SUPERCRITICAL FLUID EXTRACTION OF PASSION FRUIT SEEDS AND ITS PROCESSING RESIDUE (CAKE)

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Abstract. Despite the current efforts of companies to reuse process residues large amounts of passion fruit seeds are still underutilized by juice industries. Part of it has been used to produce seed oil due to its high content of unsaturated fatty acids. The seed cake is the residue derived from the cold press of the seeds from oil production and it can still contain fatty acids and phenolic compounds of interest. This study aimed to apply different extraction techniques on passion fruit seeds and seed cake evaluating their performances in terms of process yield, total phenolic content (TPC) and antioxidant activity (AA) of extracts. The raw materials were supplied by the company Extrair Óleos Naturais located in Rio de Janeiro State, Brazil. The seeds were grounded in a blender prior to extraction while the seed cake received no pre-treatment. Extraction methods used were: supercritical fluid extraction (SFE) with CO_2 (SC-CO_2), conducted at 40 °C and 50 °C with pressures of 150 and 250 bar and 0.5 kg_{CO2}/h; and the low pressure techniques (LPE) of cold maceration (MAC) and ultrasonic assisted extraction (UE) using different organic solvents. The best yield results were obtained by SFE at 250 bar and 40 °C for the seed (27 ± 1 %) and by MAC with 50 % ethanol for cake (6 ± 1 %). The cake UE performed with 50 % ethanol presented the highest TPC result determined by the Folin-Ciocalteau method (336 ± 22 $mg_{gallic\ acid\ equivalents}/g_{extract}$) and the best AA using the β-carotene bleaching method (88.8 ± 0 % AA after 120 min).

Keywords: ultrasonic assisted extraction; passion fruit oil; total phenolic content; extraction yield.

1. Introduction

Passion fruit belongs to the P*assifloraceae* family featuring over 500 species worldwide. Among these, the fruits of only about 20 varieties are edible and the most cultivated specie in the world is the yellow passion fruit (*Passiflora edulis* f. *flavicarpa*) [1]. While the origin of the passion fruit plant is unknown, it is generally believed to be native from Brazil, which is the world´s largest producer and consumer of fresh and processed yellow passion fruit, accounting for 50-60 % of the total world production. Today passion fruit grows nearly everywhere in the tropical belt, but South America is the main exporter of yellow passion fruit concentrated juice as its demand is growing worldwide [2,3]. Also, the *Passiflora* species are widely used as phytotherapeutic agents in Brazil [4].

The juice production engenders a large amount of residues such as seeds and rind. Despite the current efforts of companies to reuse process residues, large amounts of passion fruit seeds are still underutilized by the industries. Part of it has been used to produce seed oil, due to its high content of unsaturated fatty acids, especially linoleic acid (up to 70 %) finding various applications in the food, pharmaceutical and cosmetic industries [5,6]. Still, in the residue of the oil production - the seed cake - remains fatty acids, phenolic compounds and proteins of interest.

Phenolic compounds are known to present antioxidant activity that inhibits oxidative damage and may consequently prevent inflammatory conditions, ageing and neurodegenerative diseases. The literature evinces the presence of phenolic compounds in the *Passiflora edulis* plants, mainly belonging to the flavones C-

glucoside class [7]. Some studies also report the antioxidant activity of the passion fruit seed oil or from specific compounds present in the oil [5,8,9].

The supercritical fluid extraction (SFE) is based on the use of solvents in conditions above the critical point, resulting in a liquid like density, gas like viscosity and the diffusivity values within two orders of magnitude higher than that for typical liquids [10]. The supercritical carbon dioxide (SC-CO_2) extraction of the vegetable oil has attracted considerable attention as a promising alternative to low pressure solvent extraction and mechanical pressing process. The main reason is that SC-CO_2 not only has high extraction rate but also is non-toxic, non-flammable, non-explosive, cost-efficient, readily available, easy to remove from the extracted materials and presents moderate critical conditions and, thus, no thermal or chemical degradations of bioactive substances are expected [11].

Then, the aim of this work is to evaluate the use of different extraction methods to obtain passion fruit seed and seed cake extracts and compare the different extracts obtained in terms of process yield, total phenolic content and antioxidant activity.

