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Effects of Viscozyme L on the Yield of Oil Obtained from Fresh Avocado Fruit Pulp (*Persea americana* Mill.) by Three-Phase Partitioning Extraction Method

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Abstract

The applicability of Viscozyme L in extraction for recovery of avocado oil from fresh fruit pulp was studied. The effects of enzyme concentration, incubation temperature and time on the yield of avocado oil extracted by enzyme-assisted three-phase partitioning (EATPP) extraction method were investigated and the physicochemical properties of the final oil were evaluated. The highest oil yield obtained by EATPP using Viscozyme L was 39.79 ± 1.02 % on the dry basis of material, significantly higher than that extracted by TPP without enzyme (18.5 ± 0.76 %). The best conditions were 1.5 % (v/w) enzyme concentration, incubation at 50 ℃ for 1 h. Meanwhile, the yield of oil extracted by Soxhlet method was 58.10 ± 0.65 %, obviously higher than EATPP. However, EATPP gave better oil in terms of quality. The free fatty acid value of oil extracted by EATPP (0.87 ± 0.08 %) showed no significant difference compared to the oil extracted by Soxhlet method. Besides, oil extracted by EATPP had lower peroxide value (6.75 ± 0.29 meq O_2/kg oil) and higher total phenolic content (63.60 ± 2.73 mg GAE/100 g oil) than that by Soxhlet method. The fatty acid compositions of the oil extracted by EATPP mainly consist of palmitic acid, palmitoleic acid, oleic acid, and linoleic acid. The extraction of oil from fresh avocado fruit pulp utilizing EATPP can be used as an efficient method to reduce the avocado loss, thus saving cost, and increasing the product varieties from this kind of fruit.

Keywords: avocado oil, enzyme-assisted three-phase partitioning, fatty acid composition, peroxide value, total phenolic content

1. Introduction

Avocado (*Persea americana* Mill) is a subtropical-tropical tree which comes from the family *Lauraceae* (Dom ńguez *et al*., 2016). Avocado is mainly grown in Mexico, Dominican Republic, Peru, Colombia, Indonesia, and Kenya, where the conditions are favorable for its cultivation (Zafar and Sidhu, 2018). Avocado fruit consists of three parts including flesh (pulp), seed and peel. Different parts of the avocado fruit contain different amounts of functional compounds (Satriana *et al*., 2019). A 100-gram edible portion of Hass avocado fruit contained a large amount of water (72.3 g), lipid (15 g), few amounts of dietary fiber (6.8 g), total sugar (0.3 g), protein (1.96 g), and other vitamins and minerals (Dreher and Davenport, 2013). Moreover, avocado flesh is rich in bioactive phytochemicals and lipid-soluble antioxidants such as tocopherols, carotenoids (mainly lutein), phytosterols (mainly β-sitosterol) (Marco *et al*., 2014; Qin and Zhong, 2016). On the dry basis, the oil content in a Hass avocado fruit pulp can be up to 60-70 % (Ford *et al*., 2023). Avocado oil contains high level of monounsaturated fatty acids (50-60 % oleic acid and 2-11 % palmitoleic acid), low level of polyunsaturated fatty acids (12-21 % linoleic acid and 0.09-0.63 % linolenic acid) and comparatively high level of saturated fatty acids (21-25 % palmitic acid and 0.09-0.17 % stearic acid). The oil composition of avocado depends on not only the cultivar but also the ripening stage, growing location, and processing methods (Opiyo *et al*., 2023).

Avocado oil has been applied in many industries. In the food industry, avocado oil is used as a substitute for olive oil (Duarte *et al*., 2016) and is convenient for shallow pan frying due to its high smoke point (over 250 ℃) (Woolf *et al*., 2009). Avocado oil was proved to have a positive impact on the postprandial profile of total

cholesterol, low-density lipoproteins, glycemia, triglycerides, insulin, and inflammatory parameters in hypercaloric and hyperlipidic diets of healthy adults (Flores *et al*., 2019). Besides, due to high vitamin C content and unsaponifiable fractions that have regenerative properties of the epidermis, it has also been used in the cosmetic industry (Duarte *et al*., 2016).

