THE SEED'S OIL CONTENT AND FATTY ACID COMPOSITION OF CHIA (*Salvia hispanica* L.) VAR. IZTAC 1, GROWN UNDER SIX TROPICAL ECOSYSTEMS CONDITIONS

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SUMMARY

The highest known percentage of alfa-linolenic fatty acid, up to 67.8% compared to 36%, 53%, and 57% for camelina, perilla and flax, respectively, is concentrated in chia oil. In recent years, chia seeds have become important for human health and nutrition because of their high content of α-linolenic fatty acid and beneficial health effects arising from consuming the ω-3 fatty acids it contains. The objective of the present study was to determine the locations effect on lipid content, and fatty acid profiles, of a single chia genotype named Iztac-1. Seeds of chia genotype Iztac-1 grown on six locations (T_1-T_6) were tested. The α-linolenic fatty acid (ω-3) comprised the greatest percentage of fatty acids for oil the seeds from all of sites. The highest percentage was observed in oil of seeds from the Salinas de Ibarra location; however, there were no statistical differences (P<0.05) when compared to the contents in seeds from T_4, T_5, and T_6. Oil of seeds from T_2 showed a significantly (P<0.05) lower α-linolenic acid percentage. Land elevation was positively related with α-linolenic fatty acid content (R^2= 0.86; P<0.001). The α-linolenic fatty acid percentage was negatively related to palmitic (R^2= 0.78, P<0.001), oleic (R^2= 0.73, P<0.001), and linoleic percentages (R^2= 0.91. P<0.001).

Introduction

Chia (*Salvia hispanica* L.) is an annual summer herb, and a member of the Labiatae family. In pre-Columbian times it was one of the basic foods of several Central American civilizations. Tenochtitlan, the capital of the ancient Aztec Empire, received 5000-15000 tons of chia annually as a tribute from conquered nations (Codex Mendoza, 1542). Following the Spanish conquest, chia essentially disappeared for 500 years, being replaced by the crops brought from, and preferred by, Europeans (Ayerza and Coates, 2005).

Chia seeds contain an oil with the highest known percentage of α-linolenic fatty acid, up to 67.8% (Coates and Ayerza, 1996) compared to 36%, 53%, and 57% for camelina (*Camelina sativa* L.), perilla (*Perilla frutescens* L.) and flax (*Linum usitatissimum* L.), respectively (Sultana, 1996; SOFA, 2006; USDA, 2006). In recent years, chia seeds have become important for human health and nutrition because of their high content of α-linolenic fatty acid and beneficial health effects arising from consuming the ω-3 fatty acids it contains (Ayerza and Coates, 2007; Vuksan *et al*., 2007; Robbins, 2008).

As a botanical source, variability in chia seed composition could be expected between growing locations, and between years within a location, due to genotype and environment effects as well as genetic environment interactions. Chia is cultivated for its special seeds biochemical composition, and genotype variability related with crop growing ecosystems needs to be explored. Although, the ecosystem effect on chia seeds biochemical composition has been reported (Ayerza, 1995; Coates and Ayerza, 1996), all the studies were performed at the level of species. The influence at a level of variety, using that named Tzotzol, was reported recently (Ayerza, 2009). The objective of the present study was to determine the location effect on the lipid content and fatty acid profiles, of a single chia genotype named Iztac-1.

Materials and Methods

Seed samples

Seeds of chia genotype Iztac-1 grown on six locations (Table I) were tested. The seeds were collected at each growing field. In the case of seeds from the Inter-Andean Valley and Tropical Coastal Desert ecosystems, original data were utilized separately as part of other different studies by the author. One location (T_2) was in Argentina, in The Yungas Tropical Forest; and five were in Ecuador, one in the Tropical Coastal Desert (T_1), one on the Low Inter Andean Valley (T_3), and three in the High Inter-Andean Valley ecosystem (T_4, T_5, T_6). According to the soil classification system of FAO (1995), the soil types are Entic Haplustoll (Tropical Coastal Desert), Luvic Phaeozen (The Yungas Tropical Forest), and Cambisols (Low and High Inter-Andean Valleys).

