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ON INCREASE OF THE EFFICIENCY OF EXTRACTING PHENOLIC COMPOUNDS FROM PALM OIL MILL EFFLUENT

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ABSTRACT

In Malaysia, the palm oil industry rapid development has increased the environmental problems due to the discharge of large amounts of palm oil mill effluent (POME) into the water sources. However, POME consists of a high level of phenolic compounds, a potential source of natural antioxidants. An enhancement of the recovery process of these phenolic compounds from POME is required to increase the opportunity of this bioburden transformation into health and wellness applications. This study describes an improvement in the extraction efficiency of phenolic compounds through the combination of microwave-assisted extraction (MAE) and enzymatic hydrolysis. The latter proceeds in presence of Ragi tapai as it is readily available at any local market in Malaysia. Conventional maceration is performed aiming a comparison. The results show that 50 % ethanol is the best extracting solvent among those used. Besides, MAE provides an increase of the phenolic compounds extraction yield by 15.8 % at a decrease of the extraction time by 98.75 % when juxtaposed to the conventional maceration method. The coupling of enzymatic hydrolysis and MAE further increases the extraction yield by 16.3 %. The results obtained indicate also the existence of a microwave non-thermal effect at a low microwave power.

Keywords: palm oil mill effluent, enzymatic hydrolysis, microwave-assisted extraction, non-thermal effects, Ragi tapai.

INTRODUCTION

Palm oil production is the most important agriculture-based industry in Malaysia. The number of the palm oil mills had increased tremendously, from about 10 in 1960 [1] to 453 in 2016 [2]. As a result, Malaysia has become the second-largest palm oil producer in the world after Indonesia [3]. Basically, the oil palm fruits grow in bunches called Fresh Fruit Bunches (FFB). They can weigh about 45 kg - 75 kg when harvested being fully ripe. Then they are sent to the palm oil mills for processing. The extraction of the crude palm oil (CPO) starts with the sterilization of FFB to inactivate the enzymatic lipolysis and thus to prevent a further increase of free fatty acids (FFA) content in the oil [4]. Next, the fruit nuts are separated from the sterilized FFB via a threshing process. The fruit nuts are then pressed for its oil, while the empty fruit branches (EFB), which contain 20 % - 25 % stalk and 75 % - 80 % spikelet are discarded or used as biomass [5]. Generally, the extracted palm oil consists mainly of triglycerides and a variety of phytonutrients, such as carotenes, tocopherols, tocotrienols, squalene, sterols and coenzyme Q.

The rapid development of this industry has led to serious consequences on the environment, especially causing water pollution. This is due to the discharge of a large amount of untreated or partially-treated palm oil mill effluent (POME) into the water sources. POME is a liquid by-product, which is derived mainly from the sterilizer condensate and centrifugal desludging of the raw palm oil fruits from the screw press. It is an acidic

Table 1. Characteristics of raw POME and regulatory discharge limits.

Parameter	Value*	Regulatory discharge limit*
Temperature, °C	80-90	45
pH	4.7	5.0-9.0
Biochemical oxygen demand (BOD), 3 days at 30°C	25,000	100 (50)
Chemical oxygen demand (COD)	50,000	-
Total solids (TS)	40,000	-
Total suspended solids (TSS)	18,000	400
Total volatile solids (TVS)	34,000	-
Oil and grease (O&G)	4,000	50
Ammonia-nitrogen (NH_3-N)	35	150
Total Kjeldahl Nitrogen (TKN)	750	200

**All values, except pH and temperature, are expressed in mg L^{-1} [1, 6].*

brownish colloidal suspension, which contains lignocellulosic wastes and a mixture of carbohydrates and oil. There are different types of sugar in POME, such as arabinose, xylose, glucose, galactose and manose. Besides, there are oil-bearing cellulosic materials from the fruits in the suspended POME solids. Table 1 shows the typical characteristics of POME.

