Physicochemical Characterization of Cold Pressed Oil from Chia Seed (*Salvia hispanica* l.) Grown in Kenya.

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Abstract
Currently there is lot of research interest in chia worldwide due to its beneficial nutritional value.. The aim of the study was to determine the oil yield and physicochemical properties of oil obtained from chia seeds grown in Kenya. Oil from chia seeds grown in five different geographical locations in Kenya was extracted by cold pressing. The physicochemical properties were determined The refractive index of chia seed oil at 25°C was found to range from 1.481 to 1.483, specific gravity ranged from 0.962 to 0.963, acidity index and free fatty acids content ranged from 0.035-0081mg KOH/g oil and 0.174-0.406%, respectively. The matter in volatiles ranged from 0.047- 0.086%. The saponification value ranged from 162.197 – 183.379 milligrams (mg) of potassium hydroxide (KOH) per gram (g) of chia seed oil. The α-linolenic (C18:3) and linoleic acids (C18:2) were the predominant fatty acids in Kenyan chia seed oil, significant differences in oil yield, free fatty acids, specific gravity, saponification value and refractive index were reported in the chia seed oil from different locations in Kenya. The study recommends further research to confirm the effect of different agro ecological conditions in major regions in Kenya on fatty acid profile chia seed oil from Kenyan grown chia seeds.

Keywords: *Salvia hispanica* L., Chia seeds, Omega-3 fatty acid, alpha linolenic acids, polyunsaturated fatty acid, Physicochemical Characteristic

1. Introduction

Chia, *Salvia hispanica* L., has been cultivated under environments ranging from tropical to subtropical conditions and used as a food ingredient. Native from southern Mexico and northern Guatemala (Busilacchi et al., 2013). Chia seed oil is an exciting source of polyunsaturated fatty acids (PUFAs) as it contains the highest proportion of α-linolenic acid (approximately 60%) of any known vegetable source (Ayerza, 1995). The ω-6 family of fatty acids are essential for the growth and development of the human body, as they play a vital role in the prevention and treatment of coronary heart disease, hypertension, diabetes, arthritis, other inflammatory and autoimmune disorders and cancer (Simopoulos, 2008). Chia seeds oil is becoming an appealing and preferred choice for healthy food and cosmetic applications due to its lower content of saturated fatty acids (palmitic and stearic acids) and adequate concentration of linolenic fatty acids (55–60 %) and linoleic acids (18–20 %) (Ixtaina *et al.,* 2011). Both chia seeds and chia oil have been safely utilized in animal feeds to decrease the cholesterol levels and increase the polyunsaturated fatty acids and in egg and meat and products (Antruejo *et al.,* 2011). A lot of attention is being given to research on sources of alpha linolenic acids due to the recurrent mercury contamination of fish supply and depletion of ocean fisheries, that has led to reduced intake of the long-chain n-3 fatty

acids found in fish have been associated with reduced risks of coronary heart disease (Kris-Etherton et al, 2002; He et al., 2004; Albert et al., 2002.

Plant seed oils can be obtained through various extraction techniques which include solvent extraction, cold pressing and super critical extraction. Cold pressing is a method of oil recovery from oil-bearing seeds by mechanical devices such as expellers, screws and hydraulic presses, this method avoids the use of heat or chemical treatments (da Silva *et al*., 2017).

Chia was recently introduced in East Africa and is currently being cultivated in Tanzania, Rwanda, Kenya and Uganda (Katunzi-Kilewela, et al., 2021). However, although production of chia in Kenya and East African countries has gradually increased, there is limited data on the physicochemical characteristics of chia seeds oils from varying locations in Kenya. This study, therefore, aimed at the characterization of the seed oil from chia grown in different locations in Kenya.