The Iztac-1 variety seeds were originally collected in the area where descendants of the Nahuas still cultivate the crop, and then were multiplied for a number of years in experimental plots). This genotype had previously been classified as having been domesticated, based on the presence of human selected-traits, having a higher seed mass, closed calyces that prevent seed shattering and dispersal, and determinacy of flowering and seed set described by Ca-

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CONTENIDO DE ACEITE Y COMPOSICIÓN DE ÁCIDOS GRASOS DE SEMILLAS DE CHÍA (*Salvia hispanica* L.) VAR. IZTAC 1 CULTIVADAS EN SEIS ECOSISTEMAS TROPICALES DIFERENTES

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RESUMEN

El aceite de chía concentra el mayor porcentaje conocido de ácido graso α-linolénico, hasta un 68% comparado con el 36% de camelina, 53% de perilla y 57% de lino. En años recientes, las semillas de chía han cobrado importancia para la salud y la nutrición humana debido a su elevado contenido de ácido graso α-linolénico y a los efectos benéficos que tiene para la salud el consumo de los ácidos grasos ω-3 que ellas contienen. El objetivo de este trabajo fue determinar el efecto de la procedencia de la semilla en el contenido de lípidos y en el perfil de ácidos grasos de un solo genotipo de chía denominado Iztac-1. Se testeó la semilla del genotipo Iztac-1 proveniente de seis lugares diferentes (T_1-T_6). El mayor porcentaje de ácido graso α-linolénico se observó en el aceite de semillas provenientes de Salinas de Ibarra. Sin embargo, la diferencia no fue estadísticamente diferente (P<0,05) cuando se lo comparó con el aceite proveniente de T_4, T_5, y T_6. El aceite proveniente de T_2 mostró el menor porcentaje (P<0,05) de ácido graso α-linolénico. La elevación del terreno mostró una relación positiva con el contenido de ácido graso α-linolénico (R^2= 0,86; P<0,001). El porcentaje de ácido graso α-linolénico mostró una relación negativa con el porcentaje de palmítico (R^2= 0,78; P<0,001), oleico (R^2= 0,73, P<0,001) y linoleico (R^2= 0,91. P<0,001).

CONTEÚDO DE ÓLEO E COMPOSIÇÃO DE ÁCIDOS GRAXOS DE SEMENTES DE CHIA (*Salvia hispanica* L.) VAR. IZTAC 1 CULTIVADAS EM SEIS ECOSSISTEMAS TROPICAIS DIFERENTES

Ricardo Ayerza (h)

RESUMO

O óleo de chia concentra a maior porcentagem conhecida de ácido graxo α-linolênico, até 68% comparado com 36% de camelina, 53% de perila e 57% de linhaça. Nos últimos anos, as sementes de chia tem ganhado importância para a saúde e a nutrição humana devido a seu elevado conteúdo de ácido graxo α-linolênico e também devido aos efeitos benéficos para a saúde pelo consumo dos ácidos graxos ω-3 que elas contêm. O objetivo deste trabalho foi determinar o efeito da procedência da semente no conteúdo de lipídeos e no perfil de ácidos graxos de um só genótipo de chia denominado Iztac-1. Testou-se semente do genótipo Iztac-1 proveniente de seis terrenos diferentes (T_1-T_6). A maior porcentagem de ácido graxo α-linolênico foi observada no óleo de sementes provenientes de Salinas de Ibarra. No entanto, a diferença não foi estatisticamente diferente (P<0,05) quando comparado com o óleo proveniente de T_4, T_5, e T_6. O óleo proveniente de T_2 mostrou a menor porcentagem (P<0,05) de ácido graxo α-linolênico. A elevação do terreno mostrou uma relação positiva com o conteúdo de ácido graxo α-linolênico (R^2= 0,86; P<0,001). A porcentagem de ácido graxo α-linolênico mostrou uma relação negativa com a porcentagem de palmítico (R^2= 0,78; P<0,001), oléico (R^2= 0,73, P<0,001) e linoléico (R^2= 0,91. P<0,001)

hill and Provance (2002), Cahill (2004), and Hernández-Gómez *et al*. (2008).

The experimental design was a randomized complete block with three replications. Each plot consisted of three rows of eight to 10m long, spaced 0.75m apart. The plots were maintained using the same cultural and irrigation practices at all locations, with all field work carried out by hand. The exceptions were the San Pablo and Anta Muerta locations where plots were not irrigated. All the seeds from each plot were hand harvested and cleaned. A sub-sample from each treatment and each replication was obtained to conduct laboratory analyses.

