THE EFFECT OF EXTRACTION TIME ON THE PHYSICOCHEMICAL CHARACTERISTICS AND ANTIOXIDANT POTENTIAL OF AVOCADO WASTE OIL PRODUCED BY THE SOXHLETATION METHOD

Dyah Widowati[1*], Artini Pangastuti[2] and Setyaningrum Ariviani[3]

[12]Department of Bioscience – Postgraduate Program – Universitas Sebelas Maret
Jalan Ir. Sutami 36 A – Surakarta – Indonesia 57126
[3]Department of Food Science and Technology – Faculty of Agriculture – Universitas Sebelas Maret
Jalan Ir. Sutami 36 A – Surakarta – Indonesia 57126
Corresponding Author, email: dyah.mutiarso@gmail.com

ABSTRACT

The avocado peels and seeds are potentially produced into vegetable oil through the soxhletation method. The soxhlet extracted oil characteristics are influenced by extraction time. The proper extraction time generated the oil standard characteristics. This research aimed to determine the effect of extraction time on yield, physicochemical characteristics, and antioxidant potential of avocado waste oil. The research was conducted through the extraction and characterization of avocado waste oil. This study used a Completely Randomized Design with one factor (extraction time: 4, 5, and 6 hours). An increase in extraction time caused an increase in yield and specific gravity, but the hydrolytic-oxidative stability and antioxidant potential were decreased. The 4 hours of extraction time was selected as the best treatment. The characteristics of avocado peel oil extracted for 4 hours were 6.25% of yield, 0.961 g/mL of specific gravity, 0.27% of free fatty acids (FFA), 0.44 mgKOH/g of acid value (AV), 0.98 mEq/kg of peroxide value (PV), 4.63 mEq/kg of p-Anisidine value (PAV), 8.86 mEq/kg of total oxidation (TOTOX), 68.786% of radical scavenging activity, and 523.96 mgTr/g of reducing power. The characteristics of avocado seed oil extracted for 4 hours were 4.59% of yield, 0.973 g/mL of specific gravity, 0.23% of FFA, 0.33 mEq/kg of AV, 0.48 mEq/kg of PV, 3.77 mEq/kg of PAV, 5.05 mEq/kg of TOTOX, 53.95% of radical scavenging activity, and 438.05 mgTr/g of reducing power. Altogether, avocado waste oil characteristics in each extraction time met the vegetable oil requirements and potentially developed in food industries.

Keywords: Avocado Peel Oil; Avocado Seed Oil; Soxhlet Extraction Method

INTRODUCTION

The utilization of avocados mainly involves the flesh, so the avocado peel and seed have the potential to become waste (Salazar-López *et al*., 2020). Avocado fruits consist of pulp (71.89%), seed (21.22%), and peel (6.89%) (Krumreich *et al*., 2018). Every 1000 kg of avocado fruit processing generates 274 kg of avocado peels and seeds as waste (Permal *et al*., 2020). Food waste has 8% of total anthropogenic greenhouse gas emissions (FAO, 2015), which can lead to climate change. Thus, several efforts have been carried out to avoid the environmental problems that arise from avocado waste, for example, the avocado waste utilization in the textile, biopolymer, bioenergy, pharmaceutical, and food industries (Tesfaye *et al*., 2022).

Avocado waste is high in unsaturated fatty acids (UFA) and antioxidant potential. The Hass avocado displayed higher UFA content (2570 mg/100 g in avocado peel and 116.28 mg/100 g in avocado seed) than saturated fatty acids content (1061 mg/100 g in avocado peel and 85.26 mg/100 g in avocado seed) (Amado *et al*., 2019). The Total Phenolic Content of avocado seed oil was 1987-2992 mg GAE/100 g, while in avocado

peel oil was 10730 mg GAE/100 g (Páramos *et al*., 2020). The radical scavenging activity by DPPH assay of avocado peel was 46.49 mMol Trolox eq./100 g, while the avocado seed was 32.51 mMol Trolox eq./100 g. The reducing power by FRAP assay of avocado peel was 379.31 mMol Trolox eq./100 g, and the avocado seed was 256.34 mMol Trolox eq./100 g (Velderrain-Rodríguez *et al*., 2021). The avocado seed contains 3-4 wt% lipids, and the avocado peel contains 2-6 wt% lipids (Patra *et al*., 2022). These characteristics make the avocado waste potentially produced into vegetable oil used as functional food ingredients and food processing ingredients (edible oil, stabilizer agent, etc.) (Ogbuagu and Okoye, 2020; Tesfaye *et al*., 2022).

The easiest way to take advantage of UFA content and antioxidant potential in avocado waste is oil production through the soxhlet extraction method (Páramos *et al*., 2020). Ethanol has been widely used in natural sources extraction and classified as a less hazardous solvent. Ethanol has a high oil yield produced capability and is low-cost (Tekin *et al*., 2018). The avocado seed oil yield using ethanol as a solvent by soxhlet extraction was higher (9.5-10.3%) than the supercritical carbon dioxide extraction method (2.1%). The soxhlet method with ethanol was also proved to generate a higher yield in avocado peel oil (10-14%) and avocado seed oil (9.8-10.3%) than n-hexane (3.27-3.6% in avocado seed oil) or ethyl acetate (3.1-4.8% in avocado seed oil) (Páramos *et al*., 2020). The soxhletation method produced a higher avocado oil yield (55.2%) than the mechanical pressing method (42.8%) (Krumreich *et al*., 2018).

