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Protein content, oil content and fatty acid profiles as potential criteria to determine the origin of commercially grown chia (*Salvia hispanica* L.)

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ABSTRACT

Chia (*Salvia hispanica* L.), an annual herb of the Labiatae family, produces seeds which were one of the basic foods of Central American civilizations in pre-Columbian times. Chia seed contains the highest known percentage of α-linolenic fatty acid of any plant source. In recent years, chia seed has become increasingly important for human health and nutrition because of its high content of α-linolenic fatty acid, and the beneficial health effects that arise from its consumption. A study was undertaken to characterize protein and oil contents as well as fatty acid composition of chia seeds grown in some larger commercial fields, in an attempt to determine how these components are affected by location. Oil saturation tended to decrease as elevation of seed production increased, with decreasing levels of palmitic, stearic, oleic, and linoleic fatty acids found. The main constituent in the chia oil was ω-3 α-linolenic fatty acid, and ranged from 64.8% to 56.9%. Differences were significant ($P<0.05$) among locations. Significant differences in protein content and fatty acid composition were also found for the commercially grown chia originating from three ecosystems. It is possible that these differences could be used to distinguish chia's origin, if additional research was undertaken to characterize such differences.

1. Introduction

Chia (*Salvia hispanica* L.), an annual herb of the Labiatae family, produces seeds, which were one of the basic foods of Central American civilizations in pre-Columbian times (Ayerza and Coates, 2005). Along with chia, corn, beans, and amaranth were the most important foods of more than 11 million people when Columbus arrived in America. Chia was also used as an offering to the Nahua gods (Sahagun, 1579). It appears that because of religious persecution, and given the fact that it could not be grown in Europe, it essentially disappeared for 500 years.

Chia seed contains the highest known percentage of α-linolenic fatty acid of any plant source, up to 68% (Ayerza, 1995), compared to 36%, 53%, and 57% for camelina (*Camelina sativa* L.), perilla (*Perilla frutescens* L.) and flax (*Linum usitatissimum* L.), respectively (SOFA, 2006; USDA, 2006; Sultana, 1996). In recent years chia seed has become increasingly important for human health and nutrition because of its high content of α-linolenic fatty acid, and the beneficial health effects that can arise from its consumption.

From a nutritional point of view, it is important that the fatty acid profile of chia sold in the marketplace does not vary significantly, since the benefits obtained by consumption depend on composition. Early studies reported a wide range in oil content and fatty acid composition of seeds grown under different climatic conditions, and in various geographical locations (Ayerza and Coates, 2004; Ayerza, 1995). However, much of the chia that is commercially produced and sold today does not list the protein and oil contents, nor fatty acid profile on the bag labels, even though chia seed and chia oil are now being consumed in a number of countries as a functional food. Generally chia seed is simply sold on a weight basis, regardless of genotype, oil content or fatty acid profile.

This paper reports on the fatty acid composition, protein and oil content of chia seeds grown in some of the larger commercial fields in South America, and was written so as to begin to understand how these properties relate to the characteristics of the ecosystems in which they were grown.

2. Materials and methods

2.1. Locations and agronomic practices

Protein and lipid contents, as well as fatty acid profiles were determined for chia seed commercially grown in three South American ecosystems located in Argentina, Bolivia and Ecuador (Table 1).

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Table 1
Locations where chia was grown.

Origin	Ecosystem	Country	Elevation (m)	Annual rainfall (mm)	Mean temperature (°C)	Latitude	Longitude	Soil type
T_1	Semi-arid Chaco[a]	Argentina	1156	560	17	25°07′48″S	65°34′00″W	Calcaric rhegosols[d]
T_2	Sub-Humid Chaco[a]	Bolivia	264–266	1100	24	17°17′00″S to 17°24′00″S	62°18′00″W to 62°35′00″W	Mollic planosols[c]
T_3	Inter-Andean Valley[b]	Ecuador	1600–2200	300	16	01°18′50″S to 00°29′47″N	78°30′58″W to 78°07′56″W	Cambisols[c]

[a] Without irrigation.
[b] With irrigation.
[c] FAO (1995).
[d] Nadir and Chafatinos (1990).