Seed analysis

Oil was extracted and converted into fatty acid methyl esters using the IRAM 5-560II method (IRAM, 1982). Fatty acid methyl esters were separated and quantified by an automated gas chromatograph (Hewlett Packard Model 6890) equipped with flame ionization detectors and a 30 mH530 m i.d. capillary column (Hewlett Packard Model HP-FFAP).

Statistical analysis

Each variable was compared by analysis of variance. When the F-value was significant (P<0.05), means were separated using the Student-Newman-Keuls Test. Regression coefficient was used to assess whether a relationship existed between fatty acids percentage, and between α-linolenic fatty acid and growing land altitude (Cohort Stat, 2006).

TABLE I
LOCATIONS WHERE THE CHIA WAS GROWN

Treatment	Site	Ecosystem	Latitude	Longitude	Elevation (m)
T_1	Santa Elena	Tropical Coastal Desert[a]	02º18'00"S	83º37'00"W	48
T_2	Anta Muerta	The Yungas Tropical Forest[b]	22º53'00"S	64º24'00"W	400
T_3	Salinas de Ibarra	Low Inter-Andean Valley[a]	00º29'47"N	78º07'56"W	1621
T_4	San Pablo de Atenas	High Inter-Andean Valley[b]	01º47'90"S	70º04'03"W	2010
T_5	Patate	High Inter-Andean Valley[a]	01º18'50"S	78º30'58"W	2042
T_6	Guayllabamba	High Inter-Andean Valley[a]	00º03'26"S	78º20'58"W	2200

[a] with irrigation; [b] without irrigation.

Results

TABLE II
OIL CONTENT AND FATTY ACID COMPOSITION

Treatment	Location	Oil %[1]	Palmitic	Stearic	Oleic	Linoleic	α-Linolenic	T-6:T-3 rate	α-Linolenic g/kg seed
			%[2]						
T_1	Santa Elena	25.8 b	7.53 b	3.33 a	7.56 bc	19.23 b	61.73 b	0.31 b	159.27 b
T_2	Anta Muerta	29.3 ab	8.9 a	3.2 a	8.42	22.6 a	54.8 c	0.41 a	160.56 b
T_3	Salinas	32.07 a	6.33 c	4.78 a	6.35 c	15.7 d	66.17 a	0.24 d	212.06 a
T_4	San Pablo	34.43 a	5.9 c	3.1 a	8.23 b	18.18 bc	63.63 ab	0.29 bc	219.12 a
T_5	Patate	32.77 a	6.43 c	3.5 a	6.83 c	17.43 c	64.53 ab	0.27 c	211.41 a
T_6	Guayllabamba	29.33 ab	6.47 c	3.55a	6.8 c	17.93 bc	63.67 ab	0.28 bc	186.64 ab
	$LSD_{0.05}$.[3]	6.76	1.48	2.89	1.75	1.97	4.61	0.05	44.71

[1] percentage of dry matter; [2] percentage of total fatty acids; [3] least significant difference for P<0.05. Means in a column within a group with the same letter are not statistically different (P<0.05).

Oil content and fatty acid composition values are presented in Table II. Total oil content was significantly (P<0.05) higher in the seeds from San Pablo, Patate and Salinas than in the seeds from Santa Elena, which did not differ significantly (P<0.05) from seeds collected in Anta Muerta and Guayllabamba locations.

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.

Palmitic fatty acid, which comprised the greatest percentage of total saturated fatty acid, was significantly (P<0.05) higher in oil of seeds from Anta Muerta, compared to that of seeds from all other tested locations, followed by the oil of seeds from Santa Elena, which was significantly (P<0.05) different compared to that from another four locations. Saturated stearic fatty acid was not significantly (P<0.05) different among treatments.

Monounsaturated (ω-9) oleic fatty acid was significantly (P<0.05) higher in the oil of seeds from Anta Muerta, compared to the oil from seeds of all other locations Oleic acid percentage of seeds from San Pablo location was significantly (P<0.05) higher compared with that of seeds from Guayllabamba, Salinas, and Patate, but not with that of oil from Santa Elena. No significant (P<0.05) differences among Guayllabamba, Salinas, Patate, and San Pablo were detected.

