Justo Javier Pedroche Jiménez
Francisco Millán Rodríguez
(coords.)

CHÍA

(Salvia hispanica L.)

THE OLD FOOD OF THE FUTURE (CIRCHIA2016)

CHIA LINK
Red internacional

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

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PRÓLOGO

Este volumen recoge una colección de trabajos que tienen su origen en las conferencias plenarias y pósteres que se presentaron en la II Conferencia Internacional de la Red Chía-Link, celebrada en el Instituto de la Grasa (IG-CSIC, Sevilla, España) del 5 al 7 de Octubre de 2016 (CIRCHÍA-2016). Se trata de 30 trabajos que han sido agrupados en tres secciones: "Nuevos Alimentos", "Tecnología y Análisis" y "Alimentación y Salud". Reproducimos en formato resumen las aportaciones de algunos de los participantes en CIRCHÍA-2016 que no han podido contribuir a este volumen con un trabajo de extensión normal. Reflejando la variedad y riqueza de las aportaciones a CIRCHÍA-2016, este volumen ofrece una panorámica muy informativa y bastante completa de los temas tratados. Estos incluyen fundamentalmente la investigación básica, aplicada y tecnología sobre aceites, proteínas y coloides como componentes de los alimentos, así como la actividad biológica de proteínas alimentarias y otros componentes relacionados. No sólo es grande la variedad de temas tratados, sino también las disciplinas científicas desde las que los mismos han sido enfocados. Así, entre los autores se encuentran químicos, fisicoquímicos, biólogos, tecnólogos de alimentos, farmacéuticos, y gastrónomos, quienes utilizan la cocina como centro de creación artística para deleite de los sentidos, etc.

El congreso CIRCHÍA-2016 fue organizado conjuntamente por el Grupo de Cereales del Instituto de Agroquímica y Tecnología de Alimentos (IATA-CSIC) y el Grupo de Proteínas Vegetales del Instituto de la Grasa (IG-CSIC) en el marco de un proyecto Chía-Link 0923 denominado "Estudio físico-químico, nutricional y tecnológico de la contribución de subproductos de Chía (*Salvia hispanica*, L.) como nuevos ingredientes en Europa". Entidades públicas y privadas fueron patrocinadoras de la CIRCHÍA-2016: el Consejo Superior de Investigaciones Científicas (Instituto de Agroquímica y Tecnología de Alimentos e Instituto de la Grasa), la Universidad Pablo de Olavide de Sevilla, el Ministerio de Economía y Competitividad y la empresa Aceites 1881. A todos estos programas e instituciones les estamos muy agradecidos. También deseamos

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expresar nuestro agradecimiento a los autores de ambos lados del "charco" que con sus contribuciones han hecho posible la publicación de este libro, que esperamos sea de utilidad a los investigadores, tecnólogos e industriales interesados en productos derivados de la semilla Chía.

Sevilla, Octubre de 2016. Los editores.

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1.
NEW FOOD

2nd International Conference of Chia-Link Network

6-7 October, Seville - Spain

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NEW FOOD AND ASSESSMENT UNDER THE NOVEL FOOD REGULATION

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SUMMARY: The new food concept emerged in the seventies linked to the development by the food industry of new products and processes in response to a perceived shortage of food worldwide. This concept is also linked to developments in food security of the late twentieth century in order to provide a scientific basis for certain that a food is safe for use rightful and in accordance with the conditions expected consumptions. This background provided the basis for the development of specific legislation at European level, Regulation (EC) Nº 258/97 of the European Parliament and of the Council of 27 January 1997 concerning novel foods and novel food ingredients. Between 1997 and 2014 some 170 applications for approval were filed across the EU (between seven and ten applications per year). So far it has authorized the use of some ninety new foods. New food can be one that has been developed recently under new technologies or those from third countries whose consumption is unprecedented in Europe. Examples of new foods from third countries are chia seeds, new ingredients obtained by synthesis (synthetic zeaxanthin) or extracts obtained from existing foods (protein rapeseed). It was necessary to update the EU rules to reduce the current duration of the authorization procedure (whose average is three and a half years). Therefore, recently that regulation has been repealed and replaced by Regulation (EU) 2015/2283 of the European Parliament and of the Council of 25 November 2015 concerning novel foods. According to him, these foods must undergo a scientific assessment before authorization to ensure their safety. The scientific evaluation is now centralized in the EFSA authorization and then set its conditions of use, its designation as a food or food ingredient and labelling requirements. The new regulation specifies that engineered nanomaterials defined in the new law require novel food approval before being used in food products. Insects are also included in the definition of "novel food" as food ingredients derived from animals.

