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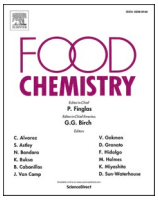

Formation of acrylamide during the roasting of chia seeds (*Salvia hispanica* L.)

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ABSTRACT

Chia is a novel food rich in health-promoting constituents. There is concern regarding the formation of process contaminants when subjected to thermal processing. Acrylamide formation in chia seeds under different roasting conditions (160–200 °C/5–30 min) and formats (whole seeds and ground seeds) was investigated. Acrylamide increased with the intensity of the thermal treatment until a maximum at 180 °C/15 min where levels of precursors were significantly reduced. The fate of acrylamide formation also depended on the physical integrity of the chia seed. Acrylamide formed rapidly on the coat of the seed but represents only 20–25 % of the total. Acrylamide was generated progressively in the inner part of the cell and efficiently retained by the pericarp. The particle size and integrity of chia seeds should be considered in food applications subjected to thermal treatment and grinding or the addition of ground seeds should be revised to reduce the exposure of acrylamide to consumers.

1. Introduction

The chia seed (*Salvia hispanica* L.) is characterized by a high lipid content (30–38 %) of which 60 % are composed of omega-3 fatty acids, proteins (15–24 %), dietary fiber (18–30 %), carbohydrates (26–41 %), and minerals (4–6 %) (Orona-Tamayo, Valverde, & Paredes-López, 2017). Chia seeds contain mucilage (5–6 %), which forms part of the soluble dietary fiber, and which is used in the food industry as a thickener, emulsifier, stabilizer, or antifreeze agent (Reyes-Caudillo, Tecante, & Valdivia-López, 2008; Muñoz, Cobos, Diaz, & Aguilera, 2013). The consumption of chia has been associated with the prevention of cardiovascular, inflammatory and nervous diseases, and diabetes (Teoh et al., 2018). It is considered a promising component of health-promoting foods with an increased biological potential (i.e. improvement of the blood lipid profile, hypotensive, hypoglycemic, antimicrobial, and immunostimulatory effects) and technological potential (i.e. substitute for emulsifiers and stabilizers, improved texture and rheological properties, and gluten-free food development) (Ullah et al., 2016; Zettel, & Hitzmann, 2018).

Chia is used in food applications as whole and ground seeds, flour, dried or soaked in water, as well as being used for its mucilage and oil (Zettel, & Hitzmann, 2018). It is included in the recipe of many foods such as baked goods, dairy, meat, and fish products to improve their nutritional and sensorial properties (Ullah et al., 2016). The reduction of the seed particle size by milling or grinding improves the extractability and bioaccessibility of phytochemicals and polyunsaturated fatty acids (Pellegrini et al., 2018; Labanca, Svelander, & Alminger, 2019). Ground seeds have been used in baked products to reduce starch retrogradation, as a fat substitute and to provide a healthier nutritional profile by increasing polyunsaturated fatty acids and fiber (Iglesias-Puig, & Haros, 2013).

The European Food Safety Authority (EFSA) evaluated whole and ground chia seeds as safe to use as a food ingredient since no evidence of adverse effects or allergenicity were recorded (EFSA, 2005, EFSA, 2009). In 2009, the European Parliament approved the use of chia seeds in bread up to 5 % in the context of the novel food regulation (European Commission, 2009). Chia seeds fall into category IV of novel food, namely food consisting of, isolated from, or produced from plants or their parts. In 2013, the EU Commission extended the use of chia in baked products, breakfast cereals and fruit, nut and seed mix up to 10 %, with a daily intake not exceeding 15 g (European Commission, 2013). In 2017, it was extended for additional food categories (fruit juice and fruit/vegetable blend beverages, pre-packaged chia seed as such fruit spreads, yogurt, sterilized ready-to-eat meals based on cereal grains, pseudocereals and/or pulses) (European Commission, 2017a). Mesias, Holgado, Márquez-Ruiz, and Morales (2016) investigated the benefits

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and potential risks of wheat-based biscuits supplemented with chia flour, and the acrylamide formation was significantly promoted as the chia flour content increased in the dough recipe. Accordingly, EFSA stated that the use of chia should be limited to non-thermally treated foods due to the possible formation of chemical process contaminants such as acrylamide (EFSA, 2019).

Acrylamide is classified as a potential carcinogen to humans (Group 2A) (IARC, 1994). Acrylamide can be naturally formed in processed foods when subjected to temperatures higher than 120 °C. It is mainly generated through the Maillard reaction between the free amino acid asparagine and the alpha-hydroxycarbonyl group of reducing sugars (Mottram, Wedzicha, & Dodson, 2002). In 2015, the EFSA stated that dietary acrylamide increased the risk of developing cancer in all age groups (EFSA, 2015). Subsequently, the European Commission established mitigation measures and benchmark levels for the reduction of its presence in foods (European Commission, 2017b).

In 2019, the EFSA promoted a public consultation to the scientific community to collect data relevant to the formation of process contaminants in chia seeds subjected to thermal processing as a novel food for extended uses. However, the EFSA concluded that information received was either limited or inconclusive, and therefore a gap remains with regard to acrylamide formation in thermally treated chia seeds (EFSA, 2020). The main objective of the present study was to provide new insights into the formation of acrylamide in chia seeds processed under different roasting conditions and structural formats (whole and ground seeds).