The heating treatment also affects the yield, physicochemical characteristics, and antioxidant activity of avocado waste oil. Pratama *et al*. (2017) presented that the avocado seed oil extracted for 3 hours resulted in a higher yield (23.53%) than 1 or 2 hours of extraction. However, the antioxidant activity of 1-hour extraction was higher (54.91%) than 2 or 3 hours of heating treatments. Ginting *et al*. (2020) explained that the increase in the amount of heat through increasing the time and temperature of extraction increased the FFA value of avocado oil. The highest FFA value (1.84%) was at 85 °C, 240 mins of heating treatment, and the lowest value (1.18%) was at 75 °C, 120 mins of heating treatment. Thus, the soxhlet method using ethanol solvent is widely used to determine the proper extraction time to optimize the yield, physicochemical characteristics, and antioxidant potential of the extracted oil.

Thermal oxidation of oil was proven to decrease nutritional safety because the oxidation products react with DNA, protein, or lipid and lead to cellular function damage. Cardiovascular disease is a high-mortality disease, including cell injury, deterioration of enzymes, and the mutagenicity of nucleic acid caused by thermal oil oxidation (Ng *et al*., 2014). Vegetable oil quality can be evaluated through FFA, AV, PV, IV, PAV, and TOTOX analysis (Martínez-Yusta *et al*., 2014; Owuna, 2020). The antioxidant potential of vegetable oil can be analyzed through the radical scavenging activity by the 2,2-Diphenyl-1picrylhydrazyl (DPPH) assay and reducing power by the Ferric Reducing Antioxidant Power (FRAP) method (Shahidi and Zhong, 2015). The acceptance requirements of the oil extracted from vegetable sources are <5% of FFA, <4 mgKOH/g of AV, <15 mEq/kg of PV, and <20 mEq/kg of TOTOX (Codex, 2017).

A previous study reported that the avocado peel and seed oil extracted for 6 hours had the highest oil yield and antioxidant activity using ethanol as a solvent (Páramos *et al*., 2020). Sathish *et al*. (2021) reported an increase in oil yield along with an increasing extraction time of avocado seed oil. Pratama *et al*. (2017) stated that the antioxidant activity decreased along with increasing extraction time due to the degradation of tocopherols and other phenolic compounds in avocado seed oil. The evaluation of the effect of extraction time on physicochemical characteristics and antioxidant potential of avocado peel and seed oil with extraction time variation of 4, 5, and 6 hours has not been reported. This study aimed to evaluate the effect of extraction time on the yield, physicochemical characteristics (specific gravity, FFA, AV, PV, PAV, and TOTOX), and antioxidant potential (radical scavenging activity and the reducing power) of avocado waste oil.

METHOD

A local avocado cultivar (Ijo Panjang) was obtained from a fruit distributor in Surakarta, Indonesia. The equipment used in this study was a soxhlet extractor (Pyrex), a vacuum pump (KnF N820, Germany), a rotary evaporator (Buchi, Indonesia), a spectrophotometer UV-Vis (Shimadzu UV-1240, Japan), oven (Memmert, UN55, Germany), grinder (Phillips, Japan), glassware (Pyrex), hotplate (Heidolph, Germany), magnetic stirrer, vortex (Gemmy, Taiwan), and hematocrit tube. The chemicals used in this study were ethanol 96% (Merck), methanol (Merck), distilled water, acetic acid (Merck), chloroform (Merck), saturated KI (Merck), Na-Thiosulfate (Merck), starch solution (Merck), NaOH (Merck), oxalic acid (Merck), phenolphthalein indicator (Merck), KOH (Merck), trimethylpentane (Merck), p-Anisidine (Merck), DPPH (Sigma Aldrich), acetic buffer (Sigma Aldrich), 2,4,6-tripyridyl-s-triazine buffer (Sigma Aldrich), HCl (Merck), $FeCl_3$ (Merck), and Trolox (Sigma Aldrich).

Avocado Waste Oil Extraction by Soxhletation Method

The extraction of avocado waste oil through the soxhletation method is based on Páramos *et al*. (2020) with modifications. Both avocado peels and seeds were cleaned and heated using an oven at 60 °C for 6 hours. Each peel and seed was crushed using a grinder and sieved with a size of 40 mesh. Each avocado peels and seeds powder (@100 g) was prepared for soxhlet extraction. Ethanol 96% was used as a solvent with the ratio of material: solvent = 1:3. The extraction was conducted at 78-80 °C. The extraction time treatments were 4, 5, and 6 hours in each avocado peel and seed and continued with the solvent evaporation at 40 °C, 30 rpm. Avocado waste oil was stored in dark storage at 10 °C until further analysis.

