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CONSOLIDATION OF DEEP EUTECTIC SOLVENTS WITH MICROWAVE FOR THE EXTRACTION OF FERULIC ACID FROM THE FRUITLETS OF OIL PALM

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ABSTRACT

*This article reports the extraction of ferulic acid from oil palm fruitlets by using deep eutectic solvent (DES) with microwave assistance. The difference in the amount of ferulic acid extracted from unsterilised and sterilised oil palm fruitlets using DES with microwave assistance was not significant, signifying that sterilisation during palm oil milling did not have much effect on the content of ferulic acid in the palm fruitlets. Acetic acid (AA) is a better hydrogen bond donor than citric acid in choline chloride (ChCl) DES whereby higher amount of ferulic acid could be extracted from oil palm fruitlets when ChCl-AA was used as the extraction medium (*ca. *40.3 mg/g versus* ca. *34.4 mg/g). The optimum conditions for the extraction of ferulic acid from palm fruitlets were by using ChCl with AA containing 30 wt.% water, heated with microwave at 60ºC for 6 min. Ferulic acid extracted from unsterilised oil palm fruitlets exhibited higher radical scavenging activity (92%-95%) than sterilised fruitlets extract (88%-92%), indicating that although the sterilisation process did not significantly affect the content of ferulic acid in the oil palm fruitlets, the bioactivity of the ferulic acid was slightly affected.*

Keywords: deep eutectic solvent, extraction, ferulic acid, microwave, palm fruitlets.

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INTRODUCTION

Palm oil milling starts with sterilisation to halt the enzyme activities responsible for the rise in free fatty acids and to soften the fruits to facilitate detachments from the stalk. However, the high temperature (130ºC to 160ºC) could also affect the non-oil components in the palm fruitlets.

Ferulic acid, a flavonoid polyphenolic compound found in several parts of the oil palm, could be one of the non-oil components affected by the sterilisation process of the palm fruits (Mohd Aanifah, 2014; Neo *et al.*, 2010; Ng, 2020; Ng and Nu'man, 2021; 2022a). Ferulic acid is a valuable component in nutraceutical, pharmaceutical and cosmeceutical industries due to its anti-microbial, antioxidative properties and could act against inflammation (Mancuso, 2007; Neo *et al.*, 2008, 2010; Ou and Kwok, 2004). Efforts have been made in the past to extract the ferulic acid from various parts of the oil palm including, fruitlets, fronds, empty fruit bunches and palm pressed mesocarp fibre by way of solvent extractions such as alkaline hydrolysis and methanolysis (Ng, 2020; Ng and Nu'man, 2022b; Stavova *et al.*, 2017; Sun *et al.*, 2001; Tang *et al.*, 2014; Zhou *et al.*, 2019).

As ferulic acid could exist in free form as well as attached to lignin and other biopolymers in plants, efforts for its extraction need to take into consideration the penetration and cleavages of these links by the extraction medium. As much as 3800-4200 mg/kg ferulic acid could be obtained from palm empty fruit bunch by way of hydrolysis with alkali while 250-514 mg/kg free ferulic acid was obtained by way of methanolic extraction (Mohd Aanifah, 2014; Ng, 2020). Ng and Nu'man (2022b) also reported the extraction of ferulic acid from palm pressed fibre by way of alkaline extraction. Although

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extraction using alcohol is relatively simpler, only ferulic acid in free form could be extracted with this method. Alkaline hydrolysis, on the other hand, involved harsh conditions and ferulic acid that was extracted using alkali cannot be termed as 'natural'. As such, there is a need for a mild technique such as using deep eutectic solvent (DES) to extract the ferulic acid from the oil palm fruits.

DES, a eutectic mixture system of Lewis or Brønsted acids and bases consisting of a variety of anionic and/or cationic species, is capable of cleaving the link of lignin and/or biopolymers while protecting the bioactives from plant materials (Cvjetko *et al.*, 2015; Paiva *et al.*, 2014). Other advantages of DESs include being made up of natural, cost effective, safe and simple compounds. These enabled the DESs to be excellent solvents for the extraction of bioactives from plants, especially phenolic compounds which dissolved better in DESs compared to water or lipids (Smith *et al*., 2014; Young *et al*., 2011). DESs could dissolve a high variety of compounds as they can both donate and accept protons and electrons (Bi *et al*., 2013; Dai *et al*., 2013a; 2013b; Woo *et al*., 2015). Another advantage of using DESs is that it offers excellent stability to the phenolic compounds extracted rendering it to be a possible novel formulation for pharmaceutical and food products (Liu *et al*., 2019).

Besides conventional heating such as reflux, DES could complement microwave heating for the extraction of phytochemicals from plant samples. The potential for microwave heating with DES for extraction was supported by the study on the extraction of hydrophilic from Radix *Salviae miltiorrhizae* whereby it was reported that the yield obtained using DES with microwave heating was higher and at a shorter duration (11 min) compared to conventional extraction techniques (Chen *et al*., 2016). Sharma and Dash (2021) also reported the completion of the extraction of certain phytochemicals from black jamun pulp in 4 min with DES under microwave assistance. This was due to the faster release of intracellular compounds as a result of plant cell rupture brought about by microwave heating and DES penetration. A study by Xie *et al.* (2019) also confirmed such phenomena whereby similar yield (~2.3 mg/g) of ferulic acid from chuanxiong could be obtained in just 20 min with microwave assisted DES extraction compared to non-microwave assisted heating which required 4 hr to be completed. Ng and Nu'man (2021; 2022a) reported a method for the extraction of ferulic acid from palm pressed fibre using microwave assistance whereby the extraction was completed in just 9 to 15 min compared to 6 hr by reflux (Ng and Nu'man, 2021; 2022a). This showed that the use of microwave heating could significantly increase extraction efficiency by drastically reducing extraction duration. Therefore, microwave assisted DES could be further developed as an effective and efficient method for the extraction of phytochemicals.

Although the use of DESs in oil palm processing is getting more popular, there is yet any study on the extraction of ferulic acid from the mesocarp of oil palm fruitlets using DES with the aid of microwave heating. This paper aimed to study the impact of sterilisation on ferulic acid, as well as the factors, such as type of DES, heating temperature and duration, that could affect the extraction of ferulic acid from oil palm fruitlets using DES heated with microwave.