2. Methods

2.1 Raw material

The raw materials were supplied by the company Extrair Óleos Naturais located in Rio de Janeiro state, Brazil. The passion fruit seed was ground in a domestic blender (Black & Decker, SP, Brazil) prior to extraction and presented moisture content of 8.50 ± 0.09 % w/w, determined according to 940.26 method from A.O.A.C. [12,13]. While the seed cake, a dry powder with moisture content of 6.26 ± 0.06 % w/w, received no pre-treatment.

2.2 Maceration (MAC)

The maceration of the raw materials was performed at room temperature. The extraction was performed with a proportion of 1 g of sample material to 5 mL of solvent for seven days, shaked once a day. The solvents applied separately were: hexane (Hx), ethyl acetate (EtOAc), ethanol (100 % EtOH) and a mixture EtOH/water (1/1, v/v) (50 % EtOH) in increasing order of polarity. The solvents of the resulting extracts were evaporated at reduced pressure in a rotary evaporator (Fisatom, 802, Brazil), supplied with cooling and vacuum control to obtain the crude extracts. After, the extracts were submitted to the inertization with nitrogen stream. All extracts were stored in sealed amber glass bottles at -18 °C. The extraction yields of all method/solvent systems were determined by the ratio between the mass of extract obtained and the mass of raw material used (wet basis), and they were presented by average ± standard deviation.

2.3 Ultrasound-assisted leaching extraction (UE)

Ultrasound-assisted leaching extractions were conducted using 3 g of raw materials and 50 mL of solvent placed inside a covered glass balloon. The extraction was performed in duplicate at room temperature during 45 min using, separately, Hx, EtOAc, EtOH and the mixture EtOH/water (1/1, v/v). The equipment used was an ultrasonic cleaner bath (USC-700/55 kHz, Unique Ltda., Indaiatuba/SP, Brasil), which operates in a frequency of 55 kHz and potency of 220 V. It was used the same procedure described in section 2.2. to eliminate the solvents, store the crude extracts and calculate yield.

2.4 Supercritical fluid extraction (SFE)

The experimental procedure for the high-pressure operation and the unit components were described by Michielin et al. [13]. The process used pure CO_2 99.9% delivered at pressure up to 60 bar (White Martins, Brazil). The fixed bed of particles was formed with 10.0 g of raw materials described on 2.1., placed slowly inside the extractor to obtain a uniform bed and avoid wall effects and channeling.

Previous studies [14,15] demonstrated that the extraction curves can be divided in three periods controlled by different mass transfer mechanisms: a) Constant Extraction Rate (CER) period, at the initial portion of the

curve, when the solute is easily accessible covering the external surface of the particles and the convection is the dominant mass transfer mechanism; b) Falling Extraction Rate (FER) period, when the diffusion mechanism starts, operating together with convection, due to failures in the external surface solute layer; c) Diffusion-controlled period, when the mass transfer occurs manly by diffusion in the bed and inside the solid particles as the external solute layer disappears, leading to a small extraction rate at the end of the curve.

Thus, the SFE assays were divided in two groups:

a) Kinetics experiment carried out at 250 bar, 40 °C and constant solvent flow rate of 0.5 kg CO_2/h, collecting the extract at pre-established time intervals and weighed in an analytical balance (OHAUS, Model AS200S ±0.0001 g, USA). The data obtained were plotted and the CER, FER and diffusional periods were fitted to determine the extraction time to be used on the following assays to determine the global yields in different SFE operational parameters applied (assays from group b). The kinetics assays were conducted until the diffusional period was completely established (240 and 270 minutes for seed and cake, respectively).

b) Global yield (X_0) assays performed at pressures of 150 and 250 bar, and temperatures of 40 and 50 °C, at constant solvent flow rate of 0.5 kg CO_2/h. The extracts were collected in amber flasks and weighted in analytical balance after 2.5 h and 3 h extracting time for the passion fruit seed and cake respectively. The inertization and the storage procedures and the yield calculation described in section 2.2. were also applied for the SFE assays.