Yield Percentage (Kamini *et al*., 2016)

Analysis was done by calculating the oil to the sample weight ratio.

$$\% \text{ Yield} = \frac{\text{produced oil weight (g)}}{\text{sample weight (g)}} \times 100\% \quad \text{.....(1)}$$

Specific Gravity (AOCS Cc 10c-95, 2017)

The oil was added to a pycnometer, and then the pycnometer was closed and weighed. The oil was discarded and changed with the water, and re-weighed. The water was discarded, and then the empty pycnometer was re-weighed.

$$\text{Specific Gravity}\left(\frac{g}{mL}\right) = \frac{(A)-(C)}{(B)-(C)} \quad \text{.....(2)}$$

Description:
A = Pycnometer + oil sample weight
B = Pycnometer + water weight
C = Empty pycnometer weight

Free Fatty Acid (AOAC Cd 8-53, 1996)

The 0.1 N NaOH was standardized by oxalic acid dissolved in 25 mL of distilled water. An indicator of PP (3 drops) was added to the solution and titrated with NaOH until the color changed (pink constant). The oil was weighed (1 gram). Then 25 mL of 96% ethanol was added to the oil sample at 40°C. An indicator of PP (2 drops) was added to the mixture and titrated with 0.1 N NaOH until the color changed (pink constant).

$$\% \text{ FFA} = \frac{\text{V. NaOH x N.NaOH x MW of oleic acid}}{\text{Sample weight (g)}} \text{x}100\% \text{ ..(3)}$$

Acid Value (AOCS Cd 3d-63, 2009)

The sample (10-50 mg) was dissolved with 96% ethanol (50 ml). The mixture was added with an indicator of PP (2 drops) and titrated with 0.1 N KOH until the color changed into pink constant.

$$\text{AV}\left(\frac{\text{mgKOH}}{\text{g}}\right) = \frac{\text{V. KOH (mL)x KOH concentration}\left(\frac{\text{mg}}{\text{mL}}\right)\text{x MWof KOH}}{\text{Sample weight (g)}} \text{.(4)}$$

Peroxide Value (AOAC 965.33-1969)

1 ml oil was put into a closed erlenmeyer. In the mixture of acetic acid: chloroform (3:2), as much as 5 ml was added to the oil and stirred until homogeneous. Saturated KI (0.1 ml) and distilled water (6 ml) were added to the mixture and titrated with 0.01 N Na-Thiosulfate until the yellow color almost disappeared. The titration was paused, and 1% starch solution (0.4 ml) was added to the mixture. The titration was continued until the blue-black color disappeared.

$$PV\ \left(\frac{mEq}{kg}\right) = \frac{V.\ NaThiosulfate \times N.NaThiosulfate \times 1000}{Sample\ weight\ (g)} .(5)$$

P-Anisidine Value (AOCS Cd 18-90, 2011)

The 1st test solution was prepared by mixing the oil sample (1 g) into trimethylpentane (25 ml). The 2nd test solution was prepared by mixing 1 mL of p-Anisidine into 5 ml of 1st test solution and stored in the dark. The standard solution was prepared by mixing p-Anisidine solution (1 ml) into trimethylpentane (5 ml) and kept in the dark. The absorbance value of the 1st test solution was measured at λ 350 nm, while the 2nd test solution was at the same wavelength but 10 mins later. The reference solution was used as compensation.

$$PAV\ \left(\frac{mEq}{kg}\right) = \frac{25\ X\ (1,2\ abs.\ of\ 1st\ test\ solution - abs\ of\ 2nd\ test\ solution)}{Sample\ weight\ (g)} ..(6)$$

Total Oxidation (Suseno *et al.*, 2016)

$$TOTOX\ \left(\frac{mEq}{kg}\right) = (2xPV) + PAV\ .....(7)$$

Radical Scavenging Activity by DPPH Assay (da Silva *et al.*, 2022)

The standard solution of DPPH was prepared by dissolving 2.7 mg of DPPH into 50 ml methanol and stored in the dark. The antioxidant activity was measured by dissolving 0.1 mg of the sample into 5 ml methanol and vortexed for 5 mins. The mixture was closed and incubated in dark conditions for 24 hours. The mixture was taken at about 0.1 ml, and then 1 ml of DPPH standard solution and 4.9 ml of methanol were added. The mixture was incubated at room temperature for 30 mins (λ=517 nm).

$$Antioxidant\ activity\ (\%) = \frac{Control\ absorbance - sample\ absorbance}{control\ absorbance} 100\% .(8)$$

Reducing Power by FRAP Assay (Velderrain-Rodríguez *et al.*, 2021)

The oil sample (150 µL) and FRAP solution were mixed and incubated at room temperature in dark conditions for 30 mins. Absorbance was measured at λ=593 nm. The standard solution was made using a Trolox solution. FRAP solution was prepared by mixing 25 ml of 300 mM acetate buffer pH 3.6; 2.5 ml 2,4,6-tripyridyl-s-triazine 10 mM in 40 mM HCl; 2.5 mL $FeCl_3$ 20 mM.

$$Reducing\ power\ \left(\frac{mgTr}{g}\right) = \frac{Sample\ concentration \times vol.\ of\ used\ extract \times dilution\ factor \times 0.001}{Sample\ weight} ……..(9)$$

Statistics Analysis

The data were presented as mean ± standard deviations (three sample replication). The statistical analysis used a completely randomized design with one factor (extraction time variation: 4, 5, and 6 hours) in each avocado peel and seed. The data were analyzed using a one-way analysis of variance and continued with Duncan's Multiple Range Test ($p<0.05$).