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2. Methods

Samples

Mixed variety of Chia (*S. hispanica*) seeds samples was collected from small holder farmers five locations. In Kirinyaga County in Mwea East Subcounty, Embu County in Mbeere Sub County, Machakos County in Masinga Subcounty, Laikipia County in Laikipia North and in Nyeri County in Nyeri Central Subcounty. The samples were collected chia seeds from the harvest of April to August 2019 planting season between August and October 2019. Five kilograms of a representative sample were drawn from each lot, manually cleaned and stored in clean airtight containers labelled with the location, and the date of sampling. The labelled samples were stored in sealed polythene bags placed on shelves at room temperatures.

Oil extraction

The chia seed oil was obtained by cold pressing. The oil was extracted in a single step with LY-169 screw oil press from China. The pressing temperature was between 40°C-50°C, constant pressure was maintained throughout the pressing. The pre-cleaned chia seeds from each location were weighed and put into the screw press hopper and extraction was carried out for 10-15 minutes. The oil press was wiped clean using cotton wool dipped in ethanol to remove oil and seed cake residue. The same cleaning method was utilized after each extraction before moving to the next sample.

Physicochemical Characterisation of Chia Oil

The weight per unit volume and specific gravity, index of refraction, saponification value, free fatty acids, acid value, iodine value, peroxide value, matter in volatiles were determined using methods described in AOAC (2016) and fatty acid profile was determined using gas chromatography using conventional saponification method

The refractive index of the chia seed oil was determined according to AOAC official method 921.08 (AOAC, 2016). Three drops of oil were placed on the refractometer prism which was then closed by tightening of screw heads. Water was circulated through the instrument for a few minutes before the readings were taken; this was to allow the equilibration of the

temperature of the test sample and refractometer. The prism was cleaned between readings by wiping off oil with a cotton pad moistened with ethyl alcohol and left to dry.

This weight per unit volume and specific gravity was determined using AOAC method 985.19 (AOAC, 2016). Clean and dry pycnometer was weighed, filled with water placed in a water bath at 20°C until it attained that temperature, the pycnometer was adjusted to ensure the water was at the level of the fixed mark. The pycnometer was removed from the bath, wiped dry, allowed to stand for a short time and re-weighed. The pycnometer was emptied and dried and later was filled with the sample of the oil previously brought near the temperature 20°C. The pycnometer was kept in a bath adjusted to 20°C until it has attained that temperature. The oil was then maintained at the temperature 20°C until no further alteration in volume occurred. The pycnometer was then removed from the water bath wiped dry and adjusted to the fixed mark. It was then allowed to stand for a short time and was weighed. This procedure was then repeated for each sample and results recorded.

The saponification number is the number of milligrams of potassium hydroxide required to neutralize the fatty acids from the complete hydrolysis of one gram of fat. Saponification number is the measure of the chain length of fatty acids. The saponification value was determined according to AOAC method 920.16(AOAC, 2016). This method involved boiling a sample under reflux with ethanolic potassium hydroxide solution, followed by titration of the excess potassium hydroxide with a standard volumetric hydrochloric acid solution.

Two grams of chia seed oil were mixed with 25 milliliters of 4 per cent alcoholic potassium hydroxide by draining from the burette. The contents were refluxed for one hour and then cooled. The cooled solution was titrated against 0.5 N hydrochloric acid using a phenolphthalein indicator. Simultaneously, a blank was run adopting a similar procedure. The saponification number was calculated as per the formula below:

$$\text{Saponification number} = \frac{28.05(\text{B} - \text{S})}{\text{Sample wt(g)}}$$

B= ml 0.5 N HCL required by blank

S= ml 0.5 N HCL required by blank

The free fatty acid content (as oleic) of the crude oil was determined using the titration method described in AOAC method 940.28(AOAC, 2016). One gram of oil was added to seven millilitres of ethanol previously neutralised by phenolphthalein, this was titrated against 0.1M sodium hydroxide (NaOH).