For identification purposes the three locations were denoted as T_1, T_2 and T_3.

Two of the growing locations were within the Chaco ecosystem, which occupies some 110 million hectares. The sub-humid Chaco is located in the northern part of this ecosystem, while the semi-arid portion is in the south. A victim of indiscriminate tree-felling and over-grazing, 95% of this region suffers from severe desertification and has become home to dense, deciduous, spiny shrubs of little forage value for livestock. Rainfall decreases from the north to the south, with the north having more and better quality trees, with the south covered by shrubs. The sub-humid Chaco never experiences frosts, while the dry Chaco has frosts ever year. This ecosystem is quite different from the Inter-Andean Valley ecosystem since the latter is densely populated, with essentially all of the native vegetation having been replaced by agricultural crops and seeded pastures.

Of the commercial fields used in this study, T_1 and T_2 were grown under rain fed conditions, while those from T_3 were produced using furrow irrigation. T_1 and T_2 were planted during the rainy season (December to April), with the seed maturing during the dry season (remainder of the year). This follows the typical cropping system used in these two ecosystems. In the case of T_2 a severe drought was intensified by atypically high temperatures for this ecosystem. The agronomic practices were those commonly used in each region with not fertilizer, herbicides or pesticides being applied. The seeding rates were similar, ranging from 5 to 6 kg/ha, with row spacing being between 600 and 700 mm. The same source of seed was used for each planting.

2.2. Seed samples

Within each region where the chia was grown, representative commercial fields were selected for sampling, with a total of 26 samples harvested. In each instance the samples were collected by the authors, cleaned by hand and sent to the laboratory for analysis. In the case of T_3 the seeds were harvested by hand, while for T_1 and T_2 they were collected from the combine after mechanical harvesting.

2.3. Determination of crude protein, total fat, and fatty acid composition

Crude nitrogen of the chia seed samples was determined by a standard micro-Kjeldahl method and was converted to protein content using a 5.71 conversion factor. Lipids were extracted and converted into fatty acid methyl esters using the IRAM 5-560II method (Instituto Argentino de Racionalización de Materiales, 1982). Fatty acid methyl esters were separated and quantified by an automated gas chromatograph (Model 6890, GC; Hewlett Packard Co., Wilmington, DE 20006) equipped with flame ionization detectors and a 30 m × 530 μm i.d. capillary column (Model HP-FFAP; Hewlett Packard Co., Wilmington, DE 20006).

2.4. Statistical analysis

The data were examined using analysis of variance, when the *F*-value was significant means were separated using Student–Newman–Keuls test. Additionally correlation and regression analyses were undertaken to develop the relationships among fatty acids, protein contents and area parameters (Cohort Stat, 2006).

3. Results

Results of the protein content, oil content, and fatty acid compositional analyses, by seed origin, are presented in Table 2. In general,

Table 2
Mean values of protein content, oil content and fatty acid composition of chia grown in three ecosystems.

Origin	Protein[a] (%)[b,d]	Lipids (%)[b]	16:0 (%)[c]	18:0 (%)[c]	18:1 (%)[c]	18:2 (%)[c]	18:3 (%)[c]	SAT (%)[c]	PUFA (%)[c]	ω-6:ω-3 (ratio)	PUFA:SAT
T_1	16.45b	33.5a	6.89b	2.36a	6.73b	22.5a	60.35b	9.26b	82.85a	0.37a	9.01a
T_2	26.03a	29.98a	7.72a	3.59a	9.12a	21.93a	56.93c	11.32a	78.87b	0.39a	6.97b
T_3	15.95b	31.47a	6.39c	3.74a	6.59b	16.99b	64.75a	10.14b	81.74a	0.26b	8.12a
LSD[e]	5.673	5.743	0.636	1.643	1.166	2.172	3.658	1.755	2.363	0.049	1.287