2. Material and methods

2.1. Reagents and chemicals

Potassium hexacyanoferrate (II) trihydrate (98 %, Carrez-I) and zinc acetate dehydrate (>99 %, Carrez-II) were obtained from Sigma (St. Louis, USA). $^{13}C_3$-labelled acrylamide (99 % isotopic purity) was obtained from Cambridge Isotope Laboratories (Andover, MA, USA). Acetonitrile and HPLC-grade methanol were obtained from Merck (Darmstadt, Germany). Formic acid (98 %), ethanol, methanol (99.5 %) and hexane were obtained from Panreac (Barcelona, Spain). Milli-Q water used was produced using an Elix3 Millipore water purification system coupled to a Milli-Q module (model Advantage10) (Millipore, Molsheim, France). Oasis HLB (polymeric reversed-phase sorbent) cartridges were obtained from Waters Millipore (Millipore, Bedford, MA, USA) and syringe filter units (0.45 μm, cellulose) from Análisis Vínicos (Tomelloso, Ciudad Real, Spain). All other chemicals, solvents and reagents were of analytical grade.

2.2. Samples

Chia seeds (*Salvia hispanica* L.) originating from Mexico and certified for organic farming (Lot PTR160412-01) were obtained from a company of food ingredients (EcoAndes Import Export, S.L., Madrid, Spain) in February 2020. The seeds used in this study showed a white pericarp and approximate diameter of 1.2 mm. Nutritional composition was obtained from the manufacturer declarations sheet and corresponded to 21 % protein, 33 % carbohydrates, 32.5 % fat, 24 % fiber, 0.02 % minerals, and 5.9 % moisture. Different formats of chia seeds were prepared before roasting: whole seeds (WS), ground seeds (GS) and defatted ground seeds (DGS). Ground chia seeds were obtained by grinding the whole chia seeds. Grinding time was selected within a 10-s time interval to prevent the excessive heating of the matrix and the formation of paste. The resulting material was size standardized by passing it through a 0.8 mm sieve. Defatted ground seeds were obtained from sieved ground chia seeds by extraction with hexane (1:10 w/v, GS:hexane, 2x), and the residue was dried for 20 h at ambient temperature. Sample codification and experimental design are summarized in **Supplementary** Fig. 1.

2.3. Roasting assays

Raw samples (WS, GS, DGS) in duplicate (10 g each) were placed in two aluminum trays (batch-1 and batch-2) and heated at 150, 160, 170, 180, 190 and 200 °C for 15 min (temperature-dependence assay) and at 180 °C for 5, 10, 15, 20, 25 and 30 min (time-dependence assay). An oven (Memmert UN 55, Germany) was used, and the temperature was externally monitored with a probe. Temperature/time combinations were selected in the range of previous studies for roasting of seeds and nuts (ie. Berk et al., 2019; Tepe et al., 2020; Hatamian et al., 2020). Temperature/time combinations would resemble two levels at moderate and two at more intense heat load above a reference. The temperature/time combination at 180 °C/15 min was selected as the reference. After heating, a portion of the roasted WS were ground using an Ultra-turrax (IKA-Werke GmbH, Staufen, Germany). Samples were passed through a mesh size sieve of 0.8 mm prior to the analytical determinations. Consequently, four groups of heated samples were obtained: WS, GS, DGS, and whole seeds ground after heating (WSG). Samples were stored at 4 °C until analysis.

2.4. Determination of weight loss after roasting

The weight of the samples was recorded before and after roasting for each trial. Percentage of weight loss related to the moisture loss was determined as follows: 100 – [sample weight after roasting (g) × 100/ sample weight before roasting (g)].

2.5. Determination of asparagine

Asparagine content in the samples before and after heating was determined by gas chromatography-flame ionization detection (GC-FID), according to Mesias, Delgado-Andrade, Holgado, and Morales (2018). A GC-FID (Agilent GC 7820A-FID) equipped with an automatic injector and an amino acid dedicated column (Zebron ZBAAA capillary; 10 × 0.25 mm) was used. The starting oven temperature was set at 110 °C and increased by 32 °C per minute until 320 °C was reached. An aliquot of the derivatized sample (1 μL) was injected in split mode (15:1) at 250 °C. The FID detector was set to 320 °C, and the carrier helium gas flow rate was maintained at 1.5 mL/min whilst in process. External calibration was carried out at five levels (20, 50, 100, 150, and 200 mmol/mL) with asparagine standard, and results were corrected for recovery with norvaline as internal standard. Free asparagine content was expressed as g/kg of sample.

2.6. Determination of reducing sugars

Glucose and fructose content in the samples before and after roasting were determined by an ion chromatography system (Metrohm AG, CH) coupled with pulsed amperometric detection (945 Professional Detector Vario, Metrohm AG, CH). The HPLC column was a Metrosep Carb2 (250 × 4 mm μm, Metrohm AG, CH), and the mobile phase was 300 mM sodium hydroxide solution and 1 mM sodium acetate. The injection volume was 10 μL and eluted at 0.5 mL/min. Glucose and fructose were quantified by an internal standard method using the sugar/sorbitol responses. The limit of quantitation was set at 10 mg/kg.