RESULTS AND DISCUSSION

The yield and specific gravity of avocado peel and seed oil were presented in Table 1. The extraction time variations affected the yield and specific gravity of avocado waste oil. The avocado peel and seed oil yield with 6 hours of extraction had a higher result than 4 or 5 hours of extraction. The oil yield of avocado peel showed a higher result than avocado seeds in each extraction time treatment. These results were in line with the study of Bullo (2021) that displayed an increase in oil yield along with the length of extraction time (5 hours had the highest oil yield than 3 or 4 hours at the same temperature). The proper solvent and extraction time would result in a high oil yield (Pratama *et al.*, 2017).

An increase in extraction time triggers an increase in oil yield. That happened because the contact between material and solvent was longer, facilitating solvent penetration into a target matrix cell. As a result, the more the oil yield would be. The increase of oil yield became slower and constant at a particular time because less and less the oil compounds in the target cell (Megawati *et al.*, 2019; Mgoma *et al.*, 2019).

The study results showed that the avocado peel has a higher oil yield than the avocado seed. That was probably due to the crop peels containing a lower cellulosic material percentage than the part of the seed. Consequently, the oil component was easily extracted from the cell, and the extraction process became more efficient (Páramos *et al.*, 2020; Qu *et al.*, 2010).

Table 1. The yield and specific gravity of avocado waste oil

Sample	Extraction time (hours)	Yield (%)	Specific gravity (g/mL)
Avocado peel oil	4	$6.25^{a} \pm 0.22$	$0.961^{a} \pm 0.0000$
	5	$9.14^{b} \pm 0.14$	$0.982^{b} \pm 0.0001$
	6	$13.14^{c} \pm 0.20$	$0.989^{c} \pm 0.0001$
Avocado seed oil	4	$4.59^{a} \pm 0.38$	$0.973^{a} \pm 0.0001$
	5	$8.05^{b} \pm 0.12$	$0.986^{b} \pm 0.0001$
	6	$12.29^{c} \pm 0.26$	$0.993^{c} \pm 0.0001$

*Values with the same superscripts are not significantly different (p>0.05)

Table 2. The chemical characteristics of avocado waste oil

Sample	Extraction time (hours)	FFA (%)	AV (mgKOH/g)	PV (mEq/kg)	PAV (mEq/kg)	TOTOX (mEq/kg)
Avocado peel oil	4	$0.27^{a} \pm 0.00$	$0.44^{a} \pm 0.04$	$0.98^{a} \pm 0.01$	$4.63^{a} \pm 0.01$	$8.86^{a} \pm 0.58$
	5	$0.36^{b} \pm 0.04$	$0.66^{b} \pm 0.04$	$1.54^{b} \pm 0.15$	$5.19^{b} \pm 0.01$	$10.22^{b} \pm 0.27$
	6	$0.42^{b} \pm 0.04$	$0.82^{e} \pm 0.04$	$2.13^{e} \pm 0.24$	$6.89^{c} \pm 0.01$	$12.26^{c} \pm 0.27$
Avocado seed oil	4	$0.23^{a} \pm 0.04$	$0.33^{a} \pm 0.04$	$0.48^{a} \pm 0.00$	$3.77^{a} \pm 0.01$	$5.05^{a} \pm 0.55$
	5	$0.32^{b} \pm 0.04$	$0.61^{b} \pm 0.04$	$1.29^{b} \pm 0.14$	$4.28^{b} \pm 0.01$	$8.46^{b} \pm 0.28$
	6	$0.38^{b} \pm 0.05$	$0.73^{c} \pm 0.04$	$2.04^{e} \pm 0.14$	$5.95^{e} \pm 0.01$	$10.84^{c} \pm 0.49$

*Values with the same superscripts are not significantly different (p>0.05)

The data in Table 1 indicated that the longer the extraction time, the higher the specific gravity. That was likely due to a lack of clarity in the avocado peel or seed oil because there was no purification stage. Rina *et al*. (2021) reported that the increase in extraction time causes gum accumulation, which consists of phosphatides, protein, carbohydrates, water, or other residues in the extracted oil. Hence, the refining process is widely used to purify the extracted oil and is proven to decrease the residue content in the final product (Kurniati and Susanto, 2015).

The free fatty acid is an analysis to measure the content of free fatty acids that formed after lipid molecules' hydrolytic degradation. Acid value analysis indicates the rancidity degree in oil hydrolysis (Prescha *et al*., 2014). P-Anisidine is an analysis that evaluates secondary lipid oxidation (Guo *et al*., 2016). Peroxide indicates an oxidative index for lipid oxidation in the early stages (Iqbal and Bhanger, 2007). Total oxidation is the overall oxidation state analyzed through primary and secondary oxidation product information (Abeyrathne *et al*., 2021).

The chemical characteristics of avocado waste oil were presented in Table 2. The 4 hours of extraction displayed a significant difference in FFA results from other extraction times. Meanwhile, the AV, PV, PAV, and TOTOX values at 4, 5, and 6 hours of extraction were significantly different in each avocado peel and seed oil. The chemicals characteristics of all avocado peel and seed oil met the standard of vegetable oil with <5% of FFA, <4 mgKOH/g of AV, <15 mEq/kg of PV, and <20 mEq/kg of TOTOX (Codex, 2017). The extraction for 4 hours generated the avocado peel oil with the lowest value of FFA (0.27%), AV (0.44 mgKOH/g), PV (0.98 mEq/kg), PAV (4.63 mEq/kg), and TOTOX (8.86 mEq/kg). The 4 hours of extraction also produced the avocado seed oil with the lowest value of FFA (0.23%), AV (0.33 mgKOH/g), PV (0.48 mEq/kg), PAV (3.77 mEq/kg), and TOTOX (5.05 mEq/kg).

