

Supercritical CO₂ Extraction of Oil from Dried Avocado (*Persea Americana* Mill.) Fruit Pulp: Oil Yield, Solubility, and the Oil Characteristics

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Received: March 31, 2023 Accepted: May 18, 2023 Online Published: May 22, 2023

doi:10.5539/jfr.v12n3p7

URL: <https://doi.org/10.5539/jfr.v12n3p7>

Abstract

The purpose of this study was to investigate the applicability of supercritical fluid technology for extraction of avocado oil. The dried avocado fruit pulp (*Persea americana* Mill.) was extracted by using a semi-pilot supercritical extraction system. Effect of several process parameters, such as CO₂ flow rate, particle size, pressure and temperature on total oil yield, free fatty acid, peroxide value, total phenolic and total flavonoid content in oil were assessed. Avocado oil was extracted within the range for flow rate of 10 and 15L/h, particle size of 2.0 and 3.0 mm, temperature of 34, 42 and 50 °C and pressure of 15, 20, 25 and 30 MPa. Kinetic curves clearly exhibited three periods of extraction (constant rate, falling rate and diffusion-controlled). Increasing flow rate, pressure, temperature or reducing particle size brought an increase in the oil yield and extraction rate. Overall, after 150 min of extraction, the oil in dried avocado was almost completely extracted. The oil yield by supercritical CO₂ method (58.97%) obtained at 50 °C and 30 MPa was higher than that by Soxhlet method (55.83%). An increase in pressure (at constant temperature) brought an increase in free fatty acid values in oil but decrease in peroxide values and total phenolic content. On the other hand, the effect of temperature on those parameters was opposite. The oil by supercritical CO₂ method was of better quality than that by Soxhlet method.

Keywords: avocado oil, supercritical carbon dioxide, oil quality, total flavonoid content, total phenolic content

1. Introduction

Avocado (*Persea americana* Mill.) is a fruit tree of the plant family Lauraceae, which is indigenous to tropical America. The avocado fruit flesh consists of around 73% water, 15% fat, 8.5% carbohydrates- mostly fibers, and 2% protein. Avocado fruit is rich in fat, fiber, minerals, fat-soluble vitamins, and considered as a food with high nutritional value (Duarte et al., 2016). The flesh of an avocado can contain up to about 60% (by dry weight) of the fruit pulp (Wong et al., 2008). The oil present in the fruit pulp is rich in bio-active compounds, with fatty acid profile similar to that in olive oil, and thus widely used in various pharmaceutical and cosmetic applications and as a functional food oil (Flores et al., 2019; Reddy et al., 2012). Avocado oil is rich in unsaturated fatty acids (USFA), in which oleic acid present at about 51%, followed by linoleic acid at 14%. Among saturated fatty acids (SFA), palmitic acid is present in oil at 28%, and just little of stearic acid (Ranade and Thiagarajan, 2015).

Many methods for avocado oil extraction have been investigated. Extraction with organic solvents (commonly hexane) presents high oil yield, however; the process can cause negative environmental impacts. Mechanical pressing or centrifugation (usually assisted with enzyme, ultrasound, microwave, etc.) are the alternatives of green technologies, however; the extraction yield is usually low (Tan, 2019; Qin and Zhong, 2016).

In recent decades, supercritical fluid extraction has attracted great interest from researchers, as a green alternative to conventional separation methods. Supercritical fluids have a good solvent power because of their density (controllable by manipulation of pressure and temperature), low viscosity, high diffusivity, and low surface tension, which enhance mass transfer inside a solid matrix (Pereira and Meireles, 2010). Supercritical CO₂ (SC-CO₂) became a favorable solvent in this technology because of being non-toxic, non-explosive, inflammable, cheap and readily available. It is suitable for extracting non-polar compounds such as oils and fats

with high extraction yield, product free of any traces of organic solvents and metals. Extracts from SC-CO₂ treatments can be regarded as all natural, and the products allowed for food applications with the GRAS status (Corzzini et al., 2017; Díaz-Reinoso et al., 2006).

