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Metabolites Profiling of Heat Treated Whole Palm Oil Extract

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Abstract: The chemically complex and diverse nature of the plant metabolome require several platform technologies to profile the entire range of metabolites. An ultra-performance liquid chromatography-electrospray ionization tandem mass spectrometry (UPLC-ESI-MS/MS) technique was used to profile and identify a set of small-molecule metabolites found in heat treated whole palm oil extract. An investigation was carried out on the effect of heat treatment on the yield, quality and metabolites profile for whole palm oil extract. Palm fruits were collected, cleaned and sterilized for 0, 20, 40 and 60 min. The pulps were then stripped from the sterilized fruits and later was pressed using laboratory scale expeller. The resulting puree was centrifuged at 4000 rpm for 20 min. The result shows that there was a significantly difference between sterilization time of 0 and 40 min in yield and quality. Of all, the highest oil yield of 19.9±0.21% (w/w) was obtained at 40 min of sterilization with DOBI value of 5.95±0.08 and FFA of 1.44±0.22%. The MarkerView software version 1.2.0.1 analysis of the UPLC-ESI-MS/MS preliminary experimental data demonstrated the distribution and identity of several compounds in the whole palm oil extract for 40 min sterilization and 0 min sterilization. This study have demonstrated the potential of UPLC-ESI-MS/MS to identify, characterize and profile the metabolites in heat treated whole palm oil extract for further research in developing health application of phytochemicals from palm oil.

Key words: UPLC-MS/MS, metabolites profiling, whole palm oil extract, sterilization time

INTRODUCTION

Palm oil is plant edible oil derived from the fleshy mesocarp of the oil palm fruits, *Elaeis guineensis*. About 80% of palm oil production is destined for human consumption with the balance going to animal feed and to various industries. Harvesting, handling and processing methods used are known to influence the quality of the extracted palm oil. Fruit sterilization is one of the basic operations to obtain palm oil besides of fruit loosening, fruit digestion, oil extraction and oil clarification. Sterilization is a heat rendering operation involves steaming of fruits and reported as an important process because it determines the efficiency and effectiveness of the downstream and the refining processes in producing high grade palm oil. Increased in sterilization time and temperature has been found to increase yield of palm oil (Akusu *et al*., 2000; Abbas *et al*., 2006; Owolarafe and Faborode, 2008). Thermal treatment reduced significantly the value of fracturability, hardness and adhesiveness of the palm fruitlets which resulted in better strippability of palm fruits from the bunch and easier separation of mesocarp from palm kernel nuts (Abbas *et al*., 2006). Consequently, the determination of optimum sterilization time for high quality and maximize oil recovery of palm oil yield is of high interest (Akusu *et al*., 2000; Abbas *et al*., 2006).

Plant metabolite profiling plays an important role in agrochemical development, functional genomics, crop improvement and nutrition (Trethewey, 2006). However, there are currently no methods that are even close to delivering a complete quantification of the metabolome due to limited sensitivity and low range of metabolites covered. The development in chromatographic performance using short column packed with 1.7 μm porous particles, operating at ca. 12 000 psi so-called ultra performance liquid chromatography, UPLC was manifested in improved peak resolution, enhanced retention time, together with increased operational speed and sensitivity (Wilson *et al*., 2005).

The impacts of sterilization process on phytonutrients of palm oil were well established.

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However, the quality and metabolites profile of whole palm oil extract for different sterilization time have not been investigated to similar extent. In the present study, the effect of sterilization time on the palm oil yield and quality was investigated followed by metabolites identification using ultra performance liquid chromatography tandem mass spectrometry (UPLC-ESI-MS/MS) associated with Marker view software version 1.2.0.1 analysis. Metabolite identification in the heat treated whole palm oil extract is an important step for further research in developing health application of phytochemicals from palm oil. This is a beneficial work for health promotion and disease prevention in human life.

MATERIALS AND METHODS

Reagents and materials: All chemicals used were of analytical or High Performance Liquid Chromatography (HPLC) grade purchased from Fisher Scientific International Inc. (Pittsburg, PA, USA). The raw material used for the study is *Tenera* species of fresh palm fruit bunches obtained from Universiti Teknologi Malaysia's plantation, Skudai, Johor. The oil palm fruits were freshly harvested, reddish in color and of full maturity. The fruitlets have an average dimension of 4 cm in length and 2.5 cm in diameter.

