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Organically *vs* conventionally-grown dark and white chia seeds (*Salvia hispanica* L.): fatty acid composition, antioxidant activity and techno-functional properties

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SUMMARY: The effects of organic and conventional crop systems on chemical composition, antioxidant activity and functional properties were evaluated in white and dark chia (*Salvia hispanica* L.) seeds. The organic system reduced the total protein content, and increased the total carbohydrates but did not change polyunsaturated fatty acids, total phenolic or flavonoids. Organic white chia seeds showed the best techno-functional properties. The antioxidant capacity of chia extracts varied in relation to the chemical complexity and differential rate kinetics of different assays. Extractable total phenolic acids and antioxidant capacity were better in organic white chia seeds. In this first approach, we have demonstrated that the organic white chia seed has a better total antioxidant capacity measured by direct quencher approaches than its conventionally-grown counterpart. To summarize, we conclude that the organic white chia seed could be a dietary source of antioxidants with a potential to promote health benefits in systemic functions and/or microbiota and the use of its techno-functional properties for the food industry.

KEYWORDS*: Antioxidant activity; Chia seeds; Fatty acids; Organic and conventional crop systems; Polyphenols; Techno-functional properties*

RESUMEN: ***Semillas de chia oscuras y blancas cultivadas orgánicamente* vs *convencionalmente (*Salvia hispanica *L.): composición de ácidos grasos, actividad antioxidante y propiedades tecno-funcionales.*** El efecto de los sistemas de cultivo orgánico *vs* convencional sobre la composición química, la actividad antioxidante y las propiedades funcionales fueron evaluadas en semillas de chía blanca y oscura (*Salvia hispanica* L.). El sistema orgánico redujo el contenido total de proteína, aumentó los carbohidratos totales, pero no modificó los ácidos grasos poliinsaturados, fenólicos totales y flavonoides. Las semillas orgánicas de chía blanca mostraron las mejores propiedades tecno-funcionales. La capacidad antioxidante de los extractos de chía varió en relación con la complejidad química y la cinética de velocidad diferencial de los diferentes ensayos. Los ácidos fenólicos totales y la capacidad antioxidante fueron mejores en las semillas orgánicas de chía blanca. En este primer enfoque, hemos demostrado que la semilla orgánica de chía blanca tiene una mejor capacidad antioxidante total medida por métodos directos que su contraparte cultivada convencionalmente. En resumen, indicamos que las semillas orgánicas de chía blanca podría ser una fuente dietética de antioxidantes con potencial para promover beneficios saludables en la función sistémica y/o microbiota y el uso de la propiedades tecno-funcionales para la industria alimentaria.

PALABRAS CLAVE*: Ácidos grasos; Actividad antioxidante; Polifenoles; Propiedades tecno-funcionales; Semillas de chía; Sistemas de cultivo orgánico y convencional*

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1. INTRODUCTION

Chia (*Salvia hispanica* L.) seeds are high in dietary fiber, protein, and significant amounts of edible oil, which is rich in omega-3 fatty acids. In addition, they are rich in lipophilic phytochemicals such as sterols, tocopherols, squalene, carotenoids, and hydrophilic phytochemicals like caffeoyl derivatives, phenols, flavonoids, organic acids, and free amino acids, among others (da Silva *et al*., 2017). Other compounds, like abietane-type diterpenes, have also been detected. These chemicals are recognized for their health-promoting capacity, particularly their role in lowering triacylglycerol and cholesterol levels, which in turn results in low blood pressure and beneficial effects on heart-related diseases, as well as antioxidant, anti-inflammatory, antithrombotic and anticancer activities (Ma *et al*., 2015).

Consumers are concerned about the safety of what they eat and about the use of pesticides, hormones and other veterinary drugs in farming practices. The interest of consumers in organic products mainly stems from health and environmental considerations, and the supposition that such techniques deliver equally or more nutritious foods that contain less (or no) pesticide residues compared to conventional crops. They believe there is a possibility of better nutritional quality and/or bioactive compounds in organically-grown crops than in their conventionally-farmed/grown counterparts. The larger number of bioactive components produced in organically-grown crops have been confirmed in several studies on many plants, potatoes, vegetables and fruits and some processed foods (Lombardo *et al*., 2017; Faller and Fialho, 2010). Similar conclusions were presented by Mazzoncini *et al*., (2015), who showed that the antioxidant power of white flour as determined by DPPH and ABTS radicals was not significantly affected by the growth system whereas bran showed higher antioxidant values under organic than conventional growing systems. Lombardo *et al*., (2017) reported that an organic cultivation system produced tubers of higher nutritional value, exhibiting a higher total phenolic content and lower nitrate content with a more attractive color in both the peel and flesh. However, chemical compositions vary between organic and conventional produce depending on differences in the production practices and many interacting variables in both organic and conventional crops (Lombardo *et al*., 2017).

To the best of our knowledge no study has yet compared the effect organic and conventional chia seeds on their chemical composition. Therefore, this study evaluates differences in proximal chemical composition, fatty acids, antioxidant capacity and functional properties between organically and conventionally-grown dark and white chia seeds.

2. MATERIALS AND METHODS

2.1. Reagents

Acetone, ethanol and hexane were supplied by J.T. Baker (Phillipsburg, NJ, USA). 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), 2,2- diphenyl-1-picrylhydrazyl (DPPH), 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), bathocuproine disulfonic acid disodium salt (BCS), Folin-Ciocalteau reagent, sodium carbonate, potassium persulfate, sodium nitrite, aluminum chloride, copper sulphate, (+)-catechin hydrate and gallic acid were obtained from Sigma-Aldrich (Sigma-Aldrich, MO, USA). All other chemicals were of analytical grade.

2.2. Samples

Chia seeds (dark and white) (Figure 1) from conventionally-grown crops were provided by a commercial supplier (Bio Feria Surquillo, Lima, Peru) and organic seeds from Chia Gold (Product manufactured by Grains Gold of Peru, S.A.C and certified by Ceres Cert GmbH) (http://www.granosgolddelperu.com/en/granos-gold-del-peru.html). The chia seeds were ground in a universal mill M20 (IKA® Works Inc, NC, USA) and meshed through a 600 μm sieve. Chia flour was de-fatted with n-hexane in a Kimble™ Soxhlet extraction apparatus with an Allihn condenser (Kavalierglass, Prague, Czech Republic). The de-fatted flour was stored in dark freezer bags at 2 °C until analysis.

