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Seed composition of two chia (*Salvia hispanica* L.) genotypes which differ in seed color

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Abstract

The objective of this study was to investigate the relationship of seed color with protein, oil, fiber, amino acids, and antioxidants content composition of two chia (*Salvia hispanica* L.) genotypes. Study was carried out using chia genotypes known as Tzotzol and Iztac; the first has black-spotted seed, the second white seed. Results: the lack of significant ($p < 0.05$) difference on biochemical compounds between Tzotzol and Iztac genotypes found in this study could be explained by the small genetic difference between these two genotypes. Conclusion: In summary, this paper showed no relationship of the seed coat color for all measured traits, protein, oil, fiber, amino acids, and antioxidants content composition. In addition, during this work it was found secoisolaricresorcinol diglucoside, an antioxidant not previously reported for this species which perhaps contributes to the stability of chia seed oil.

Key words: Antioxidants, Chia, Lignans, Phenolics, *Salvia hispanica*

Introduction

Salvia hispanica L., with the popular name chia, is a low water user plant and well adapted to arid and semiarid climates (Ayerza, 1995). It is currently not grown on a large scale. Nevertheless, due to the universal applicability of the products, the crop deserves high attention. The specific composition of chia seeds appears very attractive. Chia oil is highly unsaturated. The content of polyunsaturated fatty acids (PUFA) amounts to about 83%. The ratio of linoleic acid (18:2n-6)-(LA) with about 18%, and α -linolenic acid (18:3n-3)-(ALA) with about 64%, is unique between the common vegetable oils, such as soya (*Glycine max* L.) oil, sunflower (*Helianthus annuus* L.) oil, rape (*Brassica napus* L.) oil, olive (*Olea europaea* L.) oil, etc. Chia oil is also qualitatively different from the less common vegetable oils with a high content of PUFA such as flax (*Linum usitatissimum* L.) with the content of 53.3% ALA (Bhatty, 1993). In addition, chia seed has a significant content and

composition of protein, antioxidants, and dietary fiber (Ayerza and Coates, 2005a).

In recent years chia seed has become increasingly important for human and animal health and nutrition because of its high content of α -linolenic fatty acid, and the beneficial health effects that can arise from its consumption (Ayerza and Coates, 2005b, 2007; Vuksan et al., 2007; Espada et al., 2007; Chicco et al., 2008; Jeong et al., 2010; Ayerza, 2011).

It is theorized that the chia seeds commercialized today were selected by Nahua botanists, but came into the twenty-first century as a mixed population. This mixture continues to be grown by the descendants of the Nahua and Maya nations living in the mountains of Southern Mexico, Northern Guatemala, and Nicaragua. Seed coat color in chia ranges from black and black spotted to white. However, chia commercialized today is mainly black-spotted, followed by a low but increasing percentage of white seeds (Ayerza and Coates, 2005a).

Recently, Ayerza (2011) reported no significant differences in oil content and fatty acids profile between spotted-black seeds and white seeds of chia grown in 5 different ecosystems. However, chia is also a good source of protein, antioxidants and fiber (Taga et al., 1984; Weber et al., 1991; Reyes-Caudillo et al., 2008) and the possibility of genotype variability for these seed components

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needs to be explored. The objective of this study was to determine if differences in protein, oil, fiber, amino acids and antioxidant concentrations occur for two chia genotypes which differ in seed color, and to identify the different antioxidants which exist in this seed.

Materials and Methods

This study was carried out using white and black-spotted chia seeds commercially grown in a Tropical Forest ecosystem located in Ecuador. The black-spotted and the white seeds belong to the Tzotzol and Iztac genotypes, respectively, as was reported by Ayerza and Coates (2009a).

Within the area where the chia was grown, representative commercial fields were selected for sampling (Figure 1). Samples were collected from the combine after mechanical harvesting, 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.



Figure 1. A view of *Salvia hispanica* L. commercial plantation.

Chemical analysis

Crude nitrogen of the chia seed samples was determined by standard micro-Kjeldahl method, and 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 30m 9 530- μ m 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).

Flavonoid 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 1.0ml/min with detection at 270 nm. Caffeic acid analysis performed using HPLC by method adapted from Adzet and Puigmacia (1985); utilizing sample extracted into acetone and subjected to chromatography on a column (150x4.5mm) of Spherisorb C18 (μ m) (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 3 μ m 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. Amino acid analysis performed using HPLC following derivatization according to the AccQtag methodology (Waters Corporation, Milford, MA, USA) using 20mM HCl, Borate buffer, and AQC reagent in acetonitrile (1:3:1, v/v/v), followed by HPLC using Waters Extera C18 column (150x3.5mm, 3 μ m) (Waters Corporation, Milford, MA, USA), 40°C, with isocratic mobile phase consisting of 20mM Potassium phosphate, pH3.0/Acetonitrile (95:5) 1.5ml/min with UV detection (254nm). Fiber analysis performed using AOAC 941.2 (1997) method. Insoluble fiber determined by difference following organic solute removal.

