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ORIGINAL PAPER

Chia Oil Extraction Coproduct as a Potential New Ingredient for the Food Industry: Chemical, Physicochemical, Techno-Functional and Antioxidant Properties

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Abstract
The aim of this work was to characterize the coproduct obtained from chia oil production (cold-pressing) with a view to its possible application in new food product development. For this characterization, the following determinations were made: proximate composition, physicochemical analysis, techno-functional properties, total phenolic and flavonoid content, polyphenolic profile and antioxidant capacity (using four different methods). Chia coproduct showed significantly higher levels of proteins and total dietary fiber and lower levels of fats than chia seeds, pointing to the promising nature of this coproduct as an ingredient of food formulations since it remains a source of high biological value proteins and total dietary fiber (as chia seeds themselves) but with a lower energy value. This chia coproduct presents similar techno-functional properties to the original chia seeds and significantly higher levels of polyphenolic compounds and, consequently, higher antioxidant activity.

Keywords Chia coproduct · Polyphenolic compounds · Antioxidant · Techno-functional properties

Introduction

Chia (*Salvia hispanica* L.) is a herbaceous plant belonging to the Lamiaceae family that counted among the main crops grown by ancient Mesoamerican cultures. Although native from southern Mexico and northern Guatemala, is increasingly cultivated for commercial purposes in most South-American countries and also in Australia [1], thus that chia seeds are now commercially available for human consumption as food supplements all over the world. Since 2009 (under regulation 2009/827/EU) the European Union (EU) has authorized the use of chia seeds as a novel food ingredient, allowing them to comprise up to 5% of the ingredients of bread. They are usually consumed ground or as whole grains and can be used in fruit juices, milk, soft drinks and salads. Recently, chia seeds have also been included in processed foods such as breakfast cereals, cookie snacks, fruit juices, cakes, breads, yogurts, ice creams, etc. [2]. The increasing interest in the study of chia seeds is due to their nutritional and health-promoting properties. Chia seeds contain about 25–38% oil by weight, which makes them the richest botanical source of omega-3 α-linolenic acid (C18:3, ALA, up to 68%) [3] of any known vegetable source; the seeds are also an important source of protein, dietary fiber, minerals and bioactive compounds (such as tocopherols and phenolic compounds) [1, 4], increasing their potential beneficial effects on human health [5]. Due to the high quantity and quality of the oil in chia seeds, several studies have looked into their extraction and marketing as a healthy vegetable oil. Dubois et al. [6] classified oils according to their FA profiles, and included chia seed oil in the PUFA class (a-linolenic + linolenic acid subclass), emphasizing the fact that it provides a good equilibrium between the two essential fatty acids. Nevertheless, in the EU, authorization for placing chia oil on the market as a novel food was not achieved until 2014. Although many oil extraction methods have been used with the seeds [7, 8] it is important to remark that the oil produced by cold-pressing is the only chia oil that has been authorized by the EU (2014/890/EU). The extraction of oil from the seeds generates defatted chia as

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a coproduct, and due to the boom in the consumption of chia seeds and chia-enriched foods, together with the recent EU authorization for chia oil commercialization, high amounts of chia coproducts are expected to be generated in the future. Taking into account the healthy properties attributed to chia seeds, it should be expected that this coproduct will also be useful as an ingredient for food production. The aim of this work, therefore, was to characterize the coproduct obtained from chia oil production (cold-pressing) with a view to its application in new food product development.

Fig. 1 Chia seeds and their oil extraction coproduct

Materials and Methods

Samples

The study was performed on two different chia samples: Chia seeds and their coproduct (obtained from a cold-pressing oil extraction process) (Fig. 1). The samples were supplied by the Spanish National Research Council (IATA-CSIC) and grounded with a mill to obtain flour.

Proximate Composition

Moisture, ash, lipid and protein were determined by the corresponding AOAC method [9]. Total dietary fiber (TDF), soluble dietary fiber (SDF) and insoluble dietary fiber (IDF) were determined using the enzymatic-gravimetric AOAC method [10]. Available carbohydrates were calculated by difference using the following equation: [100–(%Moisture+%Ash+ %Lipids+ %Protein+%TDF)]. The total energy value of the samples was estimated considering the conversion factors of 4 kcal/g protein or carbohydrate, and 9 kcal/g lipid.