2.3. Proximal composition

Using de-fatted chia flour, crude protein was calculated by the micro/Kjeldahl method, and moisture and ash contents using standard AOAC methods (AOAC, 2016). The Soxhlet extraction method was used to measure total fat content. Carbohydrate content was calculated by difference (AOAC, 2016).

2.4. Physical features

2.4.1. Fatty acid contents

The fatty acid composition was determined by gas chromatography (GC) as fatty acid methyl esters (FAMEs) according to the AOAC Standard methods 996.06, c41 (AOAC, 2016). FAMEs were produced by a methylation reaction using BF_3-Methanol (10% w/w), then extracted by a liquid-liquid procedure using hexane and dried with sodium sulfate. GC analysis was carried out using a TRACE Ultra gas chromatograph (Thermo Fisher Scientific Inc., NY, USA) equipped with a capillary

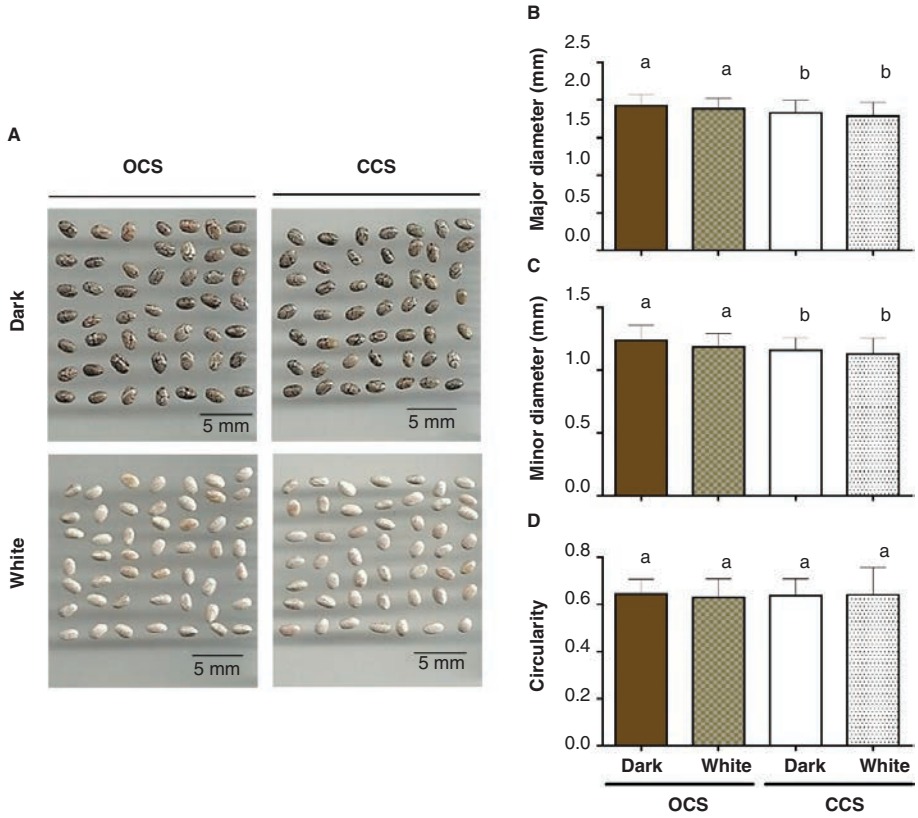

FIGURE 1. Optical analysis: A) Chia seeds of dark and white organic chia seeds (OCS) and conventional seeds (CCS); Morphological features: B) mayor diameter (mm), C) minor diameter (mm) and D) circularity. Values are mean ± SD, n=49. Mean values with different superscripts in the same row differ significantly at $p < 0.05$, according to Duncan's test.

column (70% cyanopropyl polysilphenylene-siloxane, TRACE TR-FAME, 100 m x 0.25 mm × 0.20 µm film thickness). Helium was used as carrier gas at a flow rate of 1.2 mL min^{-1}. The column temperature regime was as follows: 100 °C (held for 4 min) to 240 °C at 3 °C/min (held for 10 min). The temperatures of the injector and detector were 225 and 250 °C, respectively. The injection volume and split ratio were 2 µL and 200:1, respectively. FAMEs were identified by comparing their retention times with a standard retention time Supelco 37 Component FAME Mix (Sigma-Aldrich, MO, USA). For calculation, the fatty acids were normalized to 100%, considering the composition (moles %) from fatty acid composition data (area %).

2.4.2. Extractable phenolics and antioxidant activity

2.4.2.1. Sample extraction: De-fatted chia flour was weighed (approximately 5.0 g) and then subjected to extraction using 70% acetone (25 mL) for 1 h in an electric shaker Multi-Position Digital Stirring Hotplates (Thermo Scientific Inc., NY, USA). The extract was filtered through Whatman® quantitative filter paper (Sigma-Aldrich, MO, USA) and stored at 4 °C in the dark until analysis.

2.4.2.2. Total phenolics: The total phenolic content was measured by the Folin-Ciocalteau method (Ramos-Escudero *et al*., 2012). Acetonic extract (100 µL) or standard was reacted with 750 µL of 0.2 N Folin-Ciocalteau reagent and, after 5 minutes of reaction, 750 µL of sodium carbonate (7.5%) were added. The reaction was performed for 16 to 18 h at room temperature and in the dark. Absorbance was read at 725 nm in a Genesys 10S UV-Vis spectrophotometer (Thermo Scientific Inc., NY, USA). Phenolic content was expressed as milligrams gallic acid equivalents per gram (mg GAE/g) of sample from a gallic acid standard curve (5-100 µg/mL).

2.4.2.3. Total flavonoids: The total flavonoid content was determined by a colorimetric method as in Barreira *et al*., (2010). In a 10-mL centrifuge tube, 100 µL of acetonic extract were mixed with 0.90 mL distilled water and 75 µL $NaNO_2$ (5%) solution and the mixture was incubated for 5 min. At the end of the reaction, 150 µL of $AlCl_3.6H_2O$ (10%) solution were added, and the mixture was allowed to stand for 5 min. Finally, 0.5 mL NaOH (1 M) were added to the reaction mixture and absorbance was read at 510 nm in a Genesys 10S UV-Vis spectrophotometer (Thermo Scientific Inc., NY, USA). Flavonoid

content was estimated from a catechin standard curve (4-80 µg/mL) and expressed as milligrams catechin equivalents (CE) per gram of sample.