2.5 Determination of total phenolic content

The total phenolic content (TPC) was determined according to the Folin-Ciocalteu method [16]. The reaction mixture was composed of 0.1 mL of extracts, 7.9 mL of distilled water, 0.5 mL of Folin-Ciocalteu reagent and 1.5 mL of 20% sodium carbonate, placed in amber flasks. The flasks were agitated and allowed to stand for 2 h. The assays were performed in triplicate and the absorbance was measured at 765 nm and TPC was expressed in mg of gallic acid equivalent (GAE)/g of extracts.

2.6 β-carotene bleaching method

The antioxidant activity from the β-carotene/linoleic acid system was carried out according to the method described by Matthäus [17]. Briefly, an aliquot of 5 mL of a stable emulsion of β-carotene/linoleic acid prepared previously was added with 0.2 mL of ethanolic passion fruit seed and seed cake extract solutions (1667 mg/mL) and the absorbance was immediately measured at 470 nm against a blank consisting of the emulsion without β-carotene. The tubes were placed in a water bath at 50 °C and the absorbance was measured every 15 min up to 120 min. The β-carotene bleaching rate was determined by the difference in absorbance (470 nm) values at 0 min and at 120 min (mean of the triplicate experiments) and converted into percentage of antioxidant activity (% AA).

3. Results and discussion

3.1 SFE kinetics

The kinetics of solute extraction from natural products consist of releasing solutes from porous or cellular matrices into a solvent phase - supercritical CO_2 - by mass transfer mechanisms. The solute, linked to the solid matrix by physical or chemical forces, must be transferred to the solvent phase by dissolution or desorption. Then, the solute/solvent mixture diffuses to the solid surface and finally moves across the stagnant film around the particle to the fluid phase [18].

The kinetics assays with both raw materials were performed focusing to determine the ideal extraction times for the sequence of the study. Figure 1 presents the overall extraction curves of passion fruit seed and seed cake at 250 bar, 40 °C and constant solvent flow rate of 0.5 kg CO_2/h.

As presented in Figure 1, the CER period for the passion fruit seed was detected from 0 to approximately 40 min, followed by the FER period up to 100 min, the last period, the diffusion controlled period, started just after the FER. While for the seed cake the CER period is between 0 and 20 min and FER between 20 and 135

min when the diffusion controlled period started. Therefore, 150 min (2.5 h) and 180 min (3 h) were defined as the adequate time to estimate the extraction yield of SFE from seed and cake respectively.

3.2 Global yield (X_0)

The passion fruit seed and seed cake extractions were performed in duplicate and the mean yield results for different methods are presented in Figure 2. The global yield (X_0) is defined as the amount of solute extractable by the solvent at the established extraction conditions and it indicates quantitatively the process efficiency.

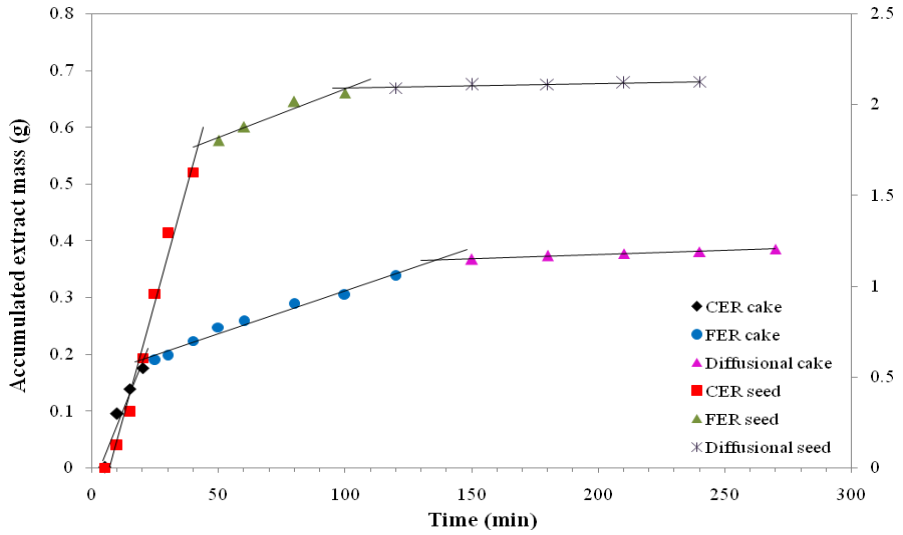

Figure 1. Overall extraction curve of SFE from passion fruit seed (right axis) and seed cake (left axis) at 250 bar, 40 °C and 0.5 kg CO_2/h.