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Parts of insects (such as legs, wings and head) are also included in this definition. Finally also it regulates all matters relating to food from third countries and data protection.

Keywords: New foods, risk assessment, traditional foods.

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CHIA (SALVIA HISPANICA) THE GOLDEN CROP OF THE 21ST CENTURY

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SUMMARY: Chia popularity has been increasing for its nutraceutical compounds and high protein content. In this work, chia proteins and peptides, antioxidant activity, inhibitory activity against angiotensin converting enzyme (ACE) and phenolic compounds were analysed. Globulins were the main protein fraction. Globulin and albumin peptides inhibited in a similar manner the ACE; it was corroborated by peptide sequentiation. The main phenolic compounds were rosmarinic, protocatechuic, caffeic and galic acids. The results show that chia peptides could be an efficient alternative to treat hypertension; they also contain functional ingredients, which possess high antioxidant potential and thus remarkable benefits for human health.

Keywords: Bioactive peptides, phenolic compounds, protein fractions.

RESUMEN: ***Chía (Salvia hispanica) el cultivo dorado del siglo XXI***. La popularidad de la chía ha incrementado debido a sus componentes nutracéuticos y al alto contenido de proteínas. En este trabajo se analizaron proteínas y péptidos, capacidad antioxidante y actividad inhibitoria de la enzima convertidora de angiotensina (ECA), también los compuestos fenólicos. Las globulinas fueron la fracción proteica principal. Péptidos de globulinas y albúminas inhiben de igual forma la ECA, esto se corroboró por secuenciación. Los principales compuestos fenólicos fueron: ácidos rosmarínico,

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protocatecuico, cafeico y gálico. Los resultados muestran que los péptidos de chía son buena alternativa contra la hipertensión y se pueden considerar ingredientes con alto potencial antioxidante y por lo tanto de gran beneficio para la salud.

Palabras clave: Péptidos bioactivos, compuestos fenólicos, fracciones de proteínas.

1. INTRODUCTION

Salvia hispanica L., commonly known as chia, is an annual plant of the Lamiaceae family that grows in arid or semiarid climates. It is an ancient crop native from southern Mexico and Guatemala, and is considered a pseudocereal (Ayerza, 1995; Mohd Ali *et al*., 2012). Chia seeds, corn, beans and amaranth were some of the main crops for native pre-Columbian people (Ayerza & Coates, 2005). Mayans and Aztecs used it as a medicine and food supplement for energy, endurance and strength needed under extreme conditions (Bueno *et al*., 2010). Chia seeds have been cultivated in Mexico for thousands of years, but their popularity has been increased because is a good source of protein, unsaturated fatty acids, dietary fibre and high amounts of natural antioxidants such as phenolic compounds and bioactive peptides (Martínez-Cruz & Paredes-López, 2014; Olivos-Lugo *et al.*, 2010; Sandoval-Oliveros & Paredes-López, 2013; Vaźquez-Ovando *et al.*, 2010).

Great advances have been observed in medicine in the last decades relating the importance of plants as source of compounds for human health. Different peptides have been used to inhibit the activity of ACE to reduce the blood pressure in hypertensive individuals, and also for their antioxidant capacity. The anti-oxidative properties are also attributed to phenolic compounds; this capacity may offer protection against some disorders such atherosclerosis, stroke, diabetes, cancer and neurodegenerative diseases such as Alzheimer's disease and Parkinsonism (Segura-Campos, *et al*., 2013; Toscano *et al*., 2014).

Chia is used in Mexico for its nutritional and medicinal properties since ancient times; and the aim of this work was to analyse protein fractions and individual peptides for antihypertensive and antioxidant activities *in vitro*, and phenolic compounds and their nutraceutical potential.

2. MATERIALS AND METHODS

2.1. Sample preparation

The seeds were soaked in water during 2 h and mucilage was removed mechanically following Olivos-Lugo *et al.* (2010). Mucilage-free seeds were

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milled and passed through a 0.5 mm mesh; then was defatted with hexane (Vaźquez-Ovando *et al*., 2010).

2.2. Proximal composition, protein extraction, fractionation procedure, sedimentation coefficient and electrophoresis separation

Proximal analysis was according AOAC (1990). Extraction and fractionation of proteins was carried out according to the method reported by Barba de la Rosa *et al.* (1996). The protein content in each fraction was determined by the BCA (Pierce) method. Sedimentation coefficient of globulin fraction was layered onto a linear sucrose density gradient (5–20% in a pH 8 buffer of 50 mM Tris-HCl + 0.3 M NaCl) and centrifuged at 218000 *g* during 24h at 4 °C. Molecular size was determined by sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE). For two-dimensional gel electrophoresis (2D-PAGE) was used 2D clean-up kit, 7 cm IPG dry strip and were focused according to the manufacturer's instructions (GE Life Sciences, Sweden).

