



# Risk/benefit considerations of a new formulation of wheat-based biscuit supplemented with different amounts of chia flour



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## ABSTRACT

The incorporation of Chia (*Salvia hispanica* L.) in the formulation of certain foods may be particularly desirable from a nutritional and healthy point of view. The effect of addition of chia flour on the nutritional properties and the formation of process contaminants in wheat flour-based biscuits was investigated. Higher percentage of chia flour in the formula increased the antioxidant capacity, phenolic compounds, protein, fiber and polyunsaturated fatty acids content, then resulting in a nutritionally enhanced product. However levels of process contaminants were also increased and thus acrylamide, hydroxymethylfurfural and furfural ranged between 151 and 1188 µg/kg, 22.8–71.4 mg/kg and 1.3–5.6 mg/kg, respectively, when chia was added in a range of 0–20% of the total weight. In parallel, the formation of dicarbonyl compounds, such as methylglyoxal and glyoxal, were significantly increased with addition of 5%. Lipid oxidation, particularly polymerization compounds, was accelerated in chia-enriched biscuits, which decreased the shelf-life of the product by promoting a rapid rancidity under accelerated storage conditions. Therefore, although nutritional properties are improved by the incorporation of chia into the biscuits, the increase in the content of process contaminants and the extent of the lipid oxidation should be carefully considered in a context of risk/benefit.

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## 1. Introduction

In the last decades the search for functional foods has been widely pursued by food companies. Consumers demand new food products not only to satisfy a physiological need but also to obtain necessary nutrients to prevent nutrition-related diseases and to improve physical and mental health. In this regard, a close link between nutrition and health has been established and functional foods containing ingredients with a specific health benefit are being developed technologically (Niva, 2007).

Chia (*Salvia hispanica* L.) is an oilseed plant used as foodstuff due to being a natural source of many nutrients. Chia seed contains significant amount of protein, fiber, vitamins, minerals and other constituents including phytoestrogens and antioxidants, such as tocopherols and phenolic compounds. In addition, chia seed stands out because of its high content of polyunsaturated fatty acids (PUFA), especially  $\alpha$ -linolenic acid. Due to its nutritional value and chemical composition, different medicinal properties have been

attributed to the chia seed and it has been considered as a new functional ingredient (Reyes-Caudillo, Tecante, & Valdivia-López, 2008). Therefore, its incorporation in the formulation of certain foods may be particularly desirable from a nutritional and healthy point of view.

Following the specifications of the European Commission (EC), chia seed has been approved to be used as a novel food ingredient in baked products but in amounts no more than 10%, since there are uncertainties with respect to its potential allergenicity (EC, 2013). However, changes in the formulation may affect, in addition to the rheological, technological and sensory parameters, the formation of process contaminants, such as acrylamide, hydroxymethylfurfural (HMF) or furfural when foods are thermally treated. Basically, these contaminants are produced by the Maillard reaction initiated by carbohydrates but also by carbonyls generated from the lipid oxidation (Zamora & Hidalgo, 2011). Acrylamide is generated as the result of the reaction between asparagine and reducing sugars as the main precursors. Recently, the European Food Safety Agency has confirmed that the presence of acrylamide in food is a public health concern, requiring continued efforts to reduce its exposure (EFSA, 2015). HMF and furfural are formed as intermediate products of the Maillard reaction and furthermore, HMF is also generated by

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the caramelization of sugars at high temperature (Morales, 2008). Based on studies in animals, HMF is suspected to have potential genotoxic and mutagenic effects through its metabolism product sulfoxymethylfurfural (Høie et al., 2015) whereas furfural may lead to hepatotoxicity (EFSA, 2005).

The purpose of this study was to investigate the effect of incorporating different amounts of chia flour in wheat-based biscuits on the nutritional properties, antioxidant content and the formation of acrylamide, HMF and furfural, assessing the risk/benefit of these new formulations. The oxidative stability in the biscuits after storage was also evaluated.

## 2. Material and methods

### 2.1. Reagents and chemicals

Chia flour was supplied by Harinas Polo (Zaragoza, Spain). Hard wheat flour (12.2% protein) and other food-grade ingredients were purchased from local supermarkets. HPLC-grade methanol was from Merck (Darmstadt, Germany). All chemicals used were obtained from Sigma Aldrich (St. Louis, MO, USA). All chemicals, solvents and reagents were of analytical grade.