2.7. Determination of CIElab color

The color of the samples was measured at room temperature using a HunterLab Spectrophotometer CM-3500D colorimeter (Hunter Associates Laboratory, Stamford, Connecticut, USA). The color CIE$L^*a^*b^*$ coordinates system was used. Three independent measurements of a^* (redness), b^* (yellowness) and L^* (lightness) parameters were carried out at different points of the samples to consider possible non-homogeneous color distributions within the same batch of samples before and after heating. The E index was calculated according to the

following equation: $E = (L^{*2} + a^{*2} + b^{*2})^{1/2}$. The equipment was calibrated with a standard calibration CR-A43 white plate (a^*/0.3156, b^*/0.3319, L^*/93.80). The delta component (ΔL^*, Δa^*, Δb^*) was calculated by subtracting the color parameter in the heated sample by their respective unheated reference. The reference for the whole seeds ground after heating was the same as GS. The delta component for the color parameters would allow the comparison of the thermal extent among samples regardless of the initial value.

2.8. LC-ESI-MS-MS determination of acrylamide

Acrylamide was determined as described by Mesias, Holgado, Marquez-Ruiz, and Morales (2015). An Agilent 1200 liquid chromatograph coupled to an Agilent-G6410B Triple Quadrupole MS detector (Agilent Technologies, Palo Alto, CA, USA) in the positive electrospray ionization was used. The sample (10 mL) was separated onto a Hypercarb (50 × 2.1 mm, 5 mm; Thermo Scientific, Bremen, Germany) at 30 °C with 0.2 % formic acid in water at a flow rate of 0.4 mL/min. The mass transitions m/z 72 > 55 and m/z 75 > 58 were used for identification and quantification of acrylamide and the isotope-labelled internal standard [$^{13}C_3$]-acrylamide, respectively. The mass transitions m/z 72 > 44, 72 > 27 for acrylamide and m/z 75 > 44 for the isotope-labelled internal standard [$^{13}C_3$]-acrylamide were used as qualifiers. The limit of the quantitation was set at 15 μg/kg. Results were expressed as μg/kg sample. The recovery rate of acrylamide spiked to the samples was between 90 and 106 %. Relative standard deviations (RSD) for the precision, repeatability and reproducibility of the analysis was calculated as 2.9 %, 2.1 % and 3.3 %, respectively. The accuracy of results is demonstrated through the participation in proficiency tests launched by the Food Analysis Performance Assessment Scheme (FAPAS) program. The latest results for the food matrices provided for FAPAS were crispbread (test 30118, January 2022), coffee (test 30117, December-2021), and potato crisps (test 30120, April-2022) yielding a z-scores of −0.1, 0.1, and 0.4 respectively.

2.9. Statistical analysis

Statistical analyses were performed using SPSS version 26 (SPSS, Chicago, IL). Data from asparagine, reducing sugars and acrylamide were measured by duplicate from two independent batches. Color was measured by triplicate from two independent batches. Data were expressed as mean ± standard deviation (SD). One-way ANOVA followed by Scheffe's test or Student's t-test were used to identify the overall significance of differences. All statistical parameters were evaluated at $p < 0.05$ significance level.

3. Results and discussion

Chia seed samples used in the present study had oval, flattened shapes (approx. 2 mm length and 1.2 mm width). To obtain GS samples, WS were subjected to a medium grinding level (particle size < 0.8 mm). This was sufficient to break up the cell wall and gain access to the inner part of the cell but not excessive so as to prevent the formation of flour. The particle size resembled table salt or sand. WS and GS samples were thermally treated under controlled conditions. The thermal treatment promoted the development of the Maillard reaction and therefore the browning and formation of chemical process contaminants (Gökmen, & Senyuva, 2006; Friedman, 2015). Previous studies have indicated the correlation between CIE-$L^*a^*b^*$ color system parameters and the acrylamide formation in different foods (i.e. almonds, hazelnuts and coffee, wheat bread, crisp bread, gingerbread, biscuits, potato-based products, among others) (Pedreschi et al., 2007; Gökmen, & Senyuva, 2006; Zhang et al., 2011; Tepe, Çebi, & Aydin, 2020). Considering these statements, the understanding of the color changes of chia seeds during the thermal treatment would gain more insight into the formation of acrylamide.

Two heating studies were designed to assess the changes in color, reducing sugars, asparagine and acrylamide content of different formats of chia seeds during roasting. In the time-dependence assay, raw WS and GS samples were heated at 180 °C up to 30 min. The weight loss of WS and GS increased rapidly to 5.29 % and 5.82 % after 5 min, respectively, and reached a maximum of 7.68 % and 7.81 % at 30 min, respectively (**Supplementary** Table 1). In the temperature-dependence assay, raw WS and GS samples were heated for 15 min at different temperatures. The weight loss was 6.57 % and 6.65 % for WS and GS, respectively, at 160 °C, and reached a maximum of 8.08 % and 7.82 %, respectively, at 200 °C (**Supplementary Table 2**).