It is worth noting that the production of 1-ton of crude palm oil (CPO) generates 3.8 m^3 of POME [7] which is usually discharged at 80°C - 90°C. As there is no chemical added during the oil extraction process, POME is considered a non-toxic waste. But it brings about environmental issues because of its high organic and nutrient content, which eventually causes oxygen depletion in the aquatic system. It has been estimated that POME contributes about 30% of the total biochemical oxygen demand (BOD) load exerted on the aquatic environment in Malaysia [8]. Thus, it has to be extensively treated prior to its discharge. Currently, the recovery of a renewable organic-based product is a new approach in managing POME, whereby the extraction of by-products such as volatile fatty acids, biogas and poly-hydroxy alkanoates can help to promote the sustainability of the palm oil industry.

Many epidemiological studies have concluded that the consumption of fruits and vegetables decreases the risk of degenerative diseases [9 - 11]. This beneficial effect can be ascribed to the antioxidant activities of minor phytochemical components, including phenolic compounds.

It is reported that oil palm fruits are a rich source of phenolic compounds [12 - 17]. However, the sterilization process during CPO extraction leads to their washing away with the water and they discard in POME. Thus, the latter has a high potential to act as an alternative low-cost source of phenolics. The phenolic compounds found in POME are water soluble and can be the major source of natural antioxidants in human diet. So, a procedure to harvest these phenolics from POME has been established to provide concentrations suitable for biological applications [15]. Extraction is an essential and initial step in obtaining phytochemicals from their sources [18]. Therefore, it is vital to select a suitable procedure on the ground of the methods applied [19] including distillation, solvent extraction, enzymatic preparation, membrane separation, centrifugation, and chromatographic procedures.

Generally, the phenolic compounds are extracted by using mixed-solvents, such as dioxane/ethanol, water/acetone, water/methanol and water/ethanol and then ultra-filtrated. On the other hand, they can also be extracted via alkaline or acid (*e.g.*, hydrochloric acid) hydrolysis, which requires a careful control of the heating regime and the concentration of the base or acid used. This is because the failure in controlling these factors (too long heating period and/or too high alkali or acid concentration) could result in a destruction of the target compounds. Alternatively, the carbohydrate-hydrolyzing enzymes, such as pectinase, cellulase, hemicellulose, and glucanase, have been introduced recently in order to release the phenolic compounds that are trapped in the cell walls matrix and in the solids found in the waste-

water [20, 21]. These enzymes play an important role in disintegrating the cell wall matrix and at the same time, improving the extraction efficiency of the phenolics compounds [22-25]. Besides, as mentioned previously, the idea of phenolic compounds recovery from POME refers to the potential of the latter as a source of natural antioxidants. Besides, environmental problems are solved. Thus, this study aim at enzymatic hydrolysis as it can provide antioxidants increase through release of free phenolics [26].

There are also advanced extraction technologies [27, 28], such as ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), supercritical fluid extraction (SFE) and accelerated solvent extraction (ASE). These technologies are developed with various purposes. For example, ASE takes less extraction time, consumes less solvent and might result in better extraction quality. SFE, however, allows selective extraction. But both of these methods are conducted under high temperature and pressure, which eventually increases the extraction cost and energy consumption.

Nowadays, the need for sustainable development and green processing technologies has enhanced the interdisciplinary efforts and put the microwave processing technology in the forefront [29 - 32]. Besides its rapid extraction rate, MAE has received great attention due to its ability to provide better or similar recoveries of the target compounds with less extraction time or less solvent and energy consumption, in comparison with the conventional extraction methods [33].

Microwaves are electromagnetic waves within the frequency range of 0.3 GHz - 300 GHz. Most of the reported microwave chemistry experiments [34 - 36] are conducted at 2450 MHz (the corresponding wavelength is 12.24 cm), as the maximum microwave energy is absorbed by liquid water at a frequency close to this value. Moreover, 2450 MHz is the frequency that is approved worldwide for domestic applications, including the commercial microwave chemistry equipment, to prevent interference with the frequencies used for communication purposes. Microwaves are made up of electric and magnetic fields, that couple in such a way that they are perpendicular to each other. The microwave heating principle is based on its direct impact on the solvents and is governed by two mechanisms: (a) ionic conduction, which occurs when microwaves are applied to a solution containing ions, and (b) dipolar polarization and realignment of the dipoles with the varying electromagnetic field applied. In a microwave-assisted reaction, the ability of the solvent to absorb microwave energy and transfer the heat to the surrounding molecules is expressed by its dielectric constant and dissipation factor [37]. Polar solvents and ionic solutions, such as acids, will absorb microwave energy due to the presence of a permanent dipole moment and a mobile charged carrier. On the other hand, non-polar solvents will not heat up when exposed to microwaves. Therefore, the efficiency of MAE depends on the selected solvent, the microwave power, the reaction time and the reaction temperature.

