Effect of enzymatic pretreatment on the fatty acid profile and micronutrient contents of hydraulically pressed palm oil

Jacynthia Charmentie Anak Cha'ir[1], Yanty Noorzianna Abdul Manaf[2], Fan Hui Yin[3#]

1 Faculty of Food Science and Nutrition, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Sabah, MALAYSIA.
2 Halal Research Group, Faculty of Food Science and Nutrition, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Sabah, MALAYSIA.
3 Food Security Research Laboratory, Faculty of Food Science and Nutrition, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Sabah, MALAYSIA.
Corresponding author. E-Mail: chloefan@ums.edu.my; Tel: +6088-320000 ext 102144; Fax: +6088-320259.

ABSTRACT Palm oil is known as one of the important vegetable oils enriched with health beneficial constituents. Despite using hydraulic pressing in palm oil recovery, enzymatic pretreatment prior to pressing was investigated for its effects on the fatty acid profile and micronutrient contents. An enzyme blend of pectinase, cellulase and tannase was used to pretreat the palm mesocarp prior to hydraulic pressing. Enzymatic pretreatment significantly affected the predominant fatty acids contents in the hydraulically pressed palm oil but the overall fatty acid profile (MUFAs > SFAs > PUFAs) remained unchanged compared to the untreated palm oil sample. α-tocopherol and all four forms of tocotrienols (α-, β-, γ- and δ-) were significantly lower in enzymatic treated sample. In contrast, the enzymatic pretreatment markedly influenced the total carotene content with two-fold higher in β-carotene content in the hydraulically pressed palm oil. Additionally, enzymatically treated palm oil showed reduced cholesterol and raised β-sitosterol contents compares to the untreated sample. Overall, enzymatic pretreatment influenced micronutrient contents in hydraulically pressed palm oil without altering its fatty acid profile.

KEYWORDS: Palm oil; Fatty acid profile; Micronutrient contents; Enzymatic pretreatment; Hydraulic pressing
Received 17 December 2025 **Revised** 24 December 2025 **Accepted** 26 December 2025 **Online** 28 December 2025

Original Article

TRANSACTIONS ON SCIENCE AND TECHNOLOGY

INTRODUCTION

Palm oil (*Elaeis guineensis*) is one of the important edible oils in the oil and fats industry, attributed to the constituents recovered from palm mesocarp. Generally, the composition of fatty acids in palm oil contains about 50% saturated fatty acids, 40% unsaturated fatty acids, and 10% polyunsaturated fatty acids (Tan *et al.*, 2021). Fatty acid composition (FAC) of oils is a significant indicator of their nutritional value, as well as one of the most important components influencing oxidative stability (Permana *et al.*, 2022). Palm oil also known for containing vitamin E (tocopherol and tocotrienols) with other phytonutrients such as phytosterols, phenolic compounds, and carotenoids. Kaseke *et al.* (2021) stated that both tocopherol and tocotrienols are significant as natural antioxidants in preserving the quality of oilseeds, especially throughout processing and storing. Besides, red palm oil is found to have a high concentration of carotenoids and vitamin A. The major carotenes produced in red palm oil are α-carotene (41.3%) and β-carotene (41%) (Loganathan *et al.*, 2020). The carotenoid contents can help to control cancer and lessen the side effects of chemotherapy (Almeida *et al.*, 2018).

Mechanical pressing is reported for extracting the edible oil from oleaginous materials in which to preserve the nutrients in the extraction oil (Sorokova *et al.*, 2022). The production of high-value edible oil from mechanical pressing can improve the oxidative stability and good antioxidant properties (Zainul *et al.*, 2024). There are few types of mechanical pressing approaches to manually produce oil such as screw press and hydraulic press. Nevertheless, hydraulic pressing has been shown to preserve better the micronutrient components of oils compared to the screw press, as

screw press showed higher primary oxidation products and greater sterol degradation in recent studies (Aksu *et al*., 2020; Rabadán *et al*., 2017).