The free fatty acid (FFA) level (as oleic) was calculated using the following equation:

$$\%\ \text{FFA (oleic)} = \frac{(\text{volume})\text{alkali} \times \text{Normality of alkali} \times 28.2}{\text{Sample wt(g)} \times 1000}$$

The acid value of the chia seed oil was also calculated from the titration results using the following equation:

$$\text{Acid value} = \frac{56.1 \times \text{volume of alkali} \times \text{Normality}}{\text{Sample wt(g)}}$$

The matter in volatiles was determined using AOAC method 926.12(AOAC, 2016). 3.5 grams of the chia seed oil were put in aluminum moisture dishes (5 cm in diameter and 2 cm deep) with a tight fit slip over cover. The oil in the moisture dishes was dried in a vacuum oven maintained at 120°C - 125°C for 30 minutes until a constant weight was attained. The percentage loss in weight was reported as percentage moisture in volatiles.

The calculation was done as per the equation below.

$$\text{Moisture and matter in volatiles} = \frac{W1 \times 100}{\text{Sample wt(g)}}$$

Where,

W1= Loss in Weight in grams of the sample on drying

Fatty acid profile was determined by gas chromatography using conventional saponification procedure as described by Ichiahara et al. (1996). The fatty acids were converted into their respective methyl esters before analysis. Fifty milligrams of the oil were mixed with two milliliters of 0.5 M methanolic potassium hydroxide (KOH) in screw-capped glass tubes. These tubes were stirred for five minutes and then held at 70°C for one hour to facilitate the release of fatty acids from the triglycerides. After acidification with 0.5 ml of hydrochloric acid (HCL), the released fatty acids were then extracted twice using five millilitres of hexane, then five millilitres of boron trifluoride- methanol mixture (14 % W/V) were added into the fatty acid mixture and vortexed for five minutes at ambient temperature. The fatty acid methyl esters (FAMEs) were then extracted from the methanolic phase by the addition of four millilitres of hexane. Subsequently, 1µ of a hexane solution containing the FAMES was injected into the column of gas chromatography (Hewlett Packard 6890) equipped with capillary column DB-23 Agilent (50% cyanopropyl-methylpolysiloxane, 60m - 0.25mm, internal diameter 0.25 µm), equipped with flame ionization detector (FID). Separation was carried out at 175-220°C at (3°C per minute) with helium as the carrier gas (25.1 psi) and a flame ionization detector at 260°C (Christie, 2003). The fatty acid methyl esters (FAMEs) were then identified through a comparison of retention times of FAMES standard mixture analyzed under the same conditions, the FAMEs in the extracted chia seed oil were quantified using peak area normalization. The results were expressed as the relative percentage of each fatty acid present in the sample.

Data Analysis

Each set of chia seed oil was analysed in triplicate. The data obtained were analysed using SPSS version 25. The significance of the differences in means of refractive index, specific gravity, saponification value, free fatty acids, acid value, matter in volatiles and fatty acids for data from different regions were determined. Data were processed by one-way analysis of variance (ANOVA). The differences were considered significant at $P>0.05$.

3. RESULTS AND DISCUSSION

Oil Yield from Chia Seeds

The mean oil yield (Table 1)from the chia seeds from the five locations was 16.23%. The differences in oil yields from chia seeds from chia grown in different locations were statistically significant at ($P<0.05$). These differences could be attributed to the differences in rainfall, temperatures, soil, altitude and agronomic practices in each of the locations (Ayerza, 2011).

Table 1: Oil Yield of Kenyan Chia Seeds from different locations in Kenya

Location	Oil Yield (%)
Laikipia County	15.58±0.10c
Nyeri County	16.23±0.14c
Kirinyaga County	17.80±0.10b
Embu County	17.75±0.10b
Machakos County	12.85±0.06 a

aValues are means (± SD) of triplicate determinations. bMeans designated by different letters in a column are significantly different at ($P <0.05$).

