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Chia (*Salvia hispanica* L.) oil extraction using different organic solvents: oil yield, fatty acids profile and technological analysis of defatted meal

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Abstract

The aim of this study was to extract oil from the seeds of Chia (*Salvia hispanica* L.) using different solvents (ethyl acetate, isopropanol and *n*-hexane) and varying the seed to solvent ratio. The fatty acids profiles of the oils were determined and technological analysis of the defatted meal was carried out. In relation to the oil extraction, higher yields were obtained with *n*-hexane and ethyl acetate and it was observed that increasing the amount of solvent had little influence on the oil yield in the experimental range considered. The major components of chia oil are linolenic acid (~62%), linoleic acid (~19%), palmitic acid (~9.3%) and oleic acid (~6.2%). The fatty acids profiles of the oils obtained employing different solvents and seed to solvent ratios showed no significant differences ($p>0.05$). For the defatted meal, higher values for the oil-holding capacity and emulsification activity were observed with the removal of the oil; however, the nature of the solvent extracts did not affect these properties. Also, the water-holding capacity was not affected by the extraction process.

Introduction

Chia (*Salvia hispanica* L.) is an annual herbaceous plant which is native to southern Mexico and northern Guatemala. It is sensitive to daylight and produces small seeds which vary from white to dark brown in color. Chia has been cultivated in the region for thousands of years (Ixtaina *et al.*, 2008; Ayerza and Coates, 2009; Borneo *et al.*, 2010; Ayerza and Coates, 2011; Capitani *et al*., 2012). In recent years, chia seeds have been included in the human diet due to the health benefits associated with their composition (Borneo *et al*., 2010; Ixtaina *et al.*, 2011; Capitani *et al*., 2012). Coorey *et al*. (2012) reported that chia is an excellent food, since it is one of the largest botanical sources of linoleic acid, and it can easily be added to commercial products. According to Ixtaina *et al*. (2008) and Marineli *et al.* (2014), chia has been investigated and recommended for use due to its high percentage of fatty acids which are beneficial to health, proteins, antioxidants and dietary fiber.

Early studies on chia seeds mainly reported the high oil content and the fatty acid composition of seeds grown under different climatic conditions in various geographical locations (Palma *et al.*, 1947; Taga *et al*., 1984; Ayerza, 1995; Ayerza and Coates, 2004; Ixtaina *et al.*, 2008; Borneo *et al.*, 2010; Ayerza and Coates, 2011; Capitani *et al*., 2012; Pizarro *et al*., 2013). According to Borneo *et al.* (2010) chia seeds have 25% to 35% of oil, especially polyunsaturated fatty acids, ~22% of fiber and ~24% of protein (Ayerza and Coates, 2005; Capitani *et al*., 2012).

The major constituents of chia oil are polyunsaturated fatty acids (PUFAs, linolenic and linoleic acids) (Ayerza and Coates, 1995; Martin *et al*., 2006; Peiretti and Gai, 2009; Ixtaina *et al.*, 2011; Martínez *et al*., 2012). Essential PUFAS can not be produced by the human body and must be obtained from the diet (Pizarro *et al*., 2013). According to Ayerza (1995) and Dauksas *et al.* (2002) chia oil is a product with variable chemical composition, this being dependent on factors such as the cultivation environment and the extraction system used.

In relation to the extraction of vegetable oils, with the development of the concepts of green chemistry, the efficiency of solvents which are less harmful to the environment and provide acceptable yields when used in low quantities has become a main research challenge. Since chia oil is mainly used as a food, the extraction solvents must be compatible with the requirements of the food industry. In this regard,

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recent studies have highlighted the use of ethyl acetate (Almeida *et al.*, 2012; Tian *et al.*, 2013), ethanol (Freitas *et al.*, 2008; Dutta *et al*., 2014) and isopropanol (Seth *et al*., 2007; Dutta *et al.,* 2014; Ramluckan *et al*., 2014) for the extraction of vegetable oils. Also, emphasize the hexane to extract vegetable oils, considered a less toxic solvent and low boiling point, which decreases the decomposition of the oil (Ramalho and Suarez, 2013).

