SUPERCRITICAL FLUID EXTRACTION OF BYPRODUCT FROM PECAN [*Caryaillinoinensis (Wangenh.) K. Koch*] OIL INDUSTRY

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Abstract. Pecan nut cakes were obtained from Brazilian pecan oil industries to evaluate an alternative of reuse of this waste with supercritical technology. The literature reports that pecan nut presents antioxidant properties and according to the popular knowledge this nut could prevent cancer and cardiovascular diseases. Supercritical fluid extraction (SFE) using CO_2 is considered a clean technology, and approved for food processing. The aim of the present work is to investigate the operational parameters (pressures and temperatures) of SFE in order to determine best conditions to obtain high extraction yield and antioxidant potential and, afterwards, compare it with different low pressure extraction methods (Soxhlet and ultrasonic process). Two pecan cakes varieties were used in this study, Mahan and a mixture of Barton and Desirable. The SFE conditions applied to Mahan variety were 313.15 K and 323.15 K with pressures ranging from 100 to 300 bar. For low pressure extractions (LPE), ethanol and hexane were used as solvents. Only the best conditions of SFE and organic solvents processes were tested for the Barton/Desirable variety. Antioxidant capacity of all extracts was evaluated by DPPH radical scavenging method and β-carotene bleaching method. Mahan nut cake variety offered highest values for global yield and for antioxidant activity. Supercritical fluid extraction presented efficiency statistically equivalent of some solvent extractions, in terms of global yield. The analysis of SFE from Mahan cake showed the best conditions extractions at 300 bar and 313.15 K, considering the highest yield value and best antioxidant potential.

Keywords: pecan nut, supercritical fluid extraction, antioxidant

1. Introduction

Pecan is a nut with high phenolic contents and, among the nuts group, it has the highest antioxidant activity [1] Pecan oil has been universally used during centuries with the purpose to eliminate free radicals, strengthen cell activity and improve nerve cell function [4] The oil content in the raw material ranges from 60 to 75 %, depending on the variety, localization, year of production, composition of the soil and harvest season [5]

Pecan nut cake is an industry byproduct with remaining oil and, currently, is used for animal feeding. It is a residue derived from pecan oil industry and due to the pecan proprieties it can still be considered a source of antioxidants compounds and important fatty acids.

Generally, oil industries use organic solvents to extract their products. There is an increasing in public awareness of health, environmental and safety hazards associated with the use of organic solvents in food processing and the possible solvent contamination in the final products [6] In this scenario, supercritical fluid extraction (SFE) appears as an alternative clean technology with many advantages. Being that, the CO_2 is the most commonly used SFE solvent in food applications, as it is approved for food processing without declaration [7]

According to the known properties declared in the literature and the high potential of the supercritical technology arise the interest of study the pecan nut cake extraction by means of supercritical CO_2.

Also the biggest plantation of Latin America of this nut is located in Rio Grande do Sul State in Brazil [8] , that cropped more than 1.5 thousand tons of pecan nut in 2011 [9] , fact that motivates the present research.

Then, the purpose of this work was to investigate SFE of pecan nut cakes (Mahan and Barton/Desirable), compared with low pressure extractions (LPE) in terms of process yield and the extracts bioactivity such as antioxidant potential. Also, the SFE operational conditions that resulted in highest antioxidant activity (SFE and LPE), obtained for Mahan variety, were applied for Barton/Desirable mixture.

2. Materials and Methods

2.1 Materials

Industrial pecan cakes milled were provided from two different Brazilian industries. One cake was composed by Mahan variety and the other one by a mixture of Barton and Desirable varieties. After delivered, both cake types from the first crop from 2012 were immediately stored at 255.15 K in a domestic refrigerator. Samples were evaluated by their moisture and oil contents according to, respectively, 940.26 and 933.05 methods from A.O.A.C. [10] The raw material particle size was determined in a vertical vibratory sieve shaker (Bertel Metalurgic Ind. Ltda., Caieiras/SP, Brazil). All solvent used had analytical purity and the chemicals reagents for test antioxidant activity were from Sigma-Aldrich.

2.2 Low Pressure Extractions (LPE)

The low pressure methods applied were Soxhlet and ultrasonic process. Two solvents were chosen to be used in the low pressure extractions (LPE), selected with the intent of further comparisons with SFE: ethanol and hexane.

Sohxlet extraction: Five grams of pecan cake were extracted with 150mL of the selected organic solvent in a Sohxlet apparatus during 6 hour in solvent boiling temperature, as detailed by method 032/IV from Adolfo Lutz [11] .