Extraction of palm oil using laboratory scale expeller: Palm fruitlets were removed from the bunch. The fruit-laden spikelets were cut from the bunch with a machete. Then, the fruits were separated manually from the spikelets before cleaning. The cleaned fruitlets were sterilized for 0, 20, 40 and 60 min at constant temperature and pressure, 121°C and 4 MPa, respectively. The selection of sterilization time was set as described by Owolarafe *et al.* (2007). The pulps (mesocarp) were then stripped from the sterilized fruits and later pressed using laboratory scale stainless steel expeller. The resulting puree was centrifuge, operated at 4000 rpm for 20 min to obtain the whole palm oil extract.

The yield of whole palm oil extract was determined using Eq. 1:

$$\text{Yield}\,(\%) = \frac{\text{Mass of oil extracted (g)}}{\text{Mass of the mash (g)}} \times 100\,\% \qquad (1)$$

Extraction of palm oil using soxhlet method: A 20 g of mesocarp was weighed and transferred into a filter paper extraction thimble and then inserted into a 500 mL reflux flask. Extraction was carried out using 300 mL of hexane as a solvent at its boiling point. Extraction was terminated after 6 h. The extract was concentrated by removing hexane using rotary evaporator and left in the oven at 60°C. Soxhlet extraction was done with triplicates using the same amount of the sample and within the same duration. The method was done according to PORIM Test Method.

The oil yield was expressed in terms of mass percentage of the samples as in Eq. 2:

$$\text{Yield}\,(\%) = \frac{\text{Mass of oil extracted (g)}}{\text{Mass of sample (g)}} \times 100\% \qquad (2)$$

Quality analysis of extracted oils: All samples of whole palm oil extract obtained from the extraction were analyzed for Free Fatty Acids (FFA) according to AOAC methods, 940.28. Peroxide value (PV) was measured according to AOCS Method, Lubrizol Standard Test Procedure. Moisture content was determined using Moisture Analyzer MX-50 (A and D Company Limited, Japan). Deterioration of Bleachability Index (DOBI) of the samples was determined according to PORIM Test Method, PORIM. Each sample was analyzed in triplicate twice a month for 3 consecutive months. A mean value of triplicate samples was calculated.

UPLC-ESI-MS/MS analysis: Analyses were carried out using Aquity Ultra Performance Liquid Chromatography (UPLC) coupled with a triple quadrapole tandem mass spectrometer (Micromass ® Quattromicro™ API, Waters Corp., Mildford, MA, USA). Chromatographic separation was performed using an ACQUITY UPLC™ BEH C_{18} column (1.7 μm particle size; 2.1×50 mm) operated in Selected Ion Monitoring mode with ESI as an ion source. The source temperature, cone temperature and desolvatation temperature were 400, 20 and 200°C, respectively. The method used was adapted from Lauridsen *et al.* (2001) with some modification. The mobile phase consisted of 0.1% formic acid in water as solvent A and acetonitrile as solvent B with a flow rate of 0.2 mL min^{-1} and volume injected of 20 μL. Data acquired by Analyst 1.4.1 software (Applied Biosystems) were imported and processed by MarkerView software version 1.2.0.1 (Applied Biosystems).

RESULTS AND DISCUSSION

Yield of whole palm oil extract at different sterilization time: The highest oil yield was obtained when the fruits were sterilized at 40 min with 19.9±0.21% (w/w) yield. This followed by 60 min of sterilization (18.2±0.42% (w/w) yield), 20 min of sterilization (17.6±0.37% (w/w) yield) and 0 min of sterilization (13.0±0.29% (w/w) yield). Increase in sterilization time beyond 20 min does not increase the

Table 1: FFA and PV content of whole palm oil extract after 3 months storage for different sterilization time (min) with respect to moisture range

