Justo Javier Pedroche Jiménez
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(coords.)

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(Salvia hispanica L.)

THE OLD FOOD OF THE FUTURE

(CIRCHIA2016)

Based on presentations made at the II International Conference of the Chía-Link Network held at the Instituto de la Grasa from october 5 to 7, 2016

EDITORIAL UNIVERSIDAD DE SEVILLA

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NUTRITIONAL AND FUNCTIONAL ASSESSMENT OF CONTRIBUTION OF CHIA BY-PRODUCTS AS FOOD INGREDIENT IN BAKERY PRODUCTS. PART II: BREAD QUALITY, FIBRE ADEQUATE INTAKE AND INHIBITION OF ENZYMES

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SUMMARY: The technological and biological properties of chia as ingredient should be validated in the development of functional foods. The objective was to evaluate the technological and functional potential of chia seeds as food ingredient in bakery products. The chia ingredients (whole and semi-defatted chia flours) were added at 5, 10 and 20% on flour basis to the bread dough formula, and technological and biological properties were evaluated in the products. The inclusion of chia by-products in the bread produced significant changes in the technological parameters, and the biological activity registered suggest their use as functional food.

Keywords: Noncommunicable diseases, chia, technological quality, dietary fibre, functional food.

RESUMEN: *Evaluación nutritiva y funcional de los subproductos de la chía como ingredientes alimentarios en productos de panadería. Parte II: Calidad del pan, ingesta adecuada de fibra dietética e inhibición de enzimas.* Las propiedades tecnológicas y biológicas de la chía como ingrediente deben ser validadas en la elaboración de alimentos funcionales. El objetivo fue evaluar el potencial tecnológico y funcional de las semillas de chía

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como ingrediente alimentario en productos de panadería. La chía (harina de chía entera y semi-desgrasada) se añadió a 5, 10 y 20% sobre la harina base de la masa fórmula del pan, y las propiedades tecnológicas y biológicas se evaluaron en los productos. La inclusión de subproductos de chía en el pan produjo cambios significativos en los parámetros tecnológicos, y la actividad biológica registrada sugiere su uso como alimento funcional.

Palabras clave: Enfermedad crónica no transmisible, chía, calidad tecnológica, fibra dietética, alimento funcional.

1. INTRODUCTION

Chia seeds are widely consumed by the many health benefits that have been attributed to it consumption, especially to its ability to maintain appropriate levels of blood lipids. Although the presence of active molecules in chia seeds guarantee their health benefit, the safety and efficacy of this medicinal food or natural product needs to be validated by scientific research (Haros et al., 2014). The International Life Science lnstitute (ILSI) establishes that a food can be considered as functional if it can be proved that has a beneficial effect in one or more specific functions in the organism improves wellness and health or is capable to reduces the risk of illness (Herrera et al., 2014). The objective of this study was to evaluate the technological and functional potential of chia flours (whole and defatted) as food ingredients in bakery products.

2. MATERIALS AND METHODS

2.1. Materials

Commercial Spanish wheat flour was purchased from the local market. Chia whole flour and semi-defatted chia flour were kindly provided from the Primaria Premium Raw Materials Company (Valencia, Spain) (Fernandez-Espinar *et al.*, 2016). Compressed yeast (*Saccharomyces cerevisiae*, Levital, Spain) was used as a starter for the breadmaking process.

2.2. Bread production

The control bread dough formula consisted on wheat flour (450 g), compressed yeast (2.5% flor basis), sodium salt (1.6% flor basis) and water (261 mL). The whole or defatted chia flour was added at 5, 10 and 20% on flour basis to the bread dough formula. Breads production was performed in a bread-maker Severin 3989. Breads were obtained by duplicate.

2.3. Total dietary fibre determination

The dietary fibre content was measured by the total dietary fibre assay procedure (AOAC, 1991).

2.4. Technological parameters

The technological parameters analysed were: weight of a loaf of bread (g), height of the loaf piece (cm), moisture content (%) and crumb firmness, determined by a texture profile analysis using the TA.XT Plus Texture Analyser (Stable Micro Systems, Godalming, UK) (Sanz-Penella *et al.*, 2009). Each parameter was measured at least in triplicate. The tristimulus colour parameters L* (lightness), a* (redness to greenness) and b* (yellowness to blueness) of the baked loaves (crumb and crust) were determined using a digital colorimeter (Chroma Meter CR-400, Konika Minolta Sensing, Japan), previously calibrated with the white plate supplied by the manufacturer. The instrument settings were: illuminant C, display L* a* b*, and observer angle 10°. Each sample was measured 18 times at different points to minimize the heterogeneity produced by the chia ingredients.

