol	Crystal	84.47	0.21	N.D	18.32	37
	Filtrate	68.40	20.97	N.D	10.63	
Hexane	Crystal	87.23	0.66	N.D	12.11	47
	Filtrate	59.62	13.40	N.D	26.98	

Note: N.D - not detected.

TABLE 6. INDIVIDUAL STEROLS COMPOSITION IN PALM FATTY ACID DISTILLATE (PFAD), PHYTOSTEROLS MIXTURE FROM PFAD AND COMMERCIAL STEROLS

Sample/ Material	Individual sterols compositions (%)				Total sterols (%)
	Cholesterol	Campesterol	Stigmasterol	β-Sitosterol	
PFAD	3-9	23-25	13-14	53-60	0.2 – 0.4
Phytosterol-mix (laboratory)	1-5	19-22	17-20	57-59	84 - 100
Phytosterol-mix (mini-pilot plant)	<2	20-22	13-20	59-63	84 - 94
Commercial sterol*	<3**	20-28	16-23	40-58	>90

Note: *Corowise™ sterols. **Other minor sterols.

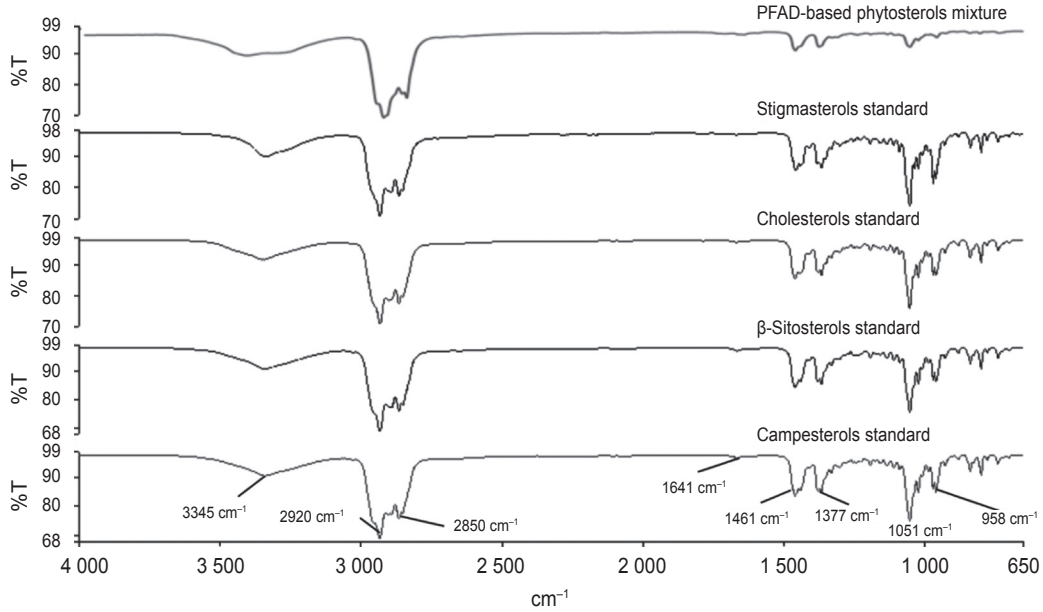

Figure 4. IR spectrum of stigmasterols, β-sitosterols, campesterols and cholesterol standard and phytosterols mixture extracted from palm fatty acid distillate (PFAD).

