Bulgarian Chemical Communications, Volume 51, Special Issue B (pp. 046 – 051) 2019 DOI:10.34049/bcc.51.B.005

Studies on extraction from Avocado's waste biomass to generate process design alternatives of valuable products

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Received: July 6, 2019; revised: August 16, 2019

Avocado (*Persea americana*) seeds of the cultivar Hass, cultivated in Brazil and Mexico, were study for extraction purposes, in order to determine the amount and the composition of valuable extracts. The avocado seeds, which represent about 23 % of fruit weight, have even higher antioxidant activity than its pulp. This study carried out the extraction of seed oil by using two extraction techniques: Soxhlet liquid-solvent extraction and supercritical fluid extraction (SFE). Soxhlet extraction (for a period of 6 hours) involved three solvents: hexane, ethanol and ethyl acetate. Ethanol presented the highest extraction yield, for both particle sizes studied and for both seed types (Mexican and Brazilian), being the highest yield of 10.3 ± 0.3% for the smaller particles, referring to the Brazilian seeds. This result indicated that the seed's extract might be high on content for polar components. The SFE was performed using supercritical carbon dioxide ($scCO_2$) as a solvent and after some preliminary experiments, ethanol revealed to be the best cosolvent. The highest extraction yield (for particle size between 0.42 and 0.60 mm) was 6.9% for the conditions of 80єC, 25 MPa and a mass ratio of 1.5:1 (referring once again to the Brazilian seed). Comparing both methods, Soxhlet reached higher yield, although it implies a greater energy, time and solvent consumption than SFE.

Key words: Avocado seed, Supercritical fluid extraction, Soxhlet extraction, yield, ethanol

INTRODUCTION

The transformation of waste biomass into valuable materials and/or energy is emerging as a powerful trend due to the depletion of natural resources, increased greenhouse emissions and awareness of the need for sustainable development in terms of safe reuse of waste and biomass [1].

A substantial quantity of biological waste (such as fruit's peels and seeds) is produced due to significant consumption and industrial processing of the edible components of the plants. One of the most useful strategies is to recover bioactive compounds, particularly phenolic compounds, making complete use of them in the food, pharmaceutical and cosmetics industries [1]. In this study, the waste biomass was Hass avocado's seeds from Mexico and Brazil, as only the avocado pulp is used for commercial purposes, while its peels and seeds are discarded. Avocado's seeds are known for its high content of phenolic compounds and high antioxidant capacity, as well as for being rich in essential fatty acids such as linoleic and linolenic acid, which are beneficial to human cardiovascular health [2].

Most biomass waste is a complicated and variable molecular combination, and separation is the main problem. Moreover, usually some of the bio-waste and the separating materials are solid, therefore, organic solvents are often involved in separation. In order to make bio-based chemical production self-sustainable, these solvents must also be bio-based and cannot be obtained from crude oil in the long term [1]. For that, it is essential to reduce extraction methods that recur to organic solvents like Soxhlet extraction. So, a more environment-friendly method is the Supercritical Fluid Extraction (SFE), in which carbon dioxide (CO_2) acts as a non-toxical and non-flammable solvent. Supercritical CO_2 ($scCO_2$) is regarded as the most common supercritical fluid due to the moderately critical temperature and pressure (31.1ºC and 7.4MPa), Its density and solvability are intermediate between gas and liquid and can be readily altered with small modifications in temperature and pressure. In addition, by merely reducing the pressure, it is totally isolated from the extract, thus achieving a product with a high degree of purity that is not feasible when using organic solvents [3-6].

This study addresses the avocado's seed usage to obtain valuable extract by using different extraction techniques: SFE (mainly CO_2 + ethanol) and Soxhlet extraction (hexane, ethanol and ethyl acetate). The extraction yield and the solvents selectivity used is compared in both e extraction techniques. The particle size was also evaluated in the Soxhlet method.