MATERIALS AND METHODS

Materials

Whole oil palm fruitlets (sterilised and unsterilised) were obtained from a palm oil mill in Malaysia. Choline chloride (ChCl), acetic acid (AA) and citric acid (CA) used in the study were of analytical or chromatography grades, obtained from Merck (Darmstadt, Germany).

Synthesis of DES

DES (choline chloride - acetic acid, ChCl-AA) was prepared following the method outlined by Abbot *et al.* (2004) with slight modifications. ChCl and AA in equal molar ratio were heated and stirred at 85°C until the mixture become homogenous and colourless. The liquid was then left to cool. Distilled water was then added into the ChCl-AA to obtain a range of DES containing 0 wt.% to up to 50 wt.% water. The same procedure was used to prepare ChCl with CA as a hydrogen bond donor (ChCl-CA).

Viscosity Analysis of DES

The viscosities of all the DESs prepared (ChCl-AA and ChCl-CA) were determined at 40ºC with a Herzog Automated Multi Range Viscometer (HVM 472).

Sample Pre-treatment

Fresh whole oil palm fruitlets were peeled to obtain their flesh (mesocarp) while the nuts therein were discarded. The mesocarp obtained from the fresh oil palm fruitlets (hence known as unsterilised fruitlets) were dried overnight at 60ºC. Thereafter, the unsterilised fruitlets were ground to *ca.* 0.3 cm. Mesocarp obtained from sterilised oil palm fruitlets (hence known as sterilised fruitlets) were treated similarly before being further used for extraction.

Influence of Water in DES on Extraction of Ferulic Acid

The influence of water in DES on the extraction of ferulic acid was studied by mixing 0.5 g finely ground sterilised palm fruitlets with neat (0 wt.% water) ChCl-AA (10 mL) in a round bottom flask. The mixture was then heated at 60ºC in a tabletop microwave oven (Panasonic NN-CD9975, 900W, 2.4GHz) for 9 min. The heating temperature was controlled by manipulating the microwave power. The mixture was then left to cool to room temperature. Thereafter, the solids were removed from the mixture first by filtration, followed by centrifugation (9000 rpm, 5 min). The supernatant was then dissolved in methanol and the ferulic acid contained therein was determined using high-performance liquid chromatography (HPLC). A similar procedure was used for ChCl-AA containing 10 wt.%, 20 wt.%, 30 wt.%, 40 wt.% and 50 wt.% water. All extractions were carried out in triplicates. The whole experiment was then repeated with ChCl-CA and with unsterilised palm fruitlets, respectively.

Influence of Microwave Heating Temperature

ChCl-AA containing 30 wt.% water (10 mL) was added into a flat bottom flask containing 0.5 g ground sterilised oil palm fruitlets. The mixture was then heated at 40ºC for 9 min using microwave. The heating temperature was controlled by manipulating the microwave power. Thereafter, the solids were removed from the mixture first by filtration, followed by centrifugation (9000 rpm, 5 min). The supernatant was then dissolved in methanol and the ferulic acid contained therein was determined using HPLC. Similar procedure was repeated for microwave heating temperatures of 50ºC, 60ºC, 70ºC and 80ºC. The whole experiment was then repeated with ChCl-CA and with unsterilised palm fruitlets, respectively. All extractions were carried out in triplicates.

HPLC Analyses of Ferulic Acid

Analyses were carried out according to the method by Ng (2020) with Waters HPLC (e2695 Separations Module) connected to a fluorescence detector (2475 FLR; 321 nm excitation, 418 nm emission). Calibrations of the ferulic acid were carried out with authentic standard.

DPPH Assay of Ferulic Acid Extracts

The ability of ferulic acid extracted from the oil palm fruitlets to scavenge free radicals was determined using DPPH radical scavenging assay outlined by Brand-Williams *et al*. (1995).

Extraction Optimisation (Temperature and Duration)

Optimisations of microwave heating temperature and duration for the extraction of ferulic acid from palm fruitlets were carried out following the procedure outlined above to observe the effect of heating temperature. Sterilised palm fruitlets in ChCl-AA containing 30 wt.% water were heated for 3 to 15 min, at 40ºC to 70ºC respectively. The experiment was repeated using ground unsterilised palm fruitlets and with ChCl-CA. All extractions were carried out in triplicates.

RESULTS AND DISCUSSION

Influence of Water in DES

DES with ChCl as base is used in this study with CA and AA as hydrogen bond donors (HBD). The higher viscosity of CA (6.50 mm^2/s) than AA (1.06 mm^2/s) was reflected in their resultant DESs (22.66 ± 0.65 mm^2/s for ChCl-CA and 9.68 ± 0.46 mm^2/s for ChCl-AA) (*Figure 1*).

Viscosity is often a limitation faced by DESs as interactions among DESs own components are strong and thus inhibited its infiltration into the substrate, causing low extraction efficiency (Hammond *et al*., 2017; Wang *et al*., 2018). The addition of water into DESs will alter their viscosities, which in turn, affect their behaviour (Ng and Nu'man, 2021; 2022a; Wang *et al*., 2018). In other words, extraction efficiency could be greatly affected by the viscosity or the amount of water in the DES.

Viscosities of ChCl-AA and ChCl-CA decreased rapidly as the amount of water contained therein increased from 0 wt.% to 10 wt.% (22.66 ± 0.65 mm^2/s to 10.22 ± 0.65 mm^2/s for ChCl-CA and 9.68 ± 0.46 mm^2/s to 5.74 ± 0.38 mm^2/s for ChCl-AA). This drastic change in viscosity was attributed to the hygroscopic nature of the DESs where even a small amount of water could alter their structures and properties (Cvjetko *et al*., 2015; Dai *et al*., 2013a; 2013b; Hammond *et al*., 2017). The viscosities of both ChCl-CA and ChCl-AA continued to drop with the increasing amount of water within up to 50 wt.%. Although there was a decline in viscosities for both DES with low water content, the drop in viscosities for both DES started to taper as the amount of water within rose beyond 30 wt.% (*Figure 1*).