Many techniques were developed and have been used to extract oil from avocado fruit. The techniques can be classified into three groups: physical, chemical, and biological methods. Physical methods include mechanical pressing, cold pressing, malaxation (Satriana *et al*., 2019) and aqueous separation. Aqueous separations using enzymatically assisted centrifugation, mechanically assisted centrifugation or mechanically assisted hot water are achievable due to their simple operation and low cost (Qin and Zhong, 2016). Chemical methods are using organic solvents with assistance of microwave or ultrasound. Biological methods are using specific enzymes in destruction of cell walls and lipid bodies to enhance extraction efficiency. It can be carried out under mild conditions in a short time without substantial energy but is not widely applied due to the costly enzyme, limited process control and the demand for further downstream processes to reduce the deterioration of the oil (Satriana *et al*., 2019).

Hexane has been widely used as solvent for the extraction of oil from plant materials. However, this solvent is flammable, non-biorenewable and can react with other pollutants to produce ozone and photo-chemical oxidants (Gandhi *et al*., 2003). In recent years, three-phase partitioning (TPP) extraction has emerged as a new method for recovering both protein and oil from plant materials (Gaur *et al*., 2007). This method is simple, expeditious and scalable, using a mixture of t-butanol and ammonium sulfate (Varakumar *et al*., 2017) to separate the plant materials into three layers: an organic upper layer, a precipitate of protein in the middle and lower aqueous layer with water-soluble solids (Gaur *et al*., 2007). The solvent t-butanol has a higher boiling point (84 ℃) than hexane (69 ℃) and thus it releases less volatile organic compounds to the environment. In addition, t-butanol was proved to have no genotoxic and reproduction toxicity and no mutagenic activity (Patocka and Kuca, 2012). For that reason, it is safe to use t-butanol in oil extraction. However, the yield of oil extracted by TPP is usually lower than that by hexane. Therefore, there is a need for some pretreatments to improve the oil yield such as enzymatic treatment. The slurry of the sample (mango kernel, soybean, or rice bran) was initially incubated with enzymes (protease) under optimal conditions, the efficiency of the technique was comparable to solvent extraction with some added advantages (Gaur *et al*., 2007). In another study, EATPP was showed to be as an efficient alternative to oleoresin extraction from turmeric, where enzymes alpha-amylase and glucoamylase were applied to release oil and resin from the matrix (Kurmudle *et al*., 2011). In addition, the enzyme-assisted three-phase partitioning (EATPP) extraction method has some advantages that are energy-saving and larger production volume which lowers the production cost.

Avocado oil bodies are distributed in avocado fruit pulp (mesocarp). The mesocarp of avocado is composed of evenly scattered idioblastic cells and mostly parenchyma cells. Idioblastic oil cells have thicker cell walls and larger diameter than the parenchyma cells. The oil exists in idioblastic cells as a single large drop and as finely dispersed oil emulsion in the parenchyma cells. The wall of idioblastic oil cell consists of three different layers: an outer inert layer made of cellulose, an intermediate layer made of suberin, and another layer of cellulose as the inner (third) layer. Viscozyme L is a multi-enzyme complex containing a wide range of carbohydrases, including arabanase, cellulase, beta-glucanase, hemicellulase and xylanase, which are effective in breaking down the cell wall, thus releasing the oil. Factors of interest in enzymatic treatment include enzyme type, enzyme concentration, substrate concentration, incubation temperature, pH, and dilution of paste to water (Qin and Zhong, 2016).

This study was carried out to evaluate the applicability of TPP method for extraction of oil from fresh avocado pulp as an alternative to solvent extraction methods, as well as the potential for improvement of oil yield by applying a hydrolytic enzyme (Viscozyme L) in the TPP method. The effect of enzyme concentration, enzyme incubation temperature and duration on the oil yield in EATPP process were investigated and discussed. The final oil extracted by EATPP was compared to that by Soxhlet method, in terms of extraction yield and some quality parameters such as free fatty acid values, peroxide values, total phenolic content, and fatty acid profiles.