	FFA content (%)			
Parameters	0 min	20 min	40 min	60 min
Moisture range (%)	0.71± 0.01 to 0.78± 0.02	0.41±0.01 to 0.52±0.02	0.44±0.03 to 0.58±0.02	0.44±0.01 to 0.48±0.01FFA
After 3 months (%)	7.76±0.89	2.59±0.87	1.44±0.09	1.83±0.24
FFA fold increase	5.22±0.58	16.17±6.50	4.17±1.28	10.17±2.73
PV (mEq kg^{-1})				

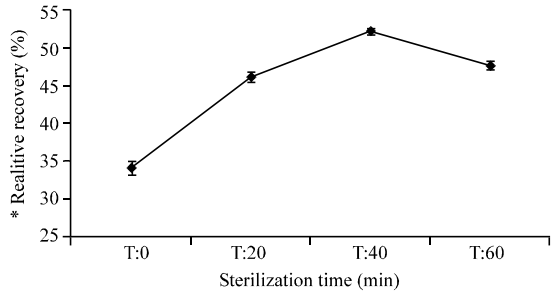

Fig. 1: Effect of sterilization time on laboratory scale expeller efficiency (error bars represent SEM of results, n = 3); *(weight of whole palm oil extract using small scale expeller/weight of whole palm oil extract using Soxhlet)×100

yield significantly. However, 0 min of sterilization gave a mean difference about 6.9% to 40 min of sterilization. Statistical evaluation showed that the yield was significantly difference between nonsterilized fruits (0 min sterilization) and sterilized fruits (20, 40 and 60 min sterilization), $p<0.05$. However, there was insignificant difference between 20 min sterilized and 60 min sterilized ($p>0.1$). The result obtained in the form of relative extraction recoveries (for Soxhlet recoveries considered as equal to 100%) was shown in Fig. 1. It observed that recovery was highest for 40 min of sterilization. The percentage of yield obtained increased slightly with increasing sterilization time. However, a decrease of recovery was noted for 60 min of sterilization. Higher oil yield for sterilized fruit compared to nonsterilized fruit was expected since sterilization is a heat rendering and moisture adsorption process which achieves the objectives of lowering the viscosity of oil as well as coagulation of protein (Owolarafe and Faborode, 2008; Lauridsen *et al*., 2001; Baryeh, 2001). Little amount of yield obtained in the nonsterilized fruits were due to fibrous and loose pounded mass fruit which are not able to squeeze out all the oil from the voids in the fibre since there was no heat applied to soften the tissues of oil-bearing material. For 60 min of sterilization, the yield was slightly reduced to 18.2±0.42% (w/w) due to the coagulation of protein which consequently reduces the viscosity of the oil to be expelled. Of all, 40 min of sterilization gave maximum oil yield compared to others at constant temperature of 121°C and constant pressure of 4 MPa.

Quality of whole palm oil extract: The quality of whole palm oil extract was determined prior to before and after three months storage at a room temperature (28-32°C). The quality parameters of the whole palm oil extract at different sterilization time was shown in Table 1. Statistical evaluation showed there was a significant difference ($p<0.05$) in moisture content between nonsterilized sample and sterilized samples, revealed that sterilization process affects the moisture content in the whole palm oil extract. However, the variation in the sterilized samples results was insignificant ($p>0.1$). It was recorded that 0 min of sterilization denotes the highest moisture content range within the three months storage period compared to other three treatments with the range from 0.71±0.01% to 0.78±0.02%. This was due to the activity of lipase enzyme which is more reactive in nonsterilized fruits since no heat was applied in order to restrict the activity of palm oil lipase (Owolarafe and Faborode, 2008). Moreover, the higher moister content catalyzes hydrolysis more. Initial moisture is an important factor affecting the rate of FFA increase (Chooi *et al*., 2006). With regard to FFA content, it was found that the variation in the results was significant ($p<0.05$) between nonsterilized fruits and sterilized fruits. Forty minutes sterilization showed the lowest content of FFA with only 1.44±0.09% compared to others. This is the most suitable treatment since the fold increase was only 4.17±1.28 even after three months storage. It was noted that change in moisture content also change the FFA content which gives affect on the hydrolysis of whole palm oil extract. As the moisture content increased, the FFA value also increased. FFA value for all samples was ranged from 0.16±0.02 to 7.76±0.89% which was lower than the value obtained by other researchers (Akusu *et al*., 2000). Besides, the reported range of free fatty acid content of crude palm oil was 2.3-6.7% (Saad *et al*., 2007) and never exceeds 10% (Chooi *et al*., 2006).