2.3. *In vitro* digestion of protein fraction

Protein digestion was based on Wang *et al.* (2008) method.

2.4. ACE inhibitory activity

The inhibitory effect of chia peptides against ACE was evaluated according to Luna-Suárez *et al.* (2010).

2.5. Phenolic extracts and compounds identification

The extraction and identification of phenolic compounds was performed according Martínez-Cruz & Paredes-López (2014).

2.6. Antioxidant activity

The scavenging activity of peptides and phenolic compounds against the DPPH radical was measured according to Martínez-Cruz & Paredes-López (2014), and against ABTS radical was performed according to the method of Zhou *et al.* (2012).

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3. RESULTS AND DISCUSSION

3.1. Proximal composition, protein extraction, fractionation procedure, sedimentation coefficient and electrophoresis separation

The values of all components of proximal composition are consistent with those reported by Ayerza & Coates (2005) and Reyes-Caudillo *et al.* (2008) (Table 1). The seeds contain low amounts of moisture, minerals and carbohydrates, as well as a large amount of total dietary fibre (33.5%), which is superior to traditional sources of fibre such as flaxseeds (22.3%), barley (17.3%), corn (13.4%), wheat (12.6%) and soybean (15%). The oil content was higher than other oilseeds of commercial importance, such as soybean and cottonseed and the protein content was similar to lentil or chickpea and higher than chan seed (14%) (Ayerza, 1995). The protein composition pattern shows some similarities with other important seeds such as peas, lupins and cotton (Table 2) (Nikokyris & Kandylis, 1997). The electrophoretic analysis by SDS-PAGE is presented in Fig. 1A. Albumins and globulins showed a large number of bands with a wide range of molecular sizes; these fractions are similar to globulins from soybeans (Liu *et al.*, 2007) and to oat albumins (Klose & Arendt, 2012). Prolamins were very difficult to identify due to the low resolution and glutelin fraction showed four bands around 20–30 kDa. Globulins were the major fraction, and the sedimentation profile revealed the presence of 11S as a major globulin, followed by 7S globulin; this pattern is similar to that observed in globulins of amaranth, sesame, barley and some other seeds. This result has been confirmed with two-dimensional gel electrophoresis in a pH range 3-10 (Fig. 1B). In this proteomic map, six proteins were observed with molecular sizes around 20-35 kDa with a range of pI between 4.5 to 7.0 (Casey *et al.*, 1993; Romero-Zepeda & Paredes-López, 1996).

3.2. *In vitro* digestion of protein fraction

Globulin fraction represents the highest concentration of peptides after gastrointestinal digestion, followed by albumin, prolamin and glutelin fractions. Electrophoretic pattern of the peptides showed an extensive hydrolysis of protein fractions, indicating that this method is appropriate to obtained chia peptides.

3.3. Angiotensin converting enzyme inhibitory activity

Albumin and globulin peptides presented similar inhibition patterns against ACE enzyme (377 and 339 g/mL, respectively); the same was observed with prolamins and glutelin, but less efficient (Fig. 2). Peptides from chia flour

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showed EC_{50} value of 516 mg/mL, producing better ACE inhibition than other seed flours such as canary seeds (Estrada-Salas *et al.*, 2014), soybean (Tsai, *et al.*, 2006), chickpea (Barbana & Boye, 2010) and wheat (Motoi & Kodama, 2003). These results shown that chia albumin and globulin fractions are the better source of peptides inhibitors against ACE.

3.4. Phenolic extracts and compounds identification

The amount of phenolic compounds in chia was 1.64 ± 0.21 mg GAE/g; 1.8-fold higher than those reported by Reyes-Caudillo *et al.* (2008) and 2.4-fold higher than the reported recently by Porras-Loaiza *et al.* (2013). The major compounds identified and quantified were: rosmarinic acid, protocatechuic ethyl ester, caffeic acid, gallic acid and the isoflavone daidzin (Table 3) (Martínez-Cruz & Paredes-López, 2014).

