Translates from Revista Aceites y Grasa 2014, Asociacion Argentina de Aceites y Grasas, Buenos Aires, Argentina, (23)3:402-407. Chaco - Ecuador (Spanish)

Antioxidants, protein, oil content and fatty acids' profiles of a single genotype of chia seeds (*Salvia hispanica* L.) growing in three different ecosystems of South America

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Abstract

Chia is a summer annual of the Lamiaceae. The objective of this study was to investigate in one genotype of chia, the effect of growing location on its antioxidants content and composition and its potential relation with its major nutritional compounds, as protein, oil, and fatty acids. This study was carried out using black-spotted chia seeds commercially grown in three different ecosystems, Tropical Rain Forest, Sub Humid Chaco, and Campo Cerrado, located in Ecuador, Bolivia and Paraguay, respectively. Flavonols quercetin, myrcetin, kaempherol, caffeic acid, and chlorogenic acid, and SDG lignan compound presence was detected by chromatographic analysis. No significant ($P<0.05$) differences between seed origins' were found. Total oil content was significantly ($P<0.05$) higher in the seeds from Ecuador than all other locations, followed by the seeds from Bolivia which was significantly ($P<0.05$) higher compared to Paraguay. The content of α-linolenic fatty acid in seeds from Ecuador was significantly ($P<0.05$) higher compared to the seeds from all three locations. The results indicate that protein content, oil content and fatty acid profile characteristics of the chia are affected by the different ecological conditions of the tested ecosystems, which not affect the polyphenols content, and composition.

Keywords: Salvia hispanica L.; chia; polyphenols; antioxidants; lignans; fatty acids; linolenic; flavonols; protein

1. Introduction

Chia (*Salvia hispanica* L.) is a summer annual of the Lamiaceae family. In pre-Columbian times it was one of the basic foods of several Central American civilizations. Tenochtitlan, the capital of the Aztec Empire, received 5–15,000 tons of chia annually as a tribute from conquered nations (Codex Mendoza, 1542). Chia seed was also part of holy ceremonies as an offering to the Aztec gods (Sahagun, 1579). It appears that because of religious

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persecution, and given the fact that it could not be grown in Europe, it essentially disappeared for 500 years (Ayerza and Coates, 2005a).

Chia oil contains one of the highest known concentrations of α-linolenic fatty acid, up to 67.8% (Coates and Ayerza, 1996). Recently, chia seed has become important for human health and nutrition because its ω-3 fatty acid content promotes beneficial health effects (Vuksan et al., 2007; Ayerza and Coates, 2005b). A number of studies have shown good oxidative stability of chia seed when used as animal feed or as a food ingredient, with this being attributed to the high antioxidant activity of the phenolic compounds it contains (Reyes-Caudillo et al., 2007; Taga et al. 1984).

Chia seed contains chlorogenic acid, caffeic acid, myricetin, quercetin and kaempferol flavonols. These compounds are both primary and synergistic antioxidants, and contribute in a major way to the strong antioxidant activity of chia (Castro-Martinez et al. 1986; Taga et al. 1984). There is evidence that phenolic substances act as antioxidants by preventing the oxidation of LDL lipoprotein, platelet aggregation, and damage of red blood cells (Cheynier, 2005). Additionally, phenolics act as: metal chelators, antimutagens and anticarcinogens, antimicrobial agents and clarifying agents (Proestos et al., 2005).

Recently (R. Ayerza unpublished) reported the detection of secoisolaricresorcinol diglucoside (SDG) compound in two genotypes of chia seeds. SDG is a lignan compound, which has an antioxidant activity (Hosseinian, 2006), and the great oxidative stability of chia oil could be attributed not just to the flavonols compounds content but to the SDG content as well. Thus, any possibility of variability in its polyphenols contents and composition needs to be explored. The objective of this study was to investigate in one genotype of chia, the effect of growing location on its antioxidants content and composition and its potential relation with its major nutritional compounds, as protein, oil, and fatty acids.

2. Material and methods

2.1. Samples

This study was carried out using black-spotted chia seeds commercially grown in three different ecosystems, Tropical Rain Forest, Sub Humid Chaco, and Campo Cerrado, located in Ecuador, Bolivia and Paraguay, respectively (Table 1). The black-spotted seeds belong to the Tzotzol variety as was reported by Ayerza and Coates (2005b)

Within the three ecosystems where the chia was grown, representative commercial fields were selected for sampling. The harvested seed was stored in 25 kg bags and random samples were taken. Samples were collected following the seed sample instructions of the Canadian Food Inspection Agency (2008). The samples were cleaned by hand and sent to the laboratory for analysis. The experimental design used was completely randomized, with six replications.