In addition, alternative technologies such as enzyme-assisted extraction methods have been proven for their significant efficiency in oil extraction, representing a promising green alternative for recovering palm oil that retains health-benefiting compounds. Particularly, the use of cell wall degrading enzymes such as cellulase, pectinase and tannase has been exclusively explored as an environmentally friendly alternative to industrial extraction processes, taking advantage of their ability to enhance phenolic content extractability and antioxidant capacity of oils (Yazdi *et al.* 2019; Teixeira *et al*., 2013). Given the lack of data on enzymatic pretreatment in hydraulic-pressed palm oil, this study focused on the feasibility of using a combined enzyme system; individual enzyme effects, dose–response, and absolute oil yield were not assessed, but consistent processing conditions enable valid comparison of effects on micronutrient and fatty acid profiles.

METHODOLOGY

Sample Collection

Oil palm fruits (*Elaeis guineensis*) were kindly supplied by Kumpulan Sawit, Kota Belud in Sabah, Malaysia. The oil palm fruits selected were of uniform size with no visible damage. All fruits were separated from a bunch and separated into mesocarp and kernel manually. Mesocarps with no visible defects were packed and stored in the freezer at -18 ℃ for further enzymatic pretreatment and the oil extraction process. Food-grade cell wall degrading enzymes of cellulase with 10000 u/g of CMCase activity, pectinase with 10000 u/g of PG activity, and tannase with 300 u/g were purchased from Sangherb Bio-Tech Inc. (Shaanxi, China). All reagents were of analytical grade.

Palm Oil Extraction

To reflect potential industrial use, the following procedures were carried out without chemicals. An amount of 40g of mesocarp was pretreated with an enzyme blend composed of cellulase, pectinase, and tannase (1:1:1) and suspended in 100 mL of distilled water (Teixeira *et al.*, 2013). Each experimental condition was conducted in triplicate using independent hydraulic pressing runs with separately prepared mesocarp samples (40 g per run). Enzymatic pretreatment was preformed using 4% (w/v) of enzyme concentration at 54 ℃ for 30 min using 1:1 (w/v) ratio of mesocarp-to-enzyme. Subsequently, pretreated mesocarp were dried at 45 ℃ for 5 h to obtain not more than 7% moisture content (Orhevba *et al*., 2013). The dried mesocarps were then subjected to hydraulic pressing using pressure at 7.5 MPa for 10 min and a 6 mm pore size of the sample cage. Hydraulically pressed palm oil after enzymatic pretreatment (ECPO), and palm oil obtained through hydraulic pressing without enzymatic pretreatment (NECPO) as control sample were kept in amber bottles at room temperature until further analysis.

Fatty Acid Composition Analysis

Fatty acid composition of palm oil samples was analyzed by gas chromatography (GC) (7820A, Agilent, Santa, CA, USA) equipped with a hydrogen flame ionization detector and capillary column (100 m × 0.25 mm i.d.) according to AOAC (2016). Helium was employed as a gas carrier with a flow through the column of 0.75 mL/min. The temperature of the injector and detector was set at 225 ℃ and 285 ℃, with an oven temperature of 240 ℃. An injection volume of 2μL was used.

Micronutrient Contents Analysis

Tocopherol and tocotrienol contents were determined by high performance liquid chromatography (HPLC) (Agilent 1100 series, Agilent Technologies, USA) equipped with a HPLC column (C18 column) (250 mm × 4 mm, 5-µm particle size) (Merck, Darmstadt, Germany), and a fluorescence detector according to ISO 9936:2016 (ISO, 2016). The separation was performed at 25 °C. The excitation and emission wavelengths were set at 295 and 330 nm, respectively. The mobile phase used was a mixed n-heptane and 2-propanol at 99:1 % (v/v) at a flow rate of 1 mL/min.

Total carotenoid contents were analyzed by HPLC in a C30 column (150 mm × 4.6 mm; 5µm) and spectrophotometry based on National Food Safety Standard GB5009.83 (2016). The mobile phase consisted of methanol/acetonitrile/dichloromethane (60/ 20/20, v/v/v) at a flow rate of 1.0 mL/min. The detection wavelength was set at 450 nm. The carotenoids were quantified using the relevant calibration curves.

