PRODUCTION OF BLACK SESAME (*Sesamum indicum L*) SEEDS EXTRACT BY SUPERCRITICAL FLUID EXTRACTION: ISOTHERMS OF GLOBAL YIELD AND DETERMINATION OF TOTAL FATTY ACIDS

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Abstract. In alternative medicine, especially amazon caboclo knowledge, black sesame (Sesamum indicum L) seeds is one of the most important ingredients at recommended tea to treat stroke victims. The objective of this work is study some process variables of supercritical fluid extraction in black sesame to generate extracts applicable in stroke research. It was explored two isothermal 40 and 60 ºC combined with pressures that ranged from 200-400 bar, at a constant mass flow rate of 2 $Lmin^{-1}$. The extraction time was about half hour in static period and 3 hours in dynamic period. The highest mass yield was 51% in dry basis, obtained in the condition of 60 ºC and 400 bar. The fatty acid composition was determined by conversion to fatty acid in methyl esters (FAMEs) based on the suggested method by Lepag and Roy (1984) and detected using gas chromatography (Varian model CP 3380, USA) equipped with a flame ionization detector and with a CP-Sil 88 capillar column. Operating conditions were: helium as carrier gas a flow rate of 0.9 mL/min, a FID detector at 280°C, a injector (split ratio 1:100) at 250°C, an injection volume of 1 μL. The temperature programmed of the column was: 175 ^oC for 8 min, followed by 2.0 ^oC/min to 180 ^oC for 28 min and then 2.0 ^oC/min to 250 ^oC for 10 min. The individual fatty acid peaks were identified by comparison of retention times with those of known mixtures of standard fatty acids (Nu-check-prep, Inc., USA) run under the same operating conditions.

Keywords: Supercritical fluid extraction; Black sesame; Process variables.

1. Introduction

World Health Organization [1] estimates that in 2008 the cerebrovascular diseases was the second leading group diseases that caused most death in the world. In these cerebrovascular diseases, brain ischemia has a great occurrence. This problem generally has devastating consequences, from difficulty walking, psychological problems in victims and people close emotionally as well as death. These facts are reason to scientific community efforts to find treatments basis in new drugs from natural extracts.

Folk knowledge suggests black sesame (Sesamum indicum L) is an indispensable ingredient in a therapeutic tea to stroke victims. Antioxidants substances like sesamol, sesamolin and sesaminol can be find in Sesamum indicum L [2].

According to Encyclopædia Britannica [3] “sesame, also called Benne, erect, annual plant (Sesamum indicum) of numerous types and varieties belonging to the family Pedaliaceae, cultivated since antiquity for its seeds, which are used as food and flavouring and from which a prized oil is extracted”.

Kiran and Asad [4] showed that seeds and oil of Sesamum indicum L have considerable healing activity when administered orally or topic. Furthermore black sesame seeds are more potential antioxidant than cream sesame seeds [5] and the black sesame coat has the greater relative antioxidant potential [6]. Figueiredo and Modesto Filho [7] showed that defatted flour of Sesamum indicum L contributes positively to the rate of glycemic control and weight in patients with diabetes mellitus. Another interesting point in sesame seed is the sesamolin. Kang et al [8] show that sesamolin and his metabolites may have an important contribution as antioxidants in seed and oil from sesame. Moreover Kim et al [9] observed that extracts of black sesame possess phenolic compounds with radical scavenging ability may be related with inducing colon cancer cell death.

According to Murray et al [10] unsaturated fatty acids are very import to fluid maintenance in the cell membrane, hence a rich diet in of polyunsaturated fatty acids to saturated fatty acids has a good expectative to coronary heart disease. Eicosanoic fatty acids are form from polyunsaturated fatty acids and in turn generates eicosanoids prostaglandins (related to inflammation, pain, sleep and also regulate blood coagulation and reproduction), thromboxanes, leukotrienes (are important in allergic reactions and inflammation because the muscle contractant and chemotactic properties), and lipoxins.

Yoshida et al [11] found that the major fatty acids determined in sesame seed were linoleic, oleic, palmitic, stearic and linolenic. These are an important tips to investigate black sesame extracts.

All efforts are fundamental to the advancement in the search for discovery and optimization of treatments for very serious problems to human health. Therefore, the main of this work was generate Sesamum indicum L extracts by supercritical fluid extraction (SFE) with the purpose of encouraging future research on treatment of focal cerebral ischemia induced in Wistar rats and determine the fatty acids composition by gas chromatography equipped with a flame ionization detector.