Craig and Sons (2004) reported that the oil extraction from chia seeds produces a subfraction with a high content of dietary fiber, which has antioxidant activity and compounds that confer functional characteristics when used in other foods. Chia meal (the seed residue remaining after oil extraction) is a good source of proteins (19.0-23.0%), dietary fiber (33.9-39.9%), and compounds with antioxidant activity (Marineli *et al*., 2014). It also exhibits some interesting functional properties which would be beneficial for its use in the food industry. Functional properties are generally associated with the presence of proteins and also dietary fiber (Reyes-Caudillo *et al*., 2008; Capitani *et al.*, 2012).

In this context, the objective of this study was to extract oil from the chia seeds using different solvents (ethyl acetate, *n*-hexane and isopropanol) and evaluate the fatty acids profile of the extracts and the technological characteristics of the defatted meal. The experiments were carried out, using an orbital shaker, with different seed to solvent ratios (1:4, 1:6 and 1:8) at 40 °C and 40 rpm and with an extraction time of 4 h.

Materials and Methods

Materials

Seed samples of *Salvia hispanica* L. were obtained from a local market in Umuarama (PR, Brazil). The proximate composition of the seeds is shown in Table 1, determined according to methods described by Adolfo Lutz Institute (2008). The seeds were milled using an electric mill (Marconi, MA 750), classified using a Tyler sieve (Bertel, ASTM) and the fraction classified as 48 mesh was used in the experiments. For the extractions the following solvents were used: ethyl acetate (Vetec), *n*-hexane (Quimex) and isopropanol (Nuclear). All solvents and reagents used in this study were of analytical grade. The standard methyl heptadecanoate (>99% purity) and derivatives of boron trifluoride-methanol were obtained from Sigma-Aldrich Chemical Co.

Oil extraction

The extraction of oil from the seeds was carried out in an orbital shaker (Marconi, MA 839/A), where five grams of chia seeds were placed in Erlenmeyer flasks with glass stoppers (250 mL) and the solvents added to give seed mass:volume of solvent ratios of 1:4, 1:6 and 1:8. The extraction temperature was 40°C, stirring speed 40 rpm and extraction time 4 h, these conditions being selected after preliminary tests. The extraction method was chosen based on the results obtained by Oliveira *et al.* (2013) and Oliveira *et al.* (2014) for oil extraction from passion fruit seeds. After the extraction period the excess solvent was evaporated in an evaporator (Marconi, MA120) and the residue remaining was dried in an oven until constant weight. The extracts were stored under refrigeration until the time of analysis, which was performed in duplicate. The oil yield was calculated as the ratio of oil mass extracted and seed mass used.

Table 1. Proximate chemical composition (%) of chia seeds

Chemical composition	Mean ± standard deviation
Moisture	7.82 ±0.06
Ash	4.77 ±0.09
Crude protein	18.65 ±0.06
Total lipids	35.80 ±0.15
Crude fiber	22.78 ±0.98

g $100g^{-1}$ dry base

Fatty acids determination

In order to determine the total fatty acids content by gas chromatography, derivatization of the oil was conducted using BF_3/methanol following the AOAC standard method Ce 2-66 (AOCS, 1990). The quantification of fatty acids in the chia oil was performed using an Agilent GC 7890 gas chromatograph coupled with a mass detector MS 8990, fitted with a capillary column (ZBWAX, 30 m x 0.25 mm x 0.25 μm) using the chromatographic method reported by Garcia *et al.* (2012). The compounds present in the chia seed oil were identified by comparing spectral data with those provided by the Wiley library. For the quantification of fatty acids, methyl heptadecanoate was used as the internal standard.

Technological analysis of defatted meal

After extraction of the oil, the meal samples were ground and then sieved through a Tyler sieve (Bertel, ASTM) and the fraction classified as fine flour (100 mesh) was used for the analysis. The values for the water-holding (WHC) and oil-holding (OHC)

Table 2. Results for oil yield extracted from chia seeds (g $100g^{-1}$ of seeds)[1]

Ratio (seeds to solvent)	Solvent		
	Ethyl Acetate	*n*-hexane	Isopropanol
1:4	30.23 ± 0.46^{aA}	30.82 ± 0.47^{aA}	23.98 ± 0.53^{aB}
1:6	28.97 ± 0.66^{aB}	31.79 ± 0.52^{aA}	24.28 ± 0.79^{abC}
1:8	29.04 ± 0.73^{aB}	33.55 ± 0.6^{bA}	25.56 ± 0.70^{bC}