Change in color is one of the most important occurrences during the thermal processing of foods. The color of raw chia seeds ranges from black and black-spotted to white (seeds without pigmentation), and it has been reported that the pigmentation has no influence on their composition and nutritional profile (Orona-Tamayo et al., 2017; Ayerza, 2013). In this investigation, unpigmented seeds were intentionally used to evaluate the progress of the browning during heating. Figs. 1 and 2 describe the profile of the color parameters (L^*, a^*, b^*) during the roasting of WS, GS, and WSG for the time-dependence and temperature-dependence assays, respectively. The dataset for each assay is summarized in the **Supplementary Tables 1 and 2**. The L^* value of the unheated samples was 65.76 ± 1.01 and 54.84 ± 0.62, the a* parameter was 2.67 ± 0.38 and 4.30 ± 0.18, and the b* parameter was 9.03 ± 0.74 and 14.24 ± 0.27 for WS and GS, respectively.

In the time-dependence assay, ΔL^* (luminosity) decreased significantly as the heating time increased for all samples (Fig. 1**a**). The darkening of heated WS with regard to the control was significantly higher ($p < 0.001$) after just 5 min compared with WSG and GS but had only minor changes after 15 min. WS lost nearly 10 units of luminosity after 5 min of heating, whereas WSG and GS decreased by 3 and 0.9 units, respectively. In contrast, ΔL^* in WSG decreased gradually from 5 to 30 min compared with the unheated sample. The different behavior of WS and WSG indicates that browning proceeds significantly faster over time in the inner part of the seed compared with the outer part. The WSG sample showed a rapid darkening as the heating time varied from 5 to 25 min. In this case, the inner part of the seed was already exposed to the thermal treatment without the protection of the cell coat. The Δa^* (redness) rose with the extent of the heating time (Fig. 1**b**). The changes proceeded slowly for WS compared with WGS that increased gradually up to 30 min of heating. In contrast, the a^* parameter reached the maximum level at 15 min for GS samples (**Supplementary Table 1**). Finally, Δb^* (yellowness) increased gradually for WS samples until 10 and 15 min in GS and WSG samples (Fig. 1**c,** Supplementary Table 1).

In the temperature-dependence assay, ΔL^* decreased for all samples as the temperature increased. However, there were no significant differences between 160 °C and 170 °C in the luminosity for WS and GS. The ΔL^* was significantly higher for WS compared with WGS for all temperatures, and the luminosity significantly decreased in WGS from 160 to 180 °C (Fig. 2**a**, **Supplementary Table 2**). The Δa^* significantly increased at temperatures higher than 180 °C for WS (Fig. 2**b, Supplementary Table 2**), whereas it increased progressively from 160 °C to 200 °C for GWS. In contrast, the Δa^* reached a maximum level at 180 °C for GS and subsequent heating up to 200 °C did not show significant differences. The Δb^* reached a maximum level at 180 °C for GS and then decreased, similarly to WGS. However, in WS this parameter increased significantly from 180 °C onwards.

Results confirmed that changes in the color of WS subjected to thermal treatment were moderate in the coat of the seed compared with the inner part, particularly for L^* and a^* parameters. Subsequently, the constituents of the inner part of the seed contributed significantly to the extent of the browning. There is no previous information in the scientific literature concerning the progress of browning of chia seed and ground chia seed during roasting. This trend was also described by Berk, Hamzalıoğlu, and Gökmen (2019) and Kahyaoğlu and Kaya (2006) in roasted sesame seeds. Berk et al. (2019) reported similar changes in

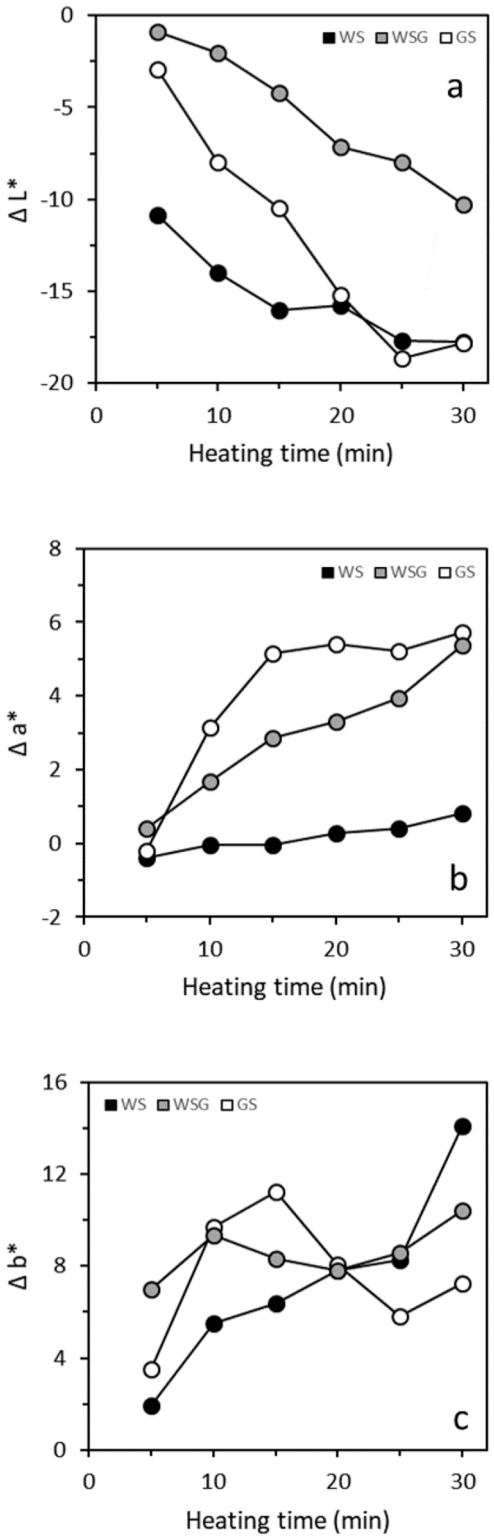

Fig. 1. Time-dependence (5, 10, 15, 20 25, 30 min) profile of the CIE $L^*a^*b^*$ parameters in whole seeds (WS, black dot), whole seeds ground after heating (WSG, grey dot), and ground seeds (GS, white dot) during roasting at 180 °C.