2. Materials and Methods

2.1 Materials and Chemicals

Fresh Hass avocado fruits, about 5-7 days after being picked up, were purchased from local markets. The whole fruits were stored in the fridge shortly before processing (about 48 h). Avocado fruits after washing under running tape water were cut into halves to remove the seed and the pulp was separated from the skin with a spoon. Next the pulp was cut into small pieces and blanched in hot water at 75 ℃ for 5 min to retain the green

color. The pulp was then ground in a food processer to make a fine paste. The ground pulp was put into clean plastic zip lock bags and stored in a freezer until the day of experiment. The moisture content of the fresh avocado, determined by rapid moisture analyzer, was 77.33 ± 0.87 %.

Viscozyme L, in liquid form, from Novozymes was used in the experiments. The optimum conditions for Viscozyme L are a pH of 3.5-5.5 and a temperature of 25-55 ℃.

2.2 Conventional Solvent Extraction

The oil from dried avocado pulp was extracted according to a method described by Krumreich *et al*. (2018) with some slight modifications. The fresh avocado pulp after collected was dried in an oven at 60 ℃ for 24 h (moisture content of 3.95 ± 0.03 %, db). The dried avocado was ground into small pieces and 20 g of which was put into a paper thimble and subjected to extraction by Soxhlet system. The extraction was carried out with 200 ml of hexane for 4 h. After extraction, the oil was collected by evaporation in a rotary vacuum evaporator at 50 ℃ for 15 min at 100 rpm.

2.3 Enzyme-assisted Three-phase Partitioning Extraction

The method was taken from Gaur *et al*. (2007) with some slight modifications. For each extraction run, 10 g of the pulp was transferred to a 50-ml falcon with the addition of 7.5 mL of water of which the desired pH 5.0 was adjusted by 0.1 M NaOH and 0.1 M HCl. Next, an appropriate quantity of enzyme (0.0-2.0 %, v/w) was added, and the slurry was incubated for a certain period (30-90 min) at different temperature (30-60 ℃). After enzyme incubation, the enzyme was inactivated by heating at 90 ℃ for 20 min in a water bath. Four grams and a half of ammonium sulphate (30 %, w/v) was then added to this slurry, followed by the addition of 15 mL of organic solvents (t-butanol) and the whole mixture was vortexed for 5 min. The mixture was allowed to stand for 1 h at 30 ℃ to form three distinct phases, upper organic phase, lower aqueous phase, and interfacial precipitate layer. These were separated by centrifugation at 4 ℃ and 7000 rpm speed for 15 min. The upper organic layer was collected and evaporated in a vacuum evaporator at 50 ℃ to obtain the oil.

2.4 Determination of Extraction Yield of Oil

Oil content was calculated as the ratio of extracted oil to the weight of sample used on the dry basis as described by Akpabio *et al*. (2011):

$$Oil\ (\%, db) = \frac{Weight\ of\ extracted\ oil\ (g)}{Weight\ of\ raw\ material, db\ (g)}\ x\ 100$$

2.5 Determination of Free Fatty Acid Value

The free fatty acid (FFA) content of avocado oil was determined using the method described by Garcá *et al*. (1996) and Lanka and Jayewardenepura (2022) with some modifications. One gram of oil was prepared in a 250 mL Erlenmeyer flask. Then 20 mL neutralized ethanol and 0.5 mL phenolphthalein indicator was added. The flask was shaken so that the mixture could dissolve completely. Titration with standard base (0.1 M NaOH) was carried out while the flask was shaken vigorously until the endpoint was reached. This was indicated by a slight pink color that persisted for 30 s. The volume of titrant used was recorded and the FFA value of each sample was calculated as follows:

$$FFA\ (\%) = \frac{V\ x\ N\ x\ 282}{W}\ x\ 100$$

where FFA (%) was the percentage of free fatty acid (g/100 g), expressed as oleic acid, V – the volume of NaOH titrant (mL), N – the normality of NaOH titrant (mol/1000 mL), 282 – the molecular weight of oleic acid (g/mol), and W – the sample mass (g).