2.4.2.4. Total phenolic acids: Total phenolic acid was determined using Arnow reagent according to the Council of Europe Directorate for the Quality of Medicines (2004). The final reaction volume amounted to 1300 µL and absorbance was measured at 505 nm in a Genesys 10S UV-Vis spectrophotometer (Thermo Scientific Inc., NY, USA). The phenolic acid content was expressed as milligram 2-hydroxycinnamic acid equivalents (HAE) per gram of sample.

2.4.3. Antioxidant capacity

2.4.3.1. Extractable: ***DPPH assay***. The DPPH assay was measured by the Brand-Williams test (Ramos-Escudero *et al*., 2012) with minor modifications. Briefly, 50 µL of extract or Trolox were mixed with 950 µL of DPPH (100 µmol/L in ethanol) and shaken vigorously in the dark for 30 min and thereafter the absorbance was measured at 515 nm in a Genesys 10S UV-Vis spectrophotometer (Thermo Scientific Inc., NY, USA). The antioxidant capacity was expressed as Trolox equivalents (TE) from a standard curve (25-400 µmol/L Trolox).

ABTS assay. The radical scavenging activity against ABTS radical cation ($ABTS^{\bullet+}$) was measured by the assay reported by Re *et al*., (1999) with some modifications. $ABTS^{\bullet+}$ was produced by reacting 7 mM ABTS with 2.6 mM potassium persulfate and allowing the mixture to stand in the dark at room temperature for 12-16 h. The reaction was run using 50 µL of extract or Trolox standard + 950 µL of $ABTS^{\bullet+}$, the mixtures were shaken vigorously and left in the dark for 30 min and thereafter the absorbance was measured at 734 nm in a Genesys 10S UV-Vis spectrophotometer (Thermo Scientific Inc., NY, USA). The antioxidant capacity was expressed as Trolox equivalents from a standard curve (25–300 µmol/L Trolox).

CUPRAC assay. A modified CUPRAC procedure was used to measure the antioxidant capacity of the extracts (Abderrahim *et al*., 2015). Briefly, 50 µL of the sample or Trolox standard were mixed in a glass tube with 950 µL of 0.25 mM BCS (dissolved in 0.1 M phosphate buffer, pH 7.4/ethanol 1:1 v/v) and 250 µL of 0.5 mM $CuSO_4$, agitated using a vortex mixer and left in the dark for 30 min. Thereafter, the absorbance was measured at 490 nm in a Genesys 10S UV-Vis spectrophotometer (Thermo Scientific Inc., NY, USA). The antioxidant capacity was expressed as Trolox equivalents from a standard curve (25-800 µmol/L Trolox)

2.4.3.2. Total: ***QUENCHER-DPPH assay***. An adapted assay to assess the total antioxidant capacity of solid foods described by Condezo-Hoyos *et al*., (2015) was used to measure the chia seeds. Briefly, 7 to 10 mg of grounded chia seed were weighed and placed in a conical centrifuge tube, and the reaction was started by adding 3 mL of DPPH solution (100 µmol/L in methanol/water 1:1 v/v). The reaction was carried out at room temperature under agitation using a MX-S vortex mixer (Importadora Andina E.I.R.L., Lima, Peru) at maximum speed for 10 min. Thereafter, the sample was centrifuged at 1700g for 5 min in a Rotofic 32A centrifuge (Hettich, Kirchlengern, Deutschland). The supernatant was put into a Quartz Suprasil 10 mm semi-micro cuvette with blackened walls (Hellman Analytics, Müllheim, Germany) and the absorbance was read at 520 nm in a Genesys 10S UV-Vis spectrophotometer (Thermo Scientific Inc., NY, USA). The total antioxidant capacity of the samples was expressed as mmol DPPH scavenged/Kg sample.

QUENCHER-CUPRAC assay. A QUENCHER-CUPRAC procedure was used to measure the antioxidant capacity of chia seeds (Abderrahim *et al*., 2015). Three to five mg of ground sample were placed in a conical centrifuge tube and reacted with 1.9 mL of 0.25 mM BCS (dissolved in 0.1 M phosphate buffer, pH 7.4/ethanol 1:1 v/v) and 500 µL 0.5 mM $CuSO_4$ under agitation using a MX-S vortex mixer (Importadora Andina E.I.R.L., Lima, Peru) at maximum speed for 20 min (room temperature). Thereafter, 500 µL of 10 mM EDTA-Na_2 were added to stop the reaction and the tubes were centrifuged at 1700g for 9 min in a Rotofic 32A centrifuge (Hettich, Kirchlengern, Deutschland). At the end of 30 min, the mixture was put in a Quartz Suprasil 10 mm semi-micro cuvette with blackened walls (Hellman Analytics, Müllheim, Germany) and the absorbance was measured at 490 nm in a Genesys 10S UV-Vis spectrophotometer (Thermo Scientific Inc., NY, USA). The total antioxidant capacity of the samples was expressed as absorbance at 490 nm scavenged/mg sample.

2.5. Functional technological properties

2.5.1. Water holding capacity (WHC)

The sample (0.10 g) was weighed and then suspended in 20 mL of distilled water and stirred for 1 min. The mixture was allowed to stand for 1 h at room temperature, and then these suspensions were centrifuged at 2200g for 30 min. WHC was expressed as mL of water held per g of sample (Segura-Campos *et al*., 2014).

2.5.2. Oil holding capacity (OHC)

OHC was determined under the same conditions as water holding capacity but using corn oil (Mazola, ACH Food Companies, Inc.). OHC was

expressed as mL of water held per g of sample (Segura-Campos *et al*., 2014).

2.5.3. Swelling capacity (SWC)

The samples (0.10 g) were hydrated in 10 mL of distilled water in a graduated cylinder at room temperature. The volume occupied by the samples in the cylinder was read directly and the SWC capacity was expressed as volume (mL) occupied by sample per gram of chia (Lou *et al*., 2009).