3.5. Antioxidant activity

Peptides from albumin and globulin showed the lowest EC_{50} values to inactivate the ABTS and DPPH (Fig. 3). Other crops with low molecular weight peptides (10 < kDa) exhibited less antioxidant activity than chia peptides, i.e., albumins and globulins of red beans and canola (Alashi *et al.*, 2014; Durak *et al.*, 2013). The antioxidant activities of chia peptides are produced by extensive hydrolysis, which results in the formation of shorter peptides that could hinder its ability as an electron donor (Siow & Gan, 2013). Also, chia is considered a seed with high antioxidant capacity due to its amount of phenolic compounds, like phenolic acids, isoflavones and anthocyanins.

4. CONCLUSIONS

Chia seeds are a good source of proteins with remarkable nutritional and nutraceutical compounds. Globulin was the major protein fraction and its peptides could be potential natural antioxidants and ACE inhibitors. On the other hand, chia seeds are very rich in phenolic compounds and isoflavones with a high antioxidant capacity, which confer health benefits. Its isoflavones may be used as a novel source of these nutraceutical to prevent various oestrogen-related disorders.

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REFERENCES

Alashi AM, Blanchard CL, Mailer RJ, Agboola SO, Mawson AJ, He R. 2014. Antioxidant properties of Australian canola meal protein hydrolysates. *Food Chem* **146**, 500-506. DOI: 10.1016/j.foodchem.2013.09.081.

Ayerza R. 1995. Oil content and fatty acid composition of oil of chia (*Salvia hispanica* L.) from five locations in northwestern Argentina. *J Am Oil Chem Soc* **72,** 1079-1081. DOI: 10.1007/BF02660727.

Ayerza R, Coates W. 2005. Ground chia seed and chia oil effects on plasma lipids and fatty acids in the rat. *Nutr Res* **25**, 995-1003. DOI: 10.1016/j.nutres.2005.09.013.

Association of Official Analytical Chemists (AOAC). Official Methods of Analysis, 15th ed.; Washington, DC, 1990.

Barba de la Rosa AP, Herrera A, Utsumi S, Paredes-Lop´ ez O. 1996. Molecular characterization, cloning and structural analysis of a cDNA encoding an amaranth globulin. *J Plant Physiol* **149**, 527-532. DOI: 10.1016/S0176-1617(96)80329-4.

Barbana C, Boye JI. 2010. Angiotensin I-converting enzyme inhibitory activity of chickpea and pea protein hydrolysates. *Food Res Intern* **43**, 1642-1649. DOI: 10.1016/j.foodres.2010.05.003.

Bueno M, Di Sapio O, Barolo M, Busilacchi H, Quiroga M, Severin C. 2010. Análisis de la calidad de los frutos de *Salvia hispanica* L. (Lamiaceae) comercializados en la ciudad de Rosario (Santa Fe, Argentina). *Bol Latinoam Caribe Plant Med Aromat* **9**, 221-227.

Casey R, Domoney C, Smith AM. 1993. Biochemistry and molecular biology of seed products. In *Peas: Genetics, Molecular Biology and Biotechnology*, CAB International pp. 121-163.

Durak A, Baraniak B, Jakubczyk A, Åswieca M. 2013. Biologically active peptides obtained by enzymatic hydrolysis of Adzuki bean seeds. *Food Chem* **141**, 2177-2183. DOI: 10.1016/j.foodchem.2013.05.012.

Estrada-Salas PA, Montero-Moran GM, Martínez-Cuevas PP, Gonzalez Barba de la Rosa AP. 2014. Characterization of antidiabetic and antihyper- tensive properties of canary seed (*Phalaris canariensis* L.) peptides. *J Agr Food Chem* **62**, 427-433. DOI: 10.1021/jf404539y.

Klose C, Arendt EK. 2012. Proteins in oats; their synthesis and changes during germination: a review. *Crit Rev Food Sci Nutr* **52**, 629-639. DOI: 10.1080/10408398.2010.504902.

Liu C, Wang H, Cui Z, Wang X, Zeng X. 2007. Optimization of extraction and isolation for 11S and 7S globulins of soybean seed storage protein. *Food Chem* **102**, 1310-1316. DOI: 10.1016/j.foodchem.2006.07.017.

Luna-Suárez S, Medina-Godoy S, Cruz-Hernández A, Paredes-López O. 2010. Modification of the amaranth 11S globulin storage protein to produce an inhibitory peptide of the angiotensin I converting enzyme, and its expression in Escherichia coli. *J Biotech* **148**, 240-247. DOI: 10.1016/j.jbiotec.2010.06.009.