2.5. ACE inhibitory activity

Angiotensin I-converting enzyme inhibitory activity in the breads was analyzed following Hayakari *et al.* (1978). Hippuryl-L-histidyl-L-leucine (HHL) was hydrolyzed by ACE to yield hippuric acid and histidyl-leucine. This method relies on the colorimetric reaction of hippuric acid with 2,4,6-trichloro-s-triazine (TT) in a 0.5 mL incubation mixture containing 40 µmol potassium phosphate buffer (pH 8.3), 300 µmol sodium chloride, 40 µmol 3% HHL in potassium phosphate buffer (pH 8.3) and 100 mU/mL ACE. The mixture was incubated at 37 °C for 45 min and the reaction terminated by adding TT (3% v/v) in dioxane and 3 mL of 0.2 M potassium phosphate buffer (pH 8.3). After centrifuging the reaction mixture at 10000 *g* for 10 min, enzymatic activity was determined in the supernatant by measuring absorbance at 382 nm. ACE inhibitory activity percentage was calculated as follows: ACE inhibitory activity (%) = (A-B/A-C) x 100. Where A represents absorbance in the presence of ACE and sample, B is absorbance of control and C is absorbance of the reaction blank. Sample concentration was 30 mg/mL.

2.6. *In vitro* alpha amylase inhibitory assay

The assay was carried out following the protocol reported by Dineshkumar *et al.* (2010). Starch (2 mg) was suspended in a tube containing 0.2 mL of 0.5 M Tris-HCl buffer (pH 6.9) with 0.01 M calcium chloride (substrate). The tube was boiled for 5 min and then pre-incubated at 37 °C for 5 min. Breads (10 mg) were dissolved with 1 mL of 0.1% of dimethyl sulfoxide in order to obtain a concentration of 10 mg/mL. Then 0.2 mL of aqueous extracts was added in the tube containing the substrate solution. Then, 0.1 mL of porcine pancreatic amylase in Tris-HCl buffer (2 U/mL) were added to the tube containing the aqueous extract and substrate solution. The process was carried out at 37 °C for 10 min. The reaction was stopped to add 0.5 mL of acetic acid (50% v/v). The reaction mixture was then centrifuged at 3000 rpm for 5 min at 4 °C. The absorbance was measured at 595 nm. The assay was performed in triplicate. The a-amylase inhibitory activity was calculated as follows:

The alpha-amylase inhibitory activity = (Ac+) - (Ac-) - (As-Ab)/(Ac+) - (Ac-) x 100.

Where, Ac+, Ac-, As and Ab are defined as the absorbance of 100% enzyme activity (only solvent with enzyme), 0% enzyme activity (only solvent without enzyme), a test sample (with enzyme) and a blank (a test sample without enzyme), respectively.

2.7. Statistical analysis

Multiple sample comparison of the means and Fisher's least significant differences (LSD) were applied to establish significant statistical differences between treatments. All statistical analyses were carried out with the Statgraphics Plus 7.1 software (Bitstream, Cambridge, MN) and differences were considered significant at $P<0.05$.

3. RESULTS AND DISCUSSION

3.1. Evaluation of bread

A decrease in the bread quality was observed by the raise of chia flours proportion in the bread formula. Chia by-products produced significant changes in the loaf weight, in the height of the loaf piece and in crumb firmness comparing to the control sample (Table 1). Opposite behavior was found by Iglesias-Puig and Haros (2013), who prepared bread with similar formulations but sponge process. These changes could be due to the lower proportion of gluten in breads with chia in spite of the presence of mucilage in chia flours in combination with a long fermentation of the current work. Furthermore, during fermentation acting amylolytic or proteolytic enzymes, leading to an impact on structure-forming components like gluten and starch. A weaker gluten network might result in breads with a worse technological quality.

The colour parameters were significantly affected by the increase of whole and defatted chia flours. As was expected, samples with higher chia flour amount showed increased darkness (lower L*) and redness, and lowered yellowness (Table 1 and Fig. 1). The total colour difference between control sample and bread with chia, ΔE, was higher than five units, indicating that significant differences are perceptible to consumers by visual observation (data not shown).