Another observation was that although ChCl-AA with or less than 20 wt.% water had significantly lower viscosity than ChCl-CA containing the same amount of water, the difference in viscosity between these two DESs was not significant as the amount of water in the DES increased beyond 30 wt.%. Both ChCl-CA and ChCl-AA containing 50 wt.% water had almost similar viscosity, 1.66 ± 0.36 mm^2/s and

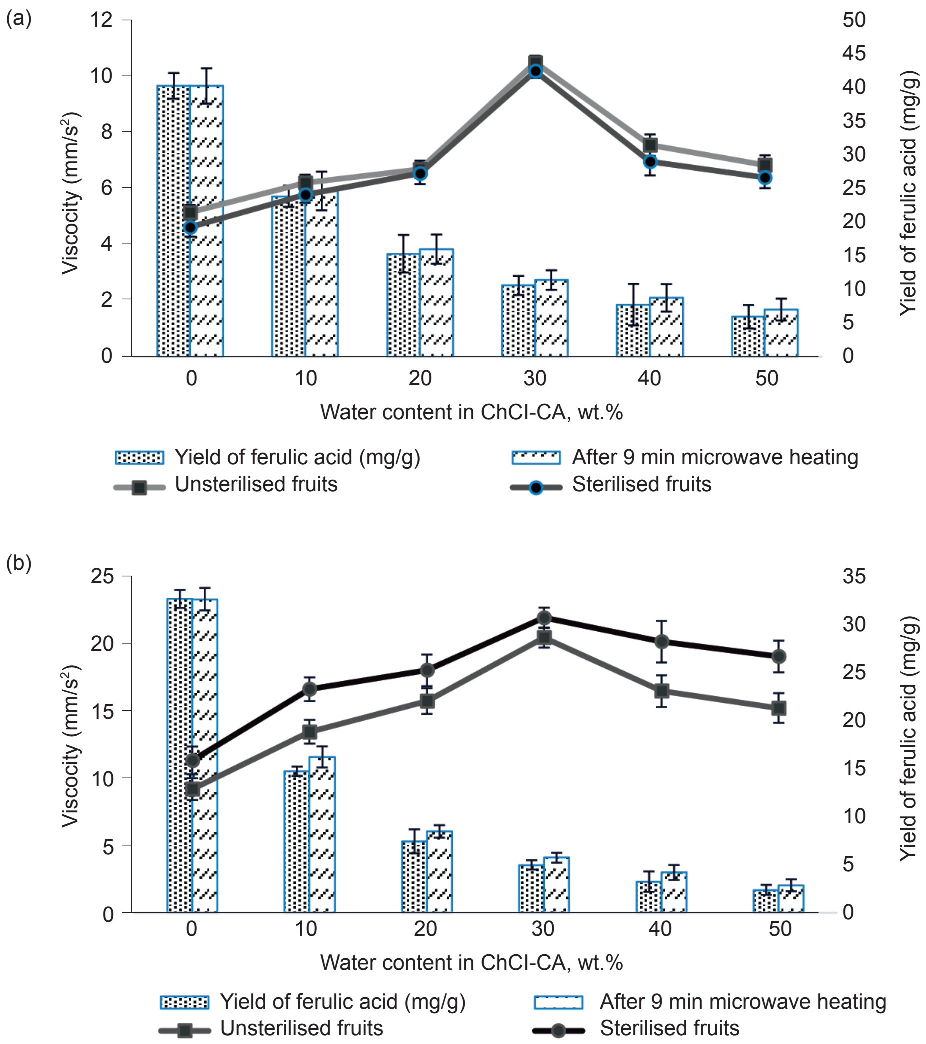


Figure 1. Viscosities of (a) ChCl-AA and (b) ChCl-CA with different amounts of water before and after microwave heating and yield of ferulic acid after 9 min heating at 5°C.

1.43 ± 0.42 mm^2/s respectively. This was because the DES started to lose its properties and lean towards aqueous behaviour in the presence of a large amount of water as reported in previous studies (Chen *et al.*, 2016; Sharma and Dash, 2021; Wang *et al.*, 2018). ChCl-based DES could tolerate up to 51 wt.% water, beyond which, the DES becomes an aqueous solution instead (Abbott *et al.*, 2005).

In general, moisture could be lost when a solution containing water is heated. Loss of moisture through microwave heating could affect the viscosity and hence the extraction efficiencies of the DES. As such, the viscosities of the DESs without palm fruitlets samples (blank) were recorded both before and after microwave heating to rule out the possibility of the DESs having undergone permanent viscosity changes due to heat treatment. Although there was a slight increase in the viscosities of both ChCl-AA and ChCl-CA after 9 min microwave heating, the changes were not significant, thus showing that heating did not have any profound effect on the DESs (*Figure 1*).

A larger amount of ferulic acid could be obtained from unsterilised palm fruitlets when extracted using ChCl-AA than ChCl-CA (*Figure 1*). However, the difference is not significant for sterilised palm fruitlets except for DES with 30 wt.% water where higher amount of ferulic acid could be obtained using ChCl-AA (42.5 ± 1.1 mg/g) than ChCl-CA (29.8 ± 1.0 mg/g). This may be due to easier penetration of DES containing certain amount of water into the sample matrices that have been pretreated with heat as were the case with sterilised palm fruitlets and supported by previous studies (Rente *et al.*, 2021; Woo *et al.*, 2015).

Although the difference in viscosity between ChCl-AA and ChCl-CA containing 40 wt.% and 50 wt.% water was not significant, the similarity in viscosities did not translate to similar extraction efficiencies of ferulic acid from unsterilised palm fruitlets. Higher amount of ferulic acid were obtained from unsterilised palm fruitlets using ChCl-AA than ChCl-CA (28.5 ± 1.5 mg/g to 31.5 ± 1.6 mg/g and 20.7 ± 1.5 mg/g to 29.1 ± 2.1 mg/g respectively) although the viscosities of both DES were quite similar. This indicated that the property or type of hydrogen bond donor (HBD) in DES is more profound than viscosity in the extraction for unsterilised palm fruitlets. However, the type of HBD did not significantly affect the extraction with sterilised palm fruitlets whereby there was not much difference in the amount of ferulic acid extracted with both types of DES containing similar amount of water (26.7 ± 1.6 mg/g to 29.1 ± 2.1 mg/g for ChCl-AA and 25.9 ± 1.6 mg/g to 27.4 ± 2.1 mg/g for ChCl-CA). A possible explanation is that DES with carboxylic acids, such as ChCl-AA, has better extraction efficiencies due to the reaction between the carboxylic functional groups and its base, ChCl (Liu *et al*., 2019; Rente *et al*., 2021). This, in turn, led to higher destruction and reconnection inhibition of the cell walls to cellulose, hemicellulose and lignin of the palm fruitlets. However, this process is more profound in unsterilised palm fruitlets which have not undergone any heat treatment compared to sterilised palm fruitlets which have been subjected to high temperatures during sterilisation. The high temperature employed during sterilisation (130ºC-160ºC) would have softened the fibre matrices rendering them to be more accessible regardless of the types of HBD present in the DES (Hammond *et al*., 2017; Ma *et al*., 2018; Rente *et al*., 2021).