Oil from flaxseeds was extracted using SC-CO₂ at a range of temperatures, pressures, and CO₂ flow rates for 3 h (Bozan and Temelli, 2002). It was found that the oil yield ranged from 21 to 25% (w/w oil/dry seed), lower than that in extraction using organic solvent (38%). The extraction curves consisted of three phases: (1) the initial linear portion corresponded to the solubility of the oil in SC-CO₂; (2) falling rate transition period, (3) diffusion-controlled rate period. Knowing the flowrate and CO₂ density, the solubility of flaxseed oil was determined from the initial linear portion. Bernardo-Gil et al. (2009) studied the process for oil extraction from fig-leaf gourd seeds using SC-CO₂ at pressures of 18-20 MPa and temperatures of 308-318K. Extraction by Soxhlet apparatus with hexane gave the oil yield of 43.5% (w/w, oil/seed), higher than that by SC-CO₂ extraction (about 41% after 150 min of extraction). The extraction kinetic followed the Sovova's model.

Effect of ripeness and drying method (freeze and oven drying) of avocado fruits on the yield of oil extracted from dried avocado flesh with SC-CO₂ was studied at 37 °C and 350 atm (Mostert et al., 2007). Extraction of the freeze-dried material gave higher yield than the oven-dried one, the same as for the ripen versus the unripe material. The average oil yield obtained from dried avocado flesh was 59.1% (w/w oil/solid) for SC-CO₂ extraction, lower than extraction with hexane (68.3%).

Corzzini et al. (2017) extracted oil from freeze-dried avocado pulp with SC-CO₂ in a range of temperatures (40, 60, 80 °C) and pressures (200, 300, 400 bar). The dried material contained 65% (w/w, oil/solid) of intrinsic oil, and the highest oil yield from SC-CO₂ extraction achieved 98% oil recovery. Supercritical CO₂ extraction of oil from dried avocado pulp did not need to operate at high temperature (>50 °C), the appropriate range of pressure was from 15 to 30 MPa, and the time of 150 min was sufficient.

In a study for co-extraction, oil from dried avocado pulp was extracted by SC-CO₂ with freeze-dried red bell pepper as co-matrix at 50 °C and 400 bar (Barros et al., 2016). As a result, 88% of the intrinsic oil from avocado and 30-50% of intrinsic capsaanthin was recovered. When dried avocado pulp was co-extracted with freeze-dried tomato pomace by supercritical CO₂ extraction (Barros et al., 2017), 80% of intrinsic oil was recovered and the oil was enriched with lycopene.

SC-CO₂ extraction of avocado oil at 313K and 25 MPa gave 40% (w/w oil/solid) yield in 150 min (Abaide et al., 2017). But the attempt of using Compressed Liquefied Petroleum gas (CLPG) at 295K and much lower pressure of 0.5 MPa gave the yield of 60% in just 10 min (much faster and with lower solvent consumption).

Although there is a variety of reports on supercritical fluid extraction for oil-seed materials, the information on SC-CO₂ extraction of avocado oil is still rare in the literature. Furthermore, most of studies on avocado oil were conducted using a laboratory scale apparatus with a small volume extractor (in order of 10 mL). This study was aimed at evaluation of the effect of several process parameters (particle size, flowrate, temperature, and pressure) in extraction for avocado oil at a considerable larger scale by using a supercritical apparatus with a 1000-mL extractor. The total oil yields were recorded and the oil solubility in SC-CO₂ calculated. Finally, the avocado oils (from SC-CO₂ extraction and by Soxhlet method) were evaluated and compared in terms of several quality parameters, such as free fatty acid value, peroxide value, total phenolic and total flavonoid content.

2. Materials and Methods

2.1 Materials and Chemicals

Fresh avocado fruits (Booth variety) with good physical integrity were obtained from a local fruit market. The fruits were cut in a half and seeds removed. The half-cut fruits were peeled and sliced into small pieces (approximate 4x4mm). Then the cut fruit pieces were transferred into a stainless-steel bag and blanched in boiled water for 60 seconds for enzyme inactivation and cooled down quickly. Following that, the fruit dices were spread on aluminum trays, dehydrated under the sun for a day, then transferred to oven drying at 40 °C for 24 h. The final moisture content of the material reached 8.0%. The dried materials were ground by a food blender, sieved through a sieve size of 2.0 and 3.38 mm (US Mesh No.10 and No. 6), kept in zipper-plastic bags (100 g each), and stored in a refrigerator for further use.