The microwave energy effect on chemical or biochemical reactions refers to: (a) a thermal effect, and (b) a non-thermal effect. In the course of MAE, the microwaves affect the polar molecules in the extraction media and eventually enhance the extraction efficiency[38]. Besides, it is concluded that MAE increases the extraction yield using a less amount of solvent.

Microwave extraction of phenolic compounds from *Rosmarinus officinalis*, *Origanum dictamnum* and *Vitexagnus* (cactus) is investigated [39]. In addition, Inglett G.E. et al. [40] study the extraction of phenolic compounds and antioxidants from buckwheat by using MAE with different solvents, such as water, ethanol, and a water–ethanol mixture. The latter is found the best. Moreover, Bourasa et al.[41] implement MAE method in extracting polyphenols and other antioxidant components from Quercus bark. They report that the extraction efficiency is increased three and two times, respectively, as compared to that of the conventional methods.

In short, it is believed that the combination of enzymatic hydrolysis and microwave-assisted extraction will result in an impressive effect due to their characteristics in terms of a high catalytic efficiency, a high specificity, mild reaction conditions and an ability to preserve the original efficiency of the active compounds. However, there is only scarce information on this approach. The application of this technology on POME has not been so far reported. Therefore, the objective of this study is to determine the optimum conditions of obtaining phenolic compounds from POME by microwave-assisted extraction coupled with enzymatic treatment.

EXPERIMENTAL

POME samples were derived from the final pond of West Palm Oil Mill, Sime Darby Plantation Sdn. Bhd. in Carey Island, Klang, Malaysia. POME (liquid form) was initially allowed to cool down to a temperature of 45°C - 50°C. Then it was poured into plastic containers (10 L). The latter were sealed tight and labelled prior to their transport to UNITEN laboratory. There POME was filtered through a 5mm sieve in order to remove the heavy suspended solids. The samples were then stored in multiple tightly-capped plastic containers (2L each) at 4°C for future usage. This was done to avoid biodegradation within POME due to microbial action.

The commercial ragi tapai (which contains hydrolytic enzymes) was purchased from the local market in Kajang, Selangor (it is cheap and readily available at any local market as it is a traditional fermented food in Malaysia and Indonesia). The moulds (*Rhizopus oryzae*, *Amylomyces rouxii, Mucor sp.* and *Candida utilis*) and yeasts were found in ragi tapai . The moulds in ragi tapai are strong amylolytic and degrade rice carbohydrate into simple sugars, which are then further decomposed by yeasts into alcoholic compounds [42].

The chemicals such as trifluoroacetic acids (TFA), follin-ciocalteu phenol reagent (2,4,6-tripridyl-s-triazine), gallic acid, and sodium carbonate, were purchased from Sigma (St. Louis, USA). Ultrapure deionized water obtained by ELGA Lab Water (UK) water purification system in the laboratory was used to prepare all the solutions.

Methodology

Determination of POME characteristics

Sufficient quantities of POME were withdrawn from the cold storage and left for a few hours to reach room temperature (25±3°C) prior use. The determination of total solids (TS), suspended solids (SS), oil and grease (O&G) and pH value of POME was carried out in accordance with the standard method used for the examination of water and wastewater [43]. Three replicates were used for each sample.

Sample preparation

Sedimentation was a common physical separation technique practiced prior any POME treatment aiming to reduce the suspended solid (SS) concentration. Firstly, POME sample was taken out from the cold (4°C) storage and brought to room temperature (25±3°C). Next, the separation was carried out under microwave irradiation at a power of 300 W for a short period (100 s). In this study, the microwave treatments were performed using a domestic microwave oven (Samsung, CE2877N, Korea). It operates at a frequency of 2450 MHz with variable power levels of 100 W, 180 W, 300 W, 450 W, 600 W and 850 W.