Martínez-Cruz O, Paredes-López O. 2014. Phytochemical profile and nutraceutical potential of chia seeds (*Salvia hispanica* L.) by ultrahigh performance liquid chromatography. *J Chrom A*, **1346**, 43-48. DOI: 10.1016/j.chroma.2014.04.007.

Mohd Ali N, Yeap SK, Ho WY, Beh BK, Tan SW, Tan SG. 2012. The promising future of chia, Salvia hispanica L. *BioMed Res Intern* **9**. Article ID 171956. DOI: 10.1155/2012/171956.

Motoi H, Kodama T. 2003. Isolation and characterization of angiotensin I-converting enzyme inhibitory peptides from wheat gliadin hydrolysate. *Nahrung* **47**, 354-358. DOI: 10.1002/food.200390081.

Nikokyris PN, Kandylis K. 1997. Feed protein fractions in various solvents of ruminant feedstuffs. *J Sci Food Agr* **75**, 198–204. DOI: DOI: 10.1002/(SICI)1097-0010(199710)75:2<198:AID-JSFA863>3.0.CO;2-C.

Olivos-Lugo BL, Valdivia-Lop´ ez MA , Tecante A. 2010. Thermal and physicochemical porpieties and nutritional value of the protein fraction of Mexican chia seed (*Salvia hispanica* L.). *Food Sci Technol Int* **1**, 1–8. DOI: 10.1177/1082013209353087.

Porras-Loaiza MT, Jiménez-Munguía ME, Sosa-Morales E, Palou A, López-Malo P. 2013. Physical properties, chemical characterization and fatty acid composition of Mexican chia (*Salvia hispanica* L.) seeds. *Int J Food Sci Technol* **49**, 571-577. DOI: 10.1111/ijfs.12339.

Reyes-Caudillo E, Tecante A, Valdivia-Lop´ ez MA. 2008. Dietary fiber content and antioxidant activity of phenolic compounds present in Mexican chia (*Salvia hispanica* L.) seeds. *Food Chem* **107**, 656–663. DOI:10.1016/j.foodchem.2007.08.

Romero-Zepeda H, Paredes-Lop´ ez O. 1996. Isolation and characterization of amarantin, the 11S amaranth seed globulin. *J Food Biochem* **19**, 329-339. DOI: 10.1111/j.1745-4514.1995.tb00538.x.

Sandoval-Oliveros MR, Paredes-López O. 2013. Isolation and characterization of proteins from chia seeds (*Salvia hispanica* L.). *J Agr Food Chem* **61**, 193-201. DOI: 10.1021/jf3034978.

Segura-Campos MR, Peralta-González F, Chel-Guerrero L, Betancur-Ancona D. 2013. Angiotensin I-converting enzyme inhibitory peptides of chia (*Salvia hispanica*) produced by enzymatic hydrolysis. *Int J Food Sci* **2013**, 1-8. DOI: 10.1155/2013/158482.

Siow HL, Gan CY. 2013. Extraction of antioxidative and antihypertensive bioactive peptides from *Parkia speciosa* seeds. *Food Chem* **141**, 3435-3442. DOI: 10.1016/j.foodchem.2013.06.030.

Toscano LT, da Silva CSO, Toscano LT, de Almeida AEM, da Cruz-Santos A, Silva AS. 2014. Chia flour supplementation reduces blood pressure in hypertensive subjects. *Plant Food Hum Nutr* **69**, 392-398. DOI: 10.1007/s11130-014-0452-7.

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Tsai JS, Lin YS, Pan BS, Chen TJ. 2006. Antihypertensive peptides and´ -aminobutyric acid from prozyme 6 facilitated lactic acid bacteria fermentation of soymilk. *Process Biochem* **41**, 1282-1288. DOI: 10.1016/j.procbio.2005.12.026.

Vaźquez-Ovando A, Rosado-Rubio G, Chel-Guerrero L, Betancur-Ancona D. 2010. Dry processing of chia (*Salvia hispanica* L.) flour: chemical characterization of fiber and protein. *J Food* **8**, 117-127. DOI: 10.1080/19476330903223580.

Wang W, Bringe NA, Berhow MA, González de Mejia E. 2008.´ -conglycinins among sources of bioactives in hydrolysates of different soybean varieties that inhibit leukemia cells *in vitro*. *J Agr Food Chem* **56**, 4012-4020. DOI: 10.1021/jf8002009.

Zhou H, Chen X, Wang C, Ye J, Chen H. 2012. Purification and characterization of a novel ~18 kDa antioxidant protein from *Ginkgo biloba* seeds. *Molecules* **17**, 14778-14794. DOI: 10.3390/molecules171214778.