Ultrasonic extraction: The extraction was performed by placing 5 g of pecan cake in 150 mL of selected organic solvent. The mixture stayed during one hour in an ultrasonic bath linked with a condenser. The technique was adapted from Vinatoru [12]

After the extraction procedures, the resulting mixtures from each technique were separated by using a rotaryevaporator (Fisatom, 802, Brazil), supplied with cooling and vacuum control, obtaining the extract. To ensure all solvent elimination the extracts were submitted to the inertization with nitrogen flush during 5 min. All extracts were stored in amber glass bottled at 255.15 K.

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 Supercritical Fluid Extraction (SFE)

The SFE was performed in a dynamic extraction unit previously described by Zetzl et al. [13] and the extraction procedure applied was completely explained by Michielin et al. [14] . Briefly, the extraction procedure consisted of placing 5 g of material inside the column to form the particles fixed bed, followed by the control of the process variables (temperature, pressure and solvent flow rate). The extraction was performed and the solute collected in amber flasks and weighted in an analytical balance (OHAUS, Model AS200S, NJ, USA). The SFE assays were performed with CO_2, using flow rate of 8.3 ± 2 g/min, during 4 h extraction. The temperatures applied were 313.15 K and 323.15 K, and the pressures of work were 100, 200 and 300 bar. The SFE assays were performed with 99.9 % pure carbon dioxide, delivered at pressure up to 60 bar (White Martins, Brazil).

The extraction yields of all SFE conditions 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.

2.4 Antioxidant Activity

DPPH radical scavenging method: The test was performed according to Mensor et al. [15] The method consisted in a 30 min reaction of the DPPH radical with the extract in ethanolic solution. Initially 0.01g of extract sample was diluted in 10 mL of ethanol. The extract solution was divided in test-tubes at pre-determined concentrations between 10 and 500 µg/mL. Then the reaction with 1 mL DPPH solution, the absorbance was measured at 517 nm. These data constructed a curve of extract concentration versus antioxidant activity and the results were expressed as maximum antioxidant activity and EC_{50} referred to the extract concentration required for 50 % antioxidant activity.

β-carotene bleaching method: The β-carotene bleaching rate was determined by the difference in absorbance (470 nm) values at 0 min and at 120 min. The antioxidant activity from the β-carotene acid system was carried out according to the method described by Matthäus [16] and Kang et al. [17] . Briefly, 40 mg of linoleic acid and 400 mg of Tween 20 were transferred into a flask, and 1 mL of a β-carotene-chloroform solution (3.34 mg/mL) was added. Chloroform was removed by rotary evaporation at 40 °C. Then 100 mL of distilled water was slowly added and vigorously agitated to form a stable emulsion. An aliquot of 5 mL of this emulsion was added with 0.2 mL of ethanolic extract solution (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 absorbance values (mean of the triplicate experiments) were converted into percentage of antioxidant activity.

2.5 Statistical Analysis

The global yield and the antioxidant activity were analyzed statistically by Analyses of variance (ANOVA one way) followed by Tukey test with 95 % of confidence level. The dates were treated in RGui statistic software version 2.15.2.

3. Results and Discussion

3.1 Pecan nut cake characterization

Mahan pecan nut cake was from the southeast region of Brazil and the results of moisture content were 5.2 ± 0.1 % and 59.4 ± 0.1 % of oil content. While the Barton/Desirable nut cake, originary from the south of Brazil, presented 5.9 ± 0.1 % of moisture content and 36 ± 1 % of oil content. Oro [5] reported the following values for Barton pecan cake variety, 7.9 % and 36.2 % for moisture and oil contents, respectively. In the same work, whole pecans presented 3.7 % of moisture content and 69.4 % of oil. The results for Barton cake from Oro work were different, probably because it was just one variety of nut and in the present work it was a mixture of others varieties.

The particle size of the pecan cakes was also analyzed. Mahan and Barton/Desirable varieties presented a particle mean diameter of 0.463 ± 0.003 mm and 0.505 ± 0.007 mm, respectively. Smaller particles increase contact surface area facilitating access of the solvent in sample.