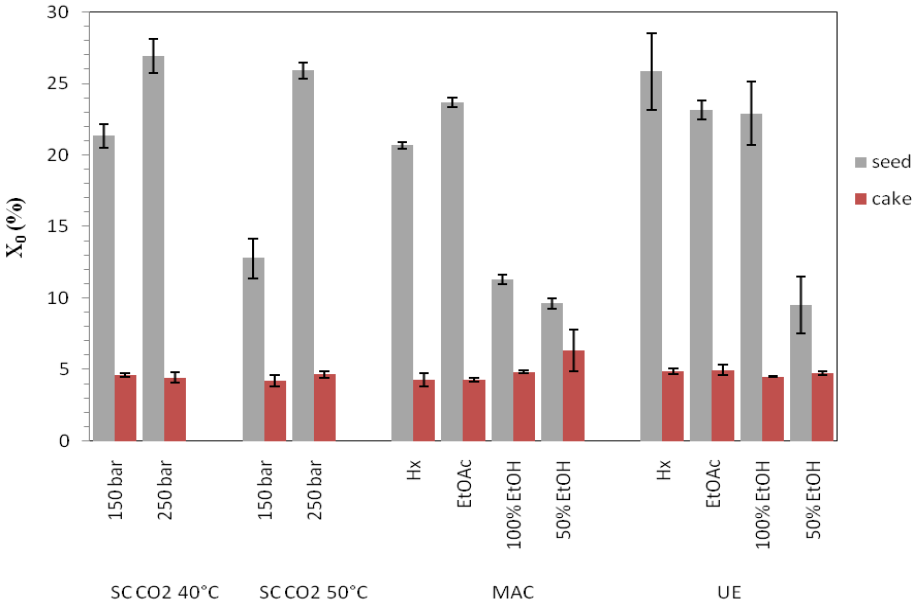

Figure 2. Global extraction yield results for passion fruit seed and seed cake extractions using different techniques: SFE with pure SC-CO_2 at 40 and 50 °C and pressures of 150 and 250 bar; MAC and UE with Hx, EtOAc, 100 % EtOH and 50 % EtOH.

3.3 Total phenolic content (TPC) and antioxidant activity (% AA)

The results of TPC obtained according to Folin-Ciocalteu method and the antioxidant activity evaluated by the β-carotene bleaching method from passion fruit seed and seed cake extracts are presented in Table 1.

TPC and % AA results of the extract obtained with the mixture EtOH/water (1/1, v/v) for both LPE methods can be highlighted as the best results, even compared to the BHT, a known commercial antioxidant used in food industry. Regarding the cake, combining yield, TPC and % AA results it is possible to imply the 50% EtOH as the best solvent, among the ones employed in the present work, to perform the passion fruit seed cake extractions.

The UE/EtOAc also presented good antioxidant activity by the β-carotene bleaching method for both raw materials. In this case, as well as the SFE at 150 bar, the antioxidant activity could not be related to a high TPC, what suggests the presence of other compounds that can be responsible for this activity to be further investigated.

For both raw materials the SFE at lower pressure provides better results, especially in terms of % AA, while the extracts obtained at 250 bar presented very low % AA. The extracts obtained with hexane also presented very low antioxidant activity. As the β-carotene bleaching method is appropriate to analyze the lipid fraction, which is easily extracted by SC-CO_2 and Hx, the AA behavior denotes that the antioxidant activity of the passion fruit seed is not related to this fraction. Finding corroborated by Oliveira et al. [23] that worked with a passion fruit industrial residue composed by seeds and rinds. According to the authors, the hexane extract of this residue did not exhibit antioxidant activity by $DPPH^{\bullet}$ and superoxide anion ($O_2^{\bullet -}$) radicals methods while its methanolic extract showed activity. This methanolic extract also presented a TPC value of 41.2 ± 4.2 mg GAE $g^{-1}_{dy\ extract}$, similiar to the ones obtained in the present work, except for the 50 % EtOH extracts mentioned above.