After microwave-assisted sedimentation, the bottom layer, the dense coagulate of suspended solid was collected. The top layer (supernatant), however, was centrifuged at 3000 g for 30 min at room temperature. After centrifugation, the bottom layer of the centrifuged samples was collected and mixed with the suspended solid collected previously. These gathered sediments from both the microwave-assisted sedimentation and centrifugation, were freeze-dried (lyophilized). A powder form of them was then prepared and stored in a vial at -20°C for the later extraction experiments.

Extraction of phenolic compounds

Maceration extraction

In this paper, maceration extraction was done at 40±1°C aiming a comparison with the microwave-assisted extraction. Four 250 mL glass bottles with caps were prepared. Each of them contained 10g of lyophilized POME samples dissolved in 150 mL of a different solvent (50 % ethanol, 80 % methanol, acetone, water). The extraction was then carried out in a shaking water bath (Protech, Malaysia) at 40ºC with a speed of 150 rpm for a different period (2 h, 4 h, 6 h, 8 h, 10 h and 12 h).

Enzymatic hydrolysis and microwave assisted extraction

A domestic microwave oven (Samsung CF3110N-5, Korea) was equipped with a magnetic stirrer, a water

Table 2. Experimental conditions for microwave-assisted extraction application.

Experimental Condition	Setting Value
Microwave Power	180W
Exposure Length	20s each cycle (15 s rest between every cycle)
Number of cycles	3, 6, 9, 12 and 15

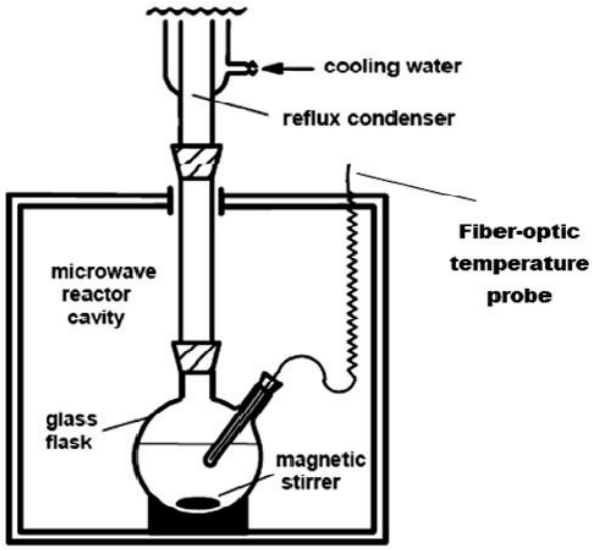

Fig. 1. Microwave irradiation extraction using a modified domestic microwave oven.

condenser, and temperature probe as shown in Fig. 1 [44]. MAE was then conducted by using this modified microwave oven. The conditions of this experiment were identical with those of the conventional maceration extraction in terms of the amounts of the lyophilized POME samples and the solvents used.

For MAE, 10 g of lyophilized POME samples were dissolved in 200 mL of the selected solvents. Then, they were subjected to microwave treatment (see Table 2) aiming extraction.

To couple the enzymatic hydrolysis and the microwave-assisted extraction, similar procedures were followed but the samples (lyophilized POME, 10 g) were initially mixed with 10 g of ragi tapai and then dissolved in 200 mL of a selected solvent. All other conditions remained identical with those of the non-enzymatic microwave-assisted extraction.

Estimation of total phenolics

The extracts obtained with the application of the methods mentioned above were filtered over a Whatman No. 1 filter paper. The methanol, ethanol and acetone filtrates were concentrated under vacuum at a temperature below 45°C in a rotary evaporator until around 5 mL of the filtrate remained. The water filtrates, however, were freeze-dried. Then, the extracts were re-dissolved in ultrapure water to a final volume of 10 mL. The final extracted samples were kept in 20 mL dark-colored bottles and stored in a refrigerator prior further analysis.

To determine the total phenolics content (TPC), Folin-Ciocalteu method was utilized. Gallic acid was used as a standard. An aliquot, 0.5mL of the final extracted samples were added to 0.5 mL of Folin-Ciocalteu's phenol reagent and vortexed for 10 s. After settling down for 2 min at room temperature, 10 mL of 7 % sodium carbonate (Na_2CO_3) were added to the mixture. The latter was then allowed to react at room temperature (28±2°C) for 60 min. After that, the absorbance of the mixture was measured at 715 nm using UV-Vis spectrophotometer (Shimadzu, Kyoto, Japan). The absorbance reading was then transformed into total phenolic content using the gallic acid standard curve and was expressed in milligrams of gallic acid equivalents (GAE) in 10 g of lyophilized POME.