Physicochemical Analysis

The pH was measured in 10% (*w*/*v*) aqueous solutions of the samples, and water activity (Aw) was determined using a Sprint TH-500 Novasina Thermoconstanter at 25 °C. Color was measured based on the CIELab color space with a CM-2600d colorimeter (Minolta Camera Co., Osaka, Japan) using illuminant D65, an observer angle of 10°, SCI mode, 11 mm aperture for illumination and 8 mm for measurement. The samples were placed in a Petri capsule for the color measurements. The following color coordinates were determined: lightness (L*), redness (a* ± red-green) and yellowness (b* ± yellow-blue). From these coordinates, hue ($h^* = \tan^{-1} b^*/a^*$) and chroma ($C^* = (a^{*2} + b^{*2})^{1/2}$) were calculated.

Techno-Functional Properties

Water and oil holding capacity (WHC and OHC, respectively) were determined following the methodology described by Robertson et al. [11]. The results were reported as g of water or oil held *per* g of sample. Emulsifying activity (EA) and emulsion stability (ES) were carried out following the methods described by Chau and Huang [12]. The EA results were calculated as the volume of the emulsified layer/total sample volume × 100. The ES was calculated as the volume of remaining emulsified layer/original emulsion volume × 100. Swelling capacity (SWC) was measured following the method described by Gómez-Ordoñez et al. [13], expressing the results as ml water *per* g of sample. Gelling capacity (GC) and precipitate in oily phase (POP) are defined as the sample's capacity to absorb fat and water in a matrix forming a gel [14]. Distilled water and sunflower oil (20 g of each one) were mixed, heated and centrifugated. Two phases, the gel phase and precipitate in oily phase, were identified in the tubes, and the relation between these two phases with respect to the total volume was calculated. The GC results were calculated as the volume of the gel layer/total sample volume × 100, and POP as the volume of the precipitate layer/total sample volume × 100.

Polyphenols Extraction Method

Samples (0.05–0.3 g) were mixed with 5–10 ml of methanol:water (80:20, *v*/v) and then sonicated for 15 min. After centrifugation for 10 min, 8000 *g* at 4 °C, the supernatants were collected and the pellets were mixed with 5–10 ml of acetone:water (70:30, *v*/v) and the same steps were repeated. Then, the supernatants were combined and evaporated to dryness. Five milliliters of methanol were added to the residue, and the mixture was thoroughly shaken in a Vortex for 2 min. The methanolic extract was filtered through a 0.45 μm filter and stored a − 20 °C until use.

Total Phenolic (TPC) and Total Flavonoid (TFC) Content

TPC was estimated using the Folin-Ciocalteu' method as proposed by Singleton and Rossi [15]. Gallic acid (GA) was used as reference standard and results were expressed as mg GA equivalents/g of sample. TFC was established by means of the method described by Blasa et al. [9]. For this, 1 ml aliquot of

appropriately diluted sample was added to a 10 ml volumetric flask. At zero time, 0.3 ml 5% $NaNO_2$ was added to the flask. After 5 min, 0.3 ml 10% $AlCl_3$ was added. At 6 min, 2 ml 1 M NaOH was added to the mixture. After 10 min the absorbance of the mixture was determined at 510 nm. The reference standard was rutin and results were expressed as mg rutin equivalents (RE)/g of sample.

Determination of Polyphenolic Compounds

Polyphenolic profiles were determined by high performance liquid chromatography (HPLC) following the methodology described by Lucas-Gonzalez et al. [16]. Polyphenolic compounds were identified by comparing UV absorption spectra and the retention times of each compound with those of pure standards injected in the same conditions.

Antioxidant Capacity

The antioxidant capacity was assessed by means of following four *in vitro* spectrophotometric assays. The DPPH assay was performed using the stable radical 2,2-diphenyl-1-picrylhydrazyl, following the method proposed by Brand-Williams et al. [17]. The ferric reducing antioxidant power (FRAP) was assessed by means of the potassium ferricyanide-ferric chloride method described by Oyaizu [18]. The TEAC-ABTS assay was applied following the method proposed by Gullón et al. [19]. In all three methods Trolox was used as reference standard and results were expressed as mg Trolox equivalents/g of sample. Finally, the ferrous ions chelating activity (FIC) was determined by establishing the inhibition of Fe^{2+}-ferrozine complex formation after adding to test material Fe^{2+} by means of the method described by Carter [20]. EDTA was used as reference standard and the results were expressed as mg EDTA/g of sample.