[a] No replication.
[b] % of seed weight.
[c] % of total fatty acids.
[d] Means in a column within a group with the same letter are not statistically different ($P<0.05$).
[e] Least significant difference for $P<0.05$.

protein content tended to decrease as altitude of the location where the seed was grown increased. The T_2 sample, which came from the Sub-Humid Chaco ecosystem, showed significantly ($P<0.05$) higher protein content (61% more) compared to the other two locations. No significant ($P<0.05$) difference in protein content was detected between the other two locations.

Oil content, as a percentage of chia seed weight, showed no significant ($P<0.05$) differences among locations. However oil composition, measured as fatty acid percentages, were significantly ($P<0.05$) affected by location.

Saturation of the oil tended to decrease as elevation of the seed production location increased. This change included increasing levels of α-linolenic and linoleic fatty acids, and decreasing levels of palmitic, stearic, oleic, and linoleic fatty acids. The palmitic fatty acid and total saturated (calculated as the sum of palmitic and stearic acids) fatty acid content of chia seeds from the T_2 location were higher than the other locations. Seeds from the T_1 location had a significantly ($P<0.05$) higher percentage of palmitic fatty acid compared to seed from the T_3 location, but did not have a significantly higher total saturated content.

Polyunsaturated ω-6 linoleic fatty acid, the second largest component of chia seed oil, tended to decrease as the level of polyunsaturated ω-3 α-linolenic fatty acid increased. Linoleic fatty acid was significantly ($P<0.05$) higher in seeds from the T_1 and T_2 locations than in seed from the T_3 location; no significant ($P<0.05$) differences were detected between T_1 and T_2.

The main constituent in the oil was polyunsaturated ω-3 α-linolenic fatty acid, ranging from 56.9% to 64.8%. The differences were significant ($P<0.05$) among locations and showed a relationship of $T_3>T_1>T_2$. When α-linolenic fatty acid content was measured as g/kg of seed and compared among locations, no significant ($P<0.05$) differences were detected. Comparing α-linolenic fatty acid content among the three locations, the maximum differences ranged from 14%, when measured as a percentage of total fatty acids, to 19%, when measured as g/kg of seed. However, no significant ($P<0.05$) differences were detected (data not shown).

Total polyunsaturated fatty acid (PUFA) percentage of seed harvested from the T_1 and T_3 locations showed no significant ($P<0.05$) differences between them, but their PUFA percentages were significantly ($P<0.05$) higher than that measured for seeds coming from T_2.

In general, as production altitude increased, the ω-6:ω-3 ratio decreased, and PUFA:SAT ratio increased. The differences, however, were not always significantly ($P<0.05$) different among locations.

Regression analyses were performed for α-linolenic vs palmitic, oleic and linoleic fatty acid contents. The regression coefficient (R^2) and significance (P) levels are presented in Fig. 1. The α-linolenic fatty acid content was negatively correlated with its precursors – palmitic ($R^2=0.83$, $P<0.0001$), oleic ($R^2=0.86$, $P<0.0001$), and linoleic ($R^2=0.89$, $P<0.0001$) fatty acids. Regression analyses of linolenic fatty acid content and protein content vs elevation was also undertaken. The oil content showed a positive correlation between α-linolenic fatty acid content and elevation ($R^2=0.94$, $P<0.0001$), and a negative correlation between protein content and elevation ($R^2=0.99$, $P<0.0001$) (Fig. 2).

4. Discussion

Although genetic differences between and within the ecosystems could have affected the results, as the genotypes were not classified at each location, the results should still be considered valid. This is because commercial multi-varietal-oil composition analyses of other crops produced in various locations have been documented by other studies as being able to determine a crop's origin (D'Imperio et al., 2007; Pires Borges et al., 2007; Mannina et al., 2001; Aparicio et al., 1994).