[1]Means followed by same lowercase letters (same solvent) and uppercase letters (same conditions) did not differ statistically ($p>0.05$).

capacity were determined according to the method of Bencini (1986). Samples (2 g) were mixed with 20 mL of distilled water or corn oil (Soya) in 50 mL centrifuge tubes. Each slurry was vortexed for 1 min, allowed to stand for 30 min and then centrifuged at 2208 x g for 30 min. The values for the water- and oil-holding capacity are expressed as the number of grams of water and oil held by 1 g of sample, respectively. All measurements were carried out in triplicate.

The emulsification activity was investigated applying the method described by Dench *et al*. (1981). Samples (5 g) were suspended in 40 ml of distilled water and the pH was adjusted to pH 7.0 using 0.1 mol L^{-1} NaOH. After stirring for 15 min, the pH was checked and adjusted if necessary, and the volume made up to 50 ml. Soya oil (50 ml) was added and blended for 3 min. The emulsion was divided between two 50 mL centrifuge tubes and centrifuged (1300 g for 5 min). The ratio of the height of the emulsified layer to the total height of the fluid was calculated and the emulsification activity expressed at this ratio x 100.

Results and Discussion

Extraction yields

The results obtained for the oil yields under the experimental conditions for the different solvents tested are shown in Table 2. The solvent with the highest extraction efficiency was *n*-hexane, followed by ethyl acetate and isopropanol, with yields of 33.55%, 30.23% and 25.56%, respectively, and it was verified that the effect of the solvent on the oil yield was statistically significant ($p<0.05$). The results for the yields are comparable with those reported in the literature using different extraction methods. Ixtaina *et al.* (2011) reported yields of 33.6% and 24.8% for oil extraction from chia seeds using solvent extraction at 80°C for 8 h and pressing, respectively. Ixtaina *et al.* (2010) reported, for oil extraction using carbon dioxide as the solvent under supercritical conditions, ~30% yield at 80°C and 450 bar applying 5 h of extraction.

The solvents *n*-hexane, ethyl acetate and isopropanol have dielectric constants of 1.88, 6.27 and 18, respectively. The dielectric constant of vegetable oils is in the range of 2 to 4 and these molecules have a greater affinity for molecules of low or no polarity (Damodaran *et al*., 2007). Galvão *et al*. (2013) reported that the more polar component has a lower affinity for oil. Thus, *n*-hexane, which provided the highest oil yield, has the lowest dielectric constant (Monson, 1971).

Another factor to be considered is the polarity of the solvent, *n*-hexane is a nonpolar solvent and ethyl acetate and isopropanol have polarity indexes of 4.4 and 3.9, respectively. It can thus be noted that the highest oil yields for the extraction from chia seeds appear to be associated with the use of nonpolar solvents and polar solvents with a high polarity index. The efficiency of *n*-hexane as a solvent in the extraction of vegetable oils is well known, since it is traditionally used in this process. The efficiency of ethyl acetate has been reported by Tian *et al*. (2013) for the extraction of pomegranate (*Punica granatum* L.) seed oil and the extraction yields (~20%) were similar to those obtained with *n*-hexane at 30°C with an extraction time of 30 min, ultrasonic power of 100 W and 1:10 seed mass:volume of solvent ratio.

From the results reported in Table 2 it can be observed that the amount of solvent used in the extraction has a significant effect ($p<0.05$) in the case of *n*-hexane and isopropanol, and with the use of ethyl acetate an increased in the amount of solvent in contact with the seeds did not increase the oil yield ($p>0.05$). The results indicate that oil yields of around 30% can be obtained with low amounts of solvent (1:4) when *n*-hexane and ethyl acetate are used.