Statistical Analysis

The results were expressed as the mean ± SD of two parallel trials ($n = 4$) and compared through the statistical program SPSS v. 27 for Windows (IBM, USA). The differences between the mean values of the samples were analyzed by one-way analysis of variance (ANOVA). Tukey's *post hoc* test was applied for comparisons of means and differences were considered significant at $p < 0.05$.

Results and Discussions

Proximate Composition

The proximate composition of chia seeds and corresponding coproduct is presented in Table 1. Chia seeds are good source of oil, proteins and TDF, and the values recorded were in good agreement with those reported by other authors for chia seeds from some of the largest commercial fields in South America [3, 21]. The high oil content in chia seeds allowed pressing, an extraction method only useful for oil contents higher than 20% [7]. The concentration of TDF in chia (33.04%) was to be higher than in other cereals and oilseeds such as linseed (22.30%), soy (15.00%), corn (13.40%), wheat (12.60%), and sesame (7.79%) [22]. Of the total dietary fiber content, insoluble fiber represented 92% (30.37 ± 1.02 g/100 g chia) and soluble fiber 8% (2.67 ± 0.26 g/100 g). The total dietary fiber content and the soluble/insoluble fiber ratio in chia seeds were in agreement with those reported in the literature for chia grown in different countries [4, 21]. As regards the protein content, although chia is not commercially grown as a protein source, its high protein content, especially the fact that its amino acid profile contains all amino acids essential for the diet of an adult person [21], increases its nutritional interest. In any case, the protein content is higher than that of other traditional crops such as barley, rice, oats, wheat, and corn [3]. Studies have also demonstrated that chia can be incorporated in human diets and mixed with other grains to produce a more balanced protein source [3].

Table 1 Proximate composition (g/100 g sample) and total energy value of chia seeds and their oil extraction coproduct

	Chia seeds	Chia coproduct
Moisture	5.94 ± 0.12[b]	6.84 ± 0.11[a]
Proteins	20.58 ± 0.24[b]	27.02 ± 0.08[a]
Lipids	32.33 ± 0.53[a]	7.40 ± 0.11[b]
TDF	33.04 ± 0.33[b]	48.2 ± 0.45[a]
Ash	4.81 ± 0.15[a]	5.95 ± 0.80[a]
Carbohydrates*	3.30 ± 0.25[b]	4.59 ± 0.22[a]
Total energy value (kcal/100 g)	389.49 ± 1.82[a]	193.04 ± 1.25[b]

TDF Total dietary fiber

*Carbohidrates = [100 − (%Moisture + % Ash + % Lipids + % Protein + % TDF)]

[(a-b)] Values in the same row followed with same letter are not significantly different ($p > 0.05$) according to Tukey's Multiple Range Test

After oil extraction, the chia coproduct continues to be good source of proteins and total dietary fiber. The fact that the lipid content is reduced to 7.4% means that the yield of the cold-pressing extraction method is approximately 77%. This decrease in the lipid content increases ($p < 0.05$) the proportions of protein and TDF, meaning that the energy value is considerably reduced (>50%). This would make this chia coproduct very interesting for incorporation in fiber-enriched foods or foods for weight control diets. Of the total dietary fiber content, insoluble fiber represents 91% (44.01 ± 0.99 g/100 g chia coproduct) and soluble fiber 9% (4.19 ± 0.18 g/100 g chia coproduct). The IDF/SDF ratio (10.5) of this chia

coproduct also reflects its potential for incorporation in several foods. In addition, from a technological point of view, the coproduct could act as thickening agent, gelling agent and stabilizer of foams and emulsions.