2.6 Determination of Peroxide Value

Peroxide value (PV) was measured according to a method of Garcá *et al*. (1996) with a slight modification for avocado oil. One gram of avocado oil was placed in a 250-mL Erlenmeyer flask. Each sample was shaken and dissolved in 12.5 mL of an acetic acid-chloroform solution (3:2, v/v). Next, 0.5 mL of saturated potassium iodide (KI) solution was added, and the flask was placed in darkness for 5 min. After that period, 37.5 mL of distilled water was added, and the mixture is titrated with 0.005 M sodium thiosulphate with 1 % (w/v) starch indicator solution. The result was expressed in milliequivalents of oxygen per kilogram of avocado oil (meq O_2/kg oil). Peroxide value was calculated by the equation:

$$PV = \frac{(V_1 - V_0)\, x\, T}{m}\, x\, 1000$$

where V_0 was the volume of sodium thiosulphate used for blank titration (mL), V_1 – the volume of sodium thiosulphate used for sample titration (mL), T – the normality of sodium thiosulphate (0.005 M), and m – the mass of the sample (g).

2.7 Determination of Total Phenolic Content

The total phenolic content (TPC) was carried out using the method described by Parry *et al*. (2006) and Rombaut *et al*. (2015) with some modifications. Initially, 1 g of oil was mixed with 1 mL of hexane and vortexed for 2 min. Next, 5 mL of methanol was added and vigorously vortexed for 5 min. The mixture was then centrifuged at 25 ℃ at 3500 rpm for 15 min. The oil residues were re-extracted once again with methanol. The two methanolic extracts were combined and the final volume was brought to 25 mL with methanol to obtain the testing sample solution.

Two mL of Folin-Ciocalteu reagent (diluted 10-fold with distilled water) was added to 0.4 mL of the extract. After 5 min, 1.6 mL of Na_2CO_3 solution (7.5 %, w/v) was added. The mixture was vortexed for 3 min and heated at 50 ℃ for 10 min in a water bath. After that, absorbance was measured at 750 nm using a UV–vis spectrophotometer against a blank solution made with methanol. A standard Gallic acid curve was constructed by preparing the dilutions in methanol from 1 % solution of Gallic acid.

2.8 Determination of Fatty Acid Profile

The oil was converted into fatty acid methyl esters (FAMEs) and analyzed by gas chromatography GC-Shimadzu 2010 with a flame ionization detector (FID). The method (CASE.SK.0107-GC) was adopted from GC-ISO/CD 5509:94. FAMEs were analyzed on an SP-2560 column (100 x 0.25 x 0.2 μm). Nitrogen was used as a gas carrier with a pressure of 20 psi. The initial temperature was 70 ℃, then programmed to increase to 250 ℃, and the detector temperature was held at 250 ℃. The flow rate was kept constant at 20 mL/min.

2.9 Statistical Analysis

All the analyses were performed in triplicate that were reported as mean ± standard deviations. ANOVA test and t-test were used for statistical analysis of data by using statistical software SPSS Statistics 20. Significant differences between the means of parameters were determined by using Tukey's test ($p < 0.05$).

3. Results and Discussion

3.1 Effects of Process Conditions on the Total Oil Yield

Effects of five different Viscozyme L concentrations (0, 0.5, 1.0, 1.5, and 2.0 %, v/w) on oil yield were illustrated in Fig. 1A. The oil yield increased slightly from the concentration of 0 to 1.0 %, reached a peak at 1.5 % and decreased at 2.0 % concentration. At 1.5% enzyme concentration, the oil yield obtained was 34.21 ± 1.55 %, which was highest. The decrease in oil yield at 2.0 % enzyme (26.87 ± 1.32 %) was possibly due to the undesirable components extracted by enzymatic activities, which resulted in a lower oil yield. Viscozyme L, a multi-enzyme complex containing a wide range of carbohydrase, had great effects on breaking down the cell wall, thus releasing the oil. Compared to other studies, Qin and Zhong (2016) reported the highest yield of avocado oil at an enzyme concentration of 1.0 % using α-amylase. Furthermore, Viscozyme L was reported to give the highest oil yield from peanuts at the concentration of 1.25 % (Liu *et al*., 2020). A study conducted by Kurmudle *et al*. (2011) revealed the glucoamylase concentration of 2 % gave the highest yield of turmeric oleoresin. The results from those studies are slightly different from this study due to the differences in the type and density of the used enzyme and the compositions of the plant materials.

