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Research Article

Metabolite profiling of heat treated whole palm oil extract

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Abstract

The chemically complex and diverse nature of the plant metabolome require different platform technology to entire range of metabolites. An ultra-performance liquid chromatography-electrospray ionization tandem mass spectrometry (UPLC-ESI-MS/MS) technique was developed 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 minutes. The kernels were then stripped from the sterilized fruit to get the pulp (mesocarp part) and later the pulp was pressed using small scale expeller. The resulting puree was centrifuged at 4000 rpm for 20 minutes. The results show that there was a significant difference between sterilization time of 0 minute and 40 minutes in yield and quality. Of all, the highest yield of oil of 19.9% was obtained at sterilization time of 40 minutes with DOBI value of 5.95 ± 0.08 and FFA of 1.44 ± 0.10. 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 minutes sterilization and 0 minute of sterilization.

Keywords: UPLC-MS/MS, sterilization, storage, *Elaeis guineensis*, Malaysia.

Introduction

Palm oil is derived from the fleshy mesocarp of the oil palm fruit, *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 [1, 2, 3]. 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 [2]. High quality of palm oil yield can be obtained under optimum heat treatment process. Thus, an optimum condition sterilization operation should be determined in order to maximize oil recovery from palm fruit and to obtain oil of good quality [1].

This research investigates the effect of different time of sterilization on the palm oil yield and quality. In addition, the study also profile the metabolites of heat treated whole palm oil extract using ultra performance liquid chromatography tandem mass spectrometry (UPLC-ESI-MS/MS), a rapid, high sensitivity and high specificity in detecting the compounds [4]. 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. At present, there is no work reported on the effect of temperature on the metabolites of whole palm oil extract.

Materials and Methods

Materials

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 fruit was freshly harvested, reddish in colour and of full maturity. The fruitlets have an average dimension of 4 cm in length and 2.5 cm in diameter.

Chemicals

All chemicals used were of analytical or high performance liquid chromatography (HPLC) grade.

Whole palm oil extraction

Palm fruitlets were removed from the bunch. The fruit-laden spikelets were cut from the bunch with a machete. Then, the fruit were separated manually from the spikelets before cleaning. The cleaned fruitlets were sterilized for 0, 20, 40 and 60 minutes [5] at constant temperature and pressure, 121^0C and 4 MPa respectively. The fruitlets were then stripped from the sterilized fruit to get the pulp (mesocarp) and later the pulp was pressed using a small scale stainless steel extruder. The resulting puree was centrifuged, operated at 4000 rpm for 20 minutes to obtain the whole palm oil extract.

Yield of whole palm oil extract

The yield of whole palm oil extract was determined using Equation (1)

$$\text{Percentage yield (\%)} = \frac{\text{Mass of oil extracted (g)}}{\text{Mass of the mash (g)}} \times 100\% \quad (1)$$

Quality analysis of extracted oil

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 &D Company Limited, Japan). Deterioration of Bleachability Index (DOBI) of the samples was determined according to PORIM Test Method, 1995, PORIM p. 29. Each sample was analyzed in triplicate.

Metabolite profile of whole palm oil extract

Sample preparation for UPLC-ESI-MS/MS analysis

A minor modification of the sample preparation described by Lauridsen, *et al* [4] was used. 1.0 mL of crude palm oil is weighed using balance. 0.8 mL of isopropanol is mixed with 0.2 mL of hexane. Then, crude palm oil was added into the mixture of isopropanol and hexane. The solution was then mixed using vortex mixture. The sample was filtered using 0.2µm nylon filter and ready to analyze using UPLC-ESI-MS/MS.

UPLC-ESI-MS/MS analysis

The LC-MS/MS used is ABI 4000 Q-Trap, Applied Biosystems (Foster City, CA). For chromatographic conditions, the mobile phase consisted of methanol/water (97:3), operated at column temperature of 25^0C with a flow rate of 0.2 mL/min and volume injected of 20 µL. Separation was performed using an Acquity UPLC BEH C_{18} column (1.7 µm; 2.1 mm x 50 mm) operated in Selected Ion Monitoring mode with ESI as an ion source. The source temperature, cone temperature and desolvatation temperature were 400^0C, 20^0C and 200^0C respectively. Analyses were carried out on Waters Acquity UPLC^{TM} System (Milford, MA, USA) [4]. Data acquired by Analyst 1.4.1 software (Applied Biosystems) on the QTRAP 4000 were imported and processed by MarkerView software version 1.2.0.1 (Applied Biosystems).