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EXPERIMENTAL

Sample collection and pre-treatment

Avocado fruits from Mexico and Brazil, both from the cultivar Hass, were purchased from the local market, although the Mexican avocados were bought at a Portuguese market and the Brazilian avocados were bought at a market situated in Brazil. The fruits were stored at room temperature (approximately 22ºC) until they become ripe. The avocados (free from any apparent skin damage) were then washed with tap water (to remove any remaining dirt), cut open with a knife and the seeds, as well as the peels and pulp, were manually removed. The pulp was discarded, the peels were stored, and the seeds were cut manually into smaller dimensions, subsequently being oven dried with air circulation (Solab, model SL-100, Brazil) for 24 hours at 55 ± 1 ºC. Afterwards, the seeds were milled in a coffee and meat mill (Arbel, Brazil) in order to be classified, according to particle size, using a system of Tyler series sieves (Bertel, Indústria metalúrgica Ltda, Brazil). The series of sieves chosen were 9, 12, 24, 28 and 35 meshes and they were put through a vertical vibratory sieve shaker to improve particle size classification.

The seeds retained between the 28 and 35-mesh and below 35-mesh (#28 = 0.60 mm and #35 = 0.42 mm) sieves were chosen for extractions purposes, once those meshes yielded great quantity after milling. However, only the particles from 28 and 35-mesh will be used in supercritical fluid extraction. The milled samples were packaged in vacuum polyethylene bags and stored at -18ºC until required for analysis.

Seed moisture on a dry basis (45.45 ± 1.69 wt%) was determined by the gravimetric method which consists of drying the sample in the oven at 105 ± 1ºC until constant mass is obtained, as it is calculated by the loss of weight upon the dry sample.

Chemical and reagents

All the solvents used were of analytical grade.

For the conventional extraction method (Soxhlet extraction) hexane (99% purity, Êxodo Científica, Brazil), ethanol (99.8% purity, Neon, Brazil) and ethyl acetate (99.5% purity, Neon, Brazil) were used.

For the supercritical fluid extractions, it was used scCO2 (99.5% purity in the liquid phase, White Martins Gases Industriais Ltda, Brazil) as the main solvent. As cosolvents for these extractions, ethanol (99.8% purity, Neon, Brazil) and ethyl acetate (99.5% purity, Neon, Brazil) were used.

Soxhlet extraction

The extraction was held with a Soxhlet apparatus (Uniglas, Brazil) constituted by the Soxhlet condenser and extractor, in which approximately 5 g of avocado's dry seeds (Brazilian or Mexican) were transferred to a handmade filter paper bag (6.5 x 4.5 cm) and placed inside the Soxhlet extractor apparatus of 250 ml. Afterwards, a pure solvent (hexane, ethanol or ethyl acetate) was added inside the extractor, for about 180 ml, into a flask of 250 ml attached to the apparatus and heated up beyond its respective boiling point, in order to achieve a constant condensation rate. The experiments were carried out by a period of 6 h (confirmed by the kinetic study) with an average cycle time of 20 ± 3 min.

At the end of the extraction, the solvent was removed in vacuum using a rotary evaporator (RV 10 digital, IKA, Wilmington, USA) at approximately 40ºC and 30 rpm to recover the seed oil. The flask containing the oil was then kept in an oven with air circulation at 60 ± 1 ºC to guarantee full removal of residual solvent for about 48 h. Following the drying and after cooling to room temperature, the sample was weighed to determine the extraction yield, transferred into a 2 ml vial and stored under refrigeration (-18 ºC) for chemical analysis.

The extraction runs were carried out in triplicate at identical conditions and mean values were used.

The extraction yield was expressed as a mass per cent of the extracted oil in relation to the initial mass of the seeds used for extraction according to Eq. (1):

$$Yield\ (\%) = \frac{mass\ of\ extracted\ oil}{mass\ of\ avocado's\ seed\ sample} \times 100 \qquad (1)$$

Supercritical fluid extraction (SFE)

The extractions using $scCO_2$ as a solvent were performed in a bench scale unit (Fig. 1), which has been described in previous studies [3-5].