CO₂ (purity > 99.9%), sodium hydroxide (NaOH), ethanol, hydrochloric acid (HCl), acetic acid (glacial), chloroform, potassium iodide (KI), aluminum chloride, potassium acetate and sodium thiosulfate were supplied by local chemical suppliers. Rutin, Folin-Ciocalteu and Gallic acid (Sigma-Aldrich) were purchased from local agents.

2.2 Experimental Design

2.2.1 Extraction Apparatus and Procedure

Equipment: The SC-CO₂ extraction system TH12-1 (Wenzhou Chengdong Medicine Machine Co., Ltd., China) was composed of the following key components: a high-pressure plunger pump, an extractor 1L/50MPa, 2 separators 0.6L/30MPa, a back pressure regulator, different filters, purifiers and valves, a control system for temperature-pressure-flowrate (hot-water exchangers, pumps and electronic display), and a piston pump for co-solvent. The CO₂ flowrate was adjustable from 4L/h to 50 L/h.

Procedure: About 100 g of the dried avocado powder was filled into an extractor, which was inserted to a temperature-controlled chamber. A cooler was turned on to achieve at least 5 °C for the cooling medium before opening the CO₂ tank. Extraction was performed at different temperatures, pressures, and fluid flow rates – particle sizes. The oil was collected from 2 separators directly into beakers without using solvent for dissolution. Each extraction run was terminated after 270 min of operation and each treatment was carried out in triplication. Avocado oil was collected at 30-min intervals. The oil samples were kept at dark and low temperature for chemical analysis.

2.2.2 Effect of Flow Rate – Particle Size on Oil Yield

The extraction was carried out with different CO₂ flow rate and particle size combinations: 10L/h-3mm, 10L/h-2mm and 15L/h-2mm. Other process parameters were fixed, such as pressure at 25 MPa, temperature at 50 °C and duration of 270 min. The oil was collected at several intervals and the yield was recorded.

2.2.3 Effect of Pressure on Oil Yield

The extraction was carried out at different pressures: 15, 20, 25 and 30 MPa. The flowrate and size combination were chosen from the previous experiment (15 L/h-2mm). Other parameters were fixed such as temperature of 50 °C, duration of 270 min.

2.2.4 Effect of Temperature on Oil Yield

The extraction was carried out at different temperatures: 34, 42 and 50 °C. Each treatment was carried out at pressure of 30 MPa, CO₂ flow rate of 15 L/h, particle size of 2 mm and duration of 270 min.

2.3 Physico-chemical Analysis

2.3.1 Determination of Oil Yield

The yield of oil extracted is calculated as percent of raw material:

$$\text{Oil Yield (\%)} = \frac{\text{Mass of oil collected (grams)}}{\text{Mass of raw materials (grams)}} * 100\%$$

% yield of oil extracted by supercritical CO₂ was compared to % yield of oil extracted by Soxhlet extraction (control sample).

2.3.2 Free Fatty Acid Value of Oil

Free fatty acid (FFA) values of oil were determined by a titration procedure using NaOH solution (Nielsen et al., 2017). An amount of 5 g oil was weighed into a 250 mL Erlenmeyer flask, followed by adding 100 mL neutralized ethanol and 2 mL phenolphthalein indicator. After that, the mixture was shaken for complete dissolution. The homogenous mixture was then titrated with standard base (0.1 N NaOH), shaken vigorously until the endpoint was reached. The FFA value was calculated:

$$\%FFA (\text{oleic acid}) = \frac{V \times N \times 282}{W} * 100$$

Where: % FFA = percent free fatty acid expressed as oleic acid; V = volume of NaOH titrant (mL); N = normality of NaOH titrant (mol/1000 mL); 282 = MW of oleic acid (g/mol); W = sample mass (g).