The results of chemical analysis in avocado peel and seed oil increased along with the increasing extraction time. It was proved that the heating treatment accelerated the hydrolytic-oxidative reaction. Heat, air, and moisture were the factors that caused the breaking of the ester bond and releasing free fatty acid molecules. The heating treatment triggers the breaking of the unsaturated fatty acids' double bonds and forms peroxide radicals. These radicals will bind to hydrogen from

other fatty acid double bonds, producing hydroperoxides or forming new radicals (Mamuaja, 2017). The avocado peel oil had a higher value of FFA, AV, PV, PAV, and TOTOX in each extraction time variation than avocado seed oil. That was presumably caused by the higher UFA content in avocado peel oil than in avocado seed oil. Amado *et al*. (2019) proved that avocado peels contain higher UFA (2570 mg/100 g) than avocado seeds (166.28 mg/100 g). The UFA is very susceptible to oxidation, so the greater the amount, the lower the oxidative stability (Domínguez *et al*., 2019). Hence, the possibility of oxidative-hydrolytic reaction in avocado peel oil was higher than in avocado seed oil.

Table 3. Radical scavenging activity and reducing the power of avocado waste oil

Sample	Extraction time (hours)	Radical scavenging activity (%)	Reducing power (mgTr/g)
Avocado peel oil	4	$68.79^{c} \pm 0.19$	$523.96^{c} \pm 4.38$
	5	$62.68^{b} \pm 0.19$	$450.32^{b} \pm 4.38$
	6	$55.69^{a} \pm 0.19$	$398.50^{a} \pm 4.38$
Avocado seed oil	4	$53.95^{c} \pm 0.19$	$438.05^{c} \pm 4.38$
	5	$44.85^{b} \pm 0.19$	$346.68^{b} \pm 4.38$
	6	$38.49^{a} \pm 0.19$	$309.86^{a} \pm 4.38$

*Values with the same superscripts are not significantly different ($p>0.05$)

The extraction time significantly affected the antioxidant potential of avocado waste oil, and the data was presented in Table 3. Both radical scavenging activity and reducing power results were decreased along with the increasing extraction time. As expected, the lowest extraction time (4 hours) generated the highest value of radical scavenging activity and reducing power in avocado waste oil. The highest percentage of inhibition and reducing power in avocado peel oil, respectively, were 68.79% and 523.96 mgTr/g. Likewise, the highest percentage of inhibition and reducing power in avocado seed oil, respectively, were 53.95% and 438.05 mgTr/g. On the contrary, the extraction time of 6 hours showed the lowest radical scavenging activity and reducing power in avocado waste oil.

The antioxidant compounds decomposed because phenolic content was super heat-sensitive and easy to oxidize. The damage of phenolic content due to heat is responsible for decreased antioxidant activity (Che Sulaiman *et al*., 2017). The reducing power was influenced by hydroxyl groups linked with a benzene ring in the chemical structure of the antioxidant. Mostly, reducing power correlated with total phenolic compounds and antioxidant activity. The damage to phenolic compounds resulted in losses of the electron donor component, which will react with free radicals to stop the free radical chain reaction. As a result, the decrease in antioxidant activity and the reducing power (Irshad *et al*., 2012; Zhang *et al*., 2015).

Table 3 showed that the radical scavenging activity and the reducing power in avocado peel oil were higher than in avocado seed oil. This result was supported by da Silva *et al*. (2022) study that explained the radical scavenging activity in avocado peel was higher than in avocado seed because the total phenolic content in avocado peel was higher than in avocado seed (Kumar *et al*., 2014). The amount of phenolic content in the avocado seed was lower than in the avocado peel, caused by distinct exposures to environmental stress. The avocado seed was less exposed to environmental stress (e.g., UV rays of sunlight), while the avocado peel was intensely exposed. Hence, phenolic compounds synthesis was higher in the avocado peel to prevent oxidative damage in the plant's cellular structure (Oboh *et al*., 2016; Rojas-García *et al*., 2022).