3.2 Kinetics assays of Supercritical Fluid Extraction with Pure CO_2

The kinetics assays for both pecan nut cakes were performed focusing on the determination of the ideal extraction times for the sequence of the study. Figure 1 shows the kinetic curves obtained in this study and expressed in mass of the extract *versus* extraction time. According to Figure 1, in the beginning of the curves it is possible to note a stationary phase until 10 min, followed by the constant extraction rate (CER) until 90 min. From this time up to 240 min a falling extraction rate (FER) period was observed. At the time of 240 min. the diffusional period started for the assays with both cake types. Then, the time standardized for the further SFE study was of 4 hours. These results were verified by means of the software SAS for windows version 9.0. The accumulated mass obtained for the Mahan variety at the end of 4 hour extraction was 2.36 g (46.5 % yield wb) and for the Barton/Desirable variety was 1.19 g (23.1 % yield wb).

3.3 Global Yield of Low Pressure Extraction

The global extraction yield in wet basis of the different extraction methods applied for the Mahan and the Barton/Desirable nut cakes are shown in Table 1. The yields were calculated in wet bases to facilitate further comparisons with other works.

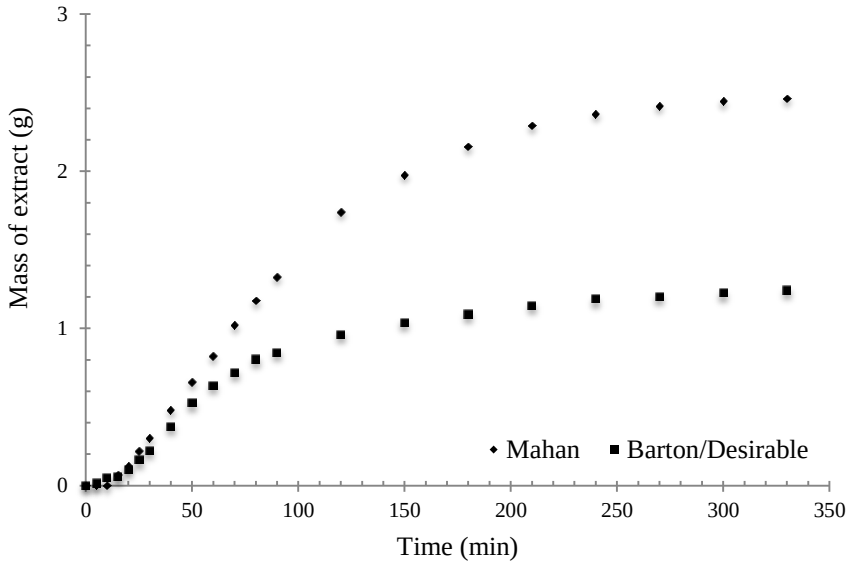

Figure 1. Supercritical fluid extraction curves of Mahan and Barton/Desirable varieties of pecannut cake 200 bar, 323.14 K, 8.3 g_{CO2}/min, using 5 g of raw material

According to Table 1, Soxhlet methods presented the highest yield results compared to the ultrasonic procedure for the same organic solvent. This behavior is probably due to the Soxhlet extraction offered higher temperature, solvent recirculation and higher interactions than ultrasonic extraction [18] . The highest LPE X_0 values were achieved from Soxhlet with ethanol for the Mahan nut cake (66.0 ± 0.3 %), and the Barton/Desirable nut cake (53.2 ± 0.4 %) (Table 1). Comparing the two varieties of nut cakes, Mahan cake presented higher yields than the Barton/Desirable cake.

Extractions from defatted pecan cake using organic solvents were performed by Prado [21] , obtaining 20% of yield when using LPE with ethanol. The result presented by Prado was lowest if compared to the yields obtained in present work because pecan cake was defatted first.

The extraction of pecans with hexane, presented by Miraliakbari and Shahidi [23] , resulted in 71.5 ± 0.4 % wb. And using polar solvents as chloroform-methanol was (73.4 ± 0.3 % wb). In this case the yields were highest because the samples were the nuts pecan, and so the material presented more oil content.

Table 1. Global Yield of extracts from Mahan nut cake and Barton/Desirable nut cake obtained by SFE and LPE