Beta-sitosterol was determined by gas chromatography equipped with a capillary column (25m length × 0.25 mm i.d.) with flame ionization detection based on Rabadán *et al.* (2017). The helium was used as a carrier gas with a flow through the column of 1.2 mL/min. The injector temperature was applied at 280 ℃, detector temperature at 290 ℃, and oven temperature at 260 ℃. A 1 µL injection volume was used.

The cholesterol contents were analyzed based on Okpuzor *et al.* (2009). The palm oil samples were first saponified with 3% ethanolic KOH. The non-saponifiable matter was then dissolved in chloroform. The HPLC analysis was done using an Agilent 1100 series, C18 column (250 mm × 4 mm, 5-µm particle size), the mobile phase was acetonitrile: water (1:1), and a UV detector at 239 nm at a flow rate of 0.4 mL/min.

Statistical Analysis

All the results were presented as means ± standard deviations (SD) (n = 3). Differences between ECPO and NECPO were analyzed using an independent-samples t-test with significance level set at $p<0.05$.

RESULTS AND DISCUSSION

Fatty acid composition (FAC) of the hydraulically pressed with enzymatic pretreatment palm oil (ECPO), and non-enzymatic palm oil (NECPO) was compared as shown in Table 1. The predominant fatty acids found in both ECPO and NECPO were oleic acid ($C_{18:1n9c}$), followed by palmitic acid ($C_{16:0}$), α-linolenic acid ($_{C18:3n3}$), linoleic acid ($C_{18:2n6c}$), and stearic acid ($C_{18:0}$). Although the NECPO sample exhibited a higher MUFA content (45.90 ± 0.41%) compared with the ECPO sample (44.79 ± 0.25%), this difference was not statistically significant ($p>0.05$). Meanwhile, NECPO had a lower SFAs content (39.35±0.42%) compared with ECPO (41.03±0.32%), but this difference was not statistically significant ($p>0.05$) as shown in Table 1. In addition, PUFAs did not differ significantly between ECPO (13.58±0.07%) and NECPO (14.24±0.13%) ($p>0.05$). Overall, enzymatic pretreatment was not significantly affecting the FAC of hydraulically pressed palm oil, with both ECPO and NECPO having unchanged distribution of MUFAs > SFAs > PUFAs. This is supported by studies reporting that enzyme combination used in seeds pretreatment has facilitated a more pronounced effect on the recovery of phytosterol and antioxidants (Prommaban *et al.*, 2021; Hu *et al.*, 2020; Tirgarian *et al.*, 2019).

Table 1. Fatty acid compositions of ECPO and NECPO obtained by hydraulically pressing.

Fatty Acid Composition	ECPO (%)	NECPO (%)
Myristic acid (C14:0)	0.71±0.09	0.79±0.09
Palmitic acid (C16:0)	36.94±0.00	34.87±0.01
Palmitoleic acid (C16:1n7)	0.18±0.02	0.22±0.02
Stearic acid (C18:0)	3.38±0.03	3.69±0.03
Oleic acid (C18:1n9c)	44.61±0.02	45.68±0.03
Linoleic acid (C18:2n6c)	12.90±0.01	13.49±0.00
α-linolenic acid (C18:3n3)	0.33±0.01	0.39±0.01
γ-linolenic acid (C18:3n6)	0.35±0.03	0.36±0.02
Σ SFAs	41.03±0.32	39.35±0.42
Σ MUFAs	44.79±0.25	45.90±0.41
Σ PUFAs	13.58±0.07	14.24±0.13
Σ TFAs	n.d	n.d

ECPO, enzymatic treated palm oil; NECPO, non-enzymatic treated palm oil. Σ SFAs, total of saturated fatty acids; Σ MUFAs, total of monounsaturated fatty acids; Σ PUFAs, total of polyunsaturated fatty acids; Σ TFAs, total of trans fatty acids; n.d, not detected. Value in the table represents means ± standard deviation (S.D.) of triplicate measurements.