The results presented herein support the contention that ecosystem has a strong effect on the protein content of chia seeds. 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 content in oil seed crops such as soybean has also been reported (Kumar et al., 2006; Thomas et al., 2003). Although the generally negative relationship between elevation and temperature is often mitigated by a number of other factors, generally air temperature decreases 1 °C/160 m (Miller, 1975). The regression analysis reported herein showed a significantly ($R^2=0.99$, $P<0.0001$) negative relationship between elevation and protein content (Fig. 1), and could be explained by this cause–effect relationship. The protein contents found in this study as well as the variations found among locations agree with those reported for another study which compared chia seed grown in tropical and subtropical ecosystems of Argentina, Bolivia, Colombia, and Peru (Ayerza and Coates, 2004).

Although chia is not commercially grown as a protein source, its amino acid profile has no limiting factors in the adult diet (Weber et al., 1991). Additionally the protein content is higher than that of other traditional crops such as wheat, corn, rice, oats, and barley (Ayerza and Coates, 2005). Studies have also demonstrated that chia can be incorporated into human diets and mixed with other grains to produce a more balanced protein source (Fernandez et al., 2005; Pallaro et al., 2004). Hence being a high-quality protein source could help reduce the commercial disadvantage that the T_2 chia exhibited by having a lower α-linolenic percentage, compared to chia from the other two locations.

The relationship between fatty acid composition and altitude, the latter often directly related to climate (in particular temperature), has been established for other oilseed crops and has been used to characterize oils by geographical origin (D'Imperio et al., 2007; Aparicio et al., 1994). Temperature has been identified as the main factor affecting oil quality (Velasco and Fernandez-Martinez, 2002). The relationship between elevation, fatty acid composition and oil saturation found for chia most likely is related to a temperature–elevation interaction since elevation has a strong