2. Material and Methods

2.1 Black sesame seeds samples

Samples of black sesame seeds were acquired as a commercial product that, according to the label, come from cultures performed in the state of São Paulo in Brazil and that seeds were grown in the town of Castanhal in the state of Pará in Brazil. A voucher specimen (MG204755) was deposited at the Emílio Goeldi Paraense Museum, Belém, Brazil. The black sesame seeds were dried in air circulation kiln at 50 °C for 12 hours to 3,95% of humidity and and crushed in a Willey mill (Tecnal), model TE-650. Particle size distribution was analyzed using a sieve series (28–16 mesh).

Figure 1. Black sesame (Sesamun indicum L) plant and seeds.

2.2 Extraction procedures: SFE

The assays were carried out in the *SPE-ED SFE* system from Applied Separations model 7071, Allentown, PA. The extractor vessel used was of 50 mL. The global yield isotherms were determined using 5g of black sesame seeds. Two levels of temperature were used (40 and 60 ºC) both above the critical temperature of carbon dioxide. Pressures of 200, 250, 300, 350, and 400 bar were used. The solvent used was carbon dioxide (99.9% purity, Gama Gases, Belém, Brazil) and the solvent flow rate used was 2 l/min. The extraction time was 3 h.

2.3 Extraction procedures: Hydrodistillation

Conventional extraction method as hydrodistillation were also performed for comparison purposes. The volatile oil was obtained using a Clevenger apparatus and according to the AOAC 962.17 method [12]. Extraction was performed for 120 min, at the solvent boiling point and with a solid/solvent ratio of 1:10 (w/v).

2.4 Determination of fatty acids in extracts

The fatty acid composition was determined by conversion to fatty acid methyl esters (FAMEs) based on the suggested method by Rodrigues et al. [13] and detected using gas chromatography (Varian model CP 3380) equipped with a flame ionization detector and with a CP-Sil 88 capillar column (length 60 m, internal diameter 0.25 mm, film thickness 0.25 μm; Varian Inc., USA). Operating conditions were: helium as carrier gas a flow rate of 0.9 ml/min, a FID detector at 250°C, a injector (split ratio 1:100) at 250°C, an injection volume of 1 μl. The temperature programmed of the column was: 4 min at 80°Cand a subsequent increase to 205°Cat 4°C/min. The individual fatty acid peaks were identified by comparison of retention times with those of known mixtures of standard fatty acids (Nu-check-prep, Inc., USA) run under the same operating conditions. Retention time and area of each peak were computed using the Varian Star 3.4.1. software. The results were expressed as relative percentages of total fatty acids.

3 Results and discussions

3.1 Hiydrodistillation and the global yield isotherms

It was not possible to obtain volatile oil by hydrodistillation the yield was negligible.

3.2 SFE Global yield isotherms

The global extraction yields obtained from black sesame seeds are shown in Figure 2. It can be observed that the highest yield was obtained at 60 ºC and 400 bar as well as the lowest yield was observed at 60 °C and 200 bar.

Isothermal compressibility tends to infinity as the critical point is approached, hence density change dramatically and the solubility of substances in supercritical CO_2 (S-CO_2) depends on the free volume differences between the solute and the S-CO_2, and the intermolecular forces in operation between S-CO_2/S-CO_2, S-CO_2/solute, and solute-solute pairs in solution [14]. Moreover the solute molecule migration from condensed phase to the supercritical phase can be classified as vaporization and the pressure and temperature effects in solubility is directly related with solute vapor pressure and density [15].

The Figure 2 shows the yields increased with pressure in 40 ºC and 60 ºC isotherms singly. For the pressures below 332,5 bar, the CO_2 density effect prevails and the 40 ºC isotherm has the higher yield. However from 332,5 to 400 bar, the vapor pressure effect predominates, hence the 60 ºC isotherm has the higher yield than 40 ºC isotherm.