To prepare the standard curve, gallic acid solutions of a varying concentration (from 0 mg/mL to 1 mg/mL) were prepared by a serial dilution. The corresponding absorbance reading was carried out within the limits of the calibration curve. Distilled water was used for the background subtraction. All measurements described above were conducted three times and average data was reported.

RESULTS AND DISCUSSION

Characteristics of palm oil mill effluent (POME)

POME samples are analysed to determine its oil and grease (O&G) and total solid (TS) content by using standard methods. POME used in this experiment has an average O&G and TS content of 4.4 g/L and 42.3 g/L, respectively. Besides, it is observed that POME is separated into top and bottom layers after the microwave treatment at 300 W for 100 s. However, no oil layer is observed.

The volume percentage of the top layer (liquid supernatant) is about 58 %, while that of the bottom layer, which was semi-solid, is about 42 %. The bottom layer appears to be a dense coagulate of suspended solids (SS). It is then re-suspended in distilled water in a ratio of 2:1 (v/v) (bottom layer:water). The mixture is stirred thoroughly by using a magnetic stirrer at a medium speed for 5 min.

On the other hand, the top layer (supernatant) is centrifuged (3000*g*) for 20 min to extract any soluble matter that is still left within the top layer. The sediments from both the initial microwave-assisted sedimentation

and centrifugation are pooled and then lyophilized.

Based on the analysis of the liquid supernatant, it is found that almost 95 % of the SS is removed from POME using microwave-assisted sedimentation. In the study done by Laohaprapanon et al. [45], the highest percentage of the top layer (56 %) is obtained by POME settled at 55°C for 24 h by floatation. Besides, SS removal percentage is 94.4 %. They conclude that the percentage of the top layer increases with settling time increase.

The comparison of the results obtained in this project and those reported by Laohaprapanon et al. [45] leads to the conclusion that microwave-assisted sedimentation can decrease the separation time (by a factor of 860) providing comparable SS percentage (95 %) removal using lower energy consumption in comparison to the conventional floatation method. Thus, microwave-assisted method is an efficient and promising technique for sedimentation.

Extraction of total phenolic compounds

Maceration extraction

The total concentration values of the phenolic compounds extracted with the solvents studied including water, 80 % methanol, 50 % ethanol and acetone, using conventional maceration method are compared in Table 3.

Due to the fact that polyphenols are mostly polar compounds, highly-polar solvents (*e.g*. water) and non-polar solvents (*e.g*. chloroform and hexane) are not suitable for extraction. Therefore, by using water as the only solvent, it is found that the extract obtained has a high content of impurities (*e.g*. organic acids, sugars, soluble proteins) along with the phenolic compounds. These impurities can interfere phenolics identification and quantification. Furthermore, using alcohol only decreases the extraction yield. However, the combination of water and another organic solvent results in a semi-polar medium providingt optimum polyphenols extraction [46].

Table 3 indicates that the extraction process with the acetone only gives the lowest total phenolic compounds content. Thus, acetone is not an appropriate solvent for polar compounds (*e.g*. phenols) extraction due to its low-polarity properties. Besides, it can be observed that the extracts from methanol and ethanol have higher phenolics content than the extract from water. In this study, ethanol (50 %) gives the best extraction yield among the solvents used. Since there are no significant differences in the phenolic concentration obtained on varying the of extraction (2 h, 4 h, 6 h, 8 h, 10 h and 12 h), it can be deduced that the optimal time of phenolic compounds extraction is 2 h with an yield of 163.2 mg GAE per 10 g of lyophilized POME.

Numerous studies conducted with other plant species report that a higher phenolics yield is obtained by

Table 3. Total phenolic compounds extracted from dried POME by the maceration method.