2.6. Statistical analysis

A statistical analysis of the results was conducted using a descriptive statistical analysis and ANOVA was done using the STATISTICA version 8.0 software package (StatSoft, Inc., Tulsa, Oklahoma, USA). A principal component analysis (PCA) was performed using the STATGRAPHICS Centurion Version 17 software package (Statpoint Technologies, Inc., Herndon, Virginia, USA). Significant differences among means were determined with Duncan's new multiple range test with $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Proximal composition

The chemical compositions of dark and white chia grown under organic and conventional systems are summarized in Table 1. The protein contents in the organic dark and white chia varieties showed lower values than those found for the conventionally-grown samples ($p < 0.05$). There are no reports on the effects of organic or conventional production systems on dark and white chia. Differences have been demonstrated for potatoes and wheat cultivated under these two systems, with a lower protein content reported for organically-grown potatoes (Lombardo *et al*., 2017) and wheat (Mazzoncini *et al*., 2015). Despite organic crops having a lower protein content in relation to their lower nitrogen supply, they are a source of better quality protein measured as essential amino acid index and nitrogen-poor molecules like polyphenols and carbohydrates (Herencia *et al*., 2011). In addition, dark organic chia showed better protein content compared to white varieties ($p < 0.05$). In conventionally-grown seeds, protein content was similar in both Peruvian chia varieties (dark = 21.78 ± 0.18% and white = 21.16 ± 0.18%, $p < 0.05$), and levels were higher than those previously reported for chia seed 18.3 ± 1.6 % (Coelho and Salas-Mellado, 2014) and chia flour 18.18 ± 1.2% wet basis (da Silva *et al*., 2017). However, a higher protein content has been reported in conventionally-grown Chilean (25.32 ± 0.21%) (Marineli *et al*., 2014) and Mexican (24.6 ± 0.3%) (Olivos-Lugo *et al*., 2010) chia seeds. In agreement with a report by Herencia *et al*., (2011), the low protein content found in organic chia compared to conventional seeds was compensated for by an increase in the total carbohydrates ($p < 0.05$) and dry matter content that was influenced by the chia variety, which were lower in dark chia and higher in the white variety. Similar to the dry matter content, the effect of organic and conventional growing systems on ash content was influenced by chia variety. Ash content was higher in conventionally-grown dark chia ($p < 0.05$) and lower in conventionally-grown white chia ($p < 0.05$). Conventionally-grown Peruvian chia seed showed a similar ash content to those found in chia from different regions ranging from 4.07 to 5.09 % (Coelho and Salas-Mellado, 2014; Coorey *et al*., 2014; da Silva *et al*., 2017; Marineli *et al*., 2014).

The organic white variety showed a higher lipid content than its counterpart dark chia seed ($p < 0.05$). This is the first comparative study to report lipid content in organically and conventionally-grown chia seeds. Organic and conventional growing systems produced chia seeds with a lipid content that ranged between 34.06 and 36.74 g/100g sample, which is comparable to that reported by Ramos *et al*., (2017), but relatively higher than that found in chia seeds from Australia (33.65%), Chile (30.22%) and Brazil (31.2%) (Coorey *et al*., 2014; da Silva *et al*., 2017; Marineli *et al*., 2014).

Table 1. Proximal composition of two varieties of chia seeds from conventional and organic crops (g/100 g)

	Organic chia seed		Conventional chia seed	
Components	**Dark**	**White**	**Dark**	**White**
Protein	20.50±0.13^{c}	17.34±0.28^{d}	21.78±0.05^{a}	21.16±0.18^{b}
Lipids	34.73±0.01^{b}	36.74±0.02^{a}	34.06±0.02^{d}	34.37±0.03^{c}
Ash	4.56±0.01^{b}	4.50±0.00^{c}	4.80±0.04^{a}	4.41±0.03^{d}
Moisture	8.74±0.01^{a}	8.54±0.01^{c}	8.67±0.04^{b}	8.61±0.01^{b}
Carbohydrates	40.21±0.11^{b}	41.43±0.30^{a}	39.35±0.11^{c}	40.07±0.18^{b}

Data are expressed as mean values ± standard deviation of triplicate (n=3) analyses. Mean values with different superscripts in the same row differ significantly at $p < 0.05$, according to Duncan's test.

3.2. Fatty acid content

The higher total lipid content found in the organically-grown chia seeds could be interesting since chia seeds have been identified as a good source of oil (from 25 to 35%), which is rich in healthy polyunsaturated fatty acids (PUFA) i.e. about 60-67% of total fatty acids (Ixtaina *et al*., 2010). However, despite a higher lipid content in organic chia seed, the PUFA content was equal to that found in conventionally-grown seeds ($p > 0.05$) (Table 2). The PUFA content found in conventionally-grown Peruvian chia was higher than that found in Australian crops (78.5 g /100 g oil) (Ding *et al*., 2018) and comparable to the PUFA content (81.0 – 82.2 g/100 g oil) previously reported in Mexican, Polish and Chilean chia seeds extracted by different techniques such as conventional Soxhlet, pressing or supercritical carbon dioxide (Ixtaina *et al*., 2010; Marineli *et al*., 2014). Similarly, the ω-3/ω-6 fatty acid ratios found in organic and conventional Peruvian chia seed crops (dark and white) were not statistically different ($p > 0.05$). These values were comparable to those found in Chilean chia seeds (3.45) (Marineli *et al*., 2014). Lower ratio values have been reported by Dabrowski *et al*., (2017) for a Polish chia seed (2.88-3.23) and Ixtaina *et al*., (2010) for a Mexican chia oil (2.98-3.13). The consumption of chia oil could balance the excess of n6 in human diets. In addition, it has been reported that n-3/n-6 ratios higher than 3.5 might reduce cholesterol levels and improve the plasma lipid profile (Morales-Medina *et al*., 2015). However, Peruvian chia seeds showed lower SFA contents than reported above (>12%) and are only comparable to Chilean chia seeds which show about 11%.