The effects of Viscozyme L incubation temperature on oil yield at four temperature levels (30, 40, 50 and 60 ℃) were illustrated in Fig. 1B. The oil yield obtained increased slightly from the temperature of 30 to 50 ℃ and then decreased to 60 ℃. The highest oil yield, obtained at 50 ℃, was 38.91 ± 0.67 %. The reduction in oil yield at 60 ℃ was possibly due to partial inactivation of enzyme at high temperature. Since the optimum temperature range for Viscozyme L was reported to be in the range of 25-55 ℃, the rate of catalysis reaction decreased at 60 ℃. According to Shah *et al*. (2005), the highest oil yield from *Jatropha* seed kernels was obtained at 40 ℃ with the use of cellulase enzyme. The highest oil yield was reported to be obtained with the enzymatic pretreatment at 50 ℃, utilizing Viscozyme L, in the oil extraction from peanut (Liu *et al*., 2020) and shea fat extraction (Otu *et al*., 2015). The incubation temperature of Viscozyme L showed significant effect on the yield of oil and the temperature of 50 ℃ could be a relevant choice for avocado fruit pulp.

Effects of Viscozyme L incubation duration on oil yield at three levels (30, 60, and 90 min) were illustrated in Fig. 1C. The highest oil yield (38.77 ± 0.44 %) was obtained at 50 ℃ in 60 min. An increase in the incubation time from 60 to 90 min did not increase the oil yield. The oil from the cell would be exhausted when the extraction time was long (Asoiro *et al*., 2019). The prolonged exposure to heat during incubation can degrade the oil (Rodr ǵuez-Miranda *et al*., 2014), thus decreasing the extracted oil. This can be used to explain the drop in oil yield after 90 min of incubation. It was reported that the highest oil yield, utilizing Viscozyme L, was obtained after 60 min of the enzymatic pretreatment in the shea fat extraction (Otu *et al*., 2015), while Liu *et al*. (2020) obtained the highest oil yield after 80 min in the oil extraction from peanut. Another study conducted by Qin and Zhong (2016) recorded an enzyme incubation time of 60 min using α-amylase in the oil extraction from avocado fruit. Therefore, an incubation duration of 60 min could be an appropriate choice for Viscozyme L in this study.