A general pattern was observed whereby larger amount of ferulic acid could be extracted from palm fruitlets when DES with up to 30 wt.% water was used for extraction. Extraction efficiencies, reflected by the concentrations of ferulic acid, dropped when the amount of water in the DES increased beyond 40 wt.%. This was observed for both types of DESs, regardless of the conditions of the palm fruitlets (unsterilised or sterilised). This could be due to the excessive water content in the DESs where it started to behave as an aqueous solution rather than as DES. This was reported in previous studies whereby the addition of an excessive amount of water into DES disrupted the hydrogen bonds between DES and water, hence reducing the interactions between the extraction medium and target compounds (Hammond *et al*., 2017; Rente *et al*., 2021; Wang *et al*., 2018). This showed that even if extraction efficiency could be increased by changing the viscosity of DESs with water, there remained a limit whereby the DESs could no longer withstand the presence of water without changing their behaviour.

The highest concentrations of ferulic acid were obtained when unsterilised and sterilised palm fruitlets were extracted using ChCl-AA containing 30 wt.% water (43.8 ± 1.0 mg/g and 42.5 ± 1.0 mg/g respectively).

Radical Scavenging Activities of Ferulic Acid Extracts

The ferulic acid extracts obtained from microwave-heated DES with varying water content were tested for a radical scavenging activity to ensure their biochemical activity is not hindered by the DES or the extraction process. Radical scavenging activity of the ferulic acid extracts from unsterilised and sterilised palm fruitlets were determined by DPPH assay with the following Equation (1).

$$\text{Scavenging activity (\%)} = \frac{(A_0 - A_1)\ \times 100\%}{A_0} \qquad (1)$$

where A_0 is the absorbance of control and, A_1 is the absorbance of extract.

Ferulic acid extracted from both types of oil palm fruitlets had good radical scavenging activity ranging from 88%-95% (*Figure 2*). Although sterilisation during palm oil milling did not have a significant effect on the content of ferulic acid whereby the difference in the amount of ferulic acid obtained from unsterilised and sterilised palm fruitlets was not significant, it caused a reduction in its antioxidative power whereby unsterilised fruitlets extracts exhibited higher radical scavenging activity compared to sterilised fruitlets extracts (92% to 95% and 88% to 92% respectively). While there was not much difference in the radical scavenging activity in unsterilised fruitlets extracts, the activity in sterilised fruit extracts seemed to increase with the escalation in the amount of water in the DES used for extraction to up to 30 wt.%. The difference in radical scavenging activity in sterilised fruitlets extracts was not significant in extracts obtained via DESs containing more than 30 wt.% water. A possible explanation for this is that the presence of water affected the extraction process as well as its physicochemical properties, which was also reported in the findings of previous study (Yuan *et al*., 2005).

Taking in concentrations as well as radical scavenging activities of ferulic acid in the extracts obtained via DES with different water content, it was perceived that DES containing 30 wt.% water was the best medium to extract ferulic acid from palm fruitlets. As such, similar DESs were used to further study the effect of heating temperature and duration of microwave irradiation on ferulic acid extraction.

Effect of Microwave Heating Temperature

Generally, higher extraction temperature leads to higher mass transfer between substrate and extraction medium (Abbott *et al*., 2004; Bi *et al*., 2013; Ng and Nu'man, 2022a). Studies on the effect of microwave heating temperature on the extraction of

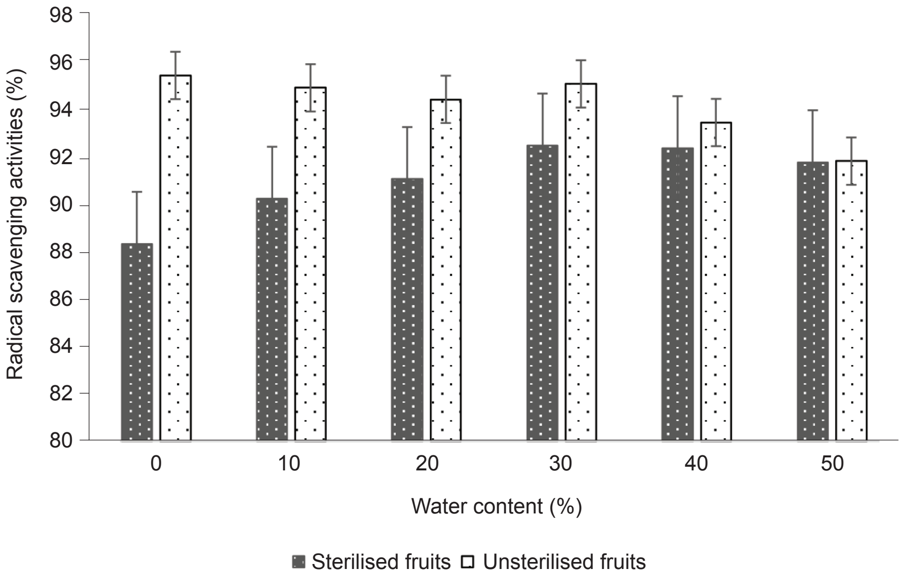


Figure 2. Free radical scavenging activities of ferulic acid extracted from oil palm fruitlets using DES with microwave heating.

ferulic acid from palm fruitlets using ChCl-CA and ChCl-AA were carried out whereby the temperature of the microwave heating increased by 10ºC with each increment, beginning with 40ºC to 80ºC while the heating duration was held constant. While palm fruitlets were charred at 80ºC, the results of the rest of the study are depicted in *Figures 3* and *4*.

At low temperature (40ºC), the efficiencies for both DESs to extract ferulic acid from both palm fruitlets were low (*Figures 3* and *4*). Although theoretically, high temperature could lead to higher extraction efficiencies, it is also important to keep in mind that most antioxidative compounds are sensitive to heat. Degradation of ferulic acid generally began at 40ºC, indicating that high temperature is not suitable for its extraction (Das and Wong, 2020; Ng and Nu'man, 2021). Nevertheless, it is worth investigating the temperature where real and significant damage occurs.