3.3 Fatty acids in extracts

In the Table 1 was demonstrated the fatty acid profile found in the extract of black sesame obtained with carbon dioxide in the supercritical state. The total oil content of black sesame extract ranged from 0.007 to 50.02 %. It was observed the presence of Caprylic acid (C8:0) in all operating conditions used to obtain the sesame extract and in the conditional of the 300bar/60ºC obtained the highest caprylic acid concentration while the lower concentration was found on the condition of the 400bar/60ºC. Traces of Capric Acid (C10:0) were obtained in the conditions of the 200, 400 bar/40ºC e 400 bar/60ºC, the same was observed to Lauric Acid (C12:0), Myristic Acid (C14:0), Palmitoleic Acid (C16:1), Margaric Acid (C17:0), Linolenic Acid (C18:3), Arachidic Acid (C20:0); Behenic Acid (C22:0) in all operating conditions of extraction. The major monounsaturated fatty acids (MUFAs) in all extractions conditions was Palmitic Acid (C16:0) followed by Stearic Acid (C18:0). Regarding polyunsaturated fatty acids the Linoleic Acid (C18:2) showed the highest concentration followed by Linolenic Acid (C18:3). Analysis of the fatty acid profile of the black sesame extract indicates a high unsaturated/saturated ratio.

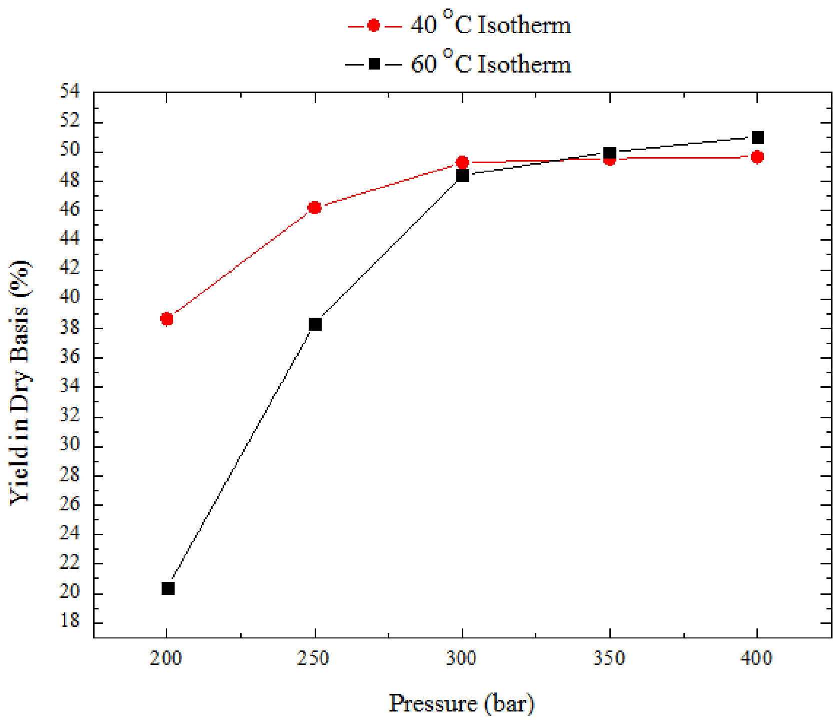

Figura 2. Isotherms yield in dry basis versus pressure.

In Figure 3 can observe the density limitation in the SFE and it is understood that the extract obtained at 60°C and 200bar had an yield too low, since the nominal density is relatively low. Once this point, the effect of density prevails in solubilization. The density nominal values were calculated by software TermoDI [16].