3.3. Extractable phenolic, flavonoid and phenolic acid contents

Organic and conventional crop systems did not influence extractable total phenolics or flavonoids in the dark and white chia seeds ($p > 0.05$) (Table 3). Although an organic management system has been associated with a lower nitrogen supply that would enhance the synthesis of N-poor molecules (e.g. polyphenols, cellulose, starch) instead of nitrogen-rich compounds like amino acids or proteins (Herencia *et al*., 2011), the effects of organic and conventional growing systems on phenolic compounds can depend on the type and variety of crops. Thus, a recent study demonstrated that the extractable amounts of phenolics and phenolic acids are not influenced by organic or conventional agricultural crop systems in the production of the winter wheat cv. 'Bologna' (Mazzoncini *et al*., 2015). In contrast, other research has found that organically-grown potatoes show a better extractable phenolic content than conventionally-grown potatoes (5.76 *vs*. 4.28 g/Kg) (Lombardo *et al*., 2017). Despite the reduction in protein content found in organic chia seeds (Table 1), neither extractable total phenolic compounds nor flavonoids were increased. On the other hand, the total phenolic compound content

Table 2. Fatty acid composition (moles %) of chia seeds from conventional and organic crops

	Organic chia seed		Conventional chia seed	
Fatty acids	**Dark**	**White**	**Dark**	**White**
Saturated fatty acids (SFA)	10.98	10.41	10.92	10.19
Myristic acid (C14:0)^{NS}	0.04±0.01	0.04±0.01	0.04±0.01	0.04±0.01
Palmitic acid (C16:0)^{NS}	6.75±0.03	6.36±0.53	6.65±0.12	6.86±0.19
Stearic acid (C18:0)^{NS}	3.19±0.03	3.11±0.14	3.28±0.10	3.29±0.11
Arachidic acid (C20:0)^{NS}	0.85±0.01	0.75±0.16	0.80±0.10	0.86±0.02
Behemic (C22:0)^{NS}	0.06±0.01	0.07±0.02	0.07±0.02	0.06±0.01
Lignoceric (C24:0)^{NS}	0.09±0.02	0.08±0.01	0.08±0.01	0.08±0.01
Monounsaturated fatty acids (MFA)	6.18	5.91	6.33	6.01
Palmitoleic acid (C16:1)^{NS}	0.12±0.01	0.02±0.01	0.02±0.01	0.02±0.01
Oleic acid (C18:1, ω-9)^{NS}	5.82±0.07	5.68±0.13	6.06±0.41	5.76±0.01
cis-11-Eicosenoic acid (C20:1)^{NS}	0.20±0.01	0.17±0.06	0.21±0.00	0.19±0.02
Ericic (C22:1)^{NS}	0.04±0.01	0.04±0.02	0.04±0.01	0.04±0.01
Polyunsaturated fatty acids (PUFA)	81.91	82.74	81.76	81.80
Linoleic acid (C18:2, ω-6)^{NS}	18.26±0.12	18.18±0.24	18.71±0.51	18.28±0.09
Linolenic acid (C18:3, ω-3)^{NS}	63.65±0.07	64.56±1.21	63.05±0.92	63.52±0.26
ω-3/ω-6 ratio	3.45	3.57	3.33	3.45

Data are expressed as means values ± standard deviation of duplicate (n=2) analyses. ANOVA did not find a significant difference among means. N.S., not significant.

Table 3. Extractable phenolic, flavonoids and phenolic acids and antioxidant capacity of two varieties of chia seeds from conventional and organic crops

	Organic chia seed		Conventional chia seed	
	Dark	**White**	**Dark**	**White**
Phenolic compounds				
Total phenolics (mg GAE/g)^{NS}	2.19±0.00	2.21±0.05	2.18±0.02	2.14±0.02
Total flavonoids (mg CE/g)^{NS}	1.57±0.05	1.50±0.11	1.54±0.03	1.56±0.03
Total phenolic acids (mg 2-HAE/g)	0.33±0.00	0.34±0.00	0.33±0.00	0.30±0.00
Antioxidant capacity				
DPPH (μmol TE/g)	459.28±7.56^{c}	457.48±8.31^{c}	528.95±6.78^{a}	505.63±7.45^{b}
ABTS (μmol TE/g)	784.95±6.71^{c}	850.54±8.72^{a}	817.92±8.03^{b}	775.99±5.29^{c}
CUPRAC (μmol TE/g)	759.25±4.40^{a}	756.50±5.87^{a}	741.39±6.35^{a}	745.36±5.31^{a}

Data are expressed as means values ± standard deviation of triplicate (n=3) analyses. Mean values with different superscripts in the same row differ significantly at $p < 0.05$, according to Duncan's test. N.S., not significant.

in organically and conventionally grown Peruvian chia seeds (2.18 mg GAE/g) was significantly higher than that observed in Mexican chia seeds (0.88-0.92 mg GAE/g) and Chilean chia seed (0.94 mg GAE/g) by Reyes-Caudillo *et al*., (2008) and Marineli *et al*., (2014), respectively. A similar total phenolic compound content has been reported for Australian chia seed (2.39 ± 0.07 mg GAE/g) (Ding *et al*., 2018). The total flavonoid content of Peruvian chia seeds was comparable to that reported for Australian chia seed (1.93 ± 0.05 mg CE/g) (Ding *et al*., 2018).

Interestingly, phenolic acids were higher in the organically-grown white chia than in its conventionally-grown counterpart ($p < 0.05$) (Table 3). Previous works reported phenolic acids like chlorogenic acid (0.04 and 0.102 mg/g) and caffeic acid (0.01 mg/g) in chia seeds (Marineli *et al*., 2014; Reyes-Caudillo *et al*., 2008). This finding could be relevant to the value of chia seeds as functional foods because phenolic acids are known to be the most-consumed phenolic compounds, with an average intake of 200 mg/day depending on diet (Acosta-Estrada *et al*., 2014).

Phenolic acids such as ferulic acid and hydroxytyrosol acetate have been detected electrochemically in chia seed methanolic extracts after being released by acid hydrolysis at 80 °C for 2 h from matrix; they significantly contribute to antioxidant activity (Oliveira-Alves *et al*., 2017). Phenolic acids are insoluble since phenolic fractions covalently bound to cell wall structural components like cellulose, hemicellulose, lignin, pectin and proteins, and thus can be released by acid hydrolysis (Acosta-Estrada *et al*., 2014). However, phenolic acid degradation or structural changes have been reported using acid hydrolysis (Shahidi and Yeo, 2016). Although the biosynthesis of bound-phenolic acids remains unclear (Shahidi and Yeo, 2016), we postulate that an increase in extractable phenolic acids would promote its bound fraction. Therefore, a rise in total antioxidant capacity, as measured by quencher approaches (Condezo-Hoyos *et al*., 2015), can be expected in organically-grown white chia.