As the extraction temperature increased to 50ºC and subsequently 60ºC, the amount of ferulic acid obtained from the reaction of sterilised fruitlets with both types of DESs was significantly higher than each previous extraction temperature. High temperature reduced the physical and chemical interactions between ferulic acid and fruitlets matrices resulting in better penetration of the DES, thus accelerating the mass transfer of ferulic acid from the fruitlets to the DES. However, as the extraction temperature escalated to 70ºC, drop in the concentrations of ferulic acid was observed. Similar pattern was reported for unsterilised fruitlets in ChCl-CA. However, when unsterilised fruitlets were reacted with ChCl-AA, the drop in the amount of ferulic acid obtained occurred at lower temperature, 60ºC instead of 70ºC as in the case for ChCl-CA.

Larger amount of ferulic acid was extracted from both types of fruitlets using ChCl-CA at 60ºC than 70ºC. The highest concentrations of ferulic acid obtained via ChCl-CA from unsterilised and sterilised fruitlets were 38.1 ± 1.2 mg/g and 37.2 ± 1.2 mg/g respectively, which occurred at 60ºC after 6 min heating. These were slightly less than what could be obtained with ChCl-AA as extraction medium, whereby the highest concentration of ferulic acid extracted from sterilised fruitlets was 46.9 ± 1.2 mg/g, under the same extraction conditions as ChCl-CA (6 min heating at 60ºC). However, lower temperature was more conducive for the extraction of ferulic acid from unsterilised palm fruitlets using ChCl-AA whereby the highest ferulic acid concentration (43.8 ± 1 mg/g) was obtained at 50ºC after 9 min heating.

Effect of Microwave Irradiation Duration

Microwave irradiation rapidly increases the temperature which enabled high extraction efficiency of heat sensitive compounds in a short period of time (Wang *et al*., 2018; Xie *et al*., 2019). Extraction of ferulic acid from palm fruitlets with DES could be accomplished in as little as 3 min of microwave heating at 40ºC. Extending the microwave heating duration at 40ºC from 3 to 15 min increased the concentration of ferulic acid extracted from unsterilised fruitlets via ChCl-CA (14.2 ± 0.7 mg/g to 20.0 ± 0.9 mg/g) and ChCl-AA (18.2 ±0.7 mg/g to 29.4 ± 0.9 mg /g). Similar increase was also observed for sterilised fruitlets reacted with ChCl-CA (12.1 ± 0.7 mg/g to 19.2 ± 0.9 mg/g) and ChCl-AA (13.5 ± 0.7 mg/g to 24.1 ± 0.9 mg /g) under the same conditions (*Figures 5* and *6*).

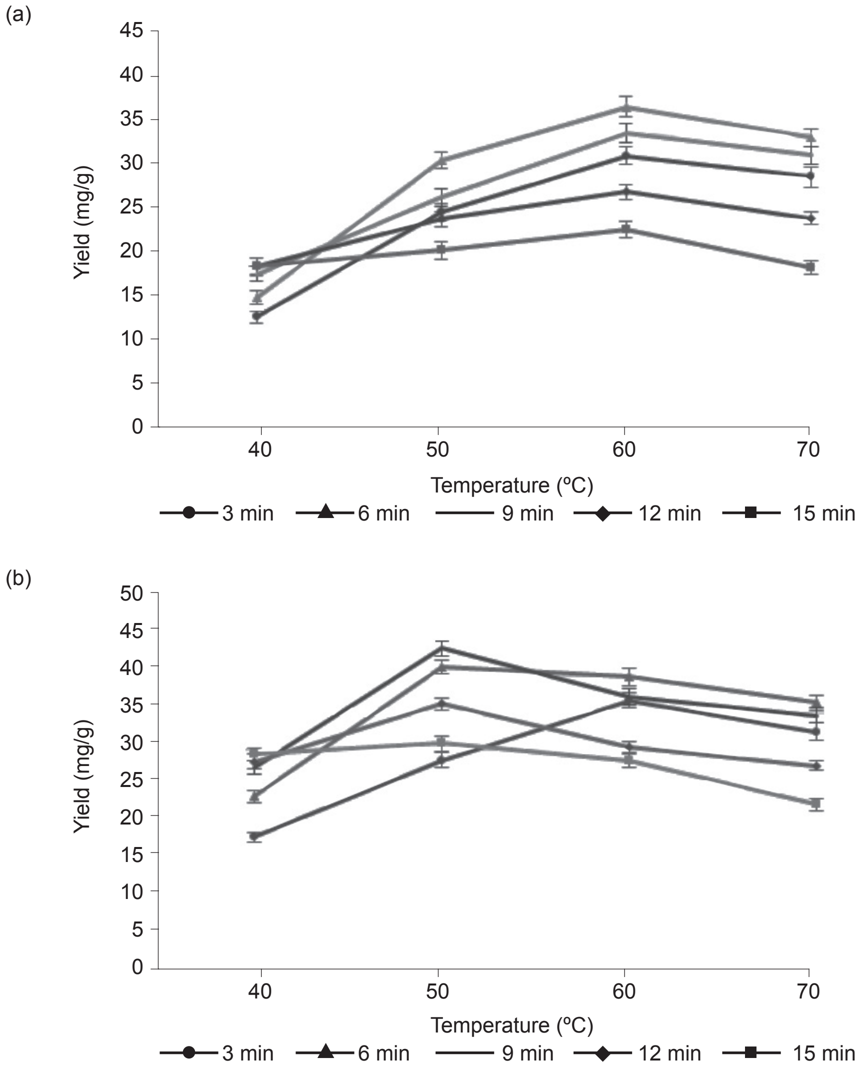


Figure 3. Yield of ferulic acid extracted from unsterilised fruitlets using (a) ChCl-CA and (b) ChCl-AA containing 30 wt.% water at different heating duration and temperatures.

Except for extractions at low temperature (40ºC), both sterilised and unsterilised fruitlets could not withstand more than 6 min microwave heating in ChCl-CA at all heating temperatures studied (*Figures 5* and *6*). Initially, extending the heating duration for both types of palm fruitlets in ChCl-CA from 3 to 6 min had a positive impact whereby the amount of ferulic acid obtained increased with the increase in heating duration (*Figures 5* and *6*). However, as the heating duration was further extended, drop in the ferulic acid yield was observed. By the end of 15 min heating at 60ºC, the concentrations of ferulic acid in unsterilised and sterilised fruitlets extracts (24.1 ± 0.9 mg/g and 23.2 ± 0.9 mg/g respectively) were less than their concentrations in extracts obtained via heating for only 3 min (32.5 ± 1.0 mg/g and 31.2 ± 1.0 mg/g respectively) at the same temperature.