Storage

Samples containing four different time of sterilization were stored at room temperature with a temperature range of 28^0C to 32^0C. Each sample was monitored for its quality twice a month for 3 consecutive months. A mean value of triplicate samples was calculated.

Results

Percentage yield of whole palm oil extract

Figure 1 shows percentage yield of whole palm oil extract at different sterilization time. In this study, highest oil yield was obtained when the fruit were sterilized at 40 min with 19.9% yield. This was followed by 60 min, 20 min and 0 min of sterilization with 18.2%, 17.6% and 13.0% yield, respectively. Increase in sterilization time beyond 20 min does not increase the yield significantly. The mean difference of yield between 20 min and 40 min of sterilized fruit is 2.3%.

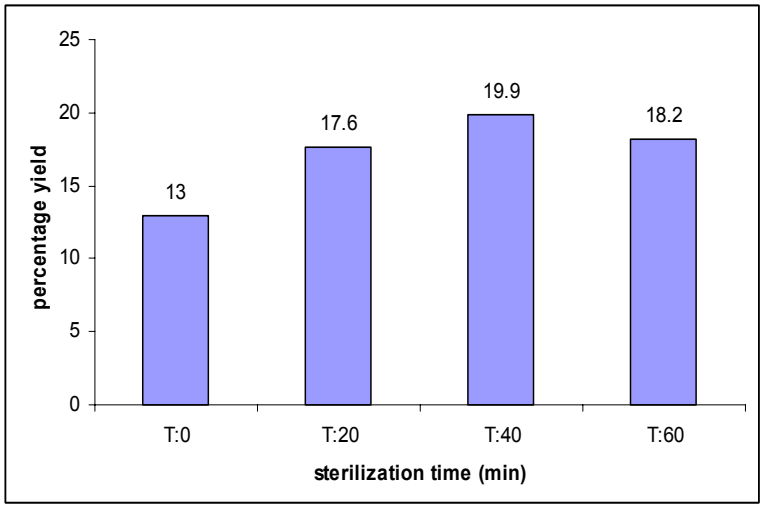


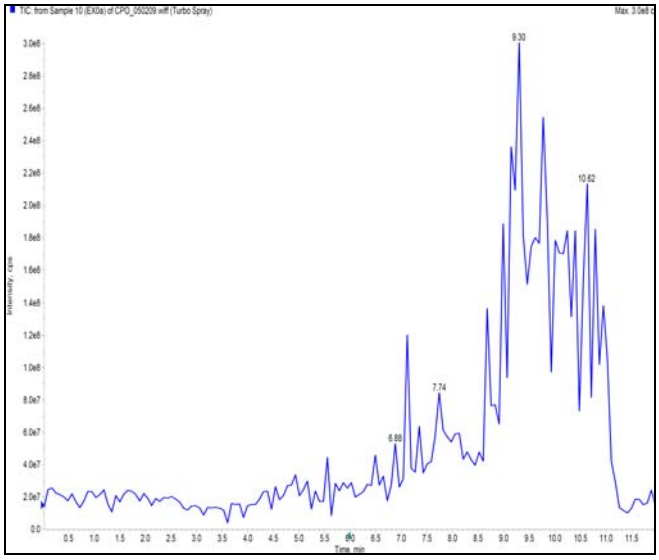
Figure 1. Percentage yield of whole palm oil extract at different times of sterilization.