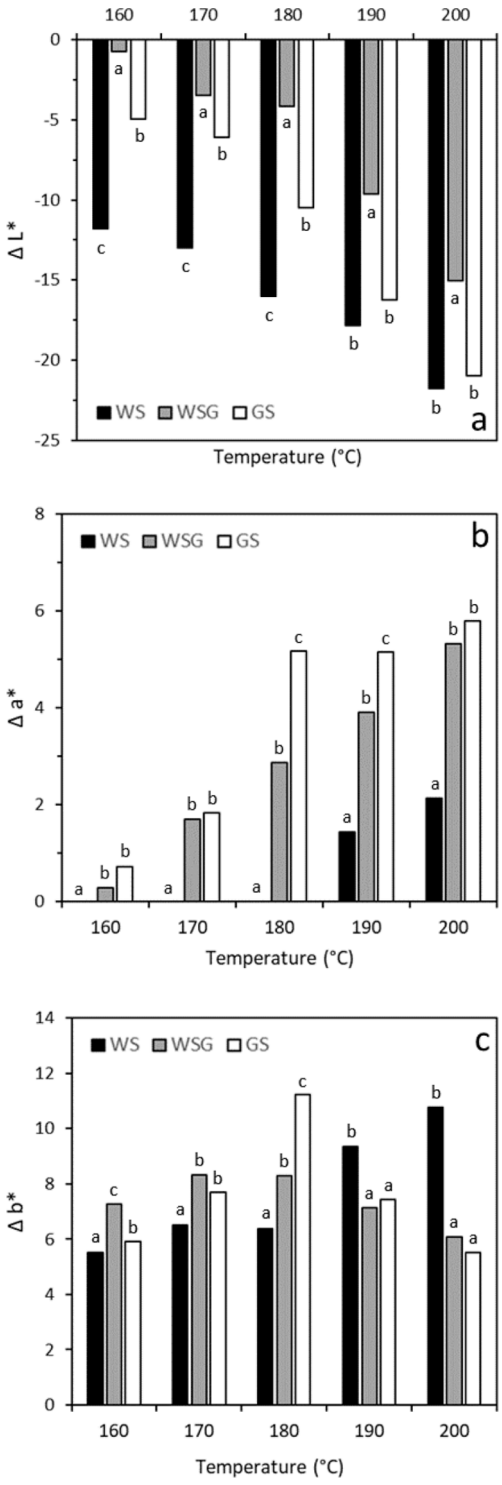

Fig. 2. Temperature-dependence (160, 170, 180, 190, 200 °C) profile of the CIE $L^*a^*b^*$ parameters in whole seeds (WS, black bar), whole seeds ground after heating (WSG, grey bar), and ground seeds (GS, white bar) roasted for 15 min. Bars with different letters in the same panel indicate significantly different values ($p < 0.05$).

parameters L^*, a^* and b^* when seeds were heated at temperatures between 150 and 220 °C for 10 min. These authors indicated significant decreases in the values of a^* when heating at 220 °C and b^* at temperatures higher than 180 °C due to the loss of yellowness and the production of brownness at these temperatures. In contrast, Kahyaoğlu and Kaya (2006) observed higher b^* values as a consequence of the increase in roasting temperatures, in accordance with results showed by whole chia seeds in the present study.

Acrylamide, reducing sugars, and asparagine content were evaluated in the samples. It should be noted that there are some analytical limitations for the determination of water-soluble constituents of whole chia seeds since the cell wall forms a mucilaginous material that expands

considerably (>10-fold) when the seed is soaked in water. Chia seed mucilage is a hydrophilic heteropolysaccharide (approximatively 93 % carbohydrate, 0.6 % fat, 2.6 % protein, 0.8 % ash, 3.9 % moisture), which contains uronic acids, xylose, glucose, arabinose, and galactose (Timilsena et al., 2016). The formation of the mucilage provides the samples with an extraordinary water holding capacity and emulsifying properties. Thus, the analytical procedures require additional verification of the performance, and the evaluation of the unheated seeds is not possible.