3.4. Antioxidant capacity

3.4.1. Extractable fraction

3.4.1.1. DPPH: Although extractable total phenolic acid levels were not different in organically and conventionally-grown chia seeds (Table 1), the antioxidant capacity measured by the DPPH assay was lower in the organic dark and white varieties compared to conventionally-grown crops ($p < 0.05$) (Table 3). Moreover, although the organic white chia variety showed a higher phenolic acid content than its conventionally grown counterpart, it did have a lower antioxidant capacity ($p < 0.05$) (Table 3). A comparative study carried out with different organically and conventionally-grown potato cultivars demonstrated that increasing phenolic compounds did not enhance the DPPH antioxidant capacity of extractable fractions (Lombardo *et al*., 2017). In another study, organic cultivation did not affect the contents of total phenolics and total phenolic acids or antioxidant power as measured by the DPPH assay (Mazzoncini *et al*., 2015). Consequently, scientific evidence suggests that the association between total phenolic compounds and antioxidant capacity is cultivar-dependent.

The DPPH-determined reactivity of phenolic antioxidants has been shown to be influenced by their phenolic chemical structure, which makes it difficult to chemically rank pure antioxidant and antioxidant from natural extracts (Xie and Schaich, 2014). Thus, the ferulic acid and chlorogenic acid found in chia seeds (Marineli *et al*., 2014; Oliveira-Alves *et al*., 2017) had a moderate reactivity with initial reaction rates in methanol of 0.96 ± 0.04 and 0.73 ± 0.10 nmol DPPH/s, respectively. These reactivity values were 2.6 times lower when ethanol was used instead of methanol as the reaction medium (Xie and Schaich, 2014). Therefore, we postulated that this moderate reactivity of chia seed phenolic acids against DPPH would explain why

organic white chia seeds showed a lower antioxidant capacity than its conventional counterpart despite the high phenolic acid levels found in the organic crop. On the other hand, the antioxidant capacity against DPPH of Peruvian chia seeds from organic or conventional cultivation ranged from 457.48 to 528.95 μmol TE/g (Table 3), similar to that found for Chilean chia seeds (436.61 μmol TE/g) (Marineli et al., 2014) and Brazilian chia seeds (466.3-478.2 μmol TEAC/g) (da Silva *et al*., 2017).

3.4.1.2. $ABTS^{\bullet+}$: The effect of organic and conventional cultivation on antioxidant capacity measured by the $ABTS^{\bullet+}$ assay was dependent on chia variety. $ABTS^{\bullet+}$ quenching was higher in the organic white chia variety than in its conventional counterpart ($p < 0.05$) (Table 3). Conversely, antioxidant capacity was higher in conventionally-farmed dark than white chia seeds ($p < 0.05$), although the total phenolic content was the same (Table 3). A comparative study carried out with different cultivars of potatoes grown organically or conventionally demonstrated that increased phenolic levels did not enhance the antioxidant capacity of extractable fractions against $ABTS^{\bullet+}$(Mazzoncini *et al*., 2015). As in the DPPH assay, the $ABTS^{\bullet+}$ quenching rate is strongly dependent on phenolic structure, for instance chlorogenic acid, a major phenolic acid in chia seeds, or Trolox react instantaneously with $ABTS^{\bullet+}$. A previous study has demonstrated that Mexican chia seed extract showed the same quenching rate as Trolox (Reyes-Caudillo *et al*., 2008). This behavior by chlorogenic acid could explain the high $ABTS^{\bullet+}$ quenching in the organic white chia variety compared to the conventional white crop. The phenolic acid content was also higher in the organic white chia variety than in conventional white chia (Table 3).

3.4.1.3. CUPRAC: The antioxidant capacity measured by the CUPRAC assay did not show a significant difference between chia seed extracts from organic and conventional crops ($p > 0.05$) (Table 3). This assay was not able to detect a difference between organic and conventional white chia varieties although the former showed a high phenolic acid content (Table 3).

3.4.2. Total antioxidant capacity: QUENCHER approaches

The measurement of antioxidant capacity in samples such as cereals, legumes, pseudo-cereals and seeds has not been limited to soluble components because insoluble substances have always shown antioxidant activity and have contributed significantly to the total antioxidant capacity (Condezo-Hoyos *et al*., 2015; Shahidi and Yeo, 2016). Insoluble phenolic acids have been found bound to macromolecules such as structural proteins, cellulose and pectin through covalent bonds via ether, ester and carbon-carbon bonds in the cell wall matrix (Shahidi and Yeo, 2016). Chia seeds have been identified as a good source of dietary fiber and protein (Olivos-Lugo *et al*., 2010), which could contain insoluble bound-phenolic compounds that contribute to antioxidant activity. In fact, a previous study has demonstrated that chia seed phenolic extract released phenolic acids after acid hydrolysis, reflecting the fact that phenolics are bound to matrix macromolecules (Oliveira-Alves *et al*., 2017). In another study, the total antioxidant capacity of chia seed was measured using several quencher approaches, which confirmed the presence of an insoluble fraction, although its contribution to the antioxidant capacity of the seed has not been clarified (Sargi *et al*., 2013). Nevertheless, the differences in total antioxidant capacity between organically and conventionally-grown chia seed have not yet been examined.