Statistical analysis

A one-way analysis of variance (ANOVA) was performed for oil, individual fatty acid content, individual amino acid content, protein content, soluble and insoluble fiber content, and peroxide value. When the F value was significant ($p < 0.05$), means were separated using the least significant difference test (LSD) (Cohort, 2006).

Results and Discussion

Total water, protein content, oil content, peroxide value, and fiber contents are summarized in Table 1. The values of all measured parameters differed very little between the two genotypes. The differences in the analytical values were not statistically significant. All these parameters had results within the limits of values reported by Ayerza (1995, 2011) and Ayerza and Coates (2005b, 1999, 2004) for the same genotypes grown in a number of different sites of Argentina, Colombia, Bolivia, Ecuador and Peru.

Chia is a good source of dietary fiber (Table 1) and can be favored compared with traditional sources of fiber as barley (17.3%), corn (13.4%), wheat (12.6%), soybeans (15%), flaxseeds (22.33%), and sesame seeds (7.79%) (Dhingra et al., 2012). The importance of food fibres has led to the development of a large and potential market for fibre-rich products and ingredients and in recent years, there is a trend to find new sources of dietary

fibre that can be used in the food industry (Chau and Huang, 2003).

Results of the fatty acid compositional analyses by genotype are presented in Table 2. Analysis of variance showed that the differences in fatty acid content were not statistically different between genotypes. The lack of significant ($p < 0.05$) difference in fatty acid profile, between these two genotypes grown in five different ecosystems was recently reported. Ayerza (2010) demonstrated that the larger differences found in oil content and fatty acid composition are due to location (because of the environmental differences) rather than chia seed coat color. In addition, the fatty acid profile found herein are similar to that reported by a number of papers (Ayerza, 1995, 2010; Ayerza and Coates, 2009a).

Antioxidant content and composition in the Tzotzol and Iztac genotypes are presented on Table 3. Analysis results of the flavonols compounds showed the presence of myrcetin, quercetin, caffeic acid and chlorogenic acid in both white and black-spotted color seeds. Statistical analysis of the data showed that antioxidants content and composition differences between both genotypes were not significant. These flavonols compounds found in chia have been shown to possess consistently strong antioxidant properties (Castro-Martinez et al., 1986; Reyes-Caudillo et al., 2008; Taga et al., 1984).

Table 1. Water, protein, oil, fiber and peroxide values of seeds from two *Salvia hispanica* L. genotypes.

Genotype	Water	Protein	Oil	Peroxide Value	Fiber (g/100g)		
	%			meq of O ₂ /kg	Soluble	Insoluble	Total
Tzotzol	5.7 ^{a1}	19 ^a	34.2 ^a	0.61 ^a	10.76 ^a	12.43 ^a	23.19 ^a
Iztac	5.4 ^a	18.8 ^a	32.1 ^a	0.82 ^a	9.68 ^a	16.27 ^a	25.94 ^a
SD ²	2.541	5.082	5.08	1.91	8.334	10.765	4.275

¹ Means in a column within a group with the same letter are not statistically different; ² Least significant difference for $p < 0.05$.

Table 2. Fatty acid composition of *Salvia hispanica* L. Tzotzol and Iztac genotypes.

Genotype	Palmitic	Stearic	Oleic	Linoleic	Linolenic	ω -6: ω -3	Linolenic
	% of total fatty acids						rate
Tzotzol	6.5 ^{a1}	3.65 ^a	6.65 ^a	17.5 ^a	64.5 ^a	0.29 ^a	22.06 ^a
Iztac	6.2 ^a	4.1 ^a	6.8 ^a	18.4 ^a	63.3 ^a	0.27 ^a	20.32 ^a
SD ²	2.541	1.27	1.272	2.541	7.624	0.032	5.886

¹ Means in a column within a group with the same letter are not statistically different; ² Least significant difference for $p < 0.05$; ³ Kilograms of seed by oil percentage by linolenic percentage.

Table 3. Antioxidant content and composition in the Tzotzol and Iztac genotypes.