The time-dependence assay for acrylamide formation showed a maximum level at 180 °C/15 min for all the samples (Fig. 3**a**). The lowest acrylamide content was recorded for WS and the highest for GS at any heating time. The acrylamide content ranged from 26 ± 5.2 (180 °C/5 min) to 79 ± 1.3 μg/kg (180 °C/15 min) in WS and from 106 ± 11.9 (180 °C/5 min) to 292 ± 7.6 μg/kg (180 °C/15 min) in WSG, which suggests that acrylamide was formed mainly in the inner part of the seed compared with that formed in the coat. The acrylamide content increased dramatically when the inner part of the chia seed was directly exposed to the thermal treatment without the protection of the coat, as was the case with GS. In this sample, concentrations ranged from 27 ± 3.4 (180 °C/5 min) to 706 ± 44.2 μg/kg (180 °C/15 min) and decreased rapidly after 30 min of heating, exhibiting levels nearly 9-fold higher than the acrylamide content in WS and 2.5-fold higher than that of WSG. Asparagine content in the unheated ground seeds was 574 ± 14.3 mg/kg. Free asparagine content could not be quantified accurately in WS due to the limitations caused by the formation of mucilage during extraction and there is no information in the literature. The time-dependence profile of the asparagine consumption in the chia seed samples heated at 180 °C is depicted in Fig. 3**b**. The initial content was rapidly consumed in all the chia seed samples during the first 15 min of heating. The asparagine concentration was linked to the acrylamide formed in the heated samples, with the highest in GS, intermediate in WSG, and the lowest in WS. The consumption of asparagine plateaued after 15 min when the highest acrylamide content was obtained. Since acrylamide content was related to changes in asparagine, it could be assumed that asparagine must be the limiting factor for the acrylamide formation in chia seeds, as in agreement with Berk et al. (2019) for roasted sesame seeds. Similarly, to asparagine, the reducing sugars were greatly consumed after 15 min of roasting at 180 °C (Fig. 3**c**).

The thermal behavior of the acrylamide, asparagine, and reducing sugar content in the temperature-dependence assay were similar to the time-dependence assay. Acrylamide peaked between 170 and 180 °C for GS (706 ± 44.2 μg/kg) and WSG (317 ± 5.7 μg/kg), whereas no significant differences were obtained for WS (Fig. 4**a**). In contrast, there was significant consumption of asparagine in GS from 170 to 180 °C, and it was undetected at 200 °C (Fig. 4**b**). Reducing sugar was gradually degraded from 160 to 190 °C, and remains above 130 mg/kg in ground seeds (Fig. 4**c**).

Thermal treatment is usually applied to improve both the nutritional, technological, and organoleptic properties of seeds such as chickpeas (Jogihalli, Singh, Kumar, & Sharanagat, 2017), barley (Sharma, & Gujral, 2011), sesame (Berk et al., 2019), and chia flour (Hatamian, Noshad, Abdanan-Mehdizadeh, & Barzegar, 2020). Our results confirm that the extent of browning, reducing sugars and asparagine consumption, and acrylamide formation depend on the physical integrity of the chia seed (whole seed and ground seed). The structural properties such as particle size can affect the stability, extractability, and the availability of bioactive compounds for uptake in the gastrointestinal tract (Labanca et al., 2019). The chia fruit is a schizocarp consisting of indehiscent locules, which separate to form four fruitlets, referred to as nutlets. According to microstructure studies by scanning electron microscopy, the nutlet is composed of a stratified pericarp, which protects the true seed inside, similarly to grains (Moon & Hong, 2006). However, the fruitlets are commercially called whole seeds. The ground chia seeds are easily separated into the cell content and cell wall. The seed wall is composed of three layers: an outer layer, formed by rectangular thin-walled cells, where presumably the mucilage is localized, a scleroid layer of long and thin cells resembling fibers, and the endocarp, a thin and inner layer (Muñoz et al., 2012). Acrylamide formation is mainly a surface phenomenon since the highest temperature is reached more quickly on the food surface than in its interior. The integrity of the seed coat is thermally resistant, and the cell content is roasted by thermal conduction. Thus, it is plausible that high amounts of acrylamide remain