For the PV, 40 min of sterilization showed the lowest increment with only 1.44±0.22 fold after storage, followed by treatment 1 (1.84±0.38), treatment 2 (2.04±0.29) and

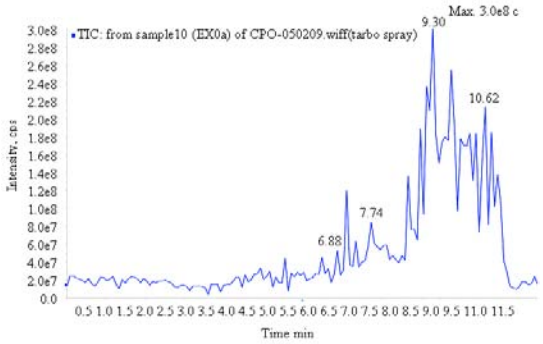

Fig. 2: Raw UPLC-MS/MS total ion chromatogram (-ESI) showing the profiles obtained for whole palm oil extract at 0 min of sterilization

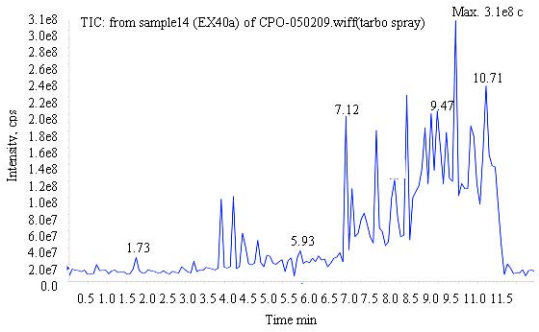

Fig. 3: Raw UPLC-MS/MS total ion chromatogram (-ESI) showing the profiles obtained for whole palm oil extract at 40 min of sterilization

treatment 4 (2.10±0.36), respectively. The result also proved that sterilization process is an important factor in determining oil quality (Gotoh and Wada, 2006) where the statistical evaluation showed nonsterilized fruits was significantly difference ($p<0.05$) with sterilized fruits. Overall, the PV in this research was found in the range of 9 to 50 mEq kg^{-1} and at the initial storage, PV for all treatments were below 10% indicates that the oil was in fresh condition. However, these values were starts to increase up to 27.50±1.04 mEq kg^{-1} until the end of storage, showing that a rancid taste was noticeable. Highest DOBI value was obtained at 40 min sterilization with 5.95±0.08 before storage and 2.77±0.02 after three months storage. Higher DOBI value in this treatment as shown by lower PV provides further support to the theory. DOBI value was higher due to reduced oxidation (Sivasothy *et al*., 2005). DOBI numerical values are observed as<1 for bad, 1-2 for poor, 2-3 for average and >3 for good crude (Tan *et al*., 2009).

Compounds distribution of whole palm oil extract using UPLC-ESI-MS/MS[a] Mean±SEM (Standard Error Method) of triplicates determinant: Based on the results obtained, this study aims to investigate the distribution of several compounds that are present in the whole palm oil extract for nonsterilized sample (0 min sterilization) and sterilized sample (40 min sterilization). UPLC-MS/MS has higher resolution separations which offering rapid analysis combined with information-rich data sets and provide a very powerful tool for metabolomics/metabonomics analysis (Gika *et al*., 2008; Dunn and Ellis, 2005). Visual inspection of the Total Ion Mass Chromatograms (TICs) for whole palm oil extract revealed a slightly difference between the samples as shown in Fig. 2 and 3. A simple visual comparison also showed that the UPLC peaks were much sharper and distinct, thus the data set was information rich. As would be expected, TICs indicates a complex profile of both samples and showed almost similar pattern of profile indicating that there is no