Table 2 presents the micronutrient contents of palm oil samples in this study. The α-tocopherol was was significantly lower in ECPO compared (0.01±0.00mg/g) compared with NECPO (0.05±0.0mg/g) (p<0.05). Tocotrienols were detected in four forms, with γ-tocotrienol predominating in both ECPO (0.21±0.01 mg/g) and NECPO (0.23±0.00 mg/g), followed by δ-tocotrienol, α-tocotrienol, and β-tocotrienol (p<0.05). The total tocotrienol contents of ECPO and NECPO denote the combined levels of all vitamin E isomers were reported as 0.32 mg/g and 0.40 mg/g, respectively.

Table 2. Micronutrient contents of ECPO and NECPO obtained by hydraulically pressing.

Micronutrients	ECPO (%)	NECPO (%)
Vitamin E		
α-tocopherol (mg/g)	0.01±0.00	0.05±0.00
α-tocotrienol (mg/g)	0.05±0.00	0.06±0.00
β-tocotrienol (mg/g)	0.02±0.00	0.03±0.00
γ-tocotrienol (mg/g)	0.21±0.01	0.23±0.00
δ-tocotrienol (mg/g)	0.05±0.00	0.07±0.00
Total tocotrienols (mg/g)[a]	0.32	0.40
Carotenoids		
α- carotene (mg/kg)	331.6±3.78	304.3±1.54
β- carotene (%)	0.41±0.02	0.21±0.02
Sterols		
Cholesterol (%)	0.12±0.02	0.20±0.03
β-sitosterol (%)	7.1±0.20	6.1±0.20

ECPO, enzymatic treated palm oil; NECPO, non-enzymatic treated palm oil. Value represents means ± standard deviation (S.D.) of triplicate measurements.

[a]The readings represent the sum of all individual vitamin E isomers, and no statistical notation is applied.

Vitamin E compounds play a crucial role in protecting lipids from oxidative degradation, which is particularly important given the high levels of unsaturated fatty acids in oil samples (Gao *et al.*, 2023). According to the result, NECPO contains vitamin E compounds higher than ECPO. The lower vitamin E content observed in ECPO may be attributed to oxidative degradation during post-pretreatment drying and pressing, as vitamin E compounds are known to be susceptible to heat- and oxygen-induced degradation during processing (Zaaboul & Liu 2022). While enzymatic pretreatment may facilitate the release of vitamin E compounds, oxygen exposure during drying

and mechanical extraction possibly influences the recovery of the final vitamin E content of the oil. Besides, Teixeira *et al.* (2013) reported that the presence of tannase has extracted other antioxidants which negatively affect the extraction of tocopherols and tocotrienols in the oil. Earlier on, Eitenmiller & Lee (2004) also reported that the natural antioxidants and synergists other than vitamin E that presence during the enzymatic pretreatment, could altered the stability of oil samples through reducing the vitamin E contents in oil.

Interestingly, the total carotene content was significantly higher in ECPO than in NECPO (p<0.05). Similarly, α-carotene was found higher in ECPO (331.6±0.02 mg/kg) compared with NECPO (304.3±0.02 mg/kg) (p<0.05). Furthermore, ECPO had a two-fold higher in β-carotene content (0.41±3.78%) compared with NECPO (0.21±1.54%) (p<0.05). This is likely due to the substantial damage to the seed cells that increased the extractability of the lipids and carotenoids, caused by the enzymatic pretreatment consists of cellulose, pectinase, and tannase in combination. Enzyme blends were reported able to disintegrate the cell wall that allowed the trapped oil globules and antioxidant compounds to be released (Mustafa *et al.*, 2022; Teixeira *et al.*, 2013). As overall, vitamin E and carotenoid contents are compared between with and without enzymatic pretreatments in this study, and potential reduction due to heat during mesocarp drying are acknowledged as a limitation of the study.