3.2. Dietary Fibre and contribution to adequate dietary intake

The incorporation of chia flours in the formulation, gradually and significantly increased the total dietary fibre (Table 2) resulting in breads with soluble/insoluble fibre ratios closer to the recommended ratio value of 1:3 (Salas-Salvadó *et al.*, 2007). Table 2 also shows the adequate intakes (AIs) for dietary fibre given by the Food and Nutrition Board of the Institute of Medicine, National Academy of Science (NAS, 2005), taking into account the World Health Organization´s recommendation of a consumption of 250 g of bread per day. For example, the substitution of 20% of wheat flour by chia flour contributed to an increase in the intakes of total dietary fibre, reaching values of 68 to 71% for men and 97 to 103% for women of AIs. Furthermore, mucilage is the main dietary fibre polysaccharides in chia, which could provide a positive influence on the post-prandial glycaemic response (Haros *et al.*, 2014). This fact, could contribute to lowering the glycaemic index after the intake of these bread products.

3.3. Angiotensin Converting Enzyme (ACE) Inhibitory activity

In relation to ACE inhibitory activity of chia products, it was observed that those products with the lowest percentage of chia incorporation showed greater inhibition of ACE; as well as statistical difference between all chia products. For both cases, chia defatted flour and whole chia flour 5% exhibited the highest inhibition percentage (57.81 and 65.32 %, respectively). Orona et al. (2015) and Segura et al. (2013) reported lower percentages of inhibition in chia peptide fractions, to those found in the present study, this could be a cause of synergy between the compounds that make up the product. Meanwhile, since the defatted products had lower inhibitory activity, it could be assumed that this process discourages biological activity.

ACE is an enzyme that acts on the renin-angiotensin-aldosterone system which regulates the cardiovascular hemodynamics and electrolyte balance in body fluids, its inhibition in patients with hypertension has been a widely studied treatment, therefore, the role playing by chia seed in this mechanism, it is a turning point for use in the treatment and prevention of diseases such as hypertension.

3.4. Alpha amylase inhibitory assay

Currently, there is significant interest in plant-based medicines and functional foods that modulate the physiological effects in the inhibition of α-glucosidase and α-amylase. In this essay, chia flour at 20% had the highest inhibitory effect (21.3%); we also found that defatted products had lower percentages of α-alpha amylase inhibition. As stated by Hui and Lei (2015), oilseeds are an important source of fat-soluble components with potential for inhibition of these enzymes, since its composition in polyunsaturated fatty acids contributes said biological effect. The above could be an explanation for which in this present study is observed a decrease of the biological effect in defatted flour.

4. CONCLUSIONS

The results registered a decrease in the technological bread quality that was observed by the raise of chia flours proportion in the tin bread formula comparing to the control sample. The incorporation of chia flours in the formulation, gradually and significantly increased the total dietary fibre for covering the AIs. The ACE inhibitory activity and alpha-amylase inhibition registered in the bread samples suggesting the use of them as functional foods in the prevention and treatment of NCDs.

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TABLES

Table 1. Effect of different amount of chia flours on technological parameters of bread[a.]

Parameter	Wheat flour %	Whole chia flour[b] %			Defatted chia flour[b] %		
	100	5	10	20	5	10	20
Technological parameters		15.2±1.1^{b}	15.1±1.4^{b}	15.9±1.2^{b}	14.2±2.1^{ab}	14.4±1.3^{ab}	12.6±0.2^{a}
Height, cm	16.4±0.7^{c}						
Loaf weight, g	634±24^{a}	638±29^{a}	652±13^{ab}	660±16^{ab}	665±20^{b}	669±21^{b}	668±19^{b}
Firmness, N	3.8±0.3^{a}	4.1±0.7^{a}	4.7±0.6^{b}	8.2±0.9^{c}	4.4±0.9^{ab}	4.6±0.9^{b}	8.1±0.2^{c}
Crust colour parameters[c]							
*L**	58±5^{a}	57±2^{b}	54±2^{b}	54±1^{b}	58±^{2}b	53±3^{b}	47.6±0.8^{a}
*a**	8±1^{b}	5.8±0.7^{a}	6±1^{ab}	5.0±0.3^{a}	5.8±1.5^{ab}	7±1^{ab}	6±2^{ab}
*b**	32±2^{c}	27±1^{b}	27±2^{b}	24.1±0.8^{a}	25±2^{a}	26±1^{ab}	22±2^{a}
Crumb colour parameters[e]							
*L**	71±2^{c}	60±1^{b}	58±1^{ab}	53±1^{a}	60±2^{b}	53±3^{a}	47±4^{a}
*a**	-1.9±0.2^{a}	0.2±0.1^{b}	0.2±0.0^{b}	1.1±0.1^{c}	1.4±0.2^{c}	2.6±0.3^{d}	3.7±0.4^{d}
*b**	15.6±0.8^{b}	14.4±0.2^{ab}	12.4±0.7^{a}	12.7±0.6^{a}	17.4±1.3^{c}	18.3±0.9^{d}	19.4±0.9^{d}