TABLES

Table 1. Proximate composition and dietary fibre of chia seed.

Component	g/100 g dry solids
Moisture	4.5 ± 0
Lipids	32.5 ± 2.7
Protein	22.7 ± 0.7
Ash	3.7 ± 0.3
Dietary fibre	
Soluble	8.2 ± 0.8
Insoluble	25.4 ± 2.2
Total	33.5 ± 2.7
Carbohydrates	3.1

Values are the mean ± SD of three determinations.

Table 2. Proportion of the protein fractions of chia seed.

Sample	g/100 g protein
Albumins	3.3 - 18.6
Globulins	52 - 54
Prolamins	7.2 - 12.7
Glutelins	6.4 - 14.5

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Table 3. Phenolic acids and isoflavone content in chia (*Salvia hispanica L.*) samples.

Compound	mg/g seed
Gallic acid	0.0115 ± 0.0000
Caffeic acid	0.0274 ± 0.0011
Ferulic acid	T
Protocatechuic ethyl ester	0.7471 ± 0.0102
Rosmarinic acid	0.9267 ± 0.0187
Isoflavons	
Daidzin	0.0066 ± 0.0007
Glycitin	0.0014 ± 0.0007
Genistin	0.0034 ± 0.0009
Glycitein	0.0005 ± 0.0000
Genistein	0.0051 ± 0.0003

Values are the mean ± SD of three determinations.
T: traces.

FIGURE CAPTIONS

Figure 1. Chia seeds protein fractions.
A. SDS-PAGE, Alb = Albumins, Glb =Globulins, Pro = Prolamins, Glu = Glutelins. MW = Molecular Weight; B. 2D-PAGE of globulins.

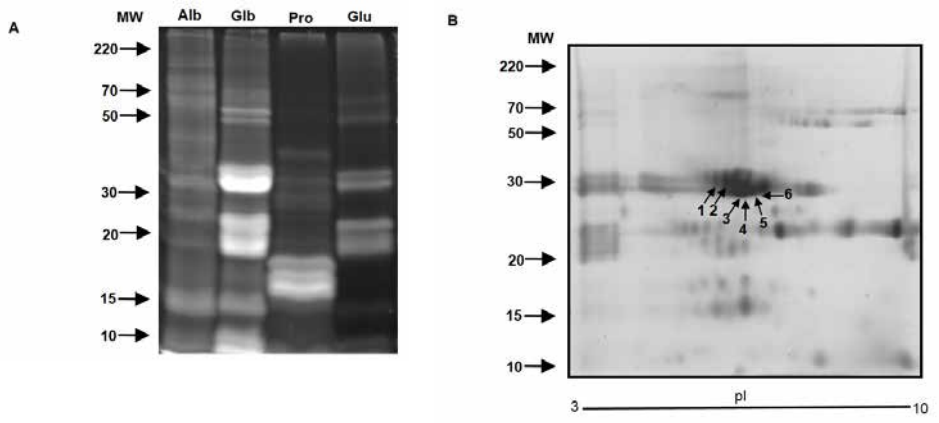

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Figure 2. Antihypertensive activities of chia peptides from protein fractions. The effective concentration inhibiting the 50 % of ACE enzyme (EC_{50}) was evaluated. Same letters mean no significant differences (P<0.05) in the same peptides fractions.

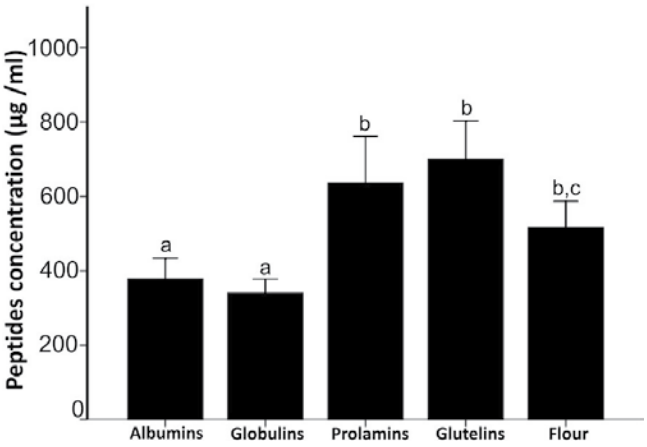
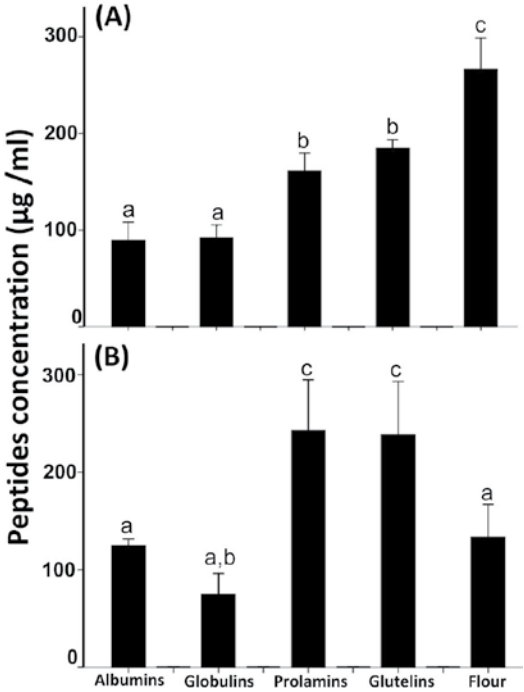