4. Conclusions

The supercritical fluid extraction with carbon dioxide provided the best yield result, for the extraction of passion fruit seed oil, at 250 bar and 40 °C, while for the seed cake the yield values were similar to the ones obtained by LPE. For SFE while the best yields were reached at 250 bar, the highest total phenolic content and antioxidant activity values were provided by the extract obtained at 150 bar. Connecting all SFE data, the selected conditions are 150 bar/40 °C for the passion fruit seed, due to the intermediate yield value and good % AA, while for the seed cake the condition of 150 bar/50 °C was selected due to good performance in yield and %AA. For both LPE methods with the seed cake, the mixture EtOH/water (1/1, v/v) presented yield and % AA values slightly better but TPC results much higher than all other extracts, which leads to consider it the best solvent to proceed these extractions with this raw material. The present work shows that SFE is an alternative to aggregate value to a residue of juice industry, the passion fruit seed for its high fat content, and also to the waste of the production of cold pressed oil from passion fruit seeds for the presence of substances of interest such as phenolic compounds.

Acknowledgements

The authors wish to thank Capes and Federal University of Santa Catarina for the financial support and Extrair Óleos Naturais for the raw material supply.

Table 1. Total phenolic content (TPC) and antioxidant activity obtained by low pressure extraction methods (LPE) and supercritical fluid extraction (SFE) for passion fruit seed and seed cake extracts

Extraction Method	**Process parameters**		**ρ CO_2[(1)] (g/cm^3)**	**Total phenolic content (TPC) (mg GAE/ g extract)**		**Antioxidant Activity (% AA after 120 min) [(4)]**	
				Cake	***Seed***	***Cake***	***Seed***
SFE SC-CO_2	40 °C	150 bar	0.781	28 ± 4	33.0 ± 0.8	7.6 ± 0.3	59.4 ± 0
		250 bar	0.880	24 ± 2	30 ± 1	4.7 ± 0	5.8 ± 0.2
	50 °C	150 bar	0.701	31 ± 2	26.4 ± 0.8	81.6 ± 0.3	52.0 ± 0
		250 bar	0.835	21 ± 2	24.3 ± 0.4	6.7 ± 0.3	4.6 ± 0.2
LPE[(2)]	**Solvents**		**SPI[(3)]**				
MAC	50 % EtOH		7.1	284 ± 5	142.4 ± 0.4	84.2 ± 0.1	68.7 ± 0.1
	100 % EtOH		5.2	27 ± 2	75 ± 2	42.2 ± 0.3	64.4 ± 0
	EtOAc		4.3	32 ± 3	24.6 ± 0.8	29.3 ± 0.5	53 ± 2
	Hx		0	31 ± 3	30 ± 1	7.3 ± 0	2.8 ± 0.4
UE	50 % EtOH		7.1	336 ± 22	61.3 ± 0.4	88.8 ± 0	67.8 ± 0.2
	100 % EtOH		5.2	39 ± 1	21.4 ± 0.4	81 ± 1	37 ± 1
	EtOAc		4.3	28.2 ± 0.8	19 ± 1	85.6 ± 0.7	61.9 ± 0.7
	Hx		0	23.8 ± 0.4	22.3 ± 0	15.6 ± 0.6	12.8 ± 0.8
BHT	-			268 ± 13		113 ± 7	

[(1)] CO_2 density [20].
[(2)] Low pressure extractions.
[(3)] SPI: solvent polarity index according to Gu et al. [21]. SPI for aqueous solution was calculated by (IA/100 × PA) + (IB/100 × PB), where IA and IB are polarity index of solvents A and B, respectively, PA and PB the percentage of solvents A and B, respectively [22].
[(4)] Antioxidant activity evaluated by the β-carotene bleaching method.

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