Similar pattern was observed for both types of fruitlets in ChCl-AA at 70ºC for up to 15 min whereby the concentration of ferulic acid obtained at the end of 15 min heating was less than after only 3 min heating. Although drop in ferulic acid concentration was observed for sterilised fruitlets extracted beyond 6 min heating at 60ºC in ChCl-AA, its concentration by the end of 15 min heating (34.2 ± 0.9 mg/g) at the same temperature was still significantly higher than when it was heated for only 3 min (24.1 ± 0.9 mg/g). This was not the case when ChCl-CA was used as extraction medium. As opposed to ChCl-CA which saw the drop in ferulic acid concentration beyond 6 min heating at 50ºC, 60ºC and 70ºC respectively, the reaction with ChCl-AA could withstand heating slightly longer at 50ºC, to up to 9 min heating before drop in ferulic acid's concentrations was observed. The drop in the ferulic acid concentrations was probably due to degradation for being exposed to high temperatures for an extended period as reported by Ng and Nu'man (2021). This indicated that while high temperature could increase extraction efficiency, heat exposure under microwave for

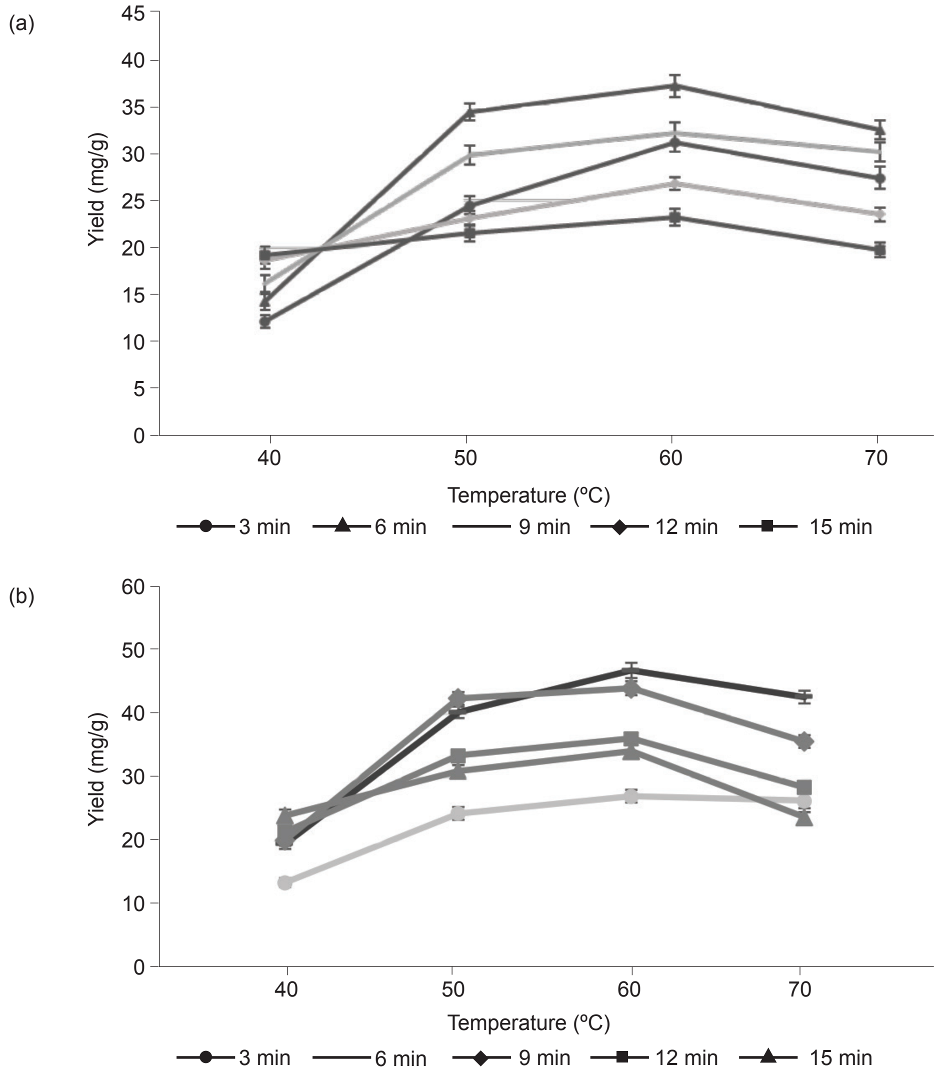


Figure 4. Yield of ferulic acid extracted from sterilised fruitlets using (a) ChCl-CA and (b) ChCl-AA containing 30 wt.% water at different heating duration and temperatures.

a long duration is not advisable. Compared to the conventional extraction method by reflux, microwave-assisted extraction could achieve similar results in a much shorter time (Ng and Nu'man, 2021; 2022a). It was therefore concluded that the optimal conditions for the extraction of ferulic acid from oil palm fruitlets were microwave heating at 60ºC for 6 min in DES containing 30 wt.% water (*Figures 7* and *8*).

Advantages of MAE DES over Other Extraction Methods

Ng (2020) reported the extraction of 0.250 mg/g and 0.668 mg/g ferulic acid from unsterilised and sterilised palm fruitlets respectively through 6 hr methanolysis. In comparison, *ca.* 46.900 mg/g of ferulic acid was obtained from this study, which was significantly higher than that achieved by Ng (2020). This could be due to efficient desolvation of lignin in palm fruitlets by DES under microwave influence, which then facilitated the transfer of ferulic acid from the fruit matrices to the DES. The high extraction efficiency was also contributed by lower DES viscosities as a result of the addition of water into the DES therein which is among the determining factor in the extraction of phenolic compounds (Dai *et al.*, 2013a; Liu *et al.*, 2019).

Through alkaline hydrolysis, Mohd Aanifah *et al.* (2014) reported the recovery of 3800-4200 ppm of ferulic acid from palm empty fruit bunch. Besides alkali, hydrolysis catalysed by cutinase had also been reported as a way to extract ferulic acid from palm empty bunches (Tang *et al.*, 2015) in which *ca.* 0.050 mg/g ferulic acid was obtained after 24 hr reaction. Oxidation of palm empty fruit bunches with nitrobenzene in 10 wt.% sodium hydroxide resulted in the extraction of *ca.* 0.045 mg/g ferulic acid in a reaction that took 180 min. However, nitrobenzene oxidation is severe and thus, could not protect the ferulic acid from degradation.