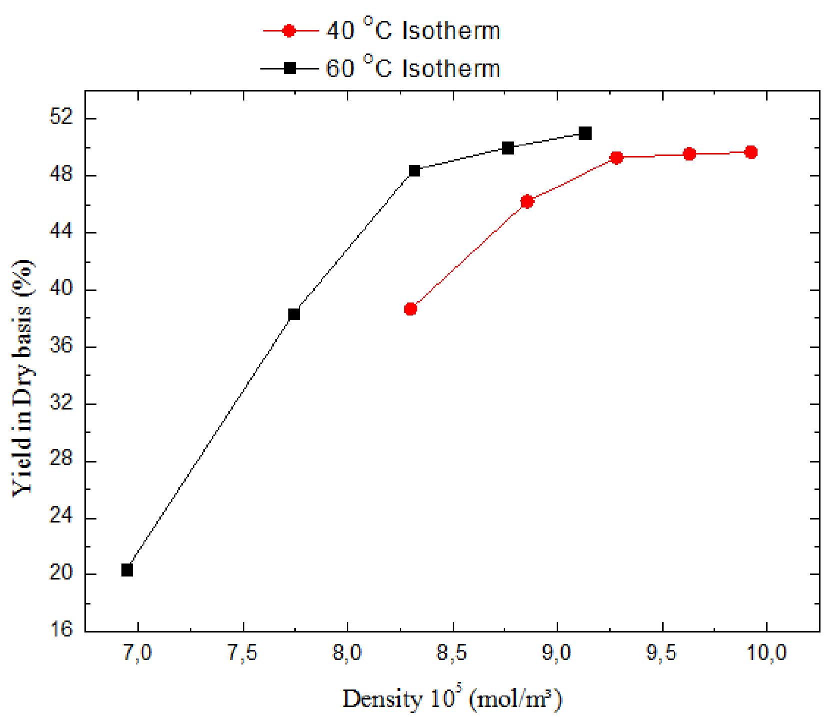

Figure 3. Yield in dry basis versus Density.

Table 1. Total oil contents of black sesame extracts

	Concentration of Fatty Acids in % g/100g									
Fatty Acids	200bar 40 ºC	250bar 40 ºC	300bar 40 ºC	350bar 40 ºC	400bar 40 ºC	200bar 60 ºC	250bar 60 ºC	300bar 60 ºC	350bar 60 ºC	400bar 60 ºC
C8:0	0,3931	0,4805	0,6480	0,5206	0,3004	0,2846	0,4456	0,8974	0,7824	0,2622
C10:0	0,0090	---------	---------	---------	0,0082	---------	---------	---------	---------	0,0032
C12:0	0,0076	0,0248	---------	0,0725	0,2020	0,0194	0,0499	0,0319	0,0408	0,0827
C14:0	0,0318	0,0573	0,0466	0,0514	0,1379	0,0506	0,0375	0,0930	0,0505	0,0672
C16:0	9,6051	9,6593	9,6540	9,6103	9,4848	10,5061	9,6768	9,6312	9,7620	9,7776
C16:1	0,1651	0,1568	0,1675	0,1763	0,1381	0,1976	0,1809	0,1802	0,1742	0,1374
C17:0	0,0301	0,0430	---------	0,0327	0,0333	0,0288	0,0390	0,0324	0,0280	0,0352
C18:0	4,9233	5,1010	4,8052	4,8820	5,6956	3,8635	4,8399	4,2787	4,6192	5,9507
C18:1	36,7273	37,2234	36,5840	36,5989	38,5455	34,9946	36,4733	35,5084	35,9182	38,7779
C18:2	47,1778	46,8822	48,0947	47,6715	44,4754	50,0238	47,8554	49,3123	48,6249	44,9059
C18:3	0,5505	---------	---------	---------	0,6690	---------	---------	---------	---------	---------
C20:0	0,3793	0,3718	---------	0,3837	0,3098	---------	0,3804	0,0344	---------	---------
C22:0	---------	---------	---------	---------	---------	0,0311	0,0213	---------	---------	---------

C8:0 (Caprylic Acid); C10:0 (Capric Acid); C12:0 (Lauric Acid); C14:0 (Myristic Acid); C16:0 (Palmitic Acid); C16:1 (Palmitoleic Acid); C17:0 (Margaric Acid); C18:0 (Stearic Acid); C18:1 (Oleic Acid); C18:2 (Linoleic Acid); C18:3 (Linolenic Acid); C20:0 (Arachidic Acid); C22:0 (Behenic Acid).

4. Conclusions

The method of extraction with carbon dioxide in supercritical state was effective in producing black sesame extract mainly in the operational condition of 60 °C and at pressures of 350 and 400bar. In the condition of 40 °C the increases in yield of extract was not as significant when the pressure was increased from 300 to 400 bar. The black sesame extract showed large amounts of fatty acids monounsaturated especially palmitic acid (C16:0) which was found in all operating conditions of extraction. However, the presence of polyunsaturated fatty acids represented by Linoleic Acid (C18: 2) and Linolenic Acid (C18: 3) was significant. According to the results observed by Kelley et al [17] certain isomers of conjugated linoleic acid are related to the anticarcinogenic effects. Kritchevsky et al [18] showed that the application of certain isomers of conjugated linoleic acid also been satisfactorily applied to the inhibition and treatment of atherosclerosis in rabbits. So this high amount of linoleic acid determined may suggest a attention to the application of extract obtained via black-sesame SFE in research for the treatment of cancer and atherosclerosis, as well as the quality and quantity in terms of isomers of linoleic acid present in extracts obtained in this work. The results shows coherent data for proceed to next steps: a) kinetics study of extraction; b) evaluate the potential to brain ischemia treatment in rat.

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