Figure 3. Free radical scavenging activity. A. ABTS; B. DPPH. Same letters mean no significant differences (P<0.05) in the same fractions.



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MECHANICAL AND OPTICAL PROPERTIES OF MUFFINS FORMULATED WITH CHIA SEEDS

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SUMMARY: Part of the traditional fat of muffins was replaced by 5% and 10% of chia seeds to evaluate the influence on their texture and colour. Hardness and gumminess significantly were lower in muffins without chia and with 10% of chia when resting time increased. Lightness, a* and b* coordinates and chroma of muffins significantly decreased with the percentage of chia, whereas hue only was higher when 10% of chia seed was added. The addition of chia seeds in muffins would be feasible since they did not imply changes in the texture of muffins although they slightly changed their colour.

Keywords: Chia seeds, colour, fat, muffins, texture.

RESUMEN: *Propiedades mecánicas y ópticas de magdalenas formuladas con semillas de chia*. Para evaluar su influencia en textura y color, parte de la grasa tradicional de magdalenas fue reemplazada por un 5% y 10% de semillas de chía. Dureza y gomosidad fueron significativamente menores, al aumentar el tiempo de reposo, en magdalenas sin chía y con 10% de chía. Luminosidad, a* y b* coordenadas y croma disminuyeron significativamente con el porcentaje de chía, mientras que el tono aumentó en magdalenas con un 10% de chía. La adición de semillas de chía en magdalenas sería factible ya que no implica cambios en su textura, aunque ligeramente varía su color.

Palabras clave: Color, grasa, magdalenas, semillas de chía, textura.

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1. INTRODUCTION

Chia (*Salvia hispanica* L.) is an annual plant of the family Lamiaceae. Chia seeds are composed of proteins (15-20 g/100 g), lipids (30-33 g/100 g), ash (4-5 g/100 g) and carbohydrates (26-41 g/100 g) and have a high fibre content (18-30 g/100 g). Furthermore, these seeds contain a large amount of antioxidants, minerals and vitamins (Ixtaina *et al.,* 2008; Iglesias-Puig and Haros, 2015).

Chia seeds can contain up to 68% omega-3 alpha-linolenic acid, thus they are among the plant sources with the highest contents of alpha-linoleic acid (Ayerza and Coates, 2011). They also contain 20% of omega-6 linoleic acid providing a good balance between the two essential fatty acids. For that, chia is a very interesting ingredient to supplement foods to design new healthy products in the sector of bakery with high content of fibre and omega-3 alpha-linolenic acid.

Thus, the aim of this work was to study the effects of incorporating different concentrations of chia seeds (0, 5 and 10%) as fat replacer in muffins considering three different resting times for the batter (0, 6 and 12 hours) on their mechanical and optical properties comparing to the traditional recipe of muffins.

2. MATERIALS AND METHODS

2.1. Components

Sunflower oil, sugar, milk, eggs, flour, ground almond, citric acid and sodium bicarbonate were blended following a recipe given by a traditional bakery. With the purpose of improve the fat profile of traditional muffins, part of the oil was replaced by chia seeds in 5 or 10%. Besides, batter remained at room temperature for 6 or 12 hours to assess this resting time on the properties of the final muffins. Therefore, three different formulations (M0, M5 and M10) were prepared using three different resting times of the batter (0, 6 and 12 hours).

2.2. Muffin preparation

All the ingredients were mixed with an electrical mixer (Kenwood, model KM240 series, UK) for 35 minutes at 4 rpm. Then, 65 g of the batter were put in paper moulds and baked at 145 ºC during 30 minutes in an electric oven. After baking, the muffins were cooled at room temperature before proceed to analysis. The batter and muffins were stored at 25 ºC with 40% of moisture.