Quantification of total fatty acids in the extracts

The fatty acids composition of the chia seed oil is reported in Table 3. On average, the fatty acids can be ranked in the following order of abundance: linolenic acid (C18:3) > linoleic acid (C18:2) > palmitic acid (C16:0) > oleic acid (C18:1) > stearic acid (C18:0). Ixtaina *et al.* (2011) reported a similar distribution between the fatty acids for chia oil obtained from

Table 3. Quantification of fatty acids in the chia seed oils obtained with different solvents

Solvent		Ethyl Acetate			*n*-Hexane			Isopropanol		
Ratio (seeds to solvent)		1:4	1:6	1:8	1:4	1:6	1:8	1:4	1:6	1:8
Fatty acids[1]	Palmitic	9.18[a]	9.95[a]	9.48[a]	9.38[a]	9.90[a]	9.51[a]	9.13[a]	9.76[a]	9.65[a]
	Stearic	2.94[a]	2.92[a]	2.98[a]	2.96[a]	2.91[a]	2.98[a]	2.93[a]	2.89[a]	2.99[a]
	Oleic	6.22[a]	6.35[a]	6.12[a]	6.38[a]	6.65[a]	6.10[a]	6.56[a]	6.87[a]	6.09[a]
	Linoleic	19.44[a]	18.13[a]	18.65[a]	19.58[a]	18.10[a]	18.89[a]	19.76[a]	18.23[a]	18.99[a]
	Linolenic	61.48[a]	62.33[a]	62.42[a]	61.68[a]	62.42[a]	62.36[a]	62.88[a]	62.92[a]	62.13[a]
SFA[2]		12.12	12.87	12.46	12.34	12.81	12.49	12.06	12.65	12.64
MUFA[3]		6.22	6.35	6.12	6.38	6.65	6.10	6.56	6.87	6.09
PUFA[4]		80.92	80.46	81.07	81.26	80.52	81.25	81.64	81.15	81.12
PUFA:SFA		6.67	6.25	6.50	6.58	6.28	6.50	6.77	6.41	6.41
ω6:ω3		0.31	0.29	0.29	0.21	0.29	0.30	0.31	0.29	0.30

[1]Results in g 100 g^{-1} of oil
[2] SFA - saturated fatty acids
[3] MUFA - monounsaturated fatty acids
[4] PUFA – polyunsaturated fatty acids

solvent extraction (*n*-hexane) and pressing.

The major components of the oils obtained under the different experimental conditions were linolenic acid (ω3) and linoleic acid (ω6) and it was reported that the nature of the solvent and the seed to solvent ratio showed no significant influence ($p>0.05$) on the composition of the extracts. The ω6:ω3 ratios obtained for the chia oils ranged from 0.28 to 0.31. According to Guimarães *et al.* (2013), the determination of the ω6:ω3 ratio is important in relation to assessing the potential health benefits, since the excessive consumption of ω6 accompanied by the ingestion of low amounts of ω3 is a risk factor for cardiovascular disorders. The WHO (1995) stipulates that to maintain a healthy condition, the human diet must provide ω6:ω3 ratios of between 5:1 and 10:1. Martin *et al*. (2006) highlights the need to reduce the ω6:ω3 ratio in modern diets due to clinical outcomes.

Ixataina *et al.* (2011) noted that the incorporation of chia seeds in the diet would be very beneficial due to the high content of PUFAs present in their composition. In this study, as reported in Table 3, the PUFA levels in the oils ranged from 80.46% to 81.64% of the total composition. According to Bowen and Clandinin (2005) the consumption of oils with high levels of PUFAs can provide health benefits. Siriwardhana *et al*. (2012) highlighted that n-3 PUFAs are known to have a variety of health benefits against cardiovascular diseases, including a well-established hypotriglyceridemic effect.

For the oil extracted from the chia seeds the polyunsaturated fatty acid/saturated fatty acid (PUFA/SFA) ratios were >6.28, which is considered suitable for food products (Matsushita *et al*., 2006, 2010; Marino *et al*., 2008; Ramos-Filho *et al.,* 2008; França *et al*., 2011). Diets with a PUFA:SFA ratio below 0.45 are considered inadequate for this purpose (London, 1984) because of their potential to increase blood cholesterol levels. Guimarães *et al.* (2013) reported a PUFA:SFA ratio of 0.79 for sesame oil and 5.25 for flaxseed oil indicating that these oils have a good fatty acids balance. In general, the values for the ω6:ω3 and PUFA:SFA ratios obtained in this study are similar to those reported in the literature, for instance, by Ixtaina *et al.* (2010), Ixtaina *et al.* (2011), Uribe *et al.* (2011) and Marineli *et al.* (2014).