[a] Mean ± SD, *n* = 3; values followed by the same letter in the same column are not significantly different at 95% confidence level.
[b] Bread formulations with 5%, 10% and 20% of chia whole flour or defatted chia flour in flour basis.
[c] Colour parameters: L*(lightness), a*(redness-greenness) and b*(yellowness-blueness) values.

Table 2. Effect of bread formulation on dietary fibre content and contribution to adequate dietary intake.

Parameter[a]	Units[b]	Wheat flour %	Whole chia flour[c] %			Defatted chia flour[c] %		
		100	5	10	20	5	10	20
Total Dietary Fibre	g/100g d.m.	6.1 ± 0.8^{a}	9.7 ± 1.0^{b}	12.6 ± 0.9^{c}	18.4 ± 3.2^{cd}	8.7 ± 0.3^{b}	12.4 ± 0.5^{c}	18.2 ± 0.8^{d}
Soluble/Insoluble Fibre Ratio, 1:3[d]	g/g	1:3.6	1:3.2	1:3.1	1:3.4	1:2.7	1:2.9	1:2.9
AI[e], Contribution	% M/W	23/35	35/53	45/69	68/103	31/47	43/66	71/97

[a] Mean ± SD, *n* = 3; values followed by the same letter in the same column are not significantly different at 95% confidence level.
[b] Dry matter, d.m.
[c] Bread formulations with 5%, 10% and 20% of chia whole flour or defatted chia flour on flour basis.
[d] 1:3 ratio of soluble/insoluble fibre (Salas-Salvadó *et al.*, 2007).
[e] AI (adequate intake) contribution (%) for a daily average intake of 250 g of bread. AI in g per day for dietary fibre in man/woman is 38/25 (these values are AI for adults between 19 and 50 years; NAS, 2005).

FIGURE CAPTIONS

Figure 1. Effect of the inclusion of chia flours on central slice and crumb structure. Bread formulations: A. White Bread; B and B'. Bread with 5 g of whole or defatted chia flour/100 g; C and C'. Bread with 10 g of chia whole or defatted flour/100 g; D and D'. Bread with 20 g of whole or defatted chia flour/100 g, respectively.