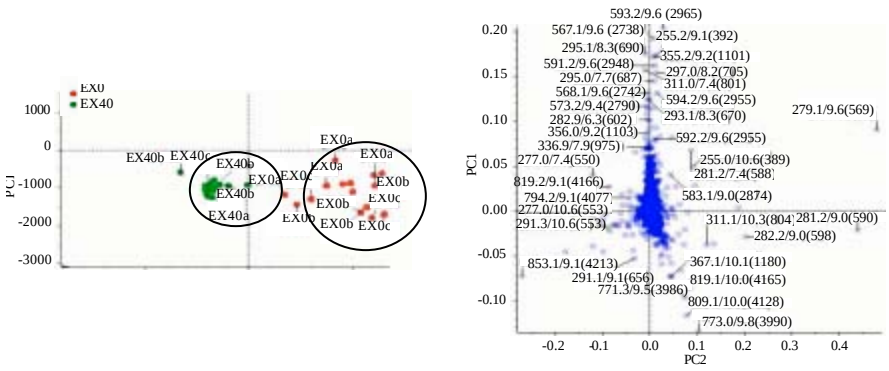

Fig. 4: MarkerView software version 1.2.0.1 constructs the (a) score plot and (b) loading plot for the data obtained

remarkable change due to the sterilization time. However, further study is required in order to identify the key metabolites due to the complex nature of the plant samples which generate huge data sets (Sumner *et al*., 2003).

Data generated were rich in information and complex, containing both chromatographic time and m/z dimension for each individual sample. Thus, they were then imported and processed by MarkerView software version 1.2.0.1 (Applied Biosystems) to construct initial score plots so that the datasets generated will be reduced and compressed. Thus, valuable information will not lose. The data clustering observed for the whole palm oil extract samples was given in Fig. 4 where there was a clear resolution of the samples into two distinct groups. The corresponding loading plot was also given in Fig. 4.

About 4954 marker ions were found for negative ESI using UPLC-MS/MS which was greater than marker ions detected by HPLC (Wilson *et al*., 2005). The MarkerView results show the possible components present in the whole palm oil extract which were depends on the m/z. Among the ions identified from the loading plot in the data set were the [M-H]-ion m/z = 228, 280, 282 and 284, eluting with a retention time from 8 to 9 min giving a proposed elemental composition of lipids group which were $C_{14}H_{28}O_2$, $C_{18}H_{32}O_2$, $C_{18}H_{34}O_2$ and $C_{14}H_{36}O_2$, respectively. From literature review, these data and chromatography standard, it was possible to identify the lipids group as fatty acid. The possible fatty acid present in both samples were myristic acid, linolic acid, linolenic acid, oleic acid and stearic acid. It was observed that samples sterilized for 0 min gave higher response than samples sterilized for 40 min. There also have potential terpenoids analyzed by Marker view. Lutein and Zeaxanthin with elemental composition of $C_{40}H_{56}O_2$ were the possible tetraterpenoids.

EX0: samples sterilized for 0 min and EX40: samples sterilized for 40 min. Presented in both samples with m/z of 568, eluting with retention time of 8.6 min in the UPLC separation. However, these tetraterpenoids were only present in nonsterilized samples. The variation in result suggested that sterilization process had an effect on the metabolite profiles in the whole palm oil extract. Future investigation and deeper study on possible metabolism in palm oil is required.

CONCLUSION

Sterilization process had been proved to provide a higher oil yield and oil quality. Sterilized fruits showed a better quality than nonsterilized fruits in terms of moisture content, FFA, PV and DOBI value. However, 20, 40 and 60 min of sterilization did not show a significant difference. In this study, 40 min of sterilization gives the most appropriate treatment with 19.9±0.21% (w/w) yield and low increase in FFA and PV with 4.17±1.28 and 1.44±0.22, respectively even after three months storage. Meanwhile, DOBI value of whole palm oil extract for 40 min shows the oil was in a good range. The preliminary data of UPLC-ESI-MS/MS demonstrated that nonsterilized samples and sterilized samples contribute to the difference in the chemical profile of the palm oil whole extract. However, more works and research need to be done to identify, characterize and profile for other possible compounds in palm oil metabolites.

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