Low Pressure Extraction	Solvent	Solvent Polarity[24]	Global Yield (%) wb	
			Mahan	Barton/Desirable
Soxhlet	Ethanol	5.2	66.0 ± 0.3^{g}	53.2 ± 0.4^{cf}
Soxhlet	Hexane	0	58.3 ± 0.4^{f}	-
Ultrasonic	Ethanol	5.2	49 ± 3^{c}	34 ± 3^{d}
Ultrasonic	Hexane	0	52 ± 2^{ce}	-
Supercritical Fluid Extraction	Solvent	ρCO_2 (g/cm^3)[25]	Global Yield (%) wb	
			Mahan	Barton/Desirable
313.15K/100bar	CO_2	0.630	3.1 ± 0.8^{a}	-
313.15K/200bar	CO_2	0.829	48 ± 1^{bc}	-
313.15K/300bar	CO_2	0.928	55.3 ± 0.2^{ef}	31 ± 1^{d}
323.15K/100bar	CO_2	0.368	0.123 ± 0.003^{a}	-
323.15K/200bar	CO_2	0.762	43 ± 2^{b}	-
323.15K/300bar	CO_2	0.879	55 ± 1^{ef}	-

-Not tested. Same letters indicated not significant difference at 5 % of significance.

3.4 Global Yield of Supercritical Fluid Extraction

The SFE yield results (X_0) obtained at different conditions of temperatures and pressures are also presented in Table 1, as well in Figure 2. The results indicated that the X_0 increased with pressure enhancement and temperature decrease. The CO_2 density (Table 1) helps to indicates that increasing the density the highest yields were obtained. The CO_2 solvation power depends on solvent density, increasing with pressure at constant temperature and decreasing with temperature at constant pressure [19] . In another study developed with native pecan [20] the X_0 enhancement with the pressure also was observed between 413 bar and 668 bar, at a constant temperature.

SFE applying pressure of 300 bar provided the highest X_0, for 313.15 K, 55.3 ± 0.2 %, and 323.15 K, 55 ± 1 %. Just the extraction of 300 bar and 313.15 K was reproduced for Barton/Desirable cake because it present highest global yield and the lowest temperature. For 300 bar, the X_0 were different for both nut cakes. The lowest X_0 were for 100 bar extractions, 3.1 ± 0.8 % and 0.123 ± 0.003 %, and because of this were not tested for antioxidant activity.

SFE can be better compared with LPE utilizing hexane due to the non-polarity of both solvents (CO_2 and hexane). Since both solvents are nonpolar, the yields of Soxhlet and ultrasonic had no significant differences to 300 bar SFE. At 300 bar SFE had higher X_0 than ultrasonic extraction with ethanol.

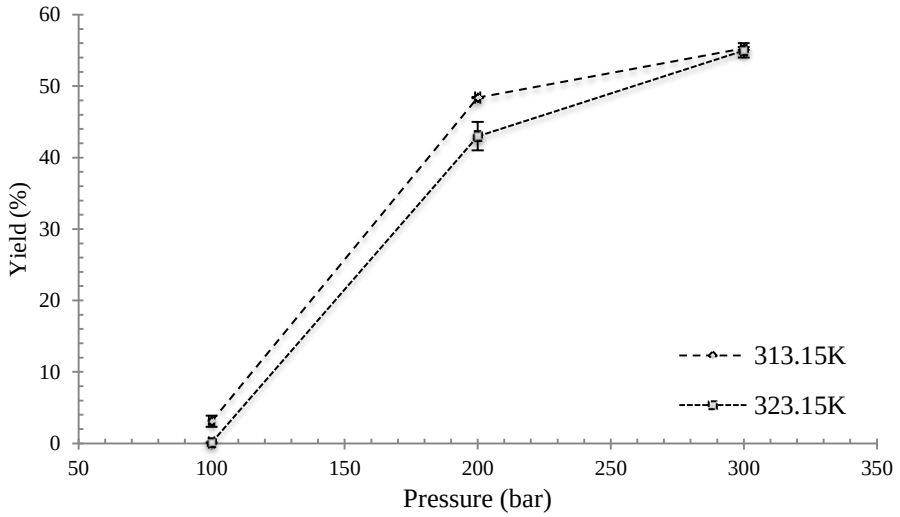

Figure 2. SFE isotherms from Mahan variety

3.5 Antioxidant Activity

The antioxidant activity of Mahan and Barton/Desirable nut cake extracts obtained by SFE and LPE are presented in Table 2, using the DPPH scavenging test and β-carotene bleaching method.

The β-carotene bleaching method showed that Mahan cake extract obtained by ultrasonic with ethanol presented the highest antioxidant activity when compared to all other extracts (79 ± 4 % - Table 2). According to the same method, Mahan variety presented better antioxidant activity for all extracts than Barton/Desirable cake (Table 2). The results for samples obtained by SFE shown an increase in antioxidant activity with pressure enhancement (from 100 bar to 300 bar) at constant temperature. This behavior is probably caused by the increase in solvent density, enlarging the extraction yield of antioxidant substances. Then, SFE extracts just had significant increase in antioxidant activity when operated at 300 bar and 313.15 K (24.2 ± 0.7 % - Table 2). At this condition, SFE extract can be compared with Soxhlet-hexane Mahan cake and Soxhlet-ethanol Barton/Desirable extracts.