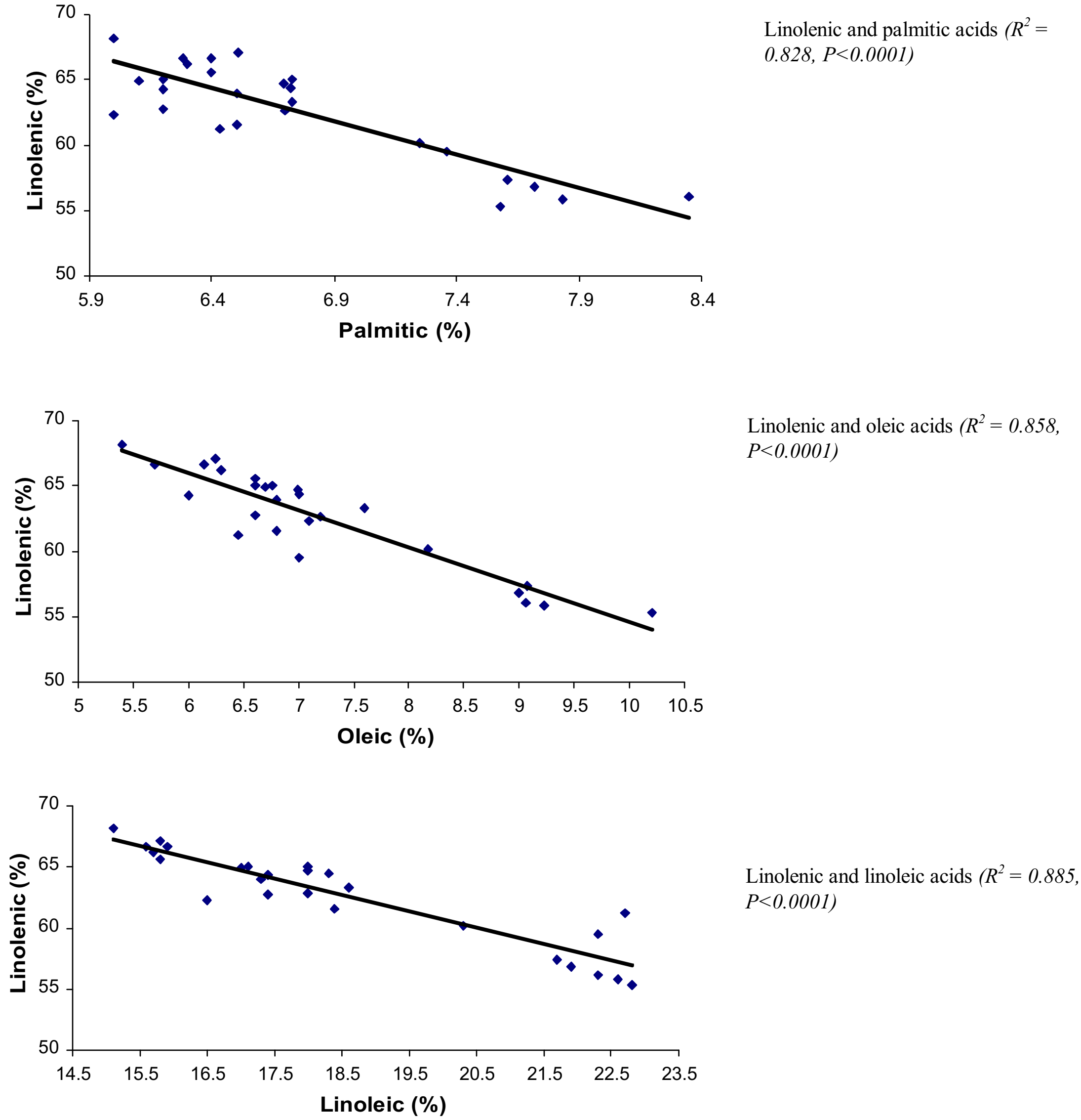


Fig. 1. Relationship between linolenic and palmitic, oleic, and linoleic fatty acid contents.

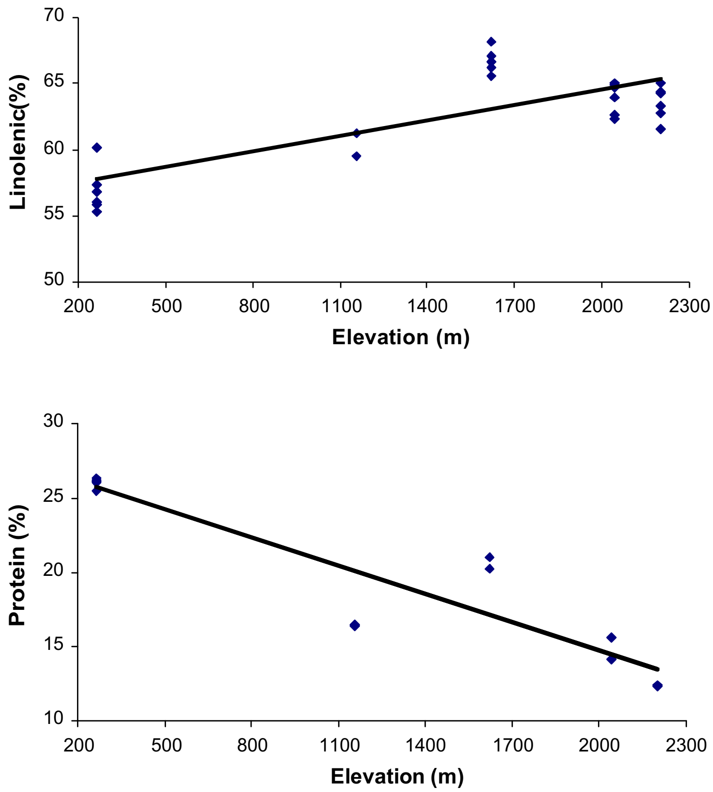
negative relationship with temperature. The correlation between elevation where the chia was grown and α-linolenic fatty acid content found herein is in agreement with previous observations by Ayerza (1995) and Ayerza and Coates (2004). That is cool temperatures generally increase the level of unsaturation of chia fatty acids, as is the case for other oilseed crops (Kumar et al., 2006; Thomas et al., 2003; Ayerza, 1995; Carver et al., 1986; Cherry et al., 1985).