Quality of Whole Palm Oil Extract

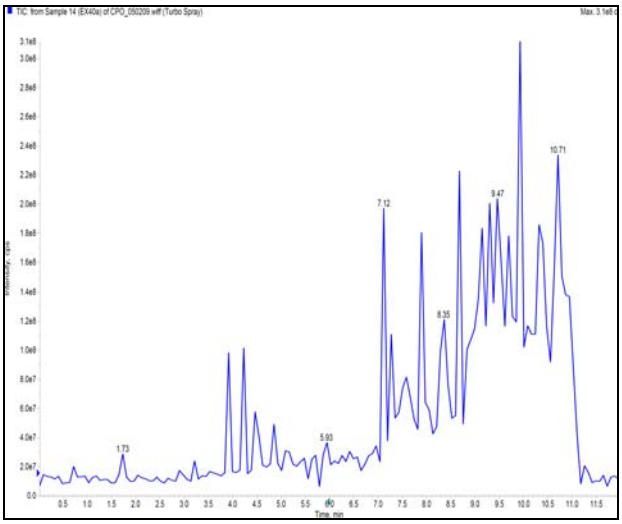
The quality of whole palm oil extract was determined prior to before and after three months storage at a room temperature as presented in Table 1. Treatment 1 results in a higher moisture content compared to other three treatments with the range of 0.71 - 0.78 %. Meanwhile, other treatments result in only a slight difference among them with the range 0.41 -0.52 %. The initial FFA content was highest in treatment 1 with 1.48 ± 0.15% followed by treatment 3, treatment 4 and treatment 2 with 0.36 ± 0.05 %, 0.18 ± 0.04 % and 0.16 ± 0.02 %, respectively. Non sterilized fruits results in the highest FFA content compared to sterilized fruits after three months storage with 7.76 ± 0.89 %. For the PV value, treatment 1 gives the lowest initial value with 8.51 ± 1.70 meq/kg followed by treatment 4, treatment 2 and treatment 3 with 8.64 ± 1.89 meq/kg, 9.06 ± 0.70 meq/kg and 9.84 ± 1.36 meq/kg, respectively. Treatment 3 has the lowest increment in PV with only 1.44 ± 0.22 fold after storage, followed by treatment 1 (1.84 ± 0.38), treatment 2 (2.04 ± 0.29) and treatment 4 (2.10± 0.36), respectively.

Metabolite profiling of heat treated whole palm oil extract

The total ion chromatogram UPLC-MS/MS of whole palm oil extract obtained from this study is as shown in Figure 2. It shows the data from injections of whole palm oil samples at 0 min of sterilization and 40 min of sterilization.



(a)



(b)

Figure 2. Raw UPLC-MS/MS total ion chromatogram (-ESI) showing the profiles obtained for whole palm oil extract (a) 0 min of sterilization and (b) 40 min of sterilization.

Data sets of whole palm oil extract generated from UPLC-MS/MS was rich in information and it was reduced and compressed using MarkerView software version 1.2.1.1. Datasets generated must be reduced and compressed so that valuable information will not lose. Besides, it is not possible to simply construct a two-dimensional data matrix for all samples. Figure 3 presents score plot and loading plot constructed from the data obtained.

Table 1: FFA content and PV value of whole palm oil extract after 3 months storage at different times of sterilization.

Sterilization Time (min)	Moisture range (%)	FFA after 3 months[a] (%)	Fold Increase[a]	PV after 3 months[a] (meq/kg)	Fold Increase[a]
0 (treatment 1)	0.71 – 0.78	7.76 ± 0.89	5.22 ± 0.58	24.13 ± 1.17	1.84 ± 0.38
20 (treatment 2)	0.41 – 0.52	2.59 ± 0.87	16.17 ± 6.50	27.50 ± 1.04	2.04 ± 0.29
40 (treatment 3)	0.44 – 0.58	1.44 ± 0.09	4.17 ± 1.28	24.05 ± 0.01	1.44 ± 0.22
60 (treatment 4)	0.44 – 0.48	1.83 ± 0.24	10.17 ± 2.73	26.77 ± 0.51	2.10 ± 0.36

[a]Mean ± SEM (n = 3)

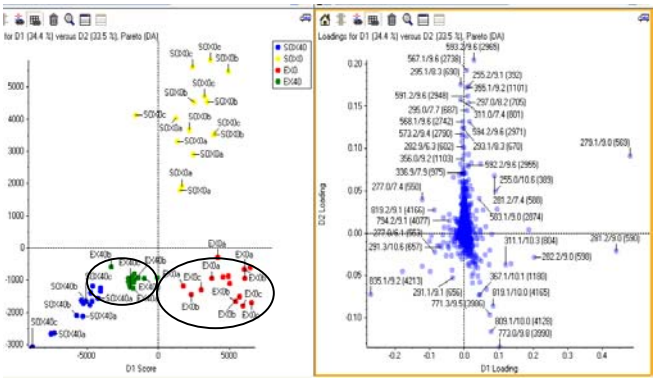


Figure 3. MarkerView software version 1.2.0.1 constructs the score plot and loading plot for the data obtained. EX0: samples sterilized for 0 min, and EX40: samples sterilized for 40 min.