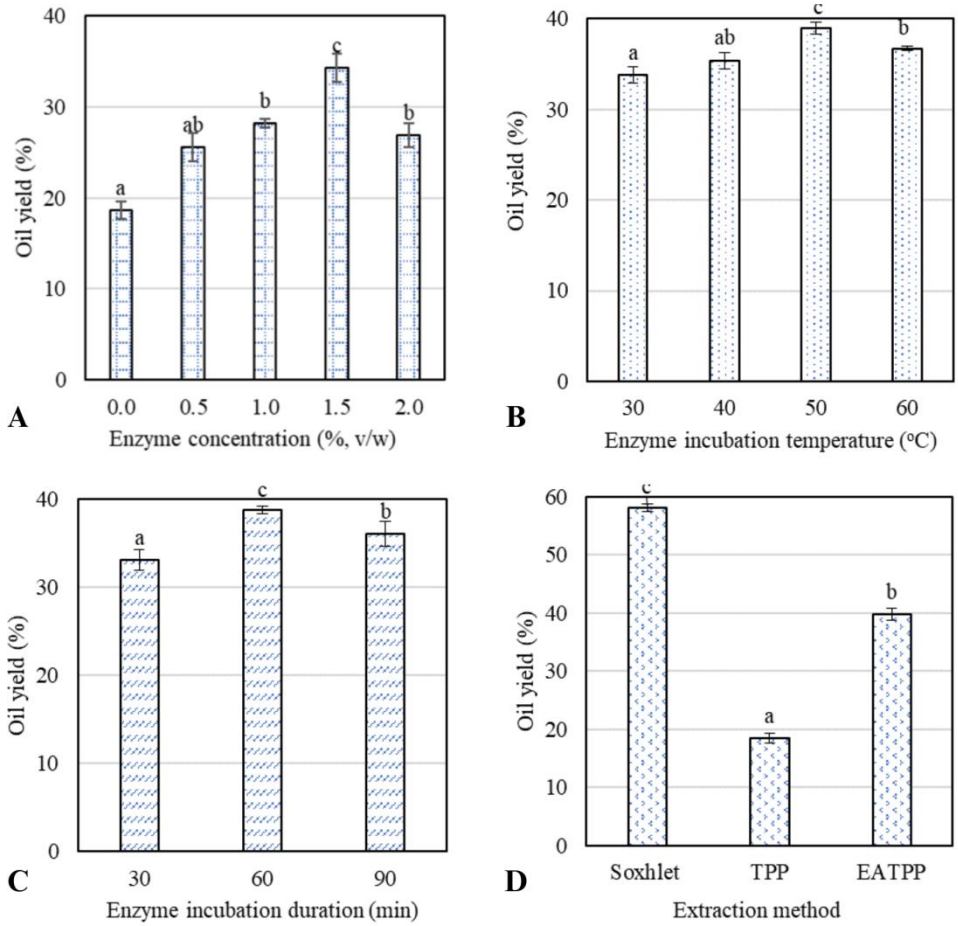

Figure 1. Effects of enzyme concentration (A), incubation temperature (B), incubation duration (C), and extraction method (D) on the yield of avocado oil

Note: Different letters indicate statistically significant differences at $p < 0.05$.

3.2 Effects of Different Extraction Methods on Oil Yield

The oil yields obtained by different extraction methods (Soxhlet, TPP, EATPP) were presented in Fig. 1D. The Soxhlet method provided the highest yield of oil (58.10 ± 0.65 %), while TPP method gave the lowest (18.50 ± 0.76 %). When Viscozyme L was applied to TPP, the oil yield increased up to 39.79 ± 1.02 %. An increase of 21.29 % of oil in the EATPP as compared to TPP showed the benefits of enzyme utilization in the oil extraction from fresh avocado fruit pulp. The same trend was also recorded in the study of Gaur *et al*. (2007) that the oil yield obtained by EATPP (around 12.64 g oil/100 g kernel) was significantly lower than that extracted by Soxhlet method (16 g oil/100 g kernel) for the oil extraction from mango kernel. Shah *et al*. (2005) also reported a significant increase up to around 40.48 g oil/100 g kernels in the yield of oil obtained by EATPP as compared to TPP alone (36.08 g oil/100 g kernels) and lower than that by Soxhlet method (44 g oil/100 g kernels) in the oil

extraction from *Jatropha curcas* L. seed kernels.

In addition, EATPP presents a potential method for oil extraction from plant materials as an alternative to conventional solvent extraction. The shorter extraction time and lower temperature in EATPP contributed to the better quality of oil. The solvent t-butanol was much safer than hexane used in the Soxhlet method as it releases less of volatile compounds to the atmosphere even if it is carried out as an open system. Therefore, EATPP can be used as a novel and ecofriendly approach in the oil extraction from fresh avocado fruit pulp.

3.3 Physicochemical Analysis

Statistical analysis results showed no significant difference in FFA value of oil extracted by Soxhlet and EATPP methods, 0.78 ± 0.01 and 0.87 ± 0.08 %, respectively (Table 1). This result was probably due to the hydrolysis of triglycerides at high temperature when the enzyme was deactivated at 90 ℃ at which triglycerides were degraded into free fatty acids and glycerol. The two values recorded in this study were both higher than the FFA value of Fuerte avocado oil reported by Orhevba and Jinadu (2011) (0.62 %). The level of FFA value depends on time, temperature and moisture content since the oil and fats can be affected by some environmental factors such as storage and processing conditions (Mahesar *et al*., 2014).