Table 2 also shows a lower cholesterol content of ECPO was detected (0.12%) than NECPO (0.20%) (p<0.05). Meanwhile, ECPO had a higher β-sitosterol (7.1%) than NECPO (6.1%, p<0.05). β-sitosterol is the principal plant sterol found in most oil seeds with cholesterol lowering properties (Kaseke *et al.*, 2021; Lomenick *et al.*, 2015). The results indicated that the enzymatic pretreatment has possibly influenced the cell wall permeability and extraction efficiency of the constituents, altering the release of cholesterol and phytosterol compared with the untreated sample, which merits additional study. It is noteworthy that oils rich in β-sitosterol and low in cholesterol have the potential to regulate the cholesterol levels, primarily lowering the risk of cardiovascular illnesses (Trautwein & McKay, 2020).

CONCLUSION

In conclusion, enzymatic pretreatment with enzyme blends of pectinase, cellulase and tannase did not alter the fatty acid profile of hydraulically pressed palm oil, which remained MUFAs > SFAs > PUFAs. Although enzymatic pretreatment of mesocarp produced palm oil with reduced vitamin E compounds but the total carotene content was remarkably influenced. Enzymatic pretreatment resulted in changes of the phytosterol contents, indicating the effectiveness in modulating the composition of palm oil recovered by hydraulic pressing. The study revealed the potential of enzymatic pretreatment using a blend of pectinase, cellulase and tannase to influence micronutrient profiles in hydraulically pressed palm oil, while maintaining an unchanged fatty acids profile.

ACKNOWLEDGEMENTS

This work was supported by Universiti Malaysia Sabah, Malaysia, under the Grant Scheme DN21096.

REFERENCES

[1] Aksu, M., Incegul, Y., Kiralan, S. S., Kiralan, M. & Ozkan, G. 2020. Cold Pressed Carrot (*Daucus carota subsp. sativus*) Seed Oil. *In*: Ramadan, M. F. (Ed.). *Cold Pressed Oils*. Massachusetts: Academic Press.

[2] Almeida, D. T. D., Viana, T. V., Costa, M. M., Silva, C. D. S. & Feitosa, S. 2018. effects of different storage conditions on the oxidative stability of crude and refined palm oil, olein and stearin (*Elaeis guineensis*). *Food Science and Technology*, 39(1), 211-217.

[3] AOAC International. 2016. *Official Method 996.06: Fat (Total, Saturated, and Unsaturated) in Foods.* (20th edition). In *Official Methods of Analysis of AOAC International.* AOAC International.

[4] Eitenmiller, R. R. & Lee, J. 2004. *Vitamin E: Food Chemistry, Composition, and Analysis*. Boca Raton: CRC Press.

[5] Gao, Z., Zhu, Y., Jin, J., Jin, Q. & Wang, X. 2023. chemical–physical properties of red palm oils and their application in the manufacture of aerated emulsions with improved whipping capabilities. *Foods*, 12(21), 3933.

[6] Hu, B., Li, Y., Song, J., Li, H., Zhou, Q., Li, C., Zhang, Z., Liu, Y., Liu, A., Zhang, Q. & Liu, S. 2020. Oil extraction from tiger nut (*Cyperus esculentus* L.) using the combination of microwave-ultrasonic assisted aqueous enzymatic method-design, optimization and quality evaluation. *Journal of Chromatography A*, 1627, 461380.

[7] Kaseke, T., Opara, U. L. & Fawole, O. A. 2021. Effects of enzymatic pretreatment of seeds on the physicochemical properties, bioactive compounds, and antioxidant activity of pomegranate seed oil. *Molecules*, 26(15), 4575.

[8] Loganathan, R., Tarmizi, A. H. A., Vethakkan, S. R. & Teng, K. T. (2020). Retention of carotenes and vitamin e, and physico-chemical changes occurring upon heating red palm olein using deep-fat fryer, microwave oven and conventional oven. *Journal of Oleo Science*, *69*(3), 167-183.

[9] Lomenick, B., Shi, H., Huang, J. & Chen, C. 2015. Identification and Characterization of *β*-sitosterol Target Proteins. *Bioorganic & Medicinal Chemistry Letters*, 25(21), 4976-4979.