2.2. Chemical Analysis

Crude nitrogen of the chia seed samples was determined by standard micro-Kjeldahl

method, then converted to protein content using a 5.71 conversion factor (AOAC, 1995).

Lipids were extracted from the samples according to the method described by Folch et al. (1957). Total lipids were then converted into fatty acid methyl esters using the IRAM 5-560II method (IRAM, 1982), which is equivalent to ISO 5509-1978 item 6 (ISO, 1978). Fatty acid methyl esters were separated and quantified by automated gas chromatography (Model 6890, GC; Hewlett Packard Co., Wilmington, DE, USA) equipped with flame ionization detectors and a 30 m 9 530-lm i.d. capillary column (Model HP-FFAPFree fatty acid phase; Hewlett Packard Co., Wilmington, DE, USA). The temperatures of the oven, injector, and detector were set at 180, 290 and 330°C, respectively. The fatty acid composition of each sample was determined by integrating the recorded peaks using Hewlett-Packard Chem-Station Software. Results were expressed as percentage of total fatty acids.

The peroxide values were determined by ISO 3960/1977 procedure; results were expressed as meq oxygen/kg (AOAC, 2002).

Flavonol analysis performed using HPLC by methodology adapted from Chang et al. (1997); utilizing water-acetonitrile (80:20) extract separated on a LiChrospher RP-18 column (Merck Chemicals, Basel, Switzerland), with mobile phase gradient elution of water-acetonitrile (0-10 min 80:20, 14-25 min 63:37) employing a flow rate of 1.0 ml/min with detection at 270 nm. Caffeic acid analysis performed using HPLC by method adapted from Adzet et al. (1985); utilizing samples extracted into acetone and subjected to chromatography on a column (150x4.5mm) of Spherisorb C18 (5 μm) (Waters Corporation, Milford, MA, USA), eluted with a gradient mobile phase of 2.5% of acetic acid in aq. methanol with a linear gradient of 13 to 43% of methanol during 30 min, and detection by photo diode array (200-40nm) with UV detection at 325 nm. Isoresorcinol analysis performed using HPLC by method adapted from Charlet et al. (2002); utilizing acid hydrolysis, necessary for the release of lignan from their complex form to form free aglycone, subjected to separation on a Waters Symmetry C18 3im column (150x4.6mm) (Waters Corporation, Milford, MA, USA) eluted with a gradient mobile phase of water (95%), acetonitrile (5%) changing linearly in 20 min to water (50%), acetonitrile (50%), with diode array detection.

2.3. Statistical Analysis

A one-way analysis of variance (ANOVA) was performed for oil, individual fatty acid content, protein content, soluble and insoluble fiber contents, and peroxide value. When the F value was significant ($P<0.05$), means were separated using Student-Newman-Keuls Test (Cohort, 2006). Additionally, correlation and regression analysis were undertaken to develop the relationship between measured parameters (Cohort, 2006).

3. Results and discussion

Total water, protein content, oil content, and peroxide value

Total water, protein content, oil content, and peroxide value, are summarized in Table 2.

Water content and peroxide value were not significantly ($P < 0.05$) affected by location. All values were lower compared to those reported by Ayerza and Coates (2004) for chia seeds from Colombia, Peru and Argentina. However, all these values are within the range of two genotypes of chia grown in five different locations of Ecuador (R. Ayerza, unpublished).

Oil and protein contents, as a percentage of chia seed weight, showed significant ($P < 0.05$) differences among locations. Total oil content was significantly ($P<0.05$) higher in the seeds from Ecuador than all other locations, followed by the oil content of seeds from Bolivia which was significantly ($P<0.05$) higher compared to that from Paraguay.

The seeds from Bolivia and Paraguay ecosystems showed significantly ($P < 0.05$) higher protein content as a percentage of chia seed weight, compared to Ecuador. No significant ($P < 0.05$) difference in protein content was detected between the other two locations.