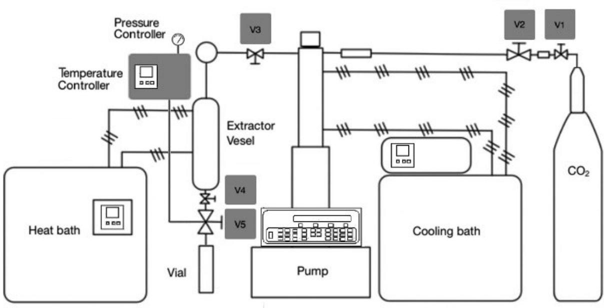

Fig.1. Schematic diagram of the extraction unit. V1 and V2: cylinder valve; V3: ball valves; V4: gas pressure regulator valve; V5: micrometric valve (adapted from [3]).

Briefly, the experimental setup consists of a stainless steel vessel extractor, with a volume of 65 cm^3, a diameter of 1.9 cm and a height of 22 cm, coupled to a heating thermostatic bath (Quimis, Model Q214S2, Brazil), a micrometric valve to control the CO2 flow rate, a syringe-type pump controller (Teledyne ISCO 500D, USA), which temperature was maintained at 10 °C for all experiments using another thermostatic bath (HipperQuímica, Brazil), temperature controller (NOVUS Produtos Eletrônicos Ltda, Brazil) and pressure controller (WIKA, Brazil).

Besides CO_2, ethanol was used as a cosolvent for the extraction, in order to increase both extractions yield and rates, since it was the most efficient cosolvent evaluated in previous tests (compared to ethyl acetate). Therefore, these extractions were named scCO_2+EtOH.

The procedure of the extraction with scCO_2+EtOH consisted of placing around 10 g of avocado's seed (Brazilian or Mexican) soaked (wetted) with ethanol, inside the extractor, at the desired ethanol to raw material mass ratio. This being said, for 10 g of avocado's seed, the amount of ethanol added was: 10 g (mass ratio of 1:1), 15 g (mass ratio of 1.5:1) and 20 g (mass ratio of 2:1), although the latter was only tested at the centre point.

Then the extractor was filled with CO_2 by injection, the pressure and temperature were set at the desired conditions and the static extraction begun for a certain confinement period (30 min using cosolvent).

After the end of static extraction, the dynamic extraction started by using compressed CO_2 at a constant flow rate around 2.0 ml/min, which was used for all extractions of this study and it was controlled by the syringe-type pump at cooling temperature and the pressure of the extractor.

The oil extracts were collected in test tubes, immediately covered with their respective taps, at 2.5 min (up to 25 min), 5 min (25-30 min) and 10 min (30-60 min).

At last, after sampling, the test tubes were placed inside an air circulating oven, in order to evaporate the ethanol until constant mass is achieved (for about 48 h) and then the dried extracts were gravimetrically quantified for each sampling and stored at −18 °C.

To determine the conditions of pressure and temperature of these experiments, a Design of Experiments (DoE) was applied (Table 1). In this study, a 2^2 full factorial experimental design with duplicate on centre points was used to analyse the impact of the independent variables: temperature (40, 60 and 80 ºC) and pressure on the extraction yield (15, 20 and 25 MPa), on both mass ratios (1:1 and 1.5:1).

The influence of density, which depends on the temperature as well as pressure, has also been evaluated. The CO_2 density values were obtained from the NIST database [7] at the temperature from the cooling bath (10ºC) and the system's pressure (15, 20 and 25 MPa).

The oil extraction yield was calculated according to Eq. (1).

RESULTS AND DISCUSSION

Soxhlet extraction

The purpose of both extractions' method (Soxhlet or SFE) is to evaluate and compare the extraction yield as well as comparing it between both samples (Mexican and Brazilian).

Soxhlet extraction was carried out in triplicate for two different particles sizes, using different Tyler's meshes. One size consisted of the particles retained from mesh 28 and 35 (0.60 mm > particle > 0.42 mm) and the other size was representative of the particles who were retained below mesh 35 (particle < 0.42 mm).

In all extractions, of both particles' sizes, three organic solvents were used: hexane, ethanol and ethyl acetate. The choice of the solvent to be used depends on the substances that are going to be extracted from the samples since the polarity of the solvent has high importance. In this study, for future purposes, polyphenol compounds are primarily of a polar type, polar solvents such as ethanol, ethyl acetate can effectively extract them. Still, hexane is well known for its efficiency in oil extraction, which may improve future extraction of fatty acids, since they are both non-polar [2-6].

