



Short Communication

Characterization of Black Chia Seed (*Salvia hispanica L*) and Oil and Quantification of β -sitosterol

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Abstract

The importance of corn, bean, amaranth and black chia seed in the Aztec's diet is well documented in the historical Florentine Codex. Black chia seed is a good source of omega-3 fatty acid, dietary fiber, proteins, as well as diverse bioelements and antioxidants. The proximal chemical analysis obtained from black chia seed shows 5.15% of moisture, 34.70% of fats, 4.10% of ashes, 17.90% of protein, 40.25% carbohydrates and 17.30% of raw fiber. The extraction technique permitted to obtain a 29% of oil with an iodine value of 197.68 cg/g, peroxides of 2.67 meq/Kg, a refraction index of 1.46 and a density of 0.89 g/mL. The oil's FTIR spectrum showed a peak at 3010.03 cm^{-1} characteristic of a =CH stretching mode. Also, the profile of fatty acids performed by, CG-MS, provides evidence of the presence of the (Z,Z,Z) 9,12,15-octadecatrienoic acid (α -linolenic acid, omega-3), γ -tocopherol as well as phytosterols: campesterol, stigmasterol and β -sitosterol. The latter was quantified obtaining a concentration of 1224.3 mg/L.

Keywords: Campesterol, chia, stigmasterol, phytosterol, polyunsaturated, sitosterol.

Introduction

By the time of the Spanish conquest, Mesoamerican peoples had domesticated at least 20 botanical species with different uses. Among those, amaranth, beans, black chia seed and corn stood out from a nutritional standpoint. The relevance of those crops in the Aztec's diet is documented in the historical Florentine Codex. In particular, black chia seed was as important as corn and in some regions even more¹. In the century 16th, the Codex Mendoza indicates that 21 of the 38 Aztec provincial states offered chia seed as annual tribute². More over, it has been determined that chia seed contains different oil amounts varying from 32% to 39%³. Chia seed oil along with linseed oil, offer the highest natural percentage of omega-3 fatty acids, which are essential in the human diet⁴. Omega-3 fatty acids help to make up the phospholipids that are fundamental components of cell membranes⁵.

Recent work has shown that hens fed with chia seed produced eggs with higher levels of polyunsaturated linolenic acid and lower cholesterol, palmitoleic and saturated acid levels in their yolks when compared with eggs produced from hens under a control diet⁶. Chia seed can be considered as a good source of dietary fiber, protein and antioxidants. Chia seed in aqueous medium is wrapped one at a mucilaginous polysaccharide which is excellent for digestion. The consumption of dietary fiber improves fecal bolus formation and proper evacuation of stool,

which helps prevent obesity, colon cancer, and high levels of cholesterol and blood glucose⁷.

Phytosterols are sterols with a structure similar to that of cholesterol with some modifications. These modifications involve the side chain and include the addition of a double bond and/or a methyl or ethyl group⁸. In general, vegetable oils (from corn, soybean and chia seed) contain large amounts of polyunsaturated fatty acids and have more phytosterols⁹. From the more than 25 different types of phytosterols the most common are: sitosterol, campesterol and stigmasterol, corresponding to 65, 30 and 3% of the daily intake of each respectively¹⁰. Some of the benefits that are attributed to a diet rich in phytosterols include anticancer effects⁸, anti-inflammatory properties¹¹, antiulcer effects¹², antioxidants effects¹³, bactericidal and antifungal effects¹⁴. Their ability to control cholesterol plasma levels in hypercholesterolemia patients was first described in 1983¹¹. In recent years, great deal of interest has been given to the role of β -sitosterol in the protection from colon cancer⁸ and their ability to prevent complications from benign prostatic hyperplasia^{15,16}.

Therefore, the objective of the present study was to identify beneficial substances to human health and quantify the β -sitosterol from black chia seed, like related substance to prevent some diseases like colon cancer, benign prostatic hyperplasia and cholesterolemia.

Material and Methods

Proximal chemical analyses were performed on Black chia seed from San Mateo Coatepec Atzitzihuacan Puebla, Mexico according to Association of Official Analytical Chemist (AOAC) techniques¹⁷. The analysis included moisture (14,003 AOAC), ash (14,006 AOAC), ether extract (7062 AOAC), protein determination (2057 AOAC), and total fiber (7068 AOAC). For the extraction, 75 grams of pulverized black chia seed were placed for four hours in a soxhlet with 750 mL of n-hexane (Merck). After the extraction, the n-hexane was removed by rotary evaporation at 60° C during 40 minutes.