The results presented herein support the contention that ecosystem has a strong effect on the protein and oil content of chia seeds (Ayerza, 2011, 2010, 1995). This has been reported for many other crops (Vollmann et al., 2007; Mohammed et al., 1987). A positive cause–effect relationship, between temperature and protein, and a negative one between temperature and oil content, in oil seed crops such as soybeans have also been reported (Kumar et al., 2006; Thomas et al., 2003).

3.1. Results of the fatty acid compositional analyses

Results of the fatty acid compositional analysis by origin are presented in Table 3. Gas chromatography analysis of the oil composition of seeds from all locations detected the presence of α-linolenic fatty acid, followed by linoleic, oleic, palmitic and stearic fatty acids. In addition, six more fatty acids were identified in all analyzed seed samples, myristic, arachidic, gadoleic, behenic, eracic, and lignoceric. However, as all of them were present just in traces, those fatty acids were omitted from this report.

Polyunsaturated ω-6 linoleic fatty acid, the second largest component of chia seed oil, was significantly ($P < 0.05$) lower in seeds from Ecuador than the other two locations; no significant ($P < 0.05$) differences were detected between seeds from Bolivia and Paraguay.

The main constituent in the oil was polyunsaturated ω-3-α-linolenic fatty acid. The seed from Ecuador showed significant ($P<0.05$) higher content of α-linolenic fatty acid comparing to all three locations. No significant ($P<0.05$) differences were detected between seeds from the other two locations. The present study confirmed that the fatty acid composition of chia oil is influenced by the effects of factors such as soil's quality and climatic and weather conditions, as it was demonstrated in an early report (Ayerza, 1995).

The seeds from Ecuador showed significantly ($P<0.05$) lower content of oleic and linoleic fatty acids, compared to seeds grown in the other two locations. Overall, the α-linolenic fatty acid was negatively correlated with linoleic and oleic fatty acids content; computed for these negative relationships, the regression coefficients (R^2) and significance levels (P) were $R^2= 0.993$ ($P=0.001$), and $R^2= 0.994$ ($P= 0.001$), respectively. The negative relationships of α-linolenic fatty acid contents with the 18-C more saturated fatty acids, linoleic and oleic, were reported for a number of crops, such as almonds (Abdallah et al., 1998), chestnuts (Pires Borges et al., 2007), soybeans (Thomas et al., 2003), flaxseed, a rich source of

α-linolenic fatty acid (Wakjira et al., 2004), and chia (Ayerza, 2011, 2009). This strong inverse relation is supported by the biosynthesis of α-linolenic fatty acid through the process of desaturation of oleic fatty acid via linoleic fatty acid by the action of desaturase enzymes (Thomas et al., 2003; Yaniv et al., 1995).

The ω-6:ω-3 ratio was significantly ($P<0.05$) lower in oils from seeds grown in Ecuador compared to that of seeds grown in the other two locations. Dietary ω-6 and ω-3 fatty acid relation has been identified as a risk factor of suffering a coronary heart disease, and a way of lowering the risk is to keep dietary ω-6:ω-3 fatty acid ratio as low as possible, the ratio of 1:1 being ideal (Simopoulos, 2003). Western diets do not provide these ratios, mainly due to their high ω-6 fatty acid content. As an ω-3 source, chia is consumed either as an oil or as whole/ground seed. The significant ($P<0.05$) lower ω-6:ω-3 rate (up to -25%), showed by seeds grown in the Ecuador location, compared with the other ones, could indicate an added health benefit for these seeds.

3.2 Polyphenols content and compositions

Polyphenols content and compositions are presented on Table 4. Chromatographic analysis found the polyphenols composition of seeds from the three locations. The presence of quercetin, myrcetin, kaempherol, caffeic acid, and chlorogenic acid flavonols, and the lignan compund SDG was detected. No significant ($P<0.05$) differences between seeds origins were found. An exception was the caffeic acid content which showed differences between seed origins; these differences were significant ($P < 0.05$) among locations and showed a relationship of Paraguay > Ecuador > Bolivia. Wether this is just an anomaly, or a result of environment is not know. The total flavonols amount found herein are not far of the 0.757 - 0.881 mg/g found for two chia sources reported by Reyes-Caudillo et al. (2007) or the 0.924 - 0.939 mg/g reported for the Totzol and Iztac genotypes, respectively (R. Ayerza, unpublished).