Table 1. Results of oil extraction yields of avocado's seed using Soxhlet with hexane, ethanol and ethyl acetate

Run	Solvent	T(°C)	PS (mm)	Extraction yield (wt%)
Mexican seed				
1	Hexane	68.00	[0.42; 0.60]	2.46 ± 0.03
2	EtOH	78.38	[0.42; 0.60]	8.2 ± 0.9
3	EtOAc	77.10	[0.42; 0.60]	3.6 ± 0.1
4	Hexane	68.00	< 0.42	3.27 ± 0.02
5	EtOH	78.38	< 0.42	9.8 ± 1.9
6	EtOAc	77.10	< 0.42	3.1 ± 0.3
Brazilian seed				
7	Hexane	68.00	[0.42; 0.60]	3.5 ± 0.1
8	EtOH	78.38	[0.42; 0.60]	9.5 ± 0.2
9	EtOAc	77.10	[0.42; 0.60]	4.6 ± 0.2
10	Hexane	68.00	< 0.42	3.6 ± 0.1
11	EtOH	78.38	< 0.42	10.3 ± 0.3
12	EtOAc	77.10	< 0.42	4.8 ± 0.4

Table 1 presents the results of Soxhlet extraction using hexane, ethanol and ethyl acetate as solvents for different particle sizes (PS), for 6 hours of extraction.

As shown in Table 1, ethanol provided higher extraction yields when compared to hexane and ethyl acetate for all particle sizes (PS) evaluated, either Mexican or Brazilian, being the highest yield referring to the Brazilian seed with the particle size being lower than 0.42 mm.

In terms of the origin of the avocado's seed, although both samples are from the same variety (Hass), the ones originated from Brazil had always a higher yield than the ones who came from Mexico, in both particles sizes (Fig.2). This difference might have to do with the composition of avocado or any other fruit being dependent on the variety, grade of ripening, climate, the composition of soil and fertilizers [2].

In terms of particles sizes, the effectiveness of all solvents was considerably improved by lower particle diameters, providing higher oil yields, except in the Mexican seed, using ethyl acetate, in which the lower particle diameter had less oil yield. However, the authors decided that the raw material with lower particle size would be used only for comparison, because for further experiments with SFE, the solid matrix should be intermediate, as over-milling can lead to too fine particles, limiting fixed-beds performance, caused by the agglomeration of particles, dead zones formation and compaction [8].

The comparison of experimental results with the ones available from the literature [9-11] can be seen in Fig.2, using the average extraction yield of both particles sizes, divided by the hours of extractions.

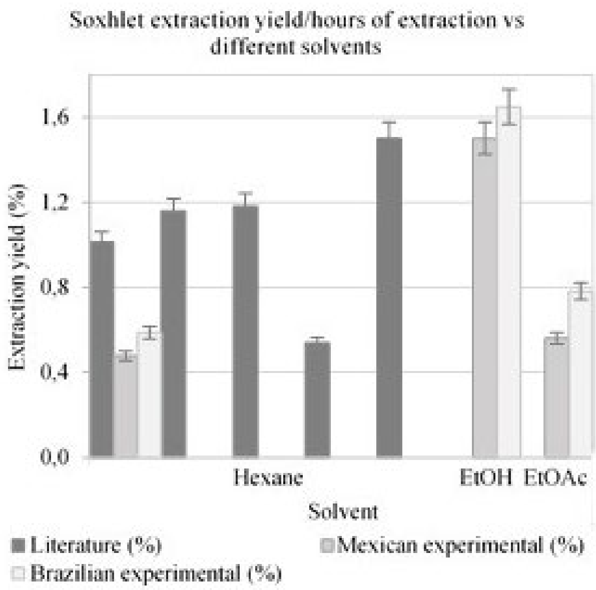

Fig.2. Experimental results from Soxhlet vs literature

Analyzing Fig.2, it was not found literature results for Soxhlet extraction using ethanol or ethyl acetate for avocado's seed, as far as the authors know. Despite that, literature results were found using hexane as a solvent for avocado's seed [9-11]. As it can be seen, taking into consideration the hours of the extraction of all experiments, most of the literature's extraction yield was higher than the ones obtained from this study with hexane, although when using ethanol, these results were similar. However, the varieties or the origins of the avocado's seed from the literature are not the same as the avocado's variety studied, which can explain the differences between yields.