Genotype	Flavonols (mg/g)					Lignans SDG ³	Total
	Myrcetin	Quercetin	Kaempferol	Chlorogenic acid	Caffeic acid		
Tzotzol	0.115 ^{a1}	0.007 ^a	0.025 ^a	0.226 ^a	0.139 ^a	0.424 ^a	0.934 ^a
Iztac	0.121 ^a	0.006 ^a	0.024 ^a	0.218 ^a	0.149 ^a	0.405 ^a	0.924 ^a
SD ²	0.014	0.002	0.005	0.042	0.032	0.097	0.153

¹Means in a column within a group with the same letter are not statistically different; ²Least significant difference for $p < 0.05$; ³Secoisolaricresorcinol diglucoside.

The most significant finding in this chia trial was the detection of secoisolaricresorcinol diglucoside (SDG) compound in both Tzotzol and Iztac chia genotypes (Table 3). This lignan compound presence in chia seed composition was not reported before. Lignans are phenolic compounds linked to many health benefits, including cancer prevention (Crosby, 2005; Morris, 2001). Once ingested, lignans are deglycosylated and converted by bacteria in the large intestine to produce enterolactone and enterodiol, the mammalian forms of plant lignans (Kitts et al., 1999). The efficacy of SDG and particularly its mammalian lignans metabolites' enterodiol (ED) and enterolactone (EL) to act as antioxidants in lipid and aqueous in vitro model systems, at relatively low concentrations (i.e. 100 microM), potentially achievable in vivo, is an evidence of a potential anticarcinogenic mechanism of lignan SDG and its mammalian metabolites ED and EL (Kitts et al., 1999).

A number of studies have shown good oxidative stability of chia seed when used as animal feed or as food ingredient, with this being attributed to the high antioxidant activity of the flavonols compounds it contains (Ayerza and Coates, 2001, 2009b; Ayerza et al., 2002; Coates and Ayerza, 2009). This stability in animal products produced when diets containing up to 30% chia seeds were attributed to the quality and quantity of these antioxidants. However, the SDG content found herein suggests that the great oxidative stability of chia oil could be attributed not only to the flavonols compounds content but also to the SDG content.

Amino acids content and composition is presented in Table 4. Amino acids' analysis results showed no significant ($p < 0.05$) differences on quantity and quality between Tzotzol and Iztac genotypes. An exception was leucine content which was 8% higher, but significantly different ($p < 0.05$), in the Tzotzol seeds than in the Iztac seed's genotype. The reason for this finding is not evident from the available data. The protein quality of chia has been demonstrated to be higher than that of common cereals and oil seeds (Weber et al., 1991).

The amino acids in chia have no limiting factors in adult diets (Fernandez et al., 2006).

Table 4. Amino acid concentration of two of chia seeds genotypes.

Genotype	Tzotzol	Iztac	SD ²
Amino acids	mg/g		
Alanine	7.41 ^{a1}	6.93 ^a	1.642
Arginine	16.34 ^a	15.79 ^a	1.613
Asparagine	12.29 ^a	13.28 ^a	1.931
Cystine	2.48 ^a	2.39 ^a	0.591
Glutamine	25.23 ^a	25.95 ^a	0.658
Glycine	6.58 ^a	7.12 ^a	0.687
Histidine	4.19 ^a	4.19 ^a	0.749
Isoleucine	5.34 ^a	5.37 ^a	0.795
Leucine	9.73 ^a	9 ^b	0.513
Lysine	7.65 ^a	7.21 ^a	1.216
Metionine	0.67 ^a	0.63 ^a	0.81
Phenylalanine	7.94 ^a	7.91 ^a	2.322
Proline	7.02 ^a	7.25 ^a	1.832
Serine	7.44 ^a	6.89 ^a	1.167
Threonine	5.54 ^a	5.92 ^a	1.208
Tryptophan	0.02 ^a	0.02 ^a	0.04
Tyrosine	4.73 ^a	4.53 ^a	0.212
Valine	8.76 ^a	8.40 ^a	0.773
Total	139.38 ^a	138.78 ^a	6.193

¹Means in a row within a group with the same letter are not statistically different;

²Least significant difference for $p < 0.05$.

The lack of significant difference on biochemical compounds between Tzotzol and Iztac genotypes found in this study could be explained by the reduced genetic variability between these two genotypes. Cahill (2004) using random polymorphic DNA markers, reported a loss of diversity accompanying chia domestication process and a near lack of diversity in modern commercial chia varieties.

Conclusion

In summary, this paper showed no effect of the seed coat color for all measured traits, protein, oil, fiber, amino acids, and antioxidants content and composition. In addition, this work found SDG, a diphenolic compound, which was not reported in chia seeds before, and suggest that this compound could be contributing to the chia seed oil stability.

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