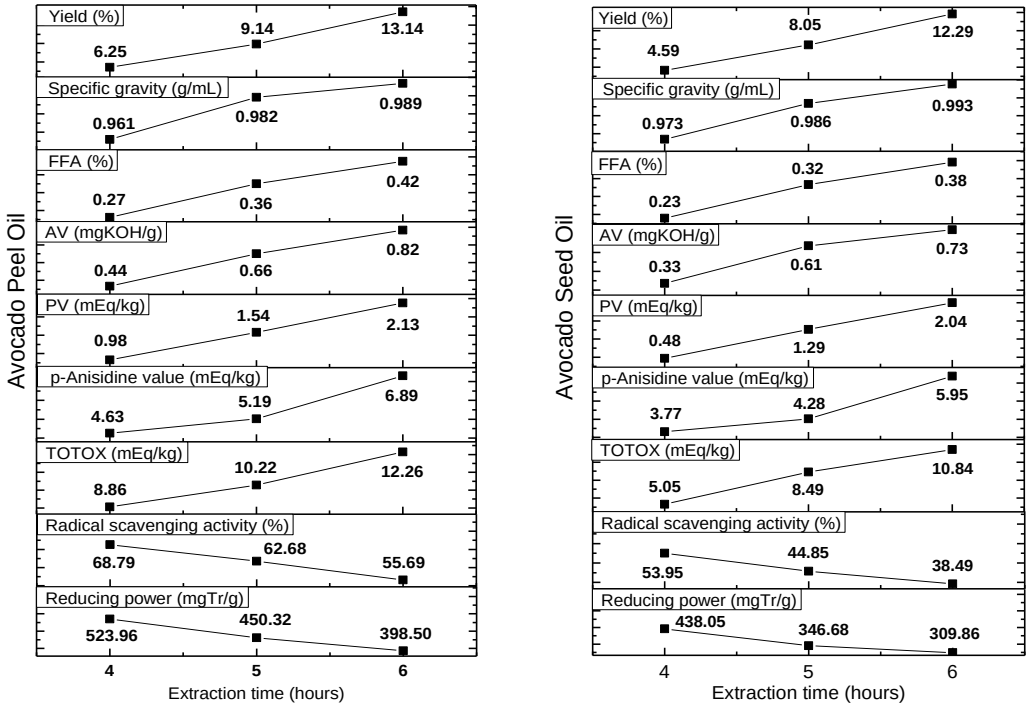

Figure 1. The Effect of Extraction Time on Avocado Peel Oil (Left) and Avocado Seed Oil (Right) Characteristics

Figure 1 indicates that the increase in extraction time definitely increases the yield and physicochemical analysis results but also decreases the antioxidant potential analysis results in avocado peel and seed oil. These results suggest that the highest extraction time (6 hours) leads to extensive decreases in oil quality as indicated by the highest values in FFA, AV, PV, PAV, and TOTOX as products of hydrolysis-oxidative reaction which indicate the level of oil damage. Based on the analysis by DeGarmo *et al*. (1984), the best extraction time treatment was 4 hours in avocado peel and seed oil. The 4 hours of extraction resulted in the avocado peel oil characteristics with 6.25% of yield, 0.961 g/ml of specific gravity, 0.27% of FFA, 0.44 mgKOH/g of AV, 0.98 mEq/kg of PV, 4.63 mEq/kg of PAV, 8.86 mEq/kg of TOTOX, 68.79% of radical scavenging activity, and 523.96 mgTr/g of reducing power. The avocado seed oil characteristics in the 4 hours of extraction were 4.59% of yield, 0.973 g/ml of specific gravity, 0.23% of FFA, 0.33 mEq/kg of AV, 0.48 mEq/kg of PV, 3.77 mEq/kg of PAV, 5.05 mEq/kg of TOTOX, 53.95% of radical scavenging activity, and 438.05 mgTr/g of reducing power.

CONCLUSION

The increase in extraction time was proved to significantly increase the yield and specific gravity of avocado peel and seed oil. Still, it decreased the hydrolytic-oxidative stability and antioxidant potential in avocado peel and seed oil. The extraction time of 4 hours was selected as the best extraction time treatment for avocado peel and seed oil with the lowest value of FFA, AV, PV, PAV, and TOTOX, and the highest radical scavenging activity and reducing power. However, all of the extraction time treatments produced the avocado waste oil characteristics which were in line with the vegetable oil requirements by the Codex Alimentarius. These studies' results provided information related to the optimization of avocado waste oil production as an effort to process plant waste into high-value products for further use in various fields.

REFERENCE

Abeyrathne, E, D, N, -S., Nam, -K., Ahn, D, -U. 2021. Analytical methods for lipid oxidation and antioxidant capacity in food systems. *Antioxidants*. 10, 1587. https://doi.org/10.3390/antiox10101587

Amado, D, A, -V., Detoni, A. -M., De Carvalho, S, L, -C., Torquato, A, -S., Martin, C. -A., Tiuman, T, -S., Aguiar, C, -M., Cottica, S, -M. 2019. Tocopherol and fatty acids content and proximal composition of four avocado cultivars (*Persea americana* Mill). *Acta Aliment*. 48, 47–55. https://doi.org/10.1556/066.2019.48.1.6

Bullo, T, A., 2021. Extraction and characterization of oil from avocado peels. *International Journal of Chemical and Molecular Engineering*. 15, 54–58. https://doi.org/10.29356/jmcs.v65i3.1511

Che Sulaiman, I, -S., Basri, -M., Fard Masoumi, H, -R., Chee, W, -J., Ashari, S, -E., Ismail, -M. 2017. Effects of temperature, time, and solvent ratio on the extraction of phenolic compounds and the anti-radical activity of *Clinacanthus nutans* Lindau leaves by response surface methodology. *Chemistry Central Journal*. 11, 54. https://doi.org/10.1186/s13065-017-0285-1

Codex. 2017. CODEX STAND 210-1999. Rev. 2017. Standard for Named Vegetable Oils

da Silva, G, -G., Pimenta, L, P, -S., Melo, J, O, -F., Mendonça, -H. de O, -P., Augusti, -R., Takahashi, J, -A. 2022. Phytochemicals of avocado residues as potential acetylcholinesterase inhibitors, antioxidants, and neuroprotective agents. *Molecules*. 27, 1892. https://doi.org/10.3390/molecules27061892

DeGarmo, EP., Sullivan, WG., Canada, JR., 1984. *Engineering Economy*, Seventh. ed. Macmillan, New York