SFE

Before a full DoE was applied, it was performed some preliminary experiments with the aim to find what cosolvent would help achieve the highest yield, in the Brazilian sample, since it had a higher yield than the Mexican sample, and also there was more quantity of it (with particle size from mesh 28 and 35 only). Since ethanol and ethyl acetate had better results as solvents in the Soxhlet extraction, they were tested in SFE. These experiments showed that pure scCO2 was not efficient enough by itself to extract the seed oil, at conditions up to 20 MPa and 60 °C (extraction yield around 3%), for 60 minutes of confinement time, being needed the addition of ethanol or ethyl acetate to optimize the extraction. Yet, ethanol obtained better results of extraction yield than ethyl acetate, being the cosolvent chosen for the full DoE (Table 2).

As presented in Table 2, the experiments are referred to the Brazilian sample. Analyzing the results obtained, the higher yields were achieved at higher pressure (25 MPa) and temperatures of 40 and 80ºC, being the highest yield of 6.9% for the mass ratio of 1.5:1 (run 20). Contrarily, the lowest yield was 1.9% for the mass ratio of 1:1, at 15 MPa and 40ºC (run 16).

These results are directly influenced by CO_2 density, in which higher density increases the extraction yield by improving CO_2 solubility power into the matrix [3,4].

For the Mexican sample, no preliminary experiments were performed, but full DoE with the mass ratios of 1:1 and 1.5:1 experiment was (table 3). The highest extraction yield was 4.4% at 15 MPa and 80 ºC (run 32) for mass ratio 1.5:1 and the lowest yield was 1.6% at 15 MPa and 40 ºC (run 33) for mass ratio 1.5:1. Contrarily to the results of the Brazilian sample, the Mexican seed had its highest yield at a lower pressure which means lower density, and for that, we can say that the temperature of extraction had a more significant effect on the yield than the pressure.

Considering Tables 2 and 3, Soxhlet extraction with ethanol is considered as a value of reference for

Table 2. Experimental conditions and results for Brazilian avocado's seed oil extraction yields for SFE using $scCO_2$ and cosolvents

Run	Condition	Pressure (MPa)	Temperature (°C)	CO_2 density (kg/m^3)	Extraction yield (wt%)
Soxhlet					
7	Hexane		68.00		3.5 ± 0.1
8	EtOH		78.38		9.5 ± 0.2
9	EtOAc		77.10		4.6 ± 0.2
SFE - preliminary experiments					
10	CO_2	25 (+1)	40 (-1)	1001.7	2.7
11	CO_2 + EtOAc	25 (+1)	40 (-1)	1001.7	2.6
12	CO_2 + EtOH	25 (+1)	40 (-1)	1001.7	4.2
17	CO_2 + EtOH (1.5:1)	20 (0)	60 (0)	980.2	5.4
18	CO_2 + EtOH (2:1)	20 (0)	60 (0)	980.2	5.1
SFE - experimental design (1:1)					
13	CO_2 + EtOH	20 (0)	60 (0)	980.2	4.4 ± 0.5
14	CO_2 + EtOH	25 (+1)	80 (+1)	1001.7	6.3
15	CO_2 + EtOH	15 (-1)	80 (+1)	954.2	2.7
16	CO_2 + EtOH	15 (-1)	40 (-1)	954.2	1.9
SFE - experimental design (1.5:1)					
19	CO_2 + EtOH	20 (0)	60 (0)	980.2	5.0 ± 0.4
20	CO_2 + EtOH	25 (+1)	80 (+1)	1001.7	6.9
21	CO_2 + EtOH	25 (+1)	40 (-1)	1001.7	5.6
22	CO_2 + EtOH	15 (-1)	80 (+1)	954.2	5.0
23	CO_2 + EtOH	15 (-1)	40 (-1)	954.2	4.6