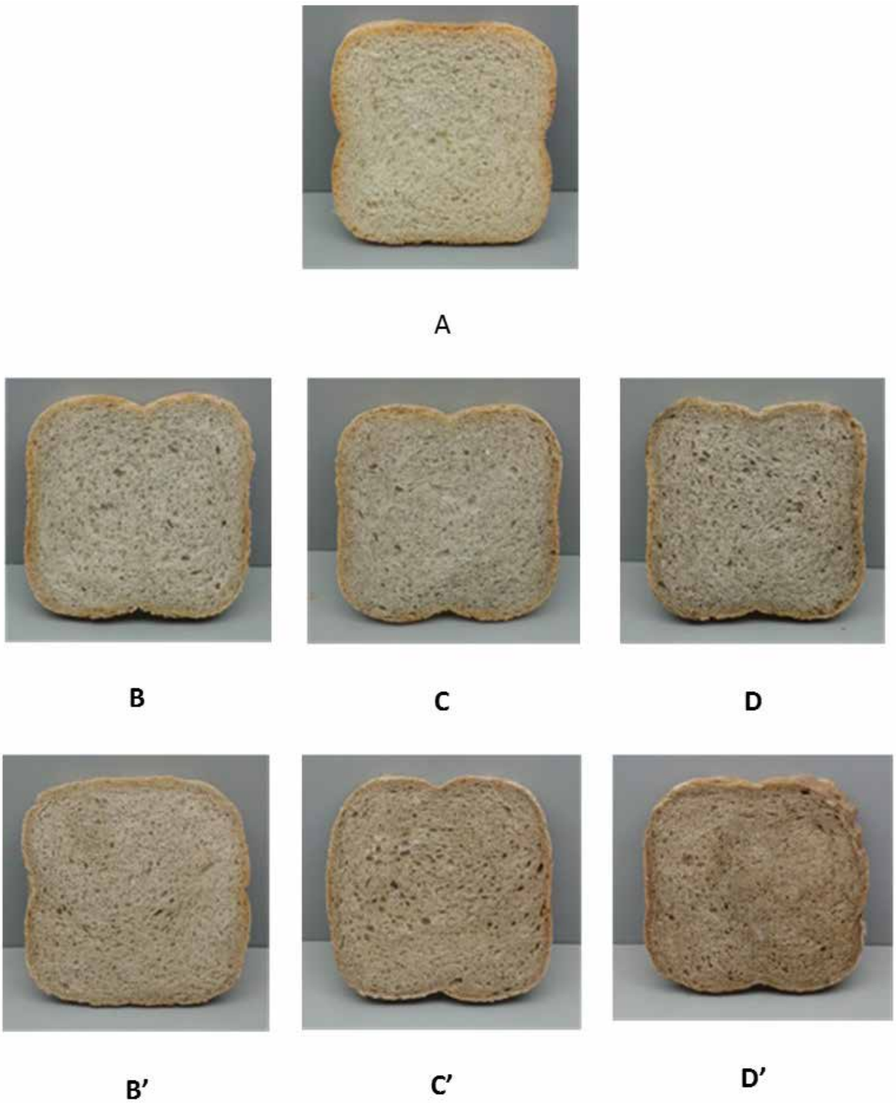


Figure 2. ACE inhibition of chia breads. Data are mean ± SD of three determinations. [a-d]Different superscript letters indicate statistical difference (*P*< 0.05).

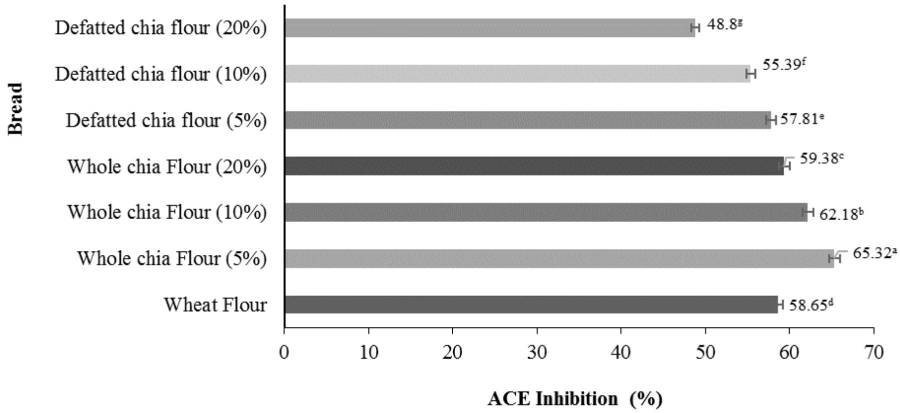
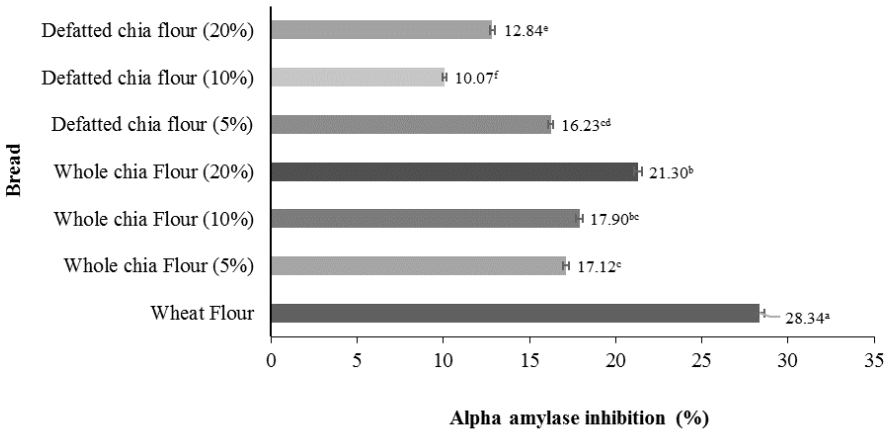


Figure 3. Inhibitory effect of breads on alpha-amylase activity. Data are mean ± SD of three determinations. [a-g]Different letters indicate statistical difference (*P*<0.05).



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