Physicochemical Properties

The physicochemical properties of chia seeds and their coproduct are presented in Table 2. As can be seen, the pH of the coproduct was slightly higher than that of the seeds, although in both cases the pH values can be considered as neutral. Similar pH values have been reported for chia seeds by other authors [23], and is very interesting since it means that their addition to foods will not greatly affect their pH. Water activity was similar ($p > 0.05$) in both the chia seeds and their coproduct and in line with the water activity values of other seeds and grains (Aw<0.6) [23]. The chia coproduct showed higher ($p < 0.05$) L* and b* values than chia seeds and similar a* values. Differences in lightness could be related to the moisture content: the higher the moisture content, the higher the L* values. This relation has been noted previously and has been attributed to an increase in light reflection due to water. It is important to remark that both samples (chia seeds and their coproduct) showed higher yellow components than red components, which is characteristic of oily seeds and vegetable oils (including chia oil) [24]. Chia seeds showed lower ($p < 0.05$) chroma values than their coproduct and similar hue values. Similarly the coproduct showed higher C* values, indicating that oil extraction increased color saturation. Both samples showed hue values included in the brown hue range.

Techno-Functional Properties

Table 2 also shows some techno-functional properties of chia seeds and their coproduct. The hydration properties of both, evaluated as their water holding capacity and swelling capacity, are very useful to determine their optimal usage levels in foods as they contribute to desirable texture properties. Chia coproduct showed lower ($p < 0.05$) WHC values than chia seeds. The WHC depends on sample processing and also on its chemical and physical structure and it is also related with the SDF content. Although the SDF content of both chia seed and coproduct samples was comparatively low, they exhibited a relatively high WHC. This may be due to the mucilages (5–6% mucilaginous polysaccharides) [25, 26] in chia seeds, which have excellent water-holding properties. They are also notable for their thickening and gelling properties, syneresis control, emulsion stabilization, etc. [25]. Saura-Calixto & García-Alonso [27] reported that mucilages do not quantify in the SDF because some components may not precipitate during the ethanol treatment for SDF determination, thus SDF could be underestimated. In addition, the oil extraction process could be modifying the number of exposed hydrophilic sites in chia coproduct and contribute to decreasing the WHC. On the other hand, the SWC is mainly related to the sample permeability in contact with water. It is known that the WHC enhances the swelling ability, which is an important function specific to proteins for preparing viscous foods such as gravies, soups, dough, and baked products. SWC values range between 0.20–0.80 have been reported in flours from different seeds [28], and in our case, both chia samples showed similar ($p > 0.05$) SWC values. The differences in WHC between both samples did not seem to affect this property, which could be explained by the changes in samples permeability due to the oil extraction process.

Both chia samples showed low and similar ($p > 0.05$) OHC values, as it has been reported by other authors for chia seeds and some of their coproducts [4]. Chia seeds should have a low OHC due to their high oil content. It means that all the lipophilic compounds would be saturated and thus, no more oil can be held. However, the fact that chia coproduct showed the same low OHC is very surprising, and could be attributed to modifications in these lipophilic compounds during the oil extraction process causing non-lipophilic

Table 2 Physicochemical [pH, water activity (Aw) and color parameters (L*, lightness; a*, red/green coordinate; b*, yellow/blue coordinate; C*, Chrome; H*, hue)] and techno-functional properties (WHC, water holding capacity; OHC, oil holding capacity; SWC, swelling capacity; GC, gelling capacity; POP, precipitate in oily phase; EA, emulsion ability; EE, emulsion stability) of chia seeds and their coproduct

	Chia seeds	Chia coproduct		Chia seeds	Chia coproduct
pH	6.51 ± 0.03^{b}	6.69 ± 0.04^{a}	WHC (g/g)	5.63 ± 0.13^{a}	3.29 ± 0.31^{b}
Aw	0.529 ± 0.007^{a}	0.517 ± 0.004a	OHC (g/g)	1.17 ± 0.09^{a}	1.15 ± 0.15^{a}
L*	47.08 ± 0.29^{b}	51.01 ± 0.12a	SWC (ml/g)	0.53 ± 0.16^{a}	0.53 ± 0.23^{a}
a*	3.11 ± 0.07^{a}	3.82 ± 0.02^{a}	GC (%)	53.64 ± 3.82^{a}	14.79 ± 6.16^{b}
b*	11.17 ± 0.12^{b}	14.84 ± 0.03^{a}	POP (%)	36.25 ± 1.02^{a}	39.22 ± 3.66^{a}
C*	11.60 ± 0.13^{a}	15.32 ± 0.03^{b}	EA (%)	56.00 ± 0.77^{a}	55.00 ± 1.15^{a}
H*	74.47 ± 0.22^{a}	75.56 ± 0.06^{a}	EE (%)	40.00 ± 0.22^{a}	42.00 ± 0.34^{a}

$^{(a-b)}$ Values in the same row followed with same letter are not significantly different ($p > 0.05$) according to Tukey's multiple range test

behavior. Due to their low OHC, both chia samples can be considered potential ingredients in fried products since they would provide a non-greasy sensation.