Domínguez, -R., Pateiro, -M., Gagaoua, -M., Barba, F, -J., Zhang, -W., Lorenzo, J, -M., 2019. A comprehensive review on lipid oxidation in meat and meat products. *Antioxidants*. 8, 429. https://doi.org/10.3390/antiox8100429

FAO. 2015. Food Wastage Footprint & Climate Change. FAO, Rome, Italy

Ginting, -Z., Nurhakim, -L., Hakim, -L. 2020. Produksi minyak alpukat dari daging buah alpukat gayo dengan metode ekstraksi menggunakan pelarut n-heksan untuk aplikasi dalam sabun mandi herbal. *Jurnal Teknologi Kimia Unimal.* 9, 58–67. https://doi.org/10.29103/jtku.v9i1.3072

Guo, -Q., Gao, -S., Sun, -Y., Gao, -Y., Wang, -X., Zhang, -Z. 2016. Antioxidant efficacy of rosemary ethanol extract in palm oil during frying and accelerated storage. *Industrial Crops and Products*. 94, 82–88. https://doi.org/10.1016/j.indcrop.2016.08.032

Iqbal, -S., Bhanger, M, -I. 2007. Stabilization of sunflower oil by garlic extract during accelerated storage. *Food Chemistry*. 100, 246–254. https://doi.org/10.1016/j.foodchem.2005.09.049

Irshad, -M., Zafaryab, -M., Singh, -M., Rizvi, M, M, -A. 2012. Comparative analysis of the antioxidant activity of *cassia fistula* extracts. *International Journal of Medicinal Chemistry*. 2012, 1–6. https://doi.org/10.1155/2012/157125

Kamini, -K., Suptijah, -P., Santoso, -J., Suseno, S, -H. 2016. Ekstraksi *dry rendering* dan karakterisasi minyak ikan dari lemak jeroan hasil samping pengolahan salai patin siam. *Jurnal Pengolahan Hasil Perikanan Indonesia*. 19, 196–205. https://doi.org/10.17844/jphpi.2016.19.3.196

Krumreich, F, -D., Borges, C, -D., Mendonça, C, R, -B., Jansen-Alves, -C., Zambiazi, R, -C. 2018. Bioactive compounds and quality parameters of avocado oil obtained by different processes. *Food Chemistry*. 257, 376–381. https://doi.org/10.1016/j.foodchem.2018.03.048

Kumar, -S., Sandhir, -R., Ojha, -S. 2014. Evaluation of antioxidant activity and total phenol in different varieties of

Lantana camara leaves. BMC Research Notes. 7, 560. https://doi.org/10.1186/1756-0500-7-560

Kurniati, -Y., Susanto, W, -H. 2015. Pengaruh basa NaOH dan kandungan ALB CPO terhadap kualitas minyak kelapa sawit pasca netralisasi. *Jurnal Pangan dan Agroindustri*. 3, 193–202. https://jpa.ub.ac.id/index.php/jpa/article/view/124

Mamuaja, CF. 2017. Lipida. Unsrat Press, Manado.

Martínez-Yusta, -A., Goicoechea, -E., Guillén, M, -D. 2014. A review of thermo-oxidative degradation of food lipids studied by ^{1}H NMR spectroscopy: Influence of degradative conditions and food lipid nature. *Comprehensive Reviews in Food Science and Food Safety*. 13, 838–859. https://doi.org/10.1111/1541-4337.12090

Megawati, Fardhyanti, D, -S., Sediawan, W, -B., Hisyam, -A. 2019. Kinetics of mace (*Myristicae arillus*) essential oil extraction using microwave assisted hydrodistillation: Effect of microwave power. *Industrial Crops and Products*. 131, 315–322. https://doi.org/10.1016/j.indcrop.2019.01.067

Mgoma, ST., Gutu, L., Basitere, M., Mshayisa, V. 2019. Effects of Different Extraction Methods and Process Conditions in the Yields of Avocado Oil, in: *16th South Africa International Conference on Agricultural, Chemical, Biological and Environmental Sciences (ACBES-19)*. Eminent Association of Pioneers (EAP), Johannesburg, pp. 271–275. https://doi.org/10.17758/eares8.eap1119138

Ng, C, -Y., Leong, X, -F., Masbah, -N., Adam, S, -K., Kamisah, -Y., Jaarin, -K. 2014. Heated vegetable oils and cardiovascular disease risk factors. *Vascular Pharmacology*. 61, 1–9. https://doi.org/10.1016/j.vph.2014.02.004

Oboh, -G., Odubanjo, V, -O., Bello, -F., Ademosun, A, -O., Oyeleye, S, -I., Nwanna, E, -E., Ademiluyi, A, -O. 2016. Aqueous extracts of avocado pear (*Persea americana* Mill.) leaves and seeds exhibit anti-cholinesterases and antioxidant activities in vitro. *Journal of Basic and Clinical Physiology and Pharmacology*. 27, 131–140. https://doi.org/10.1515/jbcpp-2015-0049

Ogbuagu, A, S, -M., Okoye, C, -I. 2020. Physico-chemical characterization of avocado (*Persea americana* Mill) oil. *Progress in Chemical and Biochemical Research*. 3, 39–45. https://doi.org/10.33945/sami/pcbr.2020.1.5

Owuna, F, -J. 2020. Stability of vegetable based oils used in the formulation of ecofriendly lubricants – A review. *Egyptian Journal of Petroleum*. 29, 251–256. https://doi.org/10.1016/j.ejpe.2020.09.003