Polyunsaturated T-6 linoleic fatty acid showed the second largest percentage detected herein. Anta Muerta oil showed a significantly (P<0.05) higher percentage of linoleic fatty acid, than the oil of seeds from all other locations. No significant (P<0.05) differences in linoleic fatty acid percentage were found between Santa Elena, San Pablo, and Guayllabamba, and between Patate, San Pablo, and Guayllabamba. The lowest α-linolenic fatty acid percentage was detected in oil of seeds from Salinas, which was significantly (P<0.05) different compared to that from all other locations.

Polyunsaturated T-3 α-linolenic fatty acid comprised the greatest percentage of fatty acids for oil of seeds from all locations. The highest percentage was observed in oil of seeds from Salinas location; however, difference was not statistically (P<0.05) different when compared to those of seeds from San Pablo, Patate, and Guayllabamba. Oil of seeds from Anta Muerta showed the significantly (P<0.05) lowest α-linolenic percentage.

The T-6:T-3 ratio was significantly (P<0.05) lower in oils from seeds grown in Salinas compared to that of oils from seeds grown in all other locations.

Regression analyses were performed for elevation *vs* α-linolenic fatty acid, expressed as g/kg of chia seed. The regression coefficient (R^2) and significance (P) level are presented in Figure 1. The land elevation was positively related with α-linolenic fatty acid content (R^2= 0.86, P<0.001).

Regression analysis was also carried out to explore the trend of associations between fatty acids, and its coefficient (R^2) and significance (P) levels are presented in Figure 1. The α-linolenic fatty acid percentage was negatively related with oleic percentage (R^2= 0.73, P<0.001) and linoleic percentage (R^2= 0.91, P<0.001).

Discussion

The results of the analysis for oil and fatty acids showed a statistically significant (P<0.05) variation in the oil content percentage and in the fatty acids profile caused by

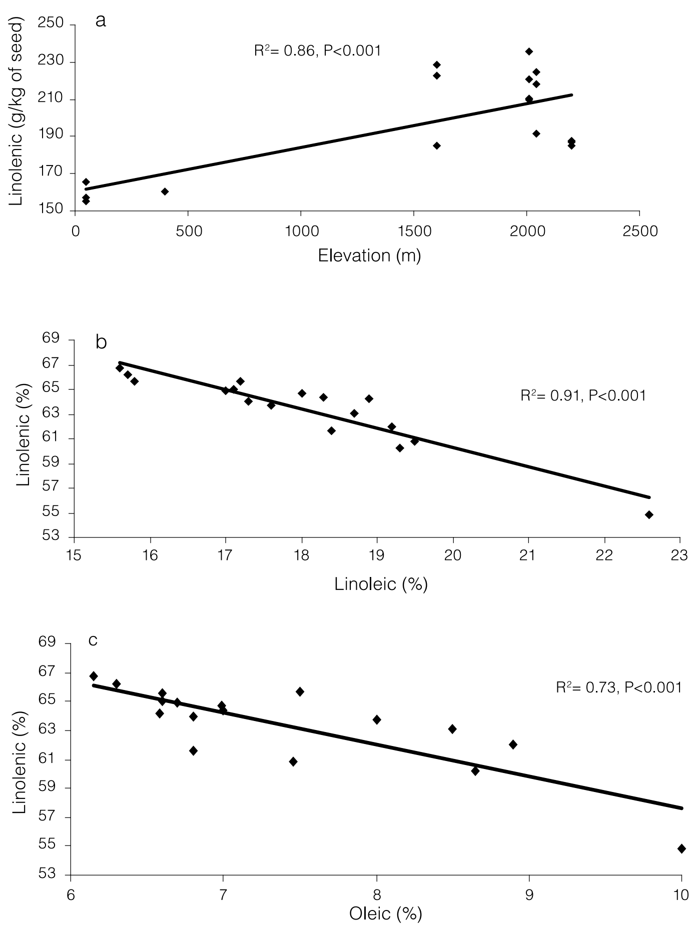

Figure 1. Relationship between α-linolenic and elevation (a), and linoleic (b) and oleic (c) fatty acid contents.

growth conditions. The present study confirmed that the chemical composition of chia oil is influenced by the effects of factors such as the quality of the soil and the climatic and weather conditions. Similar ecosystem effects were recently reported in the oil analyses of the chia variety Tzotzol from six different ecosystems, which revealed a significant ($P<0.05$) variation in the oil content and composition caused by growth conditions (Ayerza, 2009). Location mainly affected chia's duration of the growing periods and seed yields, and to a lesser degree protein and oil contents as well as fatty acid composition, and, generally, small differences between the varieties (Ayerza and Coates, 2009).