2.3.3 Peroxide Value of Oil

Peroxide value (PV) of oil was determined by a titration procedure using sodium thiosulfate solution (Nielsen et al., 2017). An amount of 5g of oil was weighed into each of two 250 mL glass-stoppered Erlenmeyer flasks, followed by adding 30 mL of the acetic acid: chloroform (3: 2) solution and swirling for dissolution. After that 0.5 mL saturated KI solution was added and let stand for 1 min with occasional shaking. Then, 30 mL distilled water was added, and the samples were slowly titrated with 0.01 N sodium thiosulfate solution, with vigorous shaking until yellow color was almost gone. 2 mL of 1% starch solution was then added and titration continued

with vigorous shaking to release all iodine from chloroform layer, until blue color just disappears. The PVs, expressed in unit of milligram-equivalent O₂ per kg oil, was calculated:

$$\text{Peroxide value (mEqO}_2\text{/kg)} = \frac{(S - B)}{W} \times N \times 100$$

Where: *S* = volume of titrant (mL) for sample; *B* = volume of titrant (mL) for blank; *N* = normality of Na₂S₂O₃ solution (mEq/mL); *W* = sample mass (g)

2.3.4 Total Phenolic Content in Oil

The method for determination of total phenolic content (TPC) in oil was modified from a procedure described by Mirón et al. (2020). Briefly, 1 g of oil was weighed and dissolved in 1 mL of hexane (to lower the sample viscosity). The diluted sample was mixed with 1 mL of a 50% (v/v) methanol solution with pure water and manually agitated for 2 min and vortexed for few seconds at the end. The mixture was finally centrifuged at 3000 rpm for 10 min. The water-methanol layer was collected, and the remaining oily fraction was re-extracted two more times and the fractions were combined. The total volume of the extract was adjusted to 10 mL with the 50% methanol solution. The extract solution was added with 0.4 mL of sodium carbonate 2.5%, followed with 0.2 mL of the Folin-Citocalteu reagent, kept in the dark for 30 min at room temperature. The absorbance of the mixture was measured at 760 nm using UV-Vis spectrophotometer (V730 Jasco, Japan). A standard curve for Gallic acid was constructed by preparing the dilutions in a range 2.0-10.0 mg/L in methanol. The TPC values were expressed in unit of milligram Gallic Acid Equivalent per 100-gram oil sample (mg GAE/100g).

2.3.5 Total Flavonoid Content in Oil

Total flavonoid content (TFC) in oil sample was determined by aluminum tri-chloride spectrophotometric method reported by Chang et al. (2002) using rutin as a standard. Briefly, 0.5 g of oil sample was dissolved in 95% ethanol (1.5 mL). Then the solution was added with 0.1 mL of 10% aluminum chloride, 0.1 mL of 1M potassium acetate, adjusted to 5 mL using distilled water and vortexed for few seconds. The tubes were kept at room temperature for 30 min. Absorbance of the reaction mixture was read at 415 nm. TFC values were determined from calibration curve, expressed in milligram rutin equivalent per 100-gram oil sample (mg RE/ 100g).

2.4 Statistical Analysis

All the treatments were performed in triplicates and data reported as mean ± standard deviations. ANOVA test was used for statistical analysis of data by using SPSS statistical software (ver 20.0). Significant difference between the means of parameters was determined by using Tukey's test (p<0.05).

3. Results and Discussion

3.1 Effect of Flow Rate and Particle Size on Oil Yield

The extraction curves for three flow rate/particle size conditions in 270 min are presented in Fig. 1. The oil yields were ranging from 14.72 to 51.24%. The higher flow rate, the higher oil yield was, as opposite to the trend for particle size. An increase in CO₂ flow rate brought an increase in the ratio of CO₂ molecules per unit weight of materials, hence increase in the interaction between CO₂ molecules and the solute, thereby increase in ability to dissolve oil. On the other hand, mass transfer rate was increased by increasing the CO₂ flow rate, thus increasing the amount of dissolved oil. Regarding particle size, the intra-particle resistance against mass transfer for decreasing particle size is smaller due to the shorter diffusion path, thus increase in the yield (Roy et al., 1996). Consequently, by increasing flow rate of CO₂ and decreasing particle size, the extraction ran faster. For the condition of 15L/h-2mm, 50 °C and 25 MPa, the extraction could practically be finished after 150 min of running, achieved the yield of 48.22%.