No significant difference ($P<0.05$) in SDG content was found among seeds origins. The lignan SDG compound amount found herein is similar to the 0.405 - 0.424 mg/g determinated by Ayerza (Unpublished) for two different genotypes of chia grown in Ecuador. Since the discovery of their physiological value, lignans have been extracted from flax and other plants, in a variety of ways. Once extracted, lignans can be added to food or taken in a concentrated form, in an attempt to take advantage of their functionality and benefits (Comin et al., 2010). This content could indicate an added commercial benefit for chia seeds.

The lack of a positive correlation (data not shown) between α-linolenic fatty acid and poly phenol compounds are somewhat surprising because flavonols and lignans are effective antioxidants in oil, and it may be expected that the plant reacts to increased polyunsaturation by producing more polyphenols to protect the oil from oxidation. The absence of a direct relationship, supports the proposition put forward by Dolde et al. (1996), that antioxidant's concentration and the fatty acid profile are not causally related but influenced differently by independent external variables such as temperature or soil type as it was reported for others seed oil crops as soybean and canola (Richards et al., 2008).

4. Conclusions

In summary, the results found herein indicate that protein content, oil content and fatty acid profile characteristics of the Tzotzol variety of chia are affected by the different ecological conditions of the ecosystems of this study, which not affected the flavonols and lignans content, and composition. Additional multi location and multi year trials are required to confirm this polyphenols compound's stability to the ecosystem's differences, and to understand the biochemical bases for these phenomena.

Acknowledges

The author acknowledges support for this project from Corporacion Internacional de Comercio y Servicios S.A. (CICS), Buenos Aires, Argentina. The author is grateful to a number of agronomists and farmers of Bolivia, Ecuador, and Paraguay for their help with field tasks.

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Tables

Table 1. Locations where *Salvia hispanica* L. was grown

Ecosystem	Country	Latitud	Elevation m	Temp Mean/year °C	Rainfall mm	Soil type
Sub-Humid Chaco	Bolivia	17°17' 00" S	265	24	1.157	Mollic planosols
Tropical Rain Forest	Ecuador	02°18' 00" S	300	25	>3,000	Regosol lateritico
Campo Cerrado	Paraguay	24°65' 59" S	168	23	1.6	Ultisol

Table 2. Fatty acid composition of *Salvia hispanica* L.

Origin	Water	Protein %	Oil	Peroxide Value meq of O_2/kg
Bolivia	5.9[a1]	23.1[a]	32.5[b]	0.425[a]
Ecuador	5.7[a]	19[b]	34.2[a]	0.605[a]
Paraguay	5.8[a]	23.25[a]	31.6[c]	0.415[a]
SD[2]	0.389	1.047	0.636	0.196

[1] Means in a column within a group with the same letter are not statistically different (P<0.05); [2] Least significant difference for P<0.05

Table 3. Fatty acid composition of *Salvia hispanica* L.

Origin	Palmitic	Stearic	Oleic	Linoleic	α-Linolenic	ω-6:ω-3 rate	α-Linolenic g/kg of seed
	% of total fatty acids						
Bolivia	6.3[a1]	0.167	8.95[a]	21.15[a]	58.5[b]	0.36[a]	19.01[b]
Ecuador	6.5[a]	3.6[b]	6.65[b]	17.5[b]	64.5[a]	0.27[b]	22.06[a]
Paraguay	7.3[a]	3.4[c]	8.85[a]	20.9[a]	59[b]	0.35[a]	18.64[b]
SD[2]	1.102	2.42	0.225	0.29	1.537	0.123	0.7

[1] Means in a column within a group with the same letter are not statistically different (P<0.05); [2] Least significant difference for P<0.05

Table 4. Antioxidant content and composition in the seeds of *Salvia hispanica* L.

Origen	Flavonols						Lignans
	Myrcetin	Quercetin	Kaempherol	Chlorogenic acid	Caffeic acid	Total	SDG
	mg/g						
Bolivia	0.119[a1]	0.006[a]	0.024[a]	0.214[a]	0.141[c]	0.914[a]	0.409[a]
Ecuador	0.121[a]	0.006[a]	0.024[a]	0.218[a]	0.149[b]	0.924[a]	0.407[a]
Paraguay	0.121[a]	0.006[a]	0.025[a]	0.235[a]	0.156[a]	0.975[a]	0.432[a]
SD[2]	0.009	---	0.003	0.055	0.003	0.101	0.06

[1] Means in a column within a group with the same letter are not statistically different (P<0.05); [2] Least significant difference for P<0.05