[10] Mustafa, A. H., Rashid, S. S., Rahim, M. H. A., Roslan, R., Musa, W. A. M., Sikder, B. H. & Sasi, A. A. 2022. Enzymatic pretreatment of lignocellulosic biomass: An overview. *Journal of Chemical Engineering and Industrial Biotechnology*, 8(1), 1-7.

[11] Okpuzor, J., Okochi, V. I., Ogbunugafor, H. A., Ogbonnia, S., Fagbayi, T. & Obidiegwu, C. 2009. Estimation of cholesterol level in different brands of vegetable oils. *Pakistan Journal of Nutrition*, *8*, 57-62.

[12] Orhevba, B. A., Chukwu, O., Osunde, Z. D. & Ogwuagwu, V. 2013. Influence of moisture content on the yield of mechanically expressed neem seed kernel oil. *Academic Research International*, 4(5), 252-257.

[13] Permana, T., Wiradjaja, N., Sutanto, H. & Surya, V. S. 2022. A Review on the Potential of Natural Antioxidant Sources to Improve Oxidative Stability in Edible Oils. *Proceedings of the 6th International Conference of Food, Agriculture, and Natural Resource (IC-FANRES 2021)*. 4–5 August, 2021, Tangerang, Indonesia. pp 117-125.

[14] Prommaban, A., Kuanchoom, R., Seepuan, N. & Chaiyana, W. 2021. Evaluation of fatty acid compositions, antioxidant, and pharmacological activities of pumpkin (*Cucurbita moschata*) seed oil from aqueous enzymatic extraction. *Plants*, 10(8), 1582.

[15] Rabadán, A., Álvarez-Ortí, M., Gómez, R., Alvarruiz, A. & Pardo, J. E. 2017. Optimization of pistachio oil extraction regarding processing parameters of screw and hydraulic presses. *LWT-Food Science and Technology*, 83, 79-85.

[16] Sorokova, N., Didur, V. & Variny, M. 2022. mathematical modeling of heat and mass transfer during moisture–heat treatment of castor beans to improve the quality of vegetable oil. *Agriculture*, 12(9), 1356.

[17] Tan, C. H., Lee, C. J., Tan, S. N., Poon, D. T. S., Chong, C. Y. E. & Pui, L. P. 2021. Red palm oil: A review on processing, health benefits and its application in food. *Journal of Oleo Science*, 70(9), 1201-1210.

[18] Teixeira, C. B., Macedo, G. A., Macedo, J. A., da Silva, L. H. M. & Rodrigues, A. M. D. C. 2013. Simultaneous extraction of oil and antioxidant compounds from oil palm fruit (*Elaeis guineensis*) by an aqueous enzymatic process. *Bioresource Technology*, 129, 575-581.

[19] Tirgarian, B., Farmani, J. & Milani, J. M. 2019. Enzyme-assisted aqueous extraction of oil and protein hydrolysate from sesame seed. *Journal of Food Measurement and Characterization*, 13(3), 2118-2129.

[20] Trautwein, E. A. & McKay, S. 2020. The role of specific components of a plant-based diet in management of dyslipidemia and the impact on cardiovascular risk. *Nutrients*, 12(9), 2671.

[21] Yazdi, G. A. P., Barzegar, M., Sahari, M. A., & Ahmadi Gavlighi, H. 2019. Optimization of the enzyme-assisted aqueous extraction of phenolic compounds from pistachio green hull. *Food Science and Nutrition*, 7(1), 356-366.

[22] Zaaboul, F. & Liu, Y. 2022. Vitamin E in foodstuff: Nutritional, analytical, and food technology aspects. *Comprehensive Reviews in Food Science and Food Safety*, 21(2), 964-998.

[23] Zainul, R., Kurniawati, D., Elida, E., Santosa, I., Adha, S. A., Taridala, A.S., Rosmawaty, R., Hidrawati, H., Purbaningsih, Y., Aisyah, D. D. & Dumasari, D. 2024. comparative analysis of virgin coconut oil (VCO) production methods and their impact on nutritional and chemical properties. *Journal of Medicinal and Chemical Sciences*, 7, 2092-2107.