Gelling capacity is an important techno-functional property of food ingredients that allows the ingredients to be formed into a matrix to form gels. Chia seeds showed a higher ($p < 0.05$) GC than the coproduct. This variation in the gelation properties can be attributed to the sizes of the various constituents, such as proteins, carbohydrates, and lipids, which, in turn, suggests that the interaction between these components may also play an important role in their functional properties. Protein gelation is very important in the development and acceptability of many foods, including vegetable and meat products. The gelation process, mechanism and gel appearance are mainly controlled by the balance between attractive hydrophobic interactions and repulsive electrostatic interactions [28]. Some authors have reported that this property in chia seeds is due to the ability of the mucilages to form a transparent mucilagenous gel when seeds come in contact with water [25].

Precipitation is induced in the oil phase when the addition of water to a system changes the solubility of some compounds in the oil phase, so that they precipitate. This is very interesting in foods or ingredients with a high fat content and is especially applicable to sterols in general [14]. Both chia samples showed similar values ($p > 0.05$) of POP.

The emulsifying capacity is related to the ability of a molecule to act as an agent that facilitates solubilization or dispersion of two immiscible liquids, and emulsion stability is the ability to maintain an emulsion along time and enhance its resistance to rupture. No differences ($p > 0.05$) in EA or EE were found between the chia seeds and coproduct samples. Several authors have reported that these two properties are related to the protein and fat contents since most proteins and lipids are strong emulsifying agents. In this respect, both chia samples are good sources of proteins and lipids (although with differences between them), in amounts that could be sufficient for them to act as good stabilizers. Supporting this idea, Bosquez [29] established that some ingredients rich in proteins are good stabilizers because they have sufficient hydrophobic groups to act as bonding points as well as hydrophilic groups that help to reduce surface tension in a liquid-liquid or liquid-gas interface. In this sense, Yadav et al. [30] established that the lipid content of these ingredients may also play an important role in the stabilization of oil-water emulsions. Other authors have also highlighted the role of carbohydrates in these properties, so their content in chia samples could be contributing to this effect. Muñoz et al. [25] reported that chia mucilage has the capacity to stabilize an emulsion, a capacity that was attributed to its ability to adsorb onto solid or liquid interfaces, thus helping to stabilize oil in water emulsions without any chemical or enzymatic modification. These findings suggest that chia seeds and their coproduct could act as good emulsifiers and stabilizers in the food industry.

Antioxidant Components

Table 3 shows the total phenolic and flavonoid content, and polyphenolic profile of chia seeds and their coproduct. Chia coproduct showed higher ($p < 0.05$) TPC and TFC than chia seeds. In both cases, the values were higher than those reported by other authors [1, 26] for chia seeds from different origins (TPC 0.5–1.2 mg GAE/g), although substantial differences were also found depending on the seed source. It is important to remark that the TPC reported for chia oil is extremely low

Table 3 Total phenolic content (TPC), total flavonoid content (TFC), polyphenolic profile and antioxidant activity values (assess by DPPH, ABTS, FRAP and FIC assays) of chia seeds and their oil extraction coproduct