Physicochemical Properties of Chia Seed Oil

The important physicochemical properties of chia seed oil are presented in Table 2. Refractive index and specific gravity are important parameters in determining authenticity of oils to detect adulteration (Timilsena et al., 2017). In this study the refractive index of chia seed oil at 250C was found to range from 1.481 to 1.4832 his was lower than what was reported in Segura-Campos et al., (2014) in which chia oil reported to have a refraction index value of 1.4761 at 250c.These findings are similar to findings by Xtaina et al., (2011) and Timilsena et al., (2017) who reported refractive at 250c of 1.48. According to as Segura-Campos et al. (2014) refractive index is directly related with the level of unsaturation of the oil and higher refractive index is associated with higher level of unsaturation.

The specific gravity in this study was found to range from 0.962 to 0.963 this was higher than was reported in other studies. The values reported in Nyeri county were higher than from Machakos and Kirinyaga Counties. this was higher than reported in other studies. Timilsena (2017) reported specific gravity of 0.93 in Austalian chia seed oil and Segura-Campos et al., (2014) who reported a specific gravity of 0.92 in Mexican chia seed oil.

The chia seed oil acidity index in this study ranged from 0.035- 0.081 mg KOH/g oil, and free fatty acids ranged from 0.174-0.406% (as oleic acids). The lowest acid value of 0.174 mg KOH/g oil and a free fatty acid value of 0.0345% (as oleic) was reported in chia seed oil from Machakos, Kirinyaga and Embu Counties. The highest levels of acid value and free fatty acids were reported in chia seed oil from Machakos, Embu and Kirinyaga Counties. The acid value and free fatty acids in these locations ranged from 0.213-0.406 mg KOH/gram of

oil and 0.042-0.081% (as oleic acid), respectively. The acid value findings were lower than was reported for chia grown in other regions. For example, the acid values of Argentinian and Mexican chia seed oils were 0.7-0.91% and 2.053%, respectively (Ixtaina et al., 2011; Segura-Campos et al., 2014). However, the results of the study were comparable to the findings of seed oil from similar oil crops such as Moringa which reported an acid value of 0.29-0.37% (Barakat & Ghazal, 2016).

In the study the matter in volatiles was reported to be 0.083%, the finds were below the requirements of Codex 210, (1999) the maximum acceptable matter in volatile is 0.2%. Matter in volatiles has been used for quality control of edible oils such as olive oil for detection of adulterants, rancidity and to determine the oil origin Guerfel et al., (2012).

The saponification values for the oil from the five locations ranged from 162.197 – 183.379 mg of potassium hydroxide (KOH) per gram of chia seed oil. The saponification values reported in this study are in the same range as had been reported for Australian chia seed oil which was 197 mg KOH/g of oil (Timilsena et al., 2017). However, they are lower than was reported for Mexican chia seed oil which was 222 mg KOH/g of oil. However, they are in the same range as has been reported for sunflower, safflower and virgin olive oil (Codex 210, 2009).

Table 2: Physicochemical Characteristics of Chia Seed Oil

Location	Free fatty acids	Acid value	Specific Gravity	Saponification Value	Refractive Index	Matter in volatiles
Kirinyaga County	0.2238±0.00a	0.0445±0.00a	0.9616±0.00a	170.44±3.81a	1.4812±0.0001a	0.07±0.00a
Embu County	0.2125±0.02a	0.0423±0.00a	0.9617±0.00a	163.75±3.08a	1.4816±0.0001b	0.06±0.02a
Machakos County	0.4061±0.01b	0.0808±0.00b	0.9616±0.00a	162.20±2.88a	1.4812±0.0001a	0.08±0.01a
Laikipia County	0.2111±0.01a	0.0420±0.00a	0.9622±0.00b	182.38±0.08b	1.4830±0.0001c	0.05±0.01a
Nyeri County	0.1736±0.01a	0.0345±0.00a	0.9629±0.00ab	180.13±1.38ab	1.4832±0.0001d	0.05±0.01a

1values are means (± SD) of duplicate determinations. 2means designated by different letters in a row are significantly different at ($P < 0.05$).