Peroxide value (PV) expresses the stability of oil towards oxidation. Therefore, lower peroxide value indicates better quality of oil. The oxidation level could be influenced by several factors such as the preservation of raw material, duration extraction or the changes of extraction process peculiarities (Santos *et al*., 2012). PV of oil extracted by Soxhlet method was significantly higher than that extracted by EATPP (7.84 ± 0.52 meq O_2/kg oil and 6.75 ± 0.29 meq O_2/kg oil, respectively) (Table 1). Similarly, in the study of Juvvi and Debnath (2020), the PV in oil from *Sesamum indicum* L. extracted by solvent (1.70 ± 0.1 meq O_2/kg oil) was higher than that by EATPP utilizing pectinase (1.40 ± 0.2 meq O_2/kg oil). The two values in this study were in the range of 5.1-12.3 meq O_2/kg oil, which was the PV range of avocado oil reported by Indriyani *et al*. (2016). On the other hand, a PV of 3.79 meq O_2/kg oil in the solvent-extracted oil was determined for the Hass variety (Krumreich *et al*., 2018).

Table 1. Effects of extraction method on the quality of oil

Method	FFA value (% as oleic acid)	Peroxide value (meq O_2/kg oil)	Total phenolic content (mg GAE/100g oil)
Soxhlet	0.78 ± 0.01[a]	7.83 ± 0.52[a]	45.13 ± 3.45[a]
EATPP	0.87 ± 0.08[a]	6.75 ± 0.29[b]	63.60 ± 2.73[b]

Note. Different letters (in the same column) indicate statistically significant difference at $p < 0.05$.

Total phenolic content (TPC) was proved to have correlation with many biological effects such as antioxidant activity. Therefore, high TPC indicates a better quality of oil. TPC of oil extracted by Soxhlet method was significantly lower than that extracted by EATPP (45.13 ± 3.45 mg GAE/100g oil and 63.60 ± 2.73 mg GAE/100g oil, respectively) (Table 1). The low level of TPC in avocado oil extracted by Soxhlet method can be explained by the long extraction time (4 h) and high extraction temperature (70 ℃), thus degrading the bioactive compounds in oil. The TPC of avocado oil in this study were both higher than that in research conducted by Krumreich *et al*. (2018) using solvent extraction (39.53 ± 3.52 mg GAE/100g oil). The difference in the TPC values was possibly due to different avocado variety and extraction methods. In addition, the TPC in avocado oil extracted by various methods was reported in the range of 4.26 – 130.17 mg GAE/100g (Tan, 2019).

3.4 Free Fatty Acid Profile

The oil extracted by EATPP contained lower percentage of total saturated fatty acids (TSFA) (26.87 %) and higher of total unsaturated fatty acids (TUFA) (72.28 %) than Soxhlet method (29.02 and 69.76 %, respectively). The predominant fatty acids found in the avocado oil extracted by the two methods (in order) were oleic acid (C18:1), palmitic acid (C16:0), linoleic acid (C18:2), and palmitoleic acid (C16:1). The oil extracted by Soxhlet method contained higher amount of palmitic acid (27.9 %) and palmitoleic acid (15.33 %) than the oil extracted by EATPP (25.92 and 13.88 %, respectively). In contrast, EATPP produced oil with higher oleic acid (39.99 %) and linoleic acid (17.26 %) than Soxhlet method (37.36 and 15.9 2%). Noticeably, there was not any trans-fatty acid reported in the oil of this study (Table 2).