		Chia seeds	Chia coproduct		Chia seeds	Chia coproduct
Phenolic acids	Gallic acid	42.56 ± 0.19^{b}	43.87 ± 0.16^{a}	TPC (mg GAE/g)	4.10 ± 0.27	5.00 ± 0.35
(μg/g)	Caffeic acid	125.36 ± 1.25^{b}	128.66 ± 1.36^{a}	TFC (mg RE/g)	48.61 ± 12.04	57.33 ± 2.72
	Ferulic acid	35.87 ± 0.23^{b}	37.04 ± 0.36^{a}	DPPH	5.63 ± 0.12^{b}	7.01 ± 0.02^{a}
	p-Coumaric	25.96 ± 0.18^{b}	26.41 ± 0.39^{a}	ABTS	4.73 ± 0.67^{b}	6.27 ± 0.42^{a}
	Rosmarinic	653.98 ± 26.98^{b}	669.88 ± 23.98^{a}	FRAP	70.1 ± 2.75^{b}	81.04 ± 3.51^{a}
				FIC	0.50 ± 0.03^{a}	0.57 ± 0.05^{a}
Flavonols	Rutin	99.88 ± 1.02^{b}	101.45 ± 0.98^{a}			
(μg/g)	Myricetin	28.88 ± 0.45^{b}	29.41 ± 0.36^{a}			
	Quercetin	285.56 ± 1.36^{b}	298.96 ± 1.56^{a}			
Isoflavones	Daizdin	457.85 ± 8.96^{b}	463.88 ± 4.87^{a}			
(μg/g)	Genistin	19.58 ± 0.09^{b}	21.56 ± 0.08^{a}			
	Genistein	55.69 ± 0.31^{b}	56.12 ± 0.35^{a}			

The DPPH, ABTS and FRAP results are expressed as mg Trolox equivalent/g fresh weight (FW), and FIC results as μg EDTA/g FW

[a-b] Values in the same row followed with same letter are not significantly different ($p > 0.05$) according to Tukey's multiple range test

(0.02 mg GAE/g), which would mean that most of these compounds remained in the coproduct, where they may even be increased due to a concentration effect. The TPC in both chia samples was higher than that reported by Irakli et al. [31] for oat (*Avena sativa*, 0.39 mg GAE/g), barley (*Hordeum vulgare*, 0.37 mg GAE/g), rice (*Oryza sativa*, 0.24 mg GAE/g) and corn (*Zea mays*, 0.15 mg GAE/g). In addition, the obtained phenolic compound content is higher than those reported for pseudocereals such as kaniwa (*Chenopodium pallidicaule*), 0.766 mg GAE/g; quinoa (*Chenopodium quinoa*), 0.876 mg GAE/g; and amaranth (*Amaranthus caudatus*), 0.249 mg GAE/g [32].

In both samples 11 polyphenolic compounds were identified, and also in this case, the values for all the polyphenolic compounds identified were higher in chia coproduct ($p < 0.05$) than in chia seeds. Regarding the polyphenolic compounds identified, rosmarinic acid was the major compound ($p < 0.05$) detected and quantified (with values of 653.98 and 669.88 μg/g in chia seeds and chia coproduct, respectively) followed by the isoflavone, daizdin and the flavonol, quercetin. Caffeic and rosmarinic acids are among the phenolic compounds already identified in chia products [4, 33].

Antioxidant Activity

Table 3 also shows the results obtained for the antioxidant capacity assays in both chia seeds and their coproduct. The highest antioxidant capacity ($p < 0.05$) in the DPPH, ABTS and FRAP assays was obtained for chia coproduct, while for metal chelating activity (FIC assay) no statistical differences ($p > 0.05$) were found between chia seeds and the chia coproduct. The values obtained in this work were lower than those mentioned in the relevant scientific literature. For example, Sargi et al. [34] reported DPPH, ABTS and FRAP values of 640.74, 430.50 and 715.82 mg TE/g, respectively, for triturated homogenized chia seeds. This high antioxidant activity in chia samples could be mainly due to the presence of caffeic and rosmarinic acids, as well as other phenolic compounds. Among the latter, is quercetine the most powerful and stable pure compound for which antioxidant activity has been evaluated [33]. Although the most important biological effect of isoflavones seems to be their estrogenic effect, an important antioxidant activity for these compounds has also been reported [35].

Conclusions

Chia coproduct showed higher levels of proteins and total dietary fiber and a lower fats content than chia seeds. This composition suggests that the coproduct could be a suitable ingredient for food formulations because it continues to be a source of high biological value proteins and total dietary fiber (as chia seeds) but with a lower energy value. Technologically, the chia coproduct has almost the same functional properties as the original chia seeds; only the water holding capacity and gelling capacity were modified (lower in the coproduct), which would have to be taken into account if used in gelled foods or cooked meat products. The chia coproduct contained a higher level of polyphenolic compounds than chia seeds and, consequently, showed higher antioxidant activity. All these properties suggest that not only chia seeds are an excellent food ingredient but that their coproduct following oil extraction has similar, or even better, properties.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest and that this article does not contain any studies with human or animal subjects.

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