Fatty Acids Profile

The free fatty acid profile of chia seed oil obtained from chia seeds grown in different locations in Kenya was determined using gas chromatography. The results are presented in Table 4. The analysis of oil from all the five locations indicated the presence of linoleic acid, oleic, α-linolenic, palmitic and stearic fatty acids. Traces of twelve more fatty acids were also detected, these included butyric, caproic, myristic, pentacyclic, palmitoleic, margaric, arachidic, eicosenoic, eicosadienoic, eicosatrienoic, behenic and lignoceric acid. These findings are consistent with other results of chia seed oil extracted from chia seeds grown in other regions of East Africa (Mihafu et al., 2020).

Differing levels of unsaturated fatty acids were found in chia seed oil from the five locations. The highest levels of oleic was reported in chia seed oil from Machakos county at 9.83%, the highest linoleic fatty acids was also reported in chia seed oil from Machakos county at had 22.90%. The highest level of linolenic fatty acid was reported in chia seed oil from Laikipia County at 61.06%. This inverse relationship could be attributed to the synthesis of α-linolenic fatty acid through the process of desaturation of oleic fatty acids, through linoleic fatty acid by desaturase enzymes (Ayerza, 2009). The levels of linoleic reported in this study are lower than 17-26 % reported in chia seed oil from other East African regions while the levels of linolenic fatty acid are higher than 50-57% reported in the same region (Mihafu et al., 2020).

The chia seed oil from chia seeds grown in Laikipia and Nyeri Counties contained 61.06% and 54.79% as linolenic fatty acid, respectively. The results from this study are lower than have been reported in previous studies. For example, Xtaina et al. (2011) reported 64.5-69.3% linoleic acid compared to this study findings of 53.32-64.04%. However, these study findings are inconsistent with the findings by Ayerza and Coates (2011) which found significant differences in chia oil fatty

Table 3: Fatty Acid Composition of Kenyan Chia Seed Samples Expressed as % Fatty Acid to Total Fatty Acids

Location	C14:0	C15:0	C16:0	C16:1	C17:0	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1	C20:2	C20:3	C22:0	C24:0
Laikipia County	0.05a	0.03a	7.30a	0.05a	0.04a	3.65a	7.26a	19.37 a	61.06a	0.28a	0.25a	0.04a	0.03a	0.09a	0.12a
Nyeri County	0.04a	0.02a	7.31a	0.06a	0.04a	2.84a	5.07a	19.40 a	64.04a	0.25a	0.28a	0.02a	0.02a	0.06a	0.09a
Kirinyaga County	0.06a	0.03a	8.06a	0.05a	0.05a	4.31a	9.74a	22.71 a	53.61a	0.33a	0.23a	0.03a	0.02a	0.12a	0.17a
Embu County	0.06a	0.03a	8.01a	0.06a	0.05a	4.31a	9.78a	22.87 a	53.98a	0.33a	0.22a	0.03a	0.02a	0.071 a	0.14a
Machakos County	0.06a	0.03a	8.13a	0.06a	0.05a	4.25a	9.83a	22.90 a	53.32a	0.33a	0.25a	0.03a	0.02a	0.07a	0.02a

aValues are means of triplicate determinations. bMeans designated by different letters in a column are significantly different at ($P < 0.05$).

Source; Research data

4. CONCLUSION

The average oil yield from chia seeds grown in the five locations was 16.23% oil. The refractive index of ranged from 1.481 to 1.483. specific gravity ranged from 0.962 to 0.963. The saponification and matter in volatiles values ranged from 162.197 to 183.79 milligrams (mg) of potassium hydroxide (KOH) per gram (g) of chia seed oil and from 0.047% to 0,086% respectively. The differences in levels of palmitic acid, oleic acid, linoleic acid and linolenic acid from different locations were not statistically significant at ($P<0.05$).

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