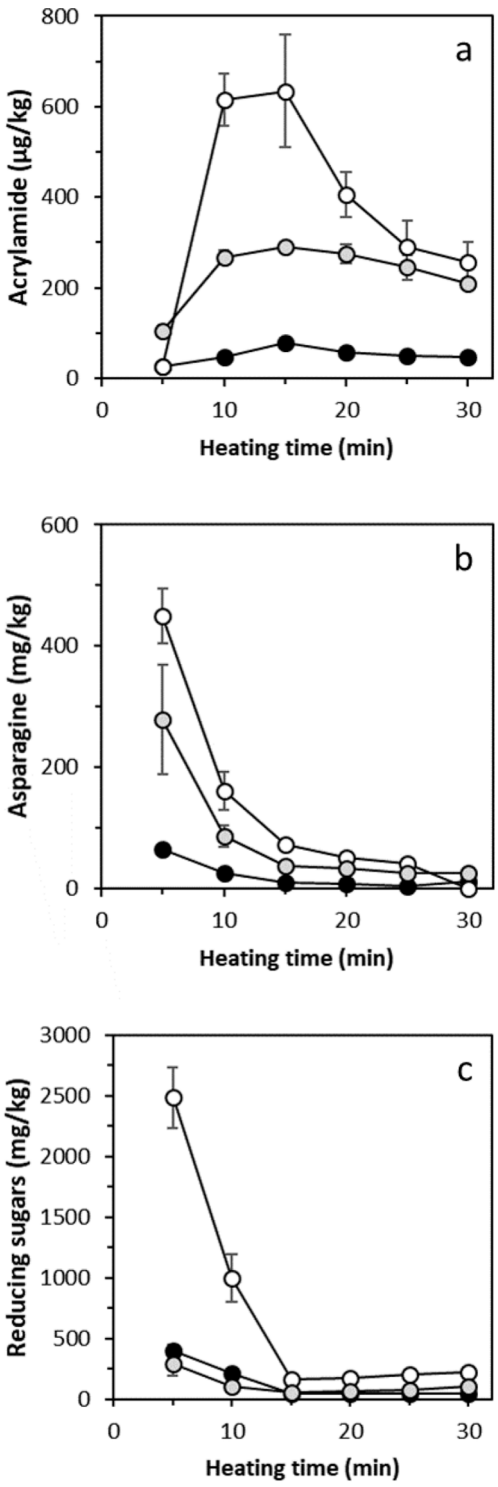

Fig. 3. Time-dependence (5, 10, 15, 20 25, 30 min) profile of the acrylamide (μg/kg, a), asparagine (mg/kg, b), and reducing sugars (mg/kg, c) content in whole seeds ((WS, black dot), whole seeds ground after heating (WSG, grey dot), and ground seeds (GS, white dot) at 180 °C.

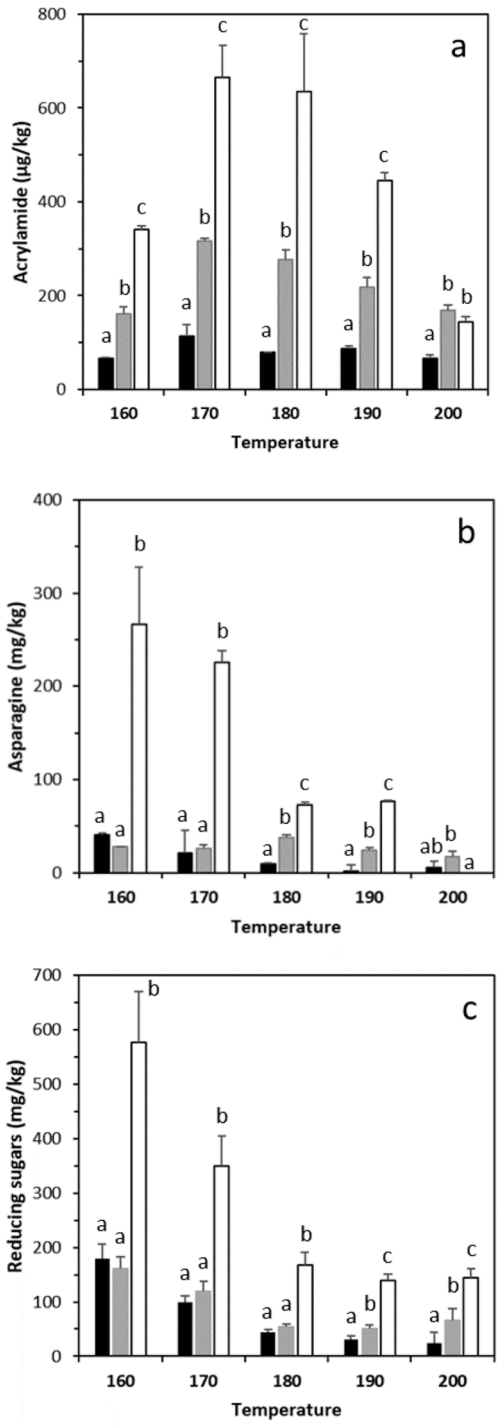

Fig. 4. Temperature-dependence (160, 170, 180, 190, 200 °C) of the acrylamide (µg/kg, a), asparagine (mg/kg, b), and reducing sugars (mg/kg, c) content of whole seeds (WS, black bar), whole seeds ground after heating (WSG, grey bar), and ground seeds (GS, white bar) roasted for 15 min. Bars with different letters in the same panel indicate significantly different values ($p < 0.05$).

inside the seed protected by the pericarp.

The grinding increased the accessibility of constituents of the inner part of the chia seed and consequently their reactivity. This effect has been previously described in the case of health-promoting constituents in unheated seeds since the reduction of the seed particle size by milling improves the extractability of phytochemicals and nutrients in grains (Brewer et al., 2014). However, when the whole seed was ground after roasting, it was possible to extract the acrylamide generated in the inner part. Comparing both models (WS *vs* WSG), the inner part of the seed would largely contribute to the formation of acrylamide in the seed, likely due to a higher concentration of acrylamide-forming precursors. Thereby, the lower acrylamide formation in the whole seeds was probably associated with the protection of the seed coat, as mentioned above, but also to the limitation of accessibility to acrylamide-forming compounds. Grinding the whole seeds exposed the constituents of the inner part, making them more accessible to the progress of the Maillard reaction. Consequently, the highest levels of acrylamide were obtained in GS since the constituents of the inner part of the chia seed were accessible and participated in the Maillard reaction. It is well known that the formation and elimination of acrylamide progress simultaneously during the heating of foods (Friedman, 2015). Acrylamide formation is predominant when precursors (asparagine and carbonyl compounds) are present. However, in their absence, elimination predominates. During the roasting of GS, acrylamide formation prevailed over elimination up to 15 min (time-dependence assay) and 180 °C (temperature-dependence assay). Similarly, maximum acrylamide levels were reached in sesame seeds roasted at 200 °C for 10 min (Berk et al., 2019) and in coffee beans roasted at 220 °C for 5 min (Kocadağli, Göncüoğlu, Hamzalıoğlu, & Gökmen, 2012). In both studies, these maximum levels were lower than those observed in the present investigation (663 µg/kg for roasted sesame seeds and 468 µg/kg for roasted coffee beans).