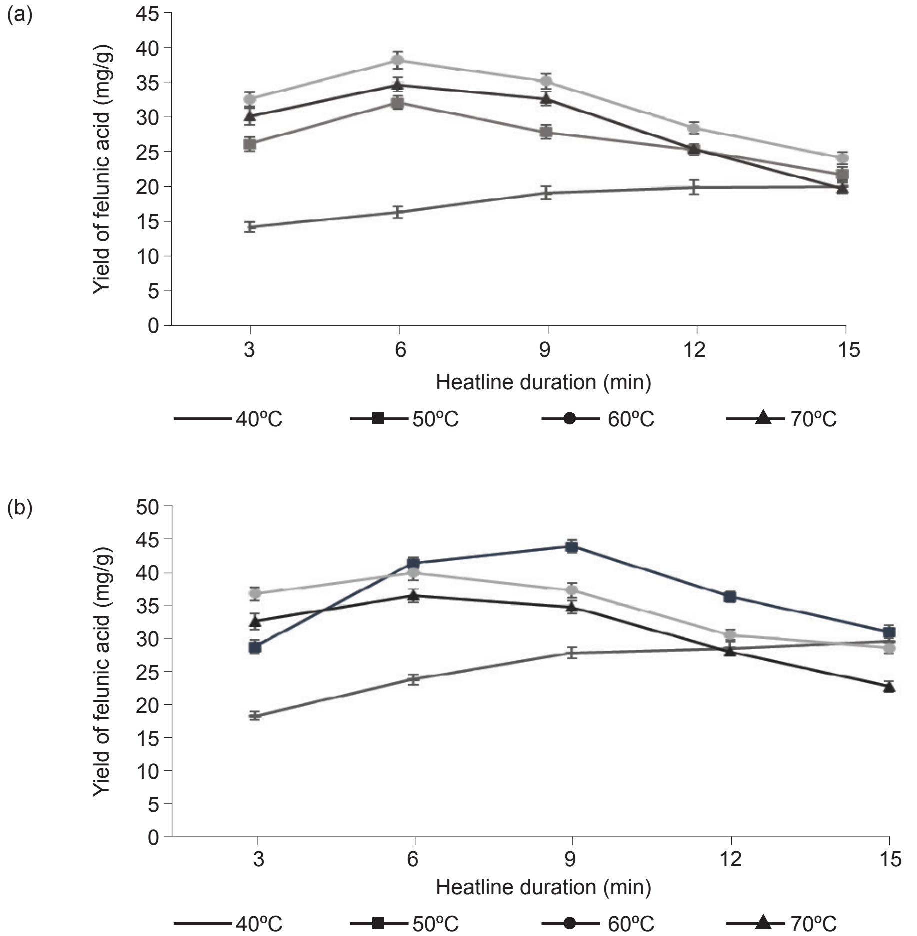


Figure 5. Yield of ferulic acid extracted from unsterilised fruitlets using (a) ChCl-CA and (b) ChCl-AA containing 30 wt.% water at different heating duration and temperature.

Wang *et al.* (2018) reported the advantages offered by microwave heating whereby dissolution and diffusion rate of baicalin from plant cells occurred at a much shorter time with microwave compared to conventional heating. Ng and Nu'man (2021) reported a yield of 0.609 - 0.617 mg/g and up to 1.123 mg/g ferulic acid when palm pressed fibre was extracted with DES under 15 min microwave heating. In another study with extraction conducted using ChCl-AA by reflux, it was reported that the optimal condition for the extraction of ferulic acid from palm pressed fibre was 6 hr heating, which was much longer compared to extractions carried out by microwave heating (Ng and Nu'man, 2022a). This shows that a combination of microwave heating with DES could greatly enhance extraction efficiency in a much shorter reaction duration. DES and microwave could complement each other by offering homogenous heating at high speed and efficiency, accelerating plant cell rupture and releasing intracellular compounds therein, resulting in superior extraction efficiency in a shorter duration.

Besides hydrolysis and microwave heating, other assisted techniques to extract ferulic acid from palm include the use of ultrasonic whereby oil palm biomass was reacted with acidified ethanol in an ultrasonic bath. As much as 0.560 mg/g of ferulic acid could be extracted from palm kernel cake after 40 min through this technique (Tsouko *et al.*, 2019). However, there was no mention of the amount of ferulic acid extracted from palm kernel shells, empty bunches, as well as pressed mesocarp fibre of the oil palm. Considering both the time required for completion of extraction as well as the amount of ferulic acid that could be obtained, DES extraction with microwave heating is a superior technique for the extraction of ferulic acid compared to other reported methods.

CONCLUSION

Extraction of ferulic acid from both unsterilised and sterilised palm fruitlets by DES could be assisted with microwave heating whereby the extraction duration could be drastically reduced compared to conventional extraction techniques. AA is a better HBD than CA in choline chloride-based DES for the extraction of ferulic acid whereby higher concentrations of ferulic acid were obtained with ChCl-AA as the extraction medium. The optimal conditions for the extraction of ferulic acid from oil