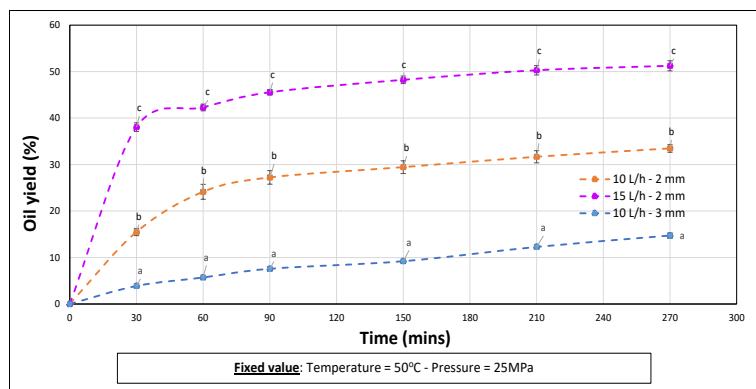


Figure 1. Effect of flow rate and particle size on oil yield

Note: letters are assigned for values at the same time for sampling

3.2 Effect of Pressure on Oil Yield

Fig. 2 presents the extraction curves of oil yields for different process pressures. The effect of pressure on the oil yield was significant. For the same extraction duration, the higher pressure was, the quicker extraction (higher yield). At 50 °C, the density of CO₂ is 699.8, 784.4, 834.4, and 870.6 g/L at 15, 20, 25, 30 MPa, respectively (Engineering ToolBox, 2018). At the same temperature, an increase in pressure of CO₂ brought to an increase in the solvent density. It is most likely to enhance the solvating power of the solvent, thus increase the solubility of the avocado oil in dense CO₂ (Tomita et al., 2013). After 270 min of extraction process, the highest oil yield was obtained at pressure of 30 MPa (58.97%). However, if the process terminated at 150 min, the yield was 54.88%, still higher than the yield after 270 min running at 25 MPa (51.24%).

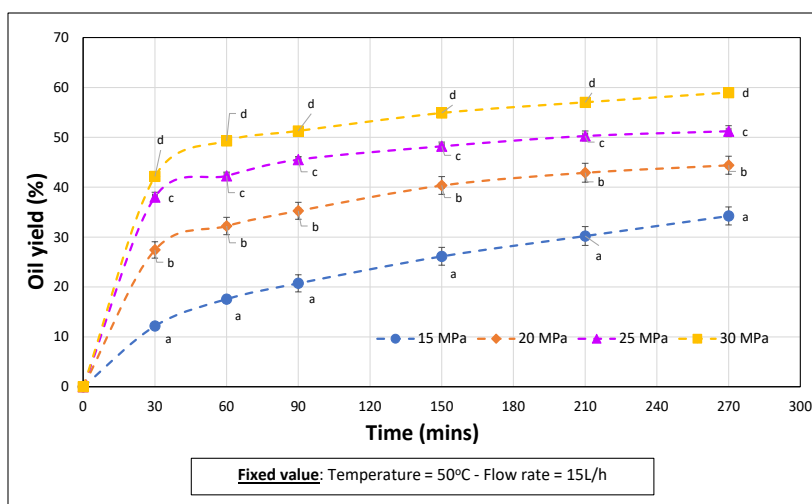


Figure 2. Effect of pressure on oil yield

Note: letters are assigned for values at the same time for sampling.

3.3 Effect of Temperature on Oil Yield

Fig. 3 presents the extraction curves of oil yields for different temperatures of 34, 42 and 50 °C. The effect of temperature on the oil yield was significant. The higher temperature, the higher yield was, at every timing of extraction. After 270 min of extraction, the yield was 57.03, 47.71, and 44.03% for 50, 42, and 34 °C, respectively. If the extraction terminated at 150 min, the yield was 54.88, 45.56, and 42.35% (w/w) for the same order. In summary, the best process conditions were determined: temperature 50 °C, pressure 30 MPa, flowrate 15 L/h and particle size 2 mm. The total running time of 150 min was recommended as practical. Even an increase in temperature brought a decrease in solvent density, however, the rise of temperature may be attributed to the enhanced solubility and mass transfer rate of oil in SC-CO₂, leading to increase in oil yield. Similar behavior was reported for supercritical CO₂ extraction of flaxseed oil by Jiao et al. (2008). It is worthy to note

that the highest oil yield achieved by using supercritical CO₂ extraction at the conditions as stated above was 58.97%, which was significantly higher than the yield by Soxhlet method (55.83%). The oil yield by Soxhlet method was like those reported by dos Santos et al. (2014), which were in a range from 45 to 57%.