Solvent* / Time, h	Water	Methanol 80%	Ethanol 50%	Acetone
2	121.8±0.2	138.4±0.4	163.2±0.4	53.5±0.6
4	123.5±0.4	139.2±0.6	163.8±0.4	54.2±0.4
6	125.3±0.3	139.8±0.3	164.1±0.6	54.5±0.3
8	125.7±0.3	139.3±0.5	164.4±0.6	53.9±0.7
10	126.1±0.4	140.1±0.7	164.1±0.4	54.2±0.4
12	125.9±0.4	140.3±0.6	164.7±0.5	54.3±0.3

**Total phenolic content expressed as mg (GAE) per 10 g of lyophilized POME.*

Table 4. Total phenolic compounds extracted from dried POME by non-enzymatic microwave-assisted extraction (MAE) method.

Solvent*	Water	Methanol	Ethanol	Acetone
Cycles of exposure		80 %	50 %	
3	129.8±0.2	140.4±0.8	176.8±0.4	55.5±0.5
6	132.6±0.4	144.2±0.7	180.6±0.3	57.2±0.4
9	135.3±0.7	148.8±0.3	188.6±0.6	58.5±0.3
12	136.1±0.3	152.1±0.5	190.7±0.5	59.0±0.8
15	136.3±0.3	153.7±0.4	190.9±0.4	59.2±0.3

**Total phenolic content expressed as mg (GAE) per 10 g of lyophilized POME*

using polar solvents when compared to that in case of non-polar solvents application [47]. This conclusion is verified by the results of this investigation as the phenolic content from water-extraction (a high polarity) is higher than that from acetone-extraction (a relative low-polarity).

Moreover, there is no particular correlation between the extraction time and the yield in this study. This is because an equilibrium is achieved between the solute concentrations in the matrix and in the bulk solution (solvent), especially after 6 h. In other words, once the equilibrium is achieved, extra extraction time will not lead to increase of phenolic compounds extracted. However, the prolonged extraction process may lead to phenolics oxidation due to their exposure to light or oxygen [48].

Microwave-assisted extraction

The effect of the solvent nature and the extraction time is studied with the application of MAE. The results are listed in Table 4.

Several parameters have be taken into consideration in selecting the appropriate extracting solvent in MAE. They refer to the target analyte solubility, the solvent-analyte interaction and the dielectric properties of the extracting solvent. Non-polar solvents are transparent to microwaves due to their lower dielectric constant and dissipation factor. Mandal V. et al. [37] find that extracting solvents of a low polarity, such as hexane, lead to a low extraction efficiency of ginger under MAE. On the other hand, polar solvents absorb microwaves and thus, provide better extraction efficiency of MAE [37] . This conclusion is verified by the results of this study. When the solvent of a lower polarity, acetone in this case, is used as an extracting solvent, the amount of the extracted phenolic compounds is the lowest.

Water is a polar solvent of a high dielectric constant, so it is good in microwave absorption. However, although ethanol and methanol have lower dielectric constants than that of water, they provide better heating efficiency when mixed with water. There is a study [40] indicating that an water-ethanol mixture is the best in extracting phenolic compounds and antioxidants from buckwheat under microwave irradiation. Similarly, in this research, ethanol (50 %) is found to be the best in extracting phenolic compounds under MAE among the solvent used. For example, after 12 cycles of microwave irradiation, 190.7mg GAE per 10g of lyophilized POME are obtained by using ethanol (50 %). Nevertheless, by using acetone as an extracting solvent, the lowest extraction yield is observed, which is 59.0 mg GAE per 10g of lyophilized POME. Similar trend is analysing the results of the conventional maceration method.

As shown in Table 4, there is no significant difference between the microwave irradiation exposure cycle and the extraction yield after 12 cycles (20 s/cycle) at a microwave power of 180 W. The highest extraction yield from microwave-assisted extraction (MAE) refers to 190.7 mg GAE per 10 g of lyophilized POME using ethanol (50 %) after 15 microwave exposure cycles (the

total time required - ca 9 min). For the conventional maceration method, the highest extraction yield is 164.7mg GAE per 10g of lyophilized POME using ethanol (50 %) after 12 h. Thus, it can be concluded that MAE can provide an increase of the extraction yield (by 15.8 %), and at the same time, a decrease of the extraction time (by 98.75 %) using 50 % ethanol as a solvent when compared to the conventional maceration method. It is shown that a 6-7 min extraction time is enough for phenolic compounds complete leaching under microwave irradiation [49]. This indicates that microwaves can diffuse all the extractable phenolics into the solvent within a short period. Besides, a shorter extraction time at decreased power consumption can decrease the deterioration of the extracted phenolic compounds. Long extraction time and high reaction temperature increase the possibility of phenolics oxidation decreasing thus the phenolics extraction yield [50].