The iodine value (Cd 8-53 method)¹⁸ and peroxide index (Cd 1-25 method)¹⁹ of the oil were determined according to the methodologies of the American Oil Chemists' Society (AOCS, 2006). The index of refraction was measured by using a refractometer (Reichert equipment, Arias 500 model) at 25°C. The density was obtained by the pycnometer method at 25°C.

Infrared spectrum was obtained in a Perkin Elmer Spectrum One FTIR Spectrometer in a range from 4000 to 400 cm⁻¹. Gas chromatography (GC-MS) was obtained by a gas chromatograph (model HP6890) with a selective detector of masses, using a capillary column (model HP5-MS) of 30 m long. The injection temperature was 200 °C in split mode with 40:1 ratio. The detector temperature was 250°C. An increase of 194°C was used during one minute until 250°C at a rate of 30°C per minute. Helium gas was used in the mobile phase and a selective detector of masses (model 5973) with mass spectra library NIST 2.0 with manual injection. Finally, β-sitosterol was quantified using a sitosterol-B standard (American Herbal Pharmacopoeia). All determinations were performed in triplicate.

Results and Discussion

Proximal chemical analysis from black chia seed was 5.15% of moisture, 34.70% of fat, 4.10% of ash, 17.90% of protein, 40.25% of carbohydrate and 17.30% of raw fiber. The compounds in major proportion were lipids, in accordance with previous studies correspond to mainly omega-3 fatty acids. Also, it was checked a protein intake and total carbohydrates, which almost 50% corresponding to raw fiber.

Extracted black chia seed oil yield was 29%. The physicochemical characterization indicates an iodine value of 197.68 cg/g corresponding to polyunsaturated fatty acids present, similar to the one reported for creole chia of 193 cgI₂/g²⁰. Black chia seed oil contains higher amounts of polyunsaturated fatty acids than with other oils such as canola (110-126 cgI₂/g), safflower (136-148 cgI₂/g), olive (118–139cgI₂/g) and soybean (118–139cgI₂/g) oils. The peroxide value obtained was 2.64 meq/Kg. The value obtained is relatively low to be unrefined oil, compared with the maximum value for refined soybean oil of 2 meq/Kg (NMX-F-252-SFC1-

2005). This low value shows that natural antioxidants that have been identified such as γ-tocopherol, protects the oxidation of omega-3 fatty acids. The refraction index was 1.46, corresponding to polyunsaturated acids. Finally, the density was determined to be 0.89 g/mL.

The functional groups identify by FTIR spectrum, corresponding to molecules from unsaturated fatty acids^{21,22} (table 1).

Table-1
Functional groups identify by FTIR

Functional groups	Wavenumber (cm ⁻¹)
= CH	3010.03
C=O	1744.27

The fatty acid profile obtained by CG-MS (table 2) showed the presence of important compounds for human metabolism that are present in the black chia seed oil from San Mateo Coatepec Atzitzihuacan, Puebla. Among those compounds Z,Z,Z-9,12,15-octadecatrienoic acid (omega-3) was found with a retention time of 21.44 minutes. The presence of this compound has relevance because of the numerous benefits associated with its consumption. For example, medical and epidemiological studies have shown that consuming lipids rich in omega-3 fatty acids reduce the risk of suffering coronary heart diseases. Another compound found was γ-tocopherol a with a retention time of 25.71 minutes. A natural antioxidant, γ-tocopherol plays an important role in the protection of the polyunsaturated compounds from oils. Phytosterols such as campesterol, stigmasterol and β-sitosterol were found at 29.06 minutes, 29.73 minutes and 31.05 minutes, respectively. The concentration of β-sitosterol was quantified as 1224.3 mg/L. The presence of β-sitosterol has been associated with prevention from prostatic and colon cancer and may help to alleviate the symptoms and urinary flow problems caused by an increase of the benign prostatic hyperplasia (BPH) and decrease cholesterol levels (HDL).

Table-2
Compounds identify by CG-MS

Compounds	Retention time (minutes)
Z,Z,Z-9,12,15-octadecatrienoic acid	21.44
γ-tocopherol	25.71
campesterol	29.06
stigmasterol	29.73
β-sitosterol	31.05

Conclusion

Proximal chemical analysis from black chia seed was 5.15% of moisture, 34.70% of fat, 4.10% of ash, 17.90% of protein, 40.25% of carbohydrate and 17.30% of raw fiber. Physicochemical characterization indicated the presence of unsaturated compounds that are stable for the presence of antioxidants.

The FTIR characterization showed the presence of polyunsaturated compounds. Finally, the profile of fatty acids by GC-MS evidenced the presence of omega-3 fatty acid, vitamin E and phytosterols such as campesterol, stigmasterol and β -sitosterol; the latter with a concentration of 1233.9 mg/L.

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