Saturated fatty acids (SAT) and the PUFA:SAT ratio (data not shown) also were significantly correlated with maximum temperature ($P < 0.001$) as well as with mean minimum and mean temperature ($P < 0.05$). The correlations were positive for the maximum and negative for the two mean temperatures, respectively. The negative correlation between temperature and unsaturated content of oilseeds appears to be associated with temperature, since high temperatures induce saturation effects on fatty acid profile which is then mediated at the level of gene expression (Thomas et al., 2003).

The statistically significant regression coefficients found between α-linolenic fatty acid and palmitic, oleic, and linoleic contents are in agreement with observations made in other crops such as almonds, chestnuts, rapeseed and mustard (Pires Borges et al., 2007; Abdallah et al., 1998; Yaniv et al., 1995). The α-linolenic fatty acid content of chia seed is controlled by the processes of desaturation and elongation of the fatty acid complex, probably as a result of the enzymatic activity, which then appears to be regulated by temperature. Such a relationship has been reported by Ichihara and Suda (2003) for perilla, and hence seems likely for chia as well.

Lack of significant ($P < 0.05$) differences in oil content between locations implies different behaviors for oil quantity, and quality. The reason for this finding is not evident from the available data, however it could be related to different temperature ranges controlling oil content and oil composition as has been reported for soybeans (Thomas et al., 2003). They reported a temperature range over which both oil and α-linolenic fatty acid percentages increased as temperatures fell, and that there was a temperature beyond which oil percentage began to decrease, while α-linolenic fatty acid continued to increase. Thus the absence of a significant relationship between oil content and α-linolenic fatty acid percentages (data not shown) found herein could be related to different temperature effects on each.

Chia seed can be considered a functional food, since it is an ω-3 fatty acid source. Considering that in a number of countries chia is consumed directly as whole seed (Ayerza and Coates, 2005), it



Linolenic acid and elevation *(R^2 = 0.937, P<0.0001)*

Protein (%) and elevation *(R^2 = 0.994, P<0.0001)*

Fig. 2. Relationship between linolenic fatty acid and protein contents and elevation.

is important to know the ω-3 content as not only a percentage of total fatty acid content, but also as grams of α-linolenic fatty acid per kilogram of seed. The α-linolenic fatty acid contents reported herein varied 14%, as a percentage of total fatty acid content, but was 20% if measured as g/kg of seed. This means that to meet the nutritional recommendations for ω-3 fatty acid for an adult male eating 2700 calories/day (British Nutrition Foundation, 1992), an individual would need to consume between 22.5 and 26.5 g/day of seeds harvested from the locations having the highest and the lowest contents, respectively. If the same individual was to consume chia oil instead of chia seed, they would have to consume between 7.9 g and 6.9 g/day, respectively.

In summary, this study showed a significant difference in protein content and fatty acid composition of chia commercially grown in different ecosystems. It is possible that these differences might be used to distinguish seed origin. Seed coming from the Inter-Andean Valley ecosystem could be promoted to consumers as the best value, because of the increased potential health benefits provided by the chia seed grown there. The results also indicate that caution needs to be exercised before chia is introduced as a crop in a new area, since location can have a significant impact on seed composition. Additional studies should be conducted to determine the environmental factors which affect formation of, and relationship between, the different components of chia seed which make up its most desirable characteristics.

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