Chia seeds have an oil content of approximately-one third of their weight. The fatty acids present in chia oil are highly unsaturated, namely, linoleic (17–26 %) and linolenic (50–57 %) acids (Orona-Tamayo et al., 2017). The acrylamide formation in foods usually involves the progress of the Maillard reaction between amino acids and carbonyl compounds (Yaylayan, & Stadler, 2005), but the potential contribution of the degradation products from lipid oxidation must also be considered (Mestdagh, Castelein, Van Peteghem, & De Meulenaer, 2008; Zamora, & Hidalgo, 2008). Thermal oxidation of polyunsaturated fatty acids can generate reactive carbonyls, which may further react with asparagine leading to the formation of acrylamide. Previous studies have indicated that thermal treatment between 160 and 180 °C for 15 min of chia flour had no significant effect on fatty acid profiles but particularly affected the linoleic and linolenic acid stability (Hatamian et al., 2020). However, Ghafoor et al. (2020) stated that tocopherols, linolenic acid, linoleic acid, and oleic acid content together with the peroxide value decreased significantly in roasted chia. To evaluate this fact, the acrylamide formation in defatted ground chia seeds (DGS) was compared with GS samples (Table 1). In the time-dependence assay, there were significant differences at 5 and 20 min and the acrylamide formation profile was similar. Acrylamide peaked faster in DGS (10 min) compared with DG (15 min), likely due to the relative higher

Table 1
Time-dependence and temperature-dependence assays for the formation of acrylamide (µg/kg) in defatted ground chia seeds (DGS) and ground seeds (GS). Different letters denote statistical differences between samples ($p < 0.05$).

Acrylamide (µg/kg)	DGS	GS
Time-dependence assay		
(180 °C)		
5 min	335 ± 73b	26 ± 4a
10 min	687 ± 140a	615 ± 58a
15 min	463 ± 184a	635 ± 124a
20 min	264 ± 3a	406 ± 51b
25 min	205 ± 36a	291 ± 57a
30 min	228 ± 52a	256 ± 46a
Temperature-dependence assay		
(15 min)		
160 °C	380 ± 33a	340 ± 8a
170 °C	604 ± 30a	664 ± 69a
180 °C	457 ± 183a	635 ± 124a
190 °C	242 ± 86a	446 ± 17b
200 °C	157 ± 9a	143 ± 11a

concentration of precursors in the defatted sample. In the temperature-assay, both DGS and GS reached the highest concentration of acrylamide at 170 °C. The reaction of a carbonyl compound with asparagine results in the corresponding *N*-glycosyl conjugate and the decarboxylated Schiff base, which is then cleaved at the C—N bond to azomethine ylide. Acrylamide is formed directly from azomethine ylide, through the β-elimination of the decarboxylated Amadori compound or through the hydrolysis of ylide followed by the deamination of 3-aminopropionamide (3-APA) (Zyzak et al., 2003). However, some oxidized lipids may potentially promote the decarboxylation of asparaginase and the subsequent deamination of the produced 3-APA. In contrast, the alternative routes of acrylamide formation in the absence of asparagine are through acrolein and ammonia, which could play a role in lipid rich foods (Yaylayan, & Stadler, 2005). Despite the contribution of non-asparagine routes to the formation of acrylamide in foods being secondary, the relevance of reactive carbonyl compounds formed during lipid oxidation cannot be ruled out as a possible route in chia seeds.

4. Conclusions

Thermal treatment is usually applied to improve the nutritional, technological, and organoleptic properties of different seeds. This is the first study reporting that roasting process of chia seeds led to the formation of acrylamide. The extent of browning, reducing sugars, and asparagine consumption and the acrylamide formation during the roasting process depend not only on the intensity of the thermal treatment but also on the physical integrity of the chia seed (whole seed and ground seed). Acrylamide content in the seeds ground before roasting was nearly 9-fold higher than in the whole seeds. Although acrylamide forms rapidly on the coat of the chia seed, the inner part of the cell is also roasted by thermal conduction and acrylamide forms progressively over time. However, the acrylamide content in the inner part of the heated whole seed became 4-fold higher than in the outer part. At 180 °C/15 min, the acrylamide levels in whole chia seeds were 79 ± 2 μg/kg for the outer part and 292 ± 7 μg/kg for the inner part. The acrylamide content in the seed coat only represented 20–25 % of the total acrylamide content in whole seeds. Since the inner part of the seed contributes to a greater extent to the acrylamide concentration of the chia seed, the grinding of thermally treated chia seeds should be avoided. Thus, the use of chia seeds in novel food applications and extensions subjected to thermal treatment should consider the particle size and avoid grinding the product or adding ground seeds in order to reduce the exposure of acrylamide to consumers.

CRediT authorship contribution statement

Marta Mesías: Conceptualization, Methodology, Validation, Investigation, Resources, Data curation, Writing – original draft, Writing – review & editing, Visualization. **Pablo Gómez:** Investigation, Data curation. **Elena Olombrada:** Investigation, Data curation. **Francisco J. Morales:** Conceptualization, Methodology, Validation, Investigation, Resources, Resources, Data curation, Data curation, Visualization, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The authors do not have permission to share data.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2022.134169.

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