Microwave-assisted extraction with enzymatic hydrolysis

Similar experimental conditions are used to extract phenolic compounds by application MAE coupled with enzymatic hydrolysis aiming a comparison. Ragi tapai is used as an enzyme. Table 5 shows the total phenolic compounds extracted from lyophilized POME in case of using different solvents and extraction times.

In analogy with the results obtained with application of MAE only, the extraction process at a microwave power of 180 W carried out within 12 cycles and 15 cycles (20 s per cycle) shows no much difference. Furthermore, 50 % ethanol gives the highest extraction yield among the solvents used. The phenolic compounds extraction yield increases by 16.3 % in case MAE is coupled with enzymatic hydrolysis. Besides, it is found that the extraction yield is by 34.7 % higher than that of the conventional maceration method.

As mentioned previously, the outcomes of the microwave-assisted process can be described by using a thermal effect and a non-thermal effect. The thermal effect, aka. kinetic effect, results from the rapid heating phenomenon observed under microwave irradiation [51]. In contrast, the non-thermal effect refers to the specific radiation effect that is not caused by different temperature regime but by the non-thermal interactions between the substrate and the microwave irradiation.

It is anticipated that microwave non-thermal effects play an important role at a low microwave power level. Under these conditions, the active site of the enzyme molecules may undergo conformational changes which favour the cleavage of the glycosidic bonds. This will eventually enhance the efficiency of the enzyme. Actually, microwave non-thermal are observed in a number of microwave-assisted catalytic or enzymatic reactions[52 - 54]. In this study, as a low microwave power is used, the microwave thermal effect is minimized and this has excludes enzyme denaturation. Besides, microwaves also help to loosen the compact matrix structure of the solids which trap some phenolic compounds. This verifies the existence of a non-thermal effect.

Table 5. Total phenolic compounds extracted from dried POME by enzymatic hydrolysis and MAE method.

Solvent* / Cycles of exposure	Water	Methanol 80%	Ethanol 50%	Acetone
3	136.6±0.2	148.3±0.8	198.3±0.2	59.5±0.5
6	141.6±0.5	154.4±0.2	207.6±0.5	63.6±0.4
9	146.3±0.5	157.2±0.3	217.4±0.6	68.7±0.5
12	148.4±0.3	159.1±0.4	221.7±0.5	69.0±0.9
15	147.9±0.2	159.7±0.7	221.9±0.4	69.2±0.4

**Total phenolic content expressed as mg (GAE) per 10 g of lyophilized POME*

With the combination of microwave and enzymatic action, all trapped phenolic compound can be released becoming readily available for extraction. This eventually leads to a higher amount of extracted phenolic compounds.

CONCLUSIONS

The present study highlights the importance of an well-controlled microwave-assisted enzymatic extraction in enhancing the overall extraction yield. It is worth pointing out some general statements: (1) Enzymatic hydrolysis of starch using typical enzymes may successfully be carried out under microwave irradiation. In this study, ragi tapai is chosen due to its high content of moulds, which is a rich source of hydrolytic enzymes; (2) The effect of microwave irradiation strongly depends on the microwave power level – a higher microwave power level may cause denaturation of the enzyme; (3) The dominant factor of the microwave-assisted reaction in this study may be ascribed to the microwave non-thermal effects. Microwaves increase the internal pressure of the solid media and thus, enhance the extraction process. Microwave-assisted extraction (MAE) provides a decrease of the extraction time by 98.75 % and at the same time an increase of the extraction yield by 15.8 % when compared to the values referring to the conventional maceration method; (4) The coupling of microwave-assisted method with enzymatic hydrolysis results in a higher extraction yield (221.9 mg GAE per 10 g of lyophilized POME) of the phenolic compounds using the same extracting solvent when compared with that of the microwave-assisted extraction without enzymatic hydrolysis (190.9 mg GAE per 10 g of lyophilized POME).

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