Technological characteristics of defatted meal

Table 4 shows the values for the water-holding capacity (WHC), oil-holding capacity (OHC) and emulsifying activity of the chia defatted meal obtained from the extraction with different solvents and without oil extraction. It can be observed that the defatted meals obtained from the extraction with the solvents *n*-hexane, ethyl acetate and isopropanol showed no significant difference in relation to their water-holding capacity (WHC), oil-holding capacity (OHC) and emulsifying activity.

WHC is the ability of a moist material to retain water when subjected to an external centrifugal gravity force or compression (Alfredo *et al*., 2009). The chia defatted meals exhibited a WHC of 9.2 to 10.13 g g^{-1} and the removal of oil did not have a significant effect on this property ($p>0.05$). These WHC values are higher than those reported by

Table 4. Water-holding capacity (WHC), oil-holding capacity (OHC) and emulsifying activity for the defatted meal and seeds without oil extraction (seed to solvent ratio of 1:8)

	Without extraction	Ethyl Acetate	*n*-Hexane	Isopropanol
WHC (g g^{-1})	9.2±0.37[a]	9.64±0.47[a]	10.13 ±0.08[a]	9.99±0.02[a]
OHC (g g^{-1})	1.94 ± 0.09[b]	2.88 ± 0.04[a]	2.75 ±0.01[a]	3.07±0.35[a]
Emulsification activity (%)	66.60[b]	100.00[a]	100.00[a]	100.00[a]

Mean followed by same letters (on the same line) did not differ statistically ($p > 0.05$).

Bolanho *et al.* (2013) for peach palm stem flour (7.36 g g^{-1}) and Joshi *et al.* (2015) for defatted seed flours obtained from black gram, peanut, soybean and rice (2.66, 2.03, 3.53 and 1.54 g g^{-1}, respectively). The high WHC values can be attributed to the presence of mucilage in the chia seeds, which has excellent water retention properties (Capitani *et al*., 2012). Alfredo *et al.* (2009) reported that the fiber structure and high proportions of hemicellulose and lignin may contribute to the high WHC values obtained for chia seeds and their derivatives. The WHC values reported in this study are similar to those reported by Capitani *et al*. (2012) for chia meal obtained after oil extraction using a solvent (*n*-hexane) in a Soxhlet apparatus at 80 °C with an extraction time of 8 h and lower than those obtained by Alfredo *et al.* (2009) for the fiber-rich fraction obtained from chia seeds. The WHC results of chia deffated meal suggest the application in products requiring hydration, viscosity development and conservation of freshness, such as baked goods.

The values for the oil-holding capacity and emulsification activity reported in Table 4 indicate that the removal of oil contributed to an increase in these properties. Alfredo *et al*. (2009) reported that the fiber-rich chia fraction showed a low oil-holding capacity (2.2 g g^{-1}). The OHC of defatted chia meals is considered to be low when compared with soya (3.66 g g^{-1}) (Chau and Huang, 2003) and high when compared with the values of 1.03, 2.17 and 1.61 g g^{-1} reported for defatted seed flours of black gram, peanut and soybean, respectively (Joshi *et al*., 2015). The OHC values for this study were below those reported for the protein isolate of chia seeds and glutelins of chia seeds, that is, 4.04 and 6.23 g g^{-1}, respectively (Olivos-Lugo *et al*., 2010).

The emulsifying activity was 100 mL^{-1}, which is similar to the value reported by Capitani *et al*. (2012) for chia meals. The high protein content of the chia meals may contribute to the emulsifying activity, since most proteins are strong emulsifying agents.

Conclusions

In summary, this study showed that higher yields for the extraction of chia oil were obtained with *n*-hexane and ethyl acetate, and the increased amount of solvent used had a low influence on the oil yield. However, the fatty acids composition of the extracts was not influenced by the solvent used. The defatted meal showed higher values for the oil-holding capacity and emulsification activity, but the process of removing the oil did not affect the water-holding capacity. It was found that the technological characteristics of the meals were not influenced by the type of solvent used for the extraction.

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