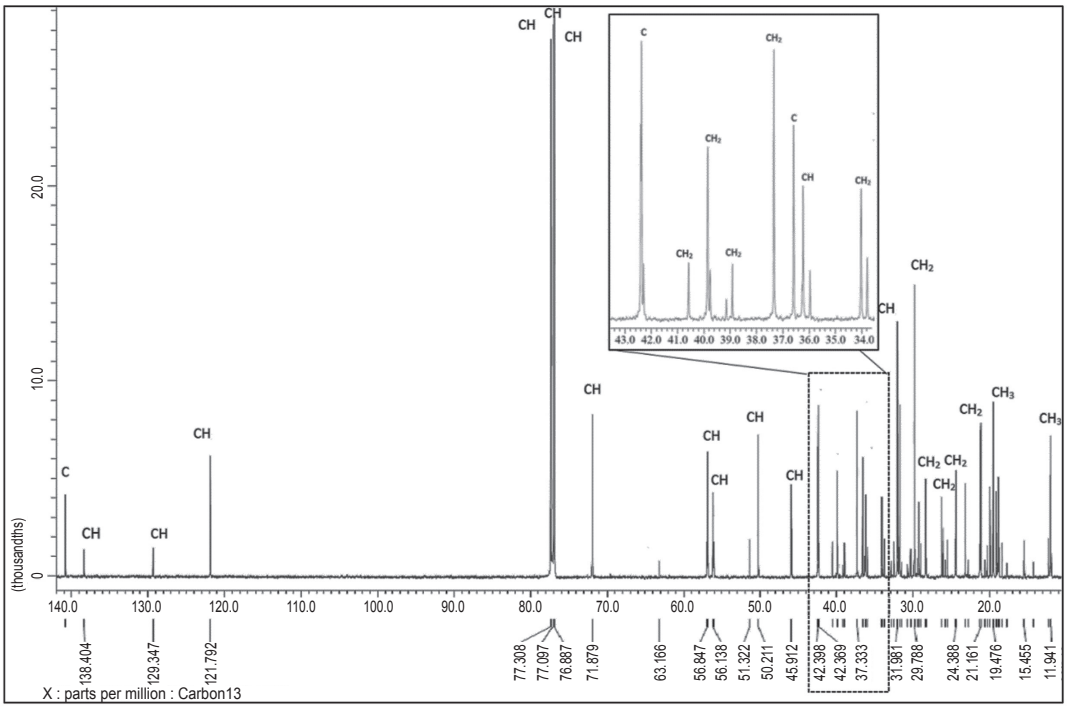

Figure 5. The ^{13}C NMR spectrum of phytosterols mixture extracted from palm fatty acid distillate (PFAD).

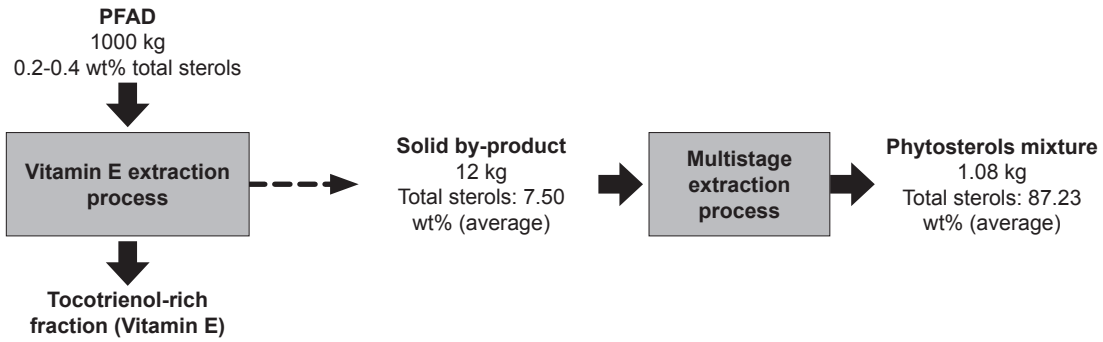

Figure 6. The overall mass balance on the multistage production of phytosterols mixture from palm fatty acid distillate (PFAD).

CONCLUSION

Extraction and purification of phytosterols from solid residue obtained after vitamin E extraction from PFAD have been successfully conducted using multistage extraction processes in laboratory scale and mini-pilot scale. Technically, the multistage extraction and purification methods in mini-pilot scale which comprised of SLE, saponification reaction, LLE, crystallisation and filtration, were capable to produce phytosterols mixture with purity of up to 94% (w/w) with individual sterols compositions of β-sitosterol (21%-22%), campesterol (13%-20%) and stigmasterol (59%-64%). The overall recovery for sterol from the PSR was 84%. FTIR, NMR and GC analysis confirmed the presence of phytosterols in the extract. This extraction process is technically feasible to extract and produce crude phytosterols from a PFAD by-product and the extraction of this minor component will adds value to the oil palm industry.

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