The fatty acids profile is an important characteristic determining the applicability of the oil. The profile of fatty acids proves that chia oil is highly unsaturated. Owing to its specific composition, chia oil appears unique among the common vegetable edible oils such as rape, corn, sunflower, soya, olive, coconut oils, etc. The highest α-linolenic fatty acid contents found in this study were similar to the highest values reported for chia commercially grown over the past 20 years (Ayerza and Coates, 2005).

The averages across the tested locations show the lowest content of α-linolenic fatty acid in seeds produced at The Yungas Tropical Forest and the Coastal Tropical Desert ecosystems (average of 159.2g/kg of seed) and the highest content in seeds grown at the Inter-Andean Valley ecosystems (average of 207.3g/kg of seed). The significant (R^2= 0.86, $P<0.001$) positive relationship of α-linolenic fatty acid content (g/kg of seed) with growing land altitudes, is in agreement with previous observations reported for other oil crops (Yaniv *et al.*, 1995; Aparicio *et al.*, 1994).

In general, increases in land elevation correspond to decreases in environmental temperature and a strong relation between growing season temperature and the number of the basic physiological metabolic process of plants have been demonstrated. It was reported for a number of crops, such as jojoba or chia, that an increase in growing temperature is related with a decrease in oil content, and an increase in oil saturation with a concomitant decrease in oil unsaturation level (Carver *et al.*, 1986; Ayerza, 1995, 2001, 2009). Thus, the higher (30%) α-linolenic fatty acid (g/kg of seed) concentration in seeds grown at the Inter-Andean Valleys region (locations T_3, T_4, T_5, and T_6) compared with that of seeds from lowland ecosystems (T_1 and T_2), could be explained, at least partially, by the difference in elevation effects between growing sites.

The lack of a significant difference ($P<0.05$) in α-linolenic acid content at all of the Inter-Andean Valley tested locations suggests that they have similar environmental conditions where the Iztac-1 variety can express its potential to produce highly α-linolenic enriched oil.

The significant negative relationships of α-linolenic fatty acid contents with the 18-C more saturated fatty acids, oleic and linoleic, are in agreement with previous observations 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, which is a rich source of α-linolenic fatty acid (Wakjira *et al.*, 2004), and chia (Ayerza, 2009). This strong inverse association found herein 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 specific desaturase enzymes (Dybing and Zimmerman, 1966).

Dietary T-6 and T-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 T-6:T-3 fatty acid ratio as low as possible, the ratio of 1:1 being ideal (Simopulous, 2003).

Western diets do not provide these ratios, mainly due to their high T-6 fatty acid content. As a T-3 source, chia is consumed either as an oil or as whole/ground seed. The significant ($P<0.05$) lower T-6:T-3 rate (up to -41.5%), showed by the oil from seeds grown in the Salinas location, compared with the other ones, could indicate an added health benefit for its seeds.

Elevated temperatures are expected to accompany an increased CO_2 because of the additional greenhouse effect of this gas in the atmosphere (IPCC, 1995). Higher temperatures could significantly affect chia seed composition, as it was demonstrated for other seed oil crops as soybean (Thomas *et al.*, 2003). Depending on the current temperature of a growing land, elevated temperature will have considerable impact on chia α-linolenic fatty acid content and on its relation with the other fatty acids constituents. The close relation between growing land elevation and environmental temperature could help predict changes in oil content and fatty acid profiles caused by climate change and elevated temperature.

In summary, the results indicate that oil content and fatty acid profile characteristics of the Iztac-1 variety of chia are affected by the different ecological conditions. Additional multi-location and multi-year trials are required to confirm the results and to understand the biochemical bases for these phenomena.

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