Interestingly, organic cultivation influenced the total antioxidant capacity measured by QUENCHER-DPPH, and white chia showed a higher value than the dark variety ($p < 0.05$). This effect was not found when dark and white chia were grown with the conventional techniques ($p > 0.05$) shown in Figure 2 (A). Although the above effect was significantly stronger for organically-grown white chia, conventional cultivation of the two varieties also showed differences between dark and white chia seeds in a QUENCHER-CUPRAC assay (Figure 2 (B)). Despite the dark variety not showing any differences between when it was grown organically or conventionally, organic white chia seed did show a higher total antioxidant capacity on QUENCHER-DPPH and QUENCHER-CUPRAC than did conventionally-cultivated white chia (Figure 2). Moreover, for organically and conventionally-grown white chia there was a positive association between QUENCHER assays and phenolic acid content and antioxidant capacity of extract as measured by the classic ABTS assay (Figure 2 and Table 3). QUENCHER-DPPH and classic DPPH did not show a similar pattern for organic white chia seed, which could be explained by the different kinetics of DPPH de-colorization shown by this radical in the methanol/water medium (1:1 v/v) used in the quencher approach compared to the kinetics in the ethanol used in the classic DPPH. The phenolic acid reactivity against DPPH in ethanol was 14 times lower than in the methanolic aqueous medium (Xie and Schaich, 2014). Peruvian organic white chia seeds showed a total antioxidant capacity QUENCHER-DPPH (19.11±1.78 mmol DPPH/Kg = 9.555 mmol Trolox/Kg) nearly 4 times that found for Brazilian chia seeds (2.56 ± 0.03 mmol Trolox/Kg) (Sargi *et al*., 2013). Therefore, organic white chia seed could be a good source of bound-phenolic compounds with healthy properties as an antioxidant and/or antioxidant

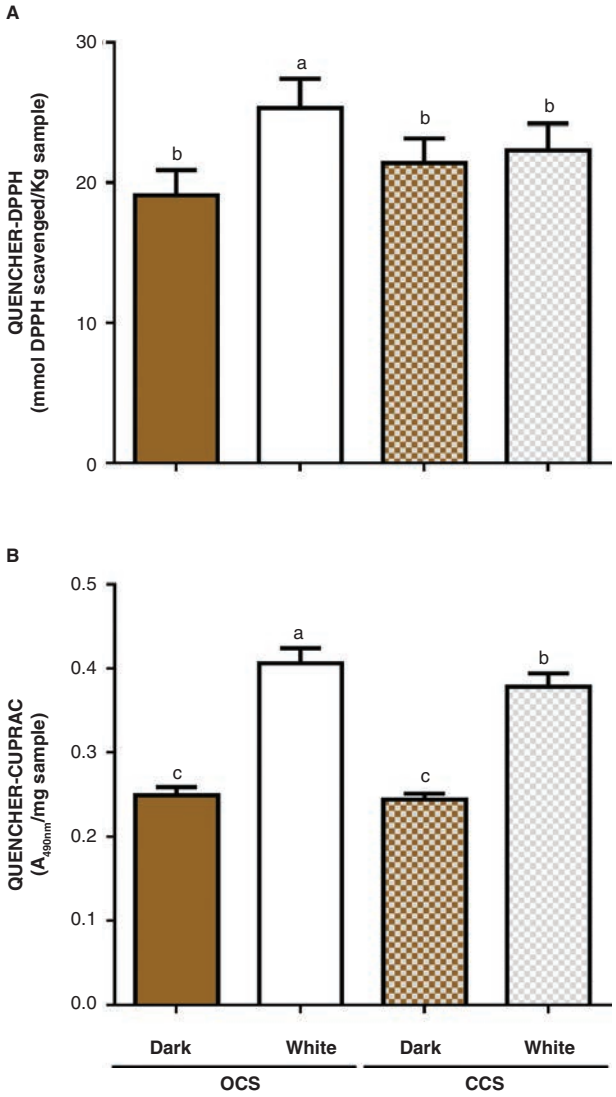

FIGURE 2. Total antioxidant capacity of dark and white organic chia seeds (OCS) and conventional seeds (CCS). Values are mean ± SD, n=3. Mean values with different superscripts in the same row differ significantly at $p < 0.05$, according to Duncan's test.

dietary supplement that could act on human microbiota (Acosta-Estrada *et al*., 2014;Shahidi and Yeo, 2016).

3.5. Functional properties

Organic dark and white chia seeds showed a higher WHC than that found in its conventional counterpart (Table 4). WHC has been associated with the presence of chia mucilage, which acts as a soluble dietary fiber, capable of holding water inside its matrix. Nevertheless, WCH is demonstrably dependent on several factors including protein (especially polar amino acid residues, which have a high affinity for water molecules) and carbohydrates (especially polysaccharides). In the case of chia seeds, it is the carbohydrate content that controls the WHC values (Table 1 and Table 4). Organic Peruvian chia seeds showed a higher WHC than that found in Brazilian seeds (Coelho and Salas-Mellado, 2014). Unlike WHC, the OHC in organic chia seeds was similar to that found in their conventional counterparts and the decrease in protein content shown in organic crops was probably compensated for by the rise in carbohydrate content (Table 1). OHC has been associated with the content in hydrophobic proteins and polysaccharides. The OHC in Peruvian seeds was lower than that found in conventionally-grown Australian chia seeds (58.61 ± 0.56 g oil retained/g of sample) (Coorey *et al*., 2014). In agreement with WHC values, the SWC in organic chia seeds was higher than that found in conventionally-grown crops (Table 4). In fact, the ability of chia seed to form gels is highly dependent on its swelling power and solubility (Ramos *et al*., 2017). Although the SWC in organic Peruvian chia seeds was lower than that found in processed chia flour (Ramos *et al*., 2017), organic chia products could be used as food ingredients for obesity prevention/weight control because they could modulate satiety and probably the microbiota composition and activity given their protein and antioxidant dietary fiber content (Acosta-Estrada *et al*., 2011). On the other hand, chia can be used in the food industry for its content in gums, and can be used for the control of viscosity, stability, texture and consistency in food systems (Capitani *et al*., 2015). In this way the techno-functional properties of the chia become an important physical-chemical property for the food industry.