Table 3. Experimental conditions and results for Mexican avocado's seed oil extraction yields for SFE using $scCO_2$ and cosolvent

Run	Condition	Pressure (MPa)	Temperature (°C)	CO_2 density (kg/m^3)	Extraction yield (wt%)
Soxhlet					
7	Hexane		68.00		2.46 ± 0.03
8	EtOH		78.38		8.2 ± 0.9
9	EtOAc		77.10		3.6 ± 0.1
SFE - experimental design (1:1)					
24	CO_2 + EtOH	20 (0)	60 (0)	980.2	2.5 ± 0.3
25	CO_2 + EtOH	25 (+1)	40 (-1)	1001.7	2.4
26	CO_2 + EtOH	25 (+1)	80 (+1)	1001.7	3.1
27	CO_2 + EtOH	15 (-1)	80 (+1)	954.2	2.7
28	CO_2 + EtOH	15 (-1)	40 (-1)	954.2	2.0
SFE - experimental design (1.5:1)					
29	CO_2 + EtOH	20 (0)	60 (0)	980.2	3.7 ± 0.6
30	CO_2 + EtOH	25 (+1)	80 (+1)	1001.7	3.9
31	CO_2 + EtOH	25 (+1)	40 (-1)	1001.7	3.6
32	CO_2 + EtOH	15 (-1)	80 (+1)	954.2	4.4
33	CO_2 + EtOH	15 (-1)	40 (-1)	954.2	1.6

its highest yield and for being a green solvent, with the right polarity for extracting a wide range of valuable compounds from the avocado's seed [3-5].

Therefore, the run from SFE that got closer to this value was run 20 from the Brazilian sample and run 32 from the Mexican sample, although the Mexican sample always presented lower yields than the Brazilian sample, for all SFE experiments, just like in the Soxhlet extraction. One reason for that is the fact the Brazilian sample was more porous than the Mexican sample and interacted strongly with the cosolvent added, consequently achieving higher yields.

Comparing both methods, Soxhlet extraction is known for its high extraction yields, justified by the fact it resorts to high temperatures that reduce the surface tension and viscosity of the solvent, improving its solubilization and increasing the number of the components soluble in the solvent phase. SFE extraction yields are lower than the results obtained by Soxhlet extraction, but still, it needs to be mentioned that the amount of solvent and energy spent in Soxhlet is much higher than the one spent in SFE (being harmful to the environment), in addition to spending much more time on the extraction than in SFE [2-6].

CONCLUSION

In order to value avocado's by-products, namely the extracts from its seeds, extraction using $scCO_2$ and Soxhlet were studied, using different solvents.

The seeds that obtained the best results were the Brazilian seeds. For them, the highest yield was obtained by using ethanol as a solvent in the Soxhlet extractor for 6 hours (9.5%). For the SFE extraction, the addition of cosolvent, in this case, ethanol as well, did increase the seed oil recovery (6.9% for the highest value, at 80 ºC, 25 MPa and mass ratio of 1.5:1) compared to extraction using only $scCO_2$ (2.7% at 60 ºC and 20 MPa), since it interacts with the polar fraction of the extract that the $scCO_2$ can not, for it is non-polar.

Even though the SFE method had lower yields results, it is of great importance because CO_2 is a more selective solvent than the organic ones.

For the Soxhlet method, different solvents were used, being the value for ethanol more than twice as high as for hexane, which confirms the role that polarity has on the extraction yield, indicating that the extract is richer in polar analytes than in non-polar. Size of particles was also a parameter evaluated, in which the smaller sizes enhanced the extraction yield (10.3% for the Brazilian seed) by increasing the surface area in contact with the solvent.

Relatively to its advantages, SFE is more economical and environmentally friendly due to less use of solvent, energy and time of extraction, when compared to Soxhlet.

The results are useful for the further development of methods to extract bioactive compounds, in order to analyse the pharmaceutical application of these extracts.

Acknowledgements: *The authors acknowledge the funding received from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 778168.*

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