Discussion

The percentage of yield obtained increased slightly with increasing sterilization time. Higher oil yield for sterilized fruit compared to nonsterilized fruit is expected since sterilization is a heat rendering and moisture adsorbtion process which achieves the objectives of lowering the viscosity of oil as well as coagulation of protein [3]. 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. At 60 min of sterilization, the yield was slightly reduced to 18.2% due to the coagulation of protein which consequently reduces the viscosity of the oil to be expelled. Of all, 40 min of sterilization gives maximum oil yield compared to others at constant temperature of 121^0C and constant pressure of 4MPa. At 60 min of sterilization, the yield was slightly reduced to. This finding was in contradiction to the finding that has been reported previously by Baryeh [6]. He claimed that oil yield increased with extraction pressure, heating time and temperature up to 25 MN/ m^2, 20 min and

100^0C. High FFA content was due to the activity of lipase enzyme which is more reactive in nonsterilized fruit since no heat was applied in order to restrict the activity of palm oil lipase [7]. Moreover, the higher moister content catalyzes hydrolysis more. Meanwhile, treatment 3 results in the lowest content of FFA with only 1.44 ± 0.09% compared to others. This is the most suitable treatment since the fold increase in the FFA was only 4.17± 1.28 even after three months storage. FFA value for all treatments in this research was ranged from 0.16 ± 0.02% to 7.76 ± 0.89% which is lower than the value obtained by other researchers [1]. This is in line with a previous report which stated that the FFA content in crude palm oil never exceeds 10% [8]. Besides, the reported range of free fatty acid content of crude palm oil was 2.3% – 6.7% [9]. Overall, the PV in this research was found in the range of 9 meq/kg to 50 meq/kg and at the initial storage, PV values for all treatments were below 10% indicates that the oil was in fresh condition. However, these values started to increase up to 27.50 ± 1.04 meq/kg until the end of storage, showing that a rancid taste is 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 [10]. DOBI numerical values are observed as<1 for bad, 1-2 for poor, 2-3 for average and >3 for good crude [11].

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 [12, 13, 14]. Based on the quality result obtained, this work aims to detect differences in the metabolites profiles that are representative in the whole palm oil extract at 0 min and 40 min of sterilization. However, this requires analysis using multivariate statistics to identify the key metabolites due to the complex nature of the samples which generate huge data sets. Visual inspection of the total ion mass chromatograms (TICs) for whole palm oil extract analysed were presented. Figure 2 shows a clear picture on the data provided by the TICs. As would be expected, it indicates a complex profile of both samples. Besides, they show almost similar profile indicating that there is no remarkable change due to the sterilization time.

Data generated were rich in information and very 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. From the score plot and loading plot, it shows the significant groupings between the two samples as represented in Figure 3. Some 4954 variables were found for –ve ESI using UPLC-MS/MS. These variables include isotopes, ions arising from ion-source fragmentation, adducts, background signals and not necessarily individual metabolites. Instead, they are based on *m/z* features so that these numbers represent an upper limit for sample component detected [12]. The MarkerView results the possible components present in the whole palm oil extract which are depends on the *m/z*. It shows that possible fatty acid present in both samples were linolic acid, linolenic acid, oleic acid, stearic acid and palmitic acid with *m/z* range of 255 to 283. However, samples sterilized at 0 min gave higher response than samples sterilized at 40 min. Potential carotene was also analyzed by MarkerView. Lutein and Zeaxanthin are possible carotenes present in both samples with *m/z* of 568. Phospholipids, tocols, phytosterols and squalene are other constituents that are interesting to investigate in this study.

Conclusion

Sterilization process had been proved to provide a higher oil yield and oil quality. Sterilized fruit showed a better quality than nonsterilized fruit (0 min of sterilization) in terms of moisture content, FFA, PV and DOBI value. However, 20 min, 40 min and 60 min of sterilization did not show a significant difference. In this study, 40 min of sterilization gave the most appropriate treatment with 19.9% 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. On the whole, the degree of heat treatment contributes to the difference in the profile of the palm oil whole extract.

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