3.6. Bi-plot analysis

A bi-plot of the multivariate relationships between the varieties of chia seeds from conventional and organic crops was carried out by comparing the PC1 and PC2 eigenvalues of PCA for both genotypes (dark and white) and the variables (Figure 3). Based on the theoretical arguments of PCA, a significant factor loading value of higher than 0.7 was used to identify the most important variables in each principal component. On the other hand, the variables with factor loadings below 0.7 were: lipids, polyphenols, ash, linoleic and palmitoleic acid. Regarding the interrelations between varieties and conventional and organic crops, the results for the first two PC axes (PC1, 39.85% and PC2, 20.11%) accounted for about 59.97% of the total variability, reflecting the complexity of the variation among the plotted components. Consequently, the first factor combines the protein (0.9471) and DPPH assay (0.8297). In general, the cultivars located on the right hand of the bi-plot (dark CCS and white CCS), indicate a high protein content and higher

TABLE 4. Techno-functional properties of two varieties of chia seeds from organic and conventional crops

	Organic chia seed		Conventional chia seed	
Functional property	**Dark**	**White**	**Dark**	**White**
WHC, mL water/g	36.16±0.04[b]	36.92±0.07[a]	35.03±0.04[d]	35.60±0.07[c]
OHC, mL oil/g[NS]	11.49±0.06[a]	11.56±0.04[a]	11.77±0.02[a]	11.65±0.06[a]
SWC, mL/g	8.54±0.04[b]	9.50±0.09[a]	6.80±0.07[d]	7.70±0.08[c]

Data are expressed as means values ± standard deviation of triplicate (n=3) analyses. Mean values with different superscripts in the same row differ significantly at $p < 0.05$, according to Duncan's test. N.S., not significant.

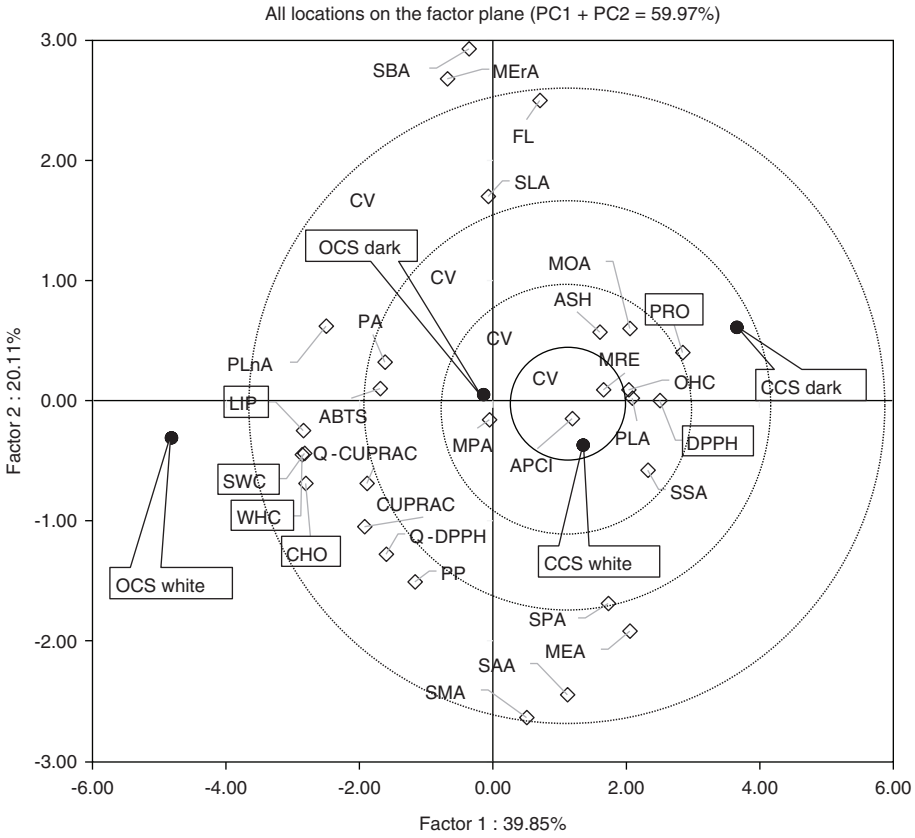

FIGURE 3. Bi-plot based on PCA among variables and varieties of chia seeds from conventional and organic crops. PRO: protein; LIP: lipids, ASH: ash; MRE: moisture; CHO: carbohydrate; SWC: swelling capacity; OHC: oil holding capacity; WHC: water holding capacity; PP: polyphenols; FL: flavonoids; DPPH: DPPH assay; ABTS: ABTS assay; CUPRAC: CUPRAC assay; SMA: myristic, SPA: palmitic, MPA: palmitoleic, SSA: stearic, MOA: oleic, PLA: linoleic; PLnA: linolenic; SAA: arachidic; MEA: eicosenoic; PA: phenolic acid; SBA: behenic; MErA: erucic; SLA: lignoceric; APCI: Antioxidant potency composite index score; Q-DPPH: QUENCHER-DPPH and Q-CUPRAC: QUENCHER-CUPRAC. Chia seeds: dark organic chia seed (dark OCS), white organic chia seed (white OCS), dark conventional chia seed (dark CCS) and white conventional chia seed (white CCS).

antioxidant activity by DPPH than those located on the left. So, dark OCS and white OCS seem to be promising candidates with high functional properties (WHC = -0.9561 and SWC = -0.9421) and nutrients such as carbohydrates (-0.9378) and lipids (-0.9486), while the second factor had the erucic, behenic, myristic, arachidic, and to a lesser extent the flavonoids, as primary elements. ABTS *vs* PA ($r = 0.8330$; $p = 0.0102$), QUENCHER-DPPH *vs* QUENCHER-CUPRAC ($r = 0.8071$; $p = 0.0155$) showed a high correlation, while CUPRAC *vs* PP showed a moderate correlation ($r = 0.5884$; $p > 0.05$). On the other hand, correlation was low between DPPH *vs* PP and DPPH *vs* FL and the antioxidant methods (DPPH, ABTS and CUPRAC) in the PCA are located in the first, second and third quadrant.

4. CONCLUSIONS

Organically-grown chia seeds showed a better carbohydrate content and lower protein content than conventionally-cultivated chia seeds, probably associated

to a rise in the dietary fiber content. Polyunsaturated fatty acid content was not changed under organic or conventional systems. An improvement in the phenolic acid content was found in organically-grown white chia seeds associated with an increase in the antioxidant capacity of the extract measured by the ABTS assay. Organic white chia seed has a better total antioxidant capacity measured by quencher approaches than its conventionally-grown counterpart. Organic white chia seeds could be a source of dietary antioxidants with a potential to promote healthy systemic and microbiota benefits.

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