In a similar study with β-carotene method [21] the authors presented values of 35.6 ± 0.8 % for the antioxidant activity for pecans extracts obtained by LPE-hexane and 79.2 ± 1.2 % for samples extracted with chloroform-methanol. The antioxidant activity of hexane extracts can be explained by hydrophobic antioxidants in pecan nuts, such as α-tocopherol, and hydrophilic compounds as galic acid when using ethanol as extraction solvent [23]

Analyzing DPPH results, SFE extracts and LPE extracts with hexane present high values of EC_{50}. The best EC_{50} result was obtained for the sample extracted using ultrasonic and ethanol (133.0 ± 0.2 µg/mL), as well as the best antioxidant activity (93.9 ± 0.3 %) (Table 2).

Both antioxidant methods applied indicated that the ultrasonic extraction was better than the Soxhlet procedure in order to reach better antioxidant activity of the extracts; also ethanol had better results than hexane. The literature indicates 323.15 K as the optimum temperature for extraction of phenolic compounds from different vegetable matrices [14] [22] , due to their thermal sensitivity and in the Soxhlet extraction the temperature employed is the boiling point of the solvent which may have degraded the compounds responsible for antioxidant activity.

Table 2. Antioxidant Activity of extracts from Mahan nut cake and Barton/Desirable nut cake

Extraction	%AA(120min)[1]		EC_{50} (µg/mL)[2]		%AA (500µg/mL)[3]	
	Mahan	Barton/ Desirable	Mahan	Barton/ Desirable	Mahan	Barton/ Desirable
Soxhlet ethanol	50 ± 4^{e}	16 ± 2^{ac}	$414 \pm 12^{\alpha}$	$238 \pm 2^{\beta}$	58 ± 1^{C}	93.5 ± 0.3^{B}
Soxhlet hexane	30 ± 4^{d}	-	>500	-	1.8 ± 0.2^{A}	-
Ultrasonic ethanol	79 ± 4^{g}	45 ± 3^{f}	$133.0 \pm 0.2^{\gamma}$	$255 \pm 1^{\beta}$	93.9 ± 0.3^{B}	91.2 ± 0.2^{D}
Ultrasonic hexane	6.4 ± 0.4^{b}	-	>500	-	3.2 ± 0.1^{A}	-
313.15K/100bar	-	-	>500	-	-	-
313.15K/200bar	10 ± 3^{ab}	-	>500	-	2.2 ± 0.3^{A}	-
313.15K/300bar	24.2 ± 0.7^{cd}	9 ± 2^{ab}	>500	>500	1.6 ± 0.4^{A}	1.1 ± 0.2^{A}
323.15K/100bar	-	-	>500	-	-	-
323.15K/200bar	8.2 ± 0.4^{ab}	-	>500	-	2.9 ± 0.4^{A}	-
323.15K/300bar	14 ± 4^{ab}	-	>500	-	1.4 ± 0.3^{A}	-

-Not tested. **1**abcdefgAntioxidant Activity after 120 minutes (β-carotene bleaching method). **2**αβγ EC_{50}extract concentration at 50% antioxidant activity (DPPH test). **3**ABCDAntioxidant Activity with 500µg/mL extracts concentration (DPPH test).Same letters indicated not significant difference at 5 % of significance. Different types of letters for different methods.

4. Conclusions

The present study shows an alternative to utilize a waste of the pecan oil industry, i.e., the pecan nut cake, which after the oil extraction still contain valuable substances worth to be submitted to extraction. The Mahan nut cake variety offered the highest values for global yield (Soxhlet with ethanol) and for antioxidant activity (ultrasonic with ethanol). The analysis of SFE from the same pecan cake showed the best extraction condition was at 300 bar and 313.15 K, considering yield and antioxidant activity, representing the highest CO_2 density applied in the SFE. The SFE presented efficiency statistically equivalent of some solvent extractions in terms of yields. SFE extracts demonstrated a lowest antioxidant activity if compare to the LPE extracts with ethanol. These results enable the future use of co-solvent in SFE. The pecan nut cake is a source of antioxidant that is better extracted with polar solvents and with high pressures and low temperatures in SFE.

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