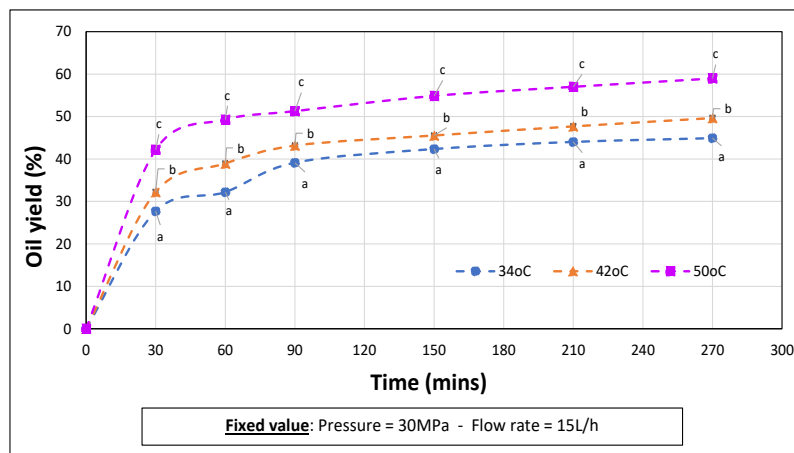


Figure 3. Effect of temperature on oil yield

Note: letters are assigned for values at the same time for sampling.

Considering extraction at 50 °C, the extraction curve was characterized by three periods: (1) a constant-extraction rate period (CER); (2) a falling-extraction rate period (FER); and (3) a diffusion-controlled rate period (DCR) (Pereira & Meireles, 2010). The first period lasted for about 30 min is limited by oil solubility and characterized by constant slope of the curve - the yield increased linearly with time (reach 42.18% w/w or 71.53% of intrinsic oil). In this period, the solute (oil) is present in large quantities on the surface of the particles of the matrix (Bernardo-Gil et al., 2009). During the CER period, approximately 50–90% of the total amount of extract is obtained as reported by different authors (Pereira & Meireles, 2010).

The second period, FER, was a transition phase, characterized by a sluggish increase in the oil yields, from 42.18 to 54.88% within 120 min. A solute layer on the surface of the solid particles is being depleted so not all of the particles are coated by the solute. In this period, the resistance to mass transfer lies in both solid and fluid phases, both diffusion and convection become significant to mass transfer, thus the rate of mass transfer decreases continuously (Talansier et al., 2008; Salinas et al., 2020).

The final period, DCR, is characterized by rapid decrease in mass transfer rate. There is an absence of free oil molecules at the particle surface, so the controlling factor for extraction rate was the diffusion of a solute in solution within the interior of the solid particles towards surface (Talansier et al., 2008; Salinas et al., 2020). Within a time span of 120 min (from the minute 150 to 270), the oil yield increased by just about 4% (from 54.88 to 58.97%). This observation recommended that the extraction of avocado oil at 50 °C and 30 MPa could be terminated after 150 min.

3.4 Calculation of Oil Solubility from Kinetics Curves

Table 1. presents the average and standard deviations of experimental solubility (g/kg) of avocado oil in CO₂, estimated from the slope of extraction curves in CER period for different pressure and temperature conditions.