Páramos, P, R, -S., Granjo, J, F, -O., Corazza, M, -L., Matos, H, -A. 2020. Extraction of high value products from avocado waste biomass. *The Journal of Supercritical Fluids*. 165, 104988. https://doi.org/10.1016/j.supflu.2020.104988

Patra, -A., Abdullah, -S., Pradhan, R, -C. 2022. Review on the extraction of bioactive compounds and characterization of fruit industry by-products. *Bioresources and Bioprocessing*. 9, 1-25. https://doi.org/10.1186/s40643-022-00498-3

Permal, -R., Leong Chang, -W., Seale, -B., Hamid, -N., Kam, -R. 2020. Converting industrial organic waste from the cold-pressed avocado oil production line into a potential food preservative. *Food Chemistry*. 306, 125635. https://doi.org/10.1016/j.foodchem.2019.125635

Pratama, R, -N., Widarta, I, W, -R., Darmayanti, L, P, -T. 2017. Pengaruh jenis pelarut dan waktu ekstraksi dengan metode soxhletasi terhadap aktivitas antioksidan minyak biji alpukat (*Persea americana* Mill.). *Media Ilmiah Teknologi Pangan*. 4, 85–93. file:///C:/Users/LAB%20MAI%20MT/Downloads/38629-1321-77483-1-10-

20180404.pdf

Prescha, -A., Grajzer, -M., Dedyk, -M., Grajeta, -H. 2014. The antioxidant activity and oxidative stability of cold-pressed oils. *Journal of the American Oil Chemists' Society*. 91, 1291–1301. https://doi.org/10.1007/s11746-014-2479-1

Qu, -W., Pan, -Z., Ma, -H. 2010. Extraction modeling and activities of antioxidants from pomegranate marc. *Journal of Food Engineering*. 99, 16–23. https://doi.org/10.1016/j.jfoodeng.2010.01.020

Rina, -D., Bahri, -S., Zulnazri, -Z. 2021. Ekstraksi minyak kacang tanah (*peanut oil*) dengan pelarut etanol dan n-heksan. *Chemical Engineering Journal Storage*. 1, 29–41. https://doi.org/10.29103/cejs.v1i1.2650

Rojas-García, -A., Fuentes, -E., Cádiz-Gurrea, M, -de la L., Rodriguez, -L., Villegas-Aguilar, M, D, -C., Palomo, -I., Arráez-Román, -D., Segura-Carretero, -A. 2022. Biological evaluation of avocado residues as a potential source of bioactive compounds. *Antioxidants*. 11, 1049. https://doi.org/10.3390/antiox11061049

Salazar-López, N, -J., Domínguez-Avila, J, -A., Yahia, E, -M., Belmonte-Herrera, B, -H., Wall-Medrano, -A., Montalvo-González, -E., González-Aguilar, G, -A. 2020. Avocado fruit and by-products as potential sources of bioactive compounds. *Food Research International*. 138, 109774. https://doi.org/10.1016/j.foodres.2020.109774

Sathish, -S., Narendrakumar, -G., Vaithyasubramanian, -S., Sinduri, -E. 2021. Mechanistic model for the batch extraction of oil from avocado seeds available for biofuel production. *International Journal of Green Energy*. 18, 1645–1657. https://doi.org/10.1080/15435075.2021.1930001

Shahidi, -F., Zhong, -Y. 2015. Measurement of antioxidant activity. *Journal of Functional Foods*. 18, 757–781. https://doi.org/10.1016/j.jff.2015.01.047

Suseno, SH., Musbah, M., Ruspatti, NP., 2016. Karakteristik Minyak Ikan Murni Sardin (*Sardinella* sp.) dan Cucut (*Centrophorus* sp.) sebagai Bahan Suplemen Makanan Kaya Omega-3 dan Squalen, in: Nugraha, WA., Siswanto, AD. (Eds.), Seminar Nasional Kelautan 2016. Universitas Trunojoyo Madura, Bangkalan, pp. 48–56.

Tekin, -K., Hao, -N., Karagoz, -S., Ragauskas, A, -J. 2018. Ethanol: A promising green solvent for the deconstruction of lignocellulose. *ChemSusChem*. 11, 3559–3575. https://doi.org/10.1002/cssc.201801291

Tesfaye, -T., Ayele, -M., Gibril, -M., Ferede, -E., Limeneh, D, -Y., Kong, -F. 2022. Beneficiation of avocado processing industry by-product: A review on future prospect. *Current Research in Green and Sustainable Chemistry*. 5, 100253. https://doi.org/10.1016/j.crgsc.2021.100253

Velderrain-Rodríguez, G, -R., Quero, -J., Osada, -J., Martín-Belloso, -O., Rodríguez-Yoldi, M, -J. 2021. Phenolic-rich extracts from avocado fruit residues as functional food ingredients with antioxidant and antiproliferative properties. *Biomolecules*. 11, 977. https://doi.org/10.3390/biom11070977

Zhang, -Y., Shen, -Y., Zhu, -Y., Xu, -Z. 2015. Assessment of the correlations between reducing power, scavenging DPPH activity and anti-lipid-oxidation capability of phenolic antioxidants. *LWT - Food Science and Technology*. 63, 569–574. https://doi.org/10.1016/j.lwt.2015.03.047