Table 2. Fatty acid compositions of avocado oil extracted by different methods

Fatty acid	Content in oil (%)				
	Soxhlet (this study)	EATPP (this study)	Soxhlet (Moreno *et al*., 2003)	Commercial refined avocado oil (Haiyan *et al*., 2007)	Cold-pressed avocado oil (Haiyan *et al*., 2007)
Myristic acid (C14:0)	0.06	-	-	-	-
Palmitic acid (C16:0)	27.90	25.92	15.71	16.30	14.10
Palmitelaidic acid (C16:1)	-	-	0.16	-	-
Palmitoleic acid (C16:1)	15.33	13.88	7.26	7.70	5.70
Cis-10-Heptadecanoic acid (C17:1)	0.12	0.06	-	-	-
Stearic acid (C18:0)	0.58	0.55	0.72	0.60	0.40
Elaidic acid (C18:1)	-	-	0.30	-	-
Oleic acid (C18:1)	37.36	39.99	60.28	62.70	69.10
Linoleic acid (C18:2)	15.92	17.26	13.66	11.40	9.60
Linolenic acid (C18:3)	0.89	0.96	1.44	0.80	0.60
Gadoleic acid (C20:1)	-	-	0.21	-	-
Arachidic acid (C20:0)	0.08	0.07	0.11	0.10	0.10
Eicosaenoicacid (20:1)	0.14	0.13	-	0.20	0.20
Heneicosanoic acid (C21:0)	0.08	-	-	-	-
Behenicacid (C22:0)	0.15	0.07	-	< 0.10	< 0.10
Lignoceric acid (C24:0)	0.17	0.26	-	-	-
Linolelaidic acid (trans 18:2)	-	-	0.04	-	-
TSFA	29.02	26.87	16.54	17.10	14.7
TUFA	69.76	72.28	82.64	82.80	85.20
MUFA	52.95	54.06	67.75	70.60	75.00
PUFA	16.81	18.22	15.10	12.20	10.20

Note. TSFA = total saturated fatty acids, TUFA = total unsaturated fatty acid, MUFA = monounsaturated fatty acid, PUFA = polyunsaturated fatty acid.

The avocado oil in this study had higher amount of TSFA and lower amount of TUFA when compared with the oil in the study of Moreno *et al*. (2003), refined oil and cold-pressed avocado oil reported by Haiyan *et al*. (2007). Of four predominant fatty acids previously mentioned, oleic acid content was much lower than other research, while content of the others three was much higher. In addition, only Moreno *et al*. (2003) reported the presence of trans-fatty acid in the oil extracted by Soxhlet method. Fatty acid composition depends on maturity, season, growing conditions of avocado trees and the ripening process of avocado fruits. As the fruits grow and mature, the triglyceride content in the flesh increases (Woolf *et al*., 2009). In comparison with commercial ripening, climacteric ripening on the trees affects both the oil yield and fatty acid compositions of avocado oil. In addition, the quantification of different analytes depends on the processing, conditions of extraction and detection limits of the analytical equipment (Flores *et al*., 2019).

In summary, the absence of trans-fatty acid in the oil extracted by EATPP indicates the suitability of it as edible oil. Moreover, the oil is rich in TUFA, especially in oleic and linoleic acids, which were reported to have a positive correlation with many health benefits (Kaur *et al*., 2014).

4. Conclusion

Recently, TPP process has attracted interest of researchers as an alternative method for recovery of plant oil and protein. In combination with enzymes, EATPP method is one of modifications from TPP method, which aims to

increase oil yield and quality of oil. The EATPP method showed a good potential for oil extraction from fresh avocado fruit pulp. With the application of Viscozyme L, the oil yield was improved and the highest yield of avocado oil (38.77 ± 0.44 %) extracted by EATPP was obtained under the conditions using 1.5 % enzyme concentration, at 50 ℃ in 1 h. The oil extracted by EATPP was of higher quality than that by Soxhlet method in term of peroxide value, total phenolic content, and free fatty acid value. The oil extracted by EATPP contained a relatively high amount of TUFA, a reasonable amount of ω-6 and no trans-fatty acids. The extraction of oil from fresh avocado fruit pulp using EATPP is a feasible method, which could avoid energy-consuming step of drying, thus saving cost, and increasing the product varieties from this kind of fruit. Though the use of enzyme in TPP gave a positive approach in the oil extraction, it still needs further research to increase the oil yield and obtain much higher quality of oil. It is recommended for further study in application of different types of enzymes, enzyme combination and optimization of the process by using experimental design methodology in extraction of oil from avocado pulp by using EATPP.

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Informed consent

Obtained.

Ethics approval

The Publication Ethics Committee of the Canadian Center of Science and Education.

The journal's policies adhere to the Core Practices established by the Committee on Publication Ethics (COPE).

Provenance and peer review

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Data availability statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Data sharing statement

No additional data are available.

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