Table 1. Solubility of avocado oil in CO₂ at different pressure and temperature conditions

Effect	Flow rate - Particle size	Temperature (°C)	Pressure (MPa)	Density of CO ₂ (kg/m ³)*	Solubility (g/kg)
Pressure	15L/h - 2mm	50	15	699.80	2.32 ± 0.05
			20	784.40	4.66 ± 0.28
			25	834.40	6.08 ± 0.15
			30	870.60	6.46 ± 0.03
Temperature	15L/h - 2mm	34	30	932.86	3.96 ± 0.04
		42		902.12	4.76 ± 0.08
		50		870.60	6.46 ± 0.03

(*): Data for density of CO₂ retrieved from Engineering ToolBox (2018). "Carbon dioxide - Density and Specific

Weight". *Resources, Tools and Basic Information for Engineering and Design of Technical Application* [online], https://www.engineeringtoolbox.com/carbon-dioxide-density-specific-weight-temperature-pressure-d_2018.html

For the effect of pressure, at constant temperature of 50 °C, the solubility increased (from 2.32 to 6.46 g/kg) when pressure increased from 15 to 30 MPa. Similar trend was observed by Corzzini et al. (2017) for avocado oil, Tomita et al. (2013) for hemp seed oil, and Zuknik et al. (2016) for virgin coconut oil. Increase in pressure brings an increase in solvent density, resulting in enhancement of solvent power and hence the solubility in SC-CO₂ (Pereira & Meireles, 2010). It is necessary to note that the data for solubility of avocado oil in SC-CO₂ reported by Corzzini et al. (2017) were comparable to those reported in this study (e.g at 40 °C, the solubility were in a range of 4.8-12.9 g/kg when pressure changed from 20 to 40 MPa).

Regarding the effect of temperature, the solubility increased from 3.96 to 6.46 g/kg) when the temperature increased (at constant pressure of 30 MPa) from 34 to 50 °C. At a higher temperature range, the behavior reported by Corzzini et al. (2017) was reverse, where the solubility decreased from 9.4 to 6.2 g/kg, when the temperature increased from 40 to 80 °C. Increase in temperature causes a decrease in solvent density, however, it raises the vapor pressure of the solute at the same time. Therefore, there exists a crossover pressure region, where the effect of temperature on solubility is reversed (Corzzini- et al., 2017).

3.5 Effect of Process Conditions on Physico-chemical Properties of Oils

Table 2 presents the values of FFA, PV, TPC, and TFC in oils obtained from different supercritical fluid conditions of pressure and temperature and from Soxhlet method.

Table 2. Physico-chemical properties of oils from different process conditions

No	Conditions	FFA (% oleic)	PV (mEqO ₂ /kg)	TPC (mg GAE/ 100g)	TFC (mg RE/ 100g)
A: Effect of Pressure (Temperature = 50 °C)					
1	15 MPa/50 C	0.19±0.01 ^a	3.65±0.21 ^a	50.38 ±0.57 ^a	18.43 ±0.78 ^a
2	20 MPa/50 C	0.24±0.02 ^{ab}	3.15±0.21 ^{ab}	48.57 ±0.86 ^{ab}	21.74 ±0.7 ^b
3	25 MPa/ 50C	0.26±0.01 ^{bc}	2.70±0.14 ^{bc}	45.33 ±0.86 ^{bc}	20.36 ±0.39 ^{ab}
4	30 MPa/ 50C	0.32±0.02 ^c	2.40±0.14 ^c	41.69 ±1.43 ^c	19.26 ±0.39 ^{ab}
B: Effect of Temperature (Pressure = 30 MPa)					
1	34°C/ 30 MPa	0.41 ±0.02 ^a	1.35 ±0.07 ^a	33.82 ±0.57 ^a	14.29 ±1.17 ^a
2	42°C/ 30 MPa	0.36 ±0.01 ^{ab}	1.80 ±0.14 ^{ab}	38.26 ±1.14 ^{ab}	23.95 ±1.56 ^b
3	50°C/ 30 MPa	0.32 ±0.02 ^{ab}	2.40 ±0.14 ^b	41.69 ±1.43 ^b	19.25 ±0.39 ^b
C: Soxhlet method					
	Hexane/69 °C	1.06 ±0.10	3.35 ±0.21	33.82 ±0.57	14.29 ±1.17

Note: values in a column, within a same section (A, B) with the same letter, are not significantly different.

3.5.1 Effect of Pressure FFA, PV, TPC, TFC

At constant temperature, the effect of pressure on FFA, PV, TPC and TFC was significant. Increase in FFA value with increasing pressure indicated that free fatty acids are more readily soluble in supercritical CO₂ than fat molecules at elevated pressure. An increase in pressure brought a decrease in PVs, but the effect of temperature on PVs was opposite. The higher pressure, the lower PVs was a trend observed in extraction of coffee oils, as reported by Muangrat and Pongsirikul (2019).