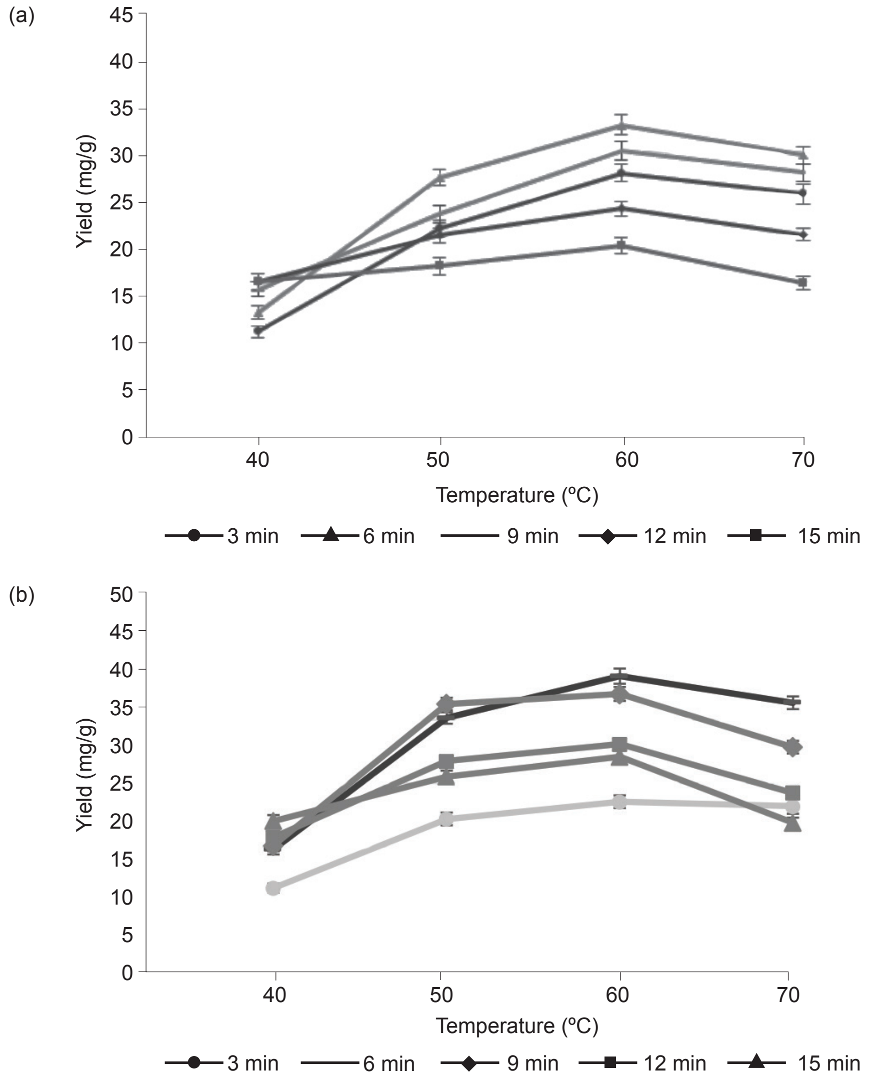
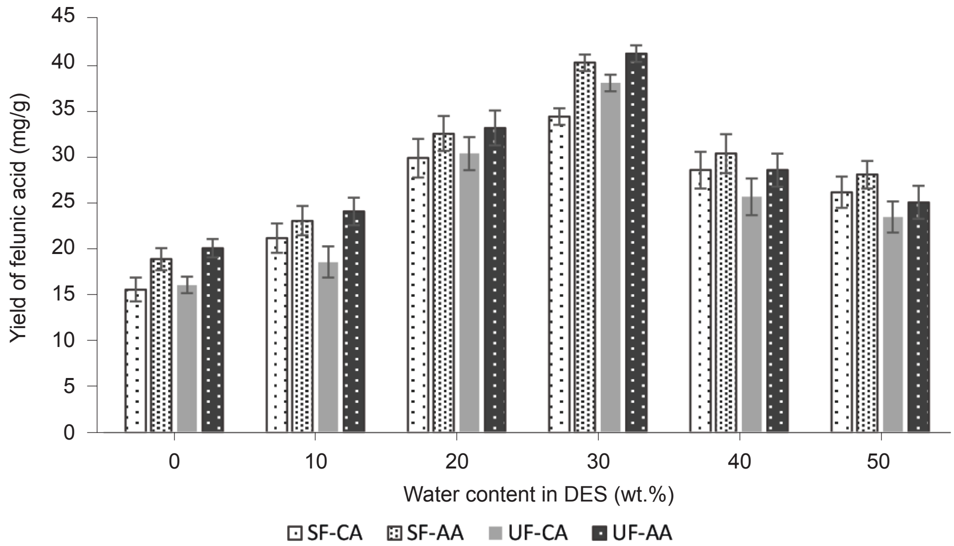
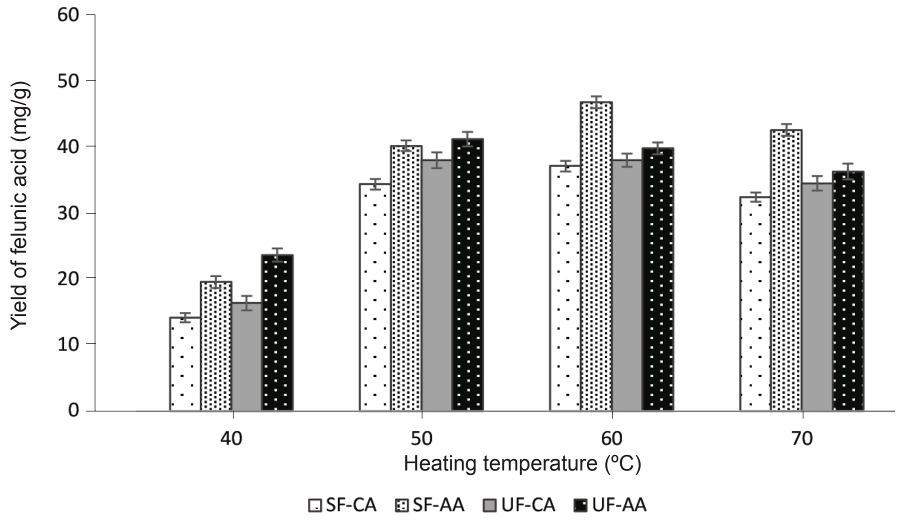


Figure 6. Yield of ferulic acid extracted from sterilised fruitlets using (a) ChCl-CA and (b) ChCl-AA containing 30 wt.% water at different heating duration and temperature.



Note: SF-CA - Sterilised fruits extracted with ChCl-CA; SF-AA - Sterilised fruits extracted with ChCl-AA;
UF-CA - Unsterilised fruits extracted with ChCl-CA; UF-AA - Unsterilised fruits extracted with ChCl-AA.

Figure 7. Yield of ferulic acid extracted with DES containing varying water content after 6 min heating at 50ºC.



Note: SF-CA - Sterilised fruits extracted with ChCl-CA; SF-AA - Sterilised fruits extracted with ChCl-AA;
UF-CA - Unsterilised fruits extracted with ChCl-CA; UF-AA - Unsterilised fruits extracted with ChCl-AA.

Figure 8. Yield of ferulic acid extracted with DES containing 30 wt.% water and 6 min microwave heating under varying temperature.

palm fruitlets were microwave heating at 60ºC for 6 min with DES containing 30 wt.% water. Sterilisation during palm oil milling did not significantly affect the amount of ferulic acid in the oil palm fruitlets as proven in this study. However, ferulic acid extracts from sterilised fruitlets had slightly lower antioxidative activities than unsterilised fruitlets extracts.

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