### 2.2. Preparation of biscuits

Both control and chia-enriched biscuits were prepared according to a recipe described in AACC (American Association of Cereal Chemists) method 10–54 (AACC, 2000) following the procedure described by Mesías, Holgado, Márquez-Ruiz and Morales (2015a). Control biscuit was formulated with wheat flour (130 g), sucrose (35 g), distilled water (30 g), sunflower oil (26 g), sodium bicarbonate (0.8 g), ammonium bicarbonate (0.4 g) and salt (1 g). Four different biscuits were formulated replacing wheat flour by chia flour so as to achieve percentages of chia in the final weight of 5% (sample A), 10% (sample B), 15% (sample C) and 20% (sample D). The final amount of solids in the dough remained the same. The ingredients were thoroughly mixed and the dough was rolled out to disks with the diameter of 6 cm and the thickness of 2 mm, and baked at 190 °C for 20 min in a conventional oven (Memmert UNE 400, Germany). Twelve biscuits per batch and two batches per formulation were prepared. Three biscuits per batch were grinded and mixed and analytical determinations were performed in duplicate for each mixture, thereby obtaining two different values from the two batches corresponding to six different biscuits per formulation. For the determination of hardness two biscuits per batch for each formulation were used. For the storage assay, nine biscuits per formulation were randomly selected and three of the biscuits were taken after 30, 60 and 90 days.

### 2.3. Determination of moisture and water activity (*A<sub>w</sub>*)

Moisture was determined gravimetrically to constant weight in an oven at 105 °C for 24 h according to the AOAC (1999) method. The water activity of biscuits was measured at 25 °C by an AquaLAB CX-2 (Decagon Devices Inc., Pullman, WA).

### 2.4. Measurement of pH

Biscuits (1 g) were mixed with 100 mL of water and vortexed for 3 min. The mixture was held at room temperature for 1 h and centrifuged to separate phases. pH of the supernatant was measured using a CG-837 pH meter (Schott, Mainz, Germany).

### 2.5. Determination of colour

The measurements were made using a HunterLab Spectrophotometer CM-3500D colorimeter (Hunter Associates laboratory, Stamford, Connecticut, USA). Three independent measurements of *a*\*(redness), *b*\*(yellowness) and *L*\*(lightness) parameters were carried out on different areas of the biscuit samples. *E* index was calculated according to the following equation:  $E = (L^2 + a^2 + b^2)^{1/2}$  (Morales & Jiménez-Pérez, 2001).

### 2.6. Determination of hardness

The hardness of the biscuits was evaluated using the Texture Analyzer TA–TXPlus (Texture Technologies Corporation, USA) equipped with a 50 kg load cell, a probe (Warner–Bratzler, HDP/BSK knife model) with a compression speed at 1 mm/s and a distance prolongation of 10 mm. The force at the first major drop in the force–deformation curve (*F*<sub>max</sub>) and deformation at maximum force were obtained for 4 replicates per sample. The results of hardness were expressed as N (Newton).

### 2.7. Determination of water retention capacity

Flours (5 g) were placed in a pre-weighed centrifuge tube to which 30 mL of water was added. The mixture was vortex for 1 min, held at room temperature for 30 min and centrifuged at 1400 g for 15 min. The non-absorbed water was discharged and the tube was weighed. Water retention capacity was calculated by the following formula: [(weight of tube with sample and water retained - weight of tube with sample)/(weight of water added)] × 100.

### 2.8. Determination of total protein content

Total protein content was determined using an automated nitrogen analyzer (FP-2000; Dumas Leco Corp., St. Joseph, MI). The nitrogen-to-protein conversion factor was  $N \times 5.7$  according to the AOAC (1999) indications. The results were expressed as g protein/100 g sample.

### 2.9. Determination of total fat content by Soxhlet extraction

Total fat content was determined by Soxhlet extraction (Soxtec System HT6, Tecator AB, Höganäs, Sweden), according to the AOAC (1999) method using petroleum ether. The results were expressed as g lipid/100 g sample.

### 2.10. Determination of carbohydrates

The total carbohydrate content was determined using the method described by Dubois, Gilles, Hamilton, Rebers, and Smith (1956). A calibration plot was drawn using a standard glucose solution in the range of 0.25–2.0 mg/mL. Results were expressed as g glucose equivalents/100 g sample.

### 2.11. Determination of reducing sugars

The reducing sugars content was determined by Miller (1959) in the range of 0.25–2.0 mg/mL. Results were expressed as g glucose equivalents/100 g sample.

### 2.12. Determination of total dietary fiber

Total dietary fiber was determined by an enzymatic–gravimetric method based on the AOAC methods 991.43 and 985.29 (Lee, Prosky, & De Vries, 1992; Prosky, Asp, Schweizer, Devries, & Furda,

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