The TPC values in oil decreased with increasing pressure, in a similar trend as reported by Gelmez et al. (2009). This could be justified by the density effect of the solvent, which increases with pressure (at constant temperature), enhancing solubility of both polyphenolics and fat (Castro-Vargas et al., 2010). In overall, pressure appeared to have larger effect on solubility of oil than polyphenols, resulting in decrease in phenolic concentrations in the extracts with increasing pressure (Gelmez et al., 2009). The TFC reached highest at pressure of 20 MPa. At higher pressures, the TFC in oil decreased, indicating that the density effect was more in favor for fat solubility rather than for flavonoids.

3.5.2 Effect of Temperature on FFA, PV, TPC, TFC

At constant pressure and in a temperature arrange of 34-50 °C, an increase in temperature brought a decrease in FFA and increase in PV and TPC values of the oils.

Similar trend was reported by Muangrat & Pongsirikul (2019) for FFA and PVs, and by Gelmez et al. (2009) for

TPC in oil. Many phenolic compounds exhibit strong antioxidant activity, so high TPC content in oil may be correlated to the its antioxidant activity (Castro-Vargas et al., 2010). The oil with high TPC (50.38 mg GAE/100g oil) was obtained at conditions of 15 MPa, 50 °C; however, it was on the expense of low oil yield. The highest TFC in oil obtained at 42 °C, which was significantly different from those in oil obtained at either lower or higher temperatures (34 or 50 °C, respectively). The similar behavior was also reported by Vyavaharkar and Mangaonkar (2016), where the TFC in the extract decreased when the temperature exceeded 40 °C. This could be due to the competitive effect of temperature and CO₂ density reduction.

The FFA values were in a range of 0.19-0.41% (oleic), which were close to those reported elsewhere for avocado oils obtained by different methods (Santana et al., 2015; Moreno et al., 2003; Tan et al., 2018; Botha, 2004). The TPC values in the oil were in a range from 33.82 to 50.38 (mg GAE/100g oil), which are close to those reported by Krumreich et al. (2018), where the avocado oil was extracted by mechanical pressing and Soxhlet method. As for TFC, the values were ranging from 18.43 to 21.74 (mg RE/100g oil), close to those in fig-leaf gourd seed oil (Tuan and Khoi, 2021) or in flax, perilla and sesame oil (Xuan et al., 2018).

Regarding FFA, PV, TPC, TFC in the oil, the oil obtained by supercritical CO₂ process (at 50 °C and 30 MPa) was of better quality that that by Soxhlet method (Table 2). Moreover, the FFA values and PVs of the oils extracted with SC-CO₂ were lower than the maximum level of 0.5% (oleic acid) and 4.0 (mEqO₂/kg) as recommended for the grade of virgin avocado oil (Wong et al., 2010; Costagli and Betti, 2015). Moreno et al. (2003) reported considerably higher PVs and FFA in avocado oil extracted with solvents such as hexane or acetone. Also, PVs and FFA in avocado oil obtained by mechanical pressing at 40 °C were reported (Krumreich et al., 2018) higher than this study.

4. Conclusion

In general, process conditions such as pressure, temperature, flowrate, and particle size presented a significant effect on the extraction yield and extraction kinetics. Increasing pressure (15 to 30 MPa) and temperature (34 to 50 °C) brought an increase in the oil yield and the extraction rate. On the other hand, increasing flow rate (10 to 15L/h), reducing particle size (3 to 2 mm) increased the extraction rate, too. The first 30 min of a run presented a CER period and the avocado oil solubility in SC-CO₂ was calculated from the oil yield during this period.

In addition, all the process conditions exhibited a significant effect on FFA, PVs, TPC, TFC of the oil. Extraction with supercritical CO₂ provided the oil of better quality than Soxhlet method with lower FFA and PVs and higher TPC and TFC values. The results showed that SC-CO₂ extraction could be considered as a favorable alternative technique for extraction of oil from dried avocado pulp.

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