For each formulation and resting time, 15 muffins were analysed after baking and after 7 days of storage.

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2.3. Determination of mechanical properties

The instrumental texture measurements were carried out using a TA.XT.plus Texture Analyser (Stable Microsystems, Godalming, UK). The muffins were cut in cylinders of 40 mm of diameter. A double compression test (TPA) was performed using a 40 mm diameter flat-ended cylindrical probe and compression to 40% of the initial height, at a speed of 1 mm/s with a 5 s waiting time between the two cycles. Values of hardness, springiness, cohesiveness and gumminess were obtained.

2.4. Determination of optical properties

The instrumental measurements of muffin crust and crumb were carried out with espectrocolourimeter (Konica Minolta, Inc., CM-3600d model, Tokyo, Japan). The results were expressed in accordance with the CIEL*a*b* system with reference to illuminant D65 and a visual angle of 10º.

2.5. Statistical analysis

Analysis of variance (ANOVA) was performed on the data using the Statgraphics Plus software (Statpoint Technologies, Inc., Centurion, Virginia, USA).

3. RESULTS AND DISCUSSION

3.1. Mechanical properties

Fig. 1 shows the results of hardness, cohesiveness, gumminess and springiness of muffins formulated with different amount of chia seeds and depending on the resting time of the batter before baking. According to the results of hardness and gumminess, there was a significant effect of the resting time in muffins without chia and with 10% of chia seeds, in which these mechanical parameters decreased with this time. After the storage time, this tendency was remained but muffins were tougher and with more gumminess as was expected.

Cohesiveness and springiness only were lower after storage time without significant differences between samples with and without chia and depending on resting time.

Therefore, the addition of chia seeds would be feasible from the mechanical point of view, since they did not imply changes in these parameters.

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3.2. Optical properties

Fig. 2 shows the chromatic planes b*-a* and the differences of the external colour of muffins at the beginning and after seven days of storage of muffins formulated with different amount of chia seeds and depending on the resting time of the batter before baking. The similar location of the b* and a* coordinates pointed out that no differences were found due to the resting time and the addition of chia and even for the storage time. Furthermore, the differences of colour were also similar in all cases.

Fig. 3 shows the chromatic planes b*-a* and the differences of the internal colour of muffins formulated with different amount of chia seeds after baking with 6 hours of resting time. There were significant decreases of lightness and a* and b* coordinates of the internal part of muffins when 10% of chia seed was used in the formulation of muffins. Consequently, chroma decreases proportionally to the percentage of the added chia, whereas hue only was higher when 10% of chia seed was added. Even though, the differences of internal colour were the same in all muffins.

4. CONCLUSIONS

The addition of chia seeds would be feasible from the mechanical point of view, since they did not imply changes in the texture of muffins. However, the highest level of chia seeds influenced colour parameters.

ACKNOWLEDGMENTS

The authors thank the Universitat Politècnica de València for providing their facilities.

REFERENCES

Ayerza R, Coates W. 2011. Protein content, oil content and fatty acid profiles as potential criteria to determine the origin of commercially grown chia. *Indl Crops Prod* **34,** 1366–1371. DOI: 10.1016/j.indcrop.2010.12.007.

Iglesias-Puig E, and Haros M. 2013. Evaluation of performance of dough and bread incorporating chia (Salvia hispanica L.). *Eur Food Res Technol* **237**, 865-874. DOI: 10.1007/s00217-013-2067-x.

Ixtaina VY, Nolasco SM, Tomás MC. 2008. Physical properties of chia (*Salvia hispanica* L.) seeds. *Ind Crops Prod* **28**, 286-293. DOI: 10.1016/j.indcrop.2008.03.009.

Sandoval-Oliveros MR, Peredes-López O. 2012. Isolation and characterization of proteins from chia seeds. *J Agr Food Chem* **61**, 193–201. DOI: 10.1021/jf3034978.

EFSA, 2009. Scientific opinion of the panel on dietetic products nutrition and allergies on a request from the European Commission on the safety of 'chia seed (*Salvia hispanica*) and ground whole chia seed' as a food ingredient. *EFSA J* **996**, 1-2.

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FIGURE CAPTIONS

Figure 1. Hardness, cohesiveness, gumminess and springiness of muffins formulated with different percentages of chia seeds (0, 5 and 10%) and depending on the resting time (0, 6 and 12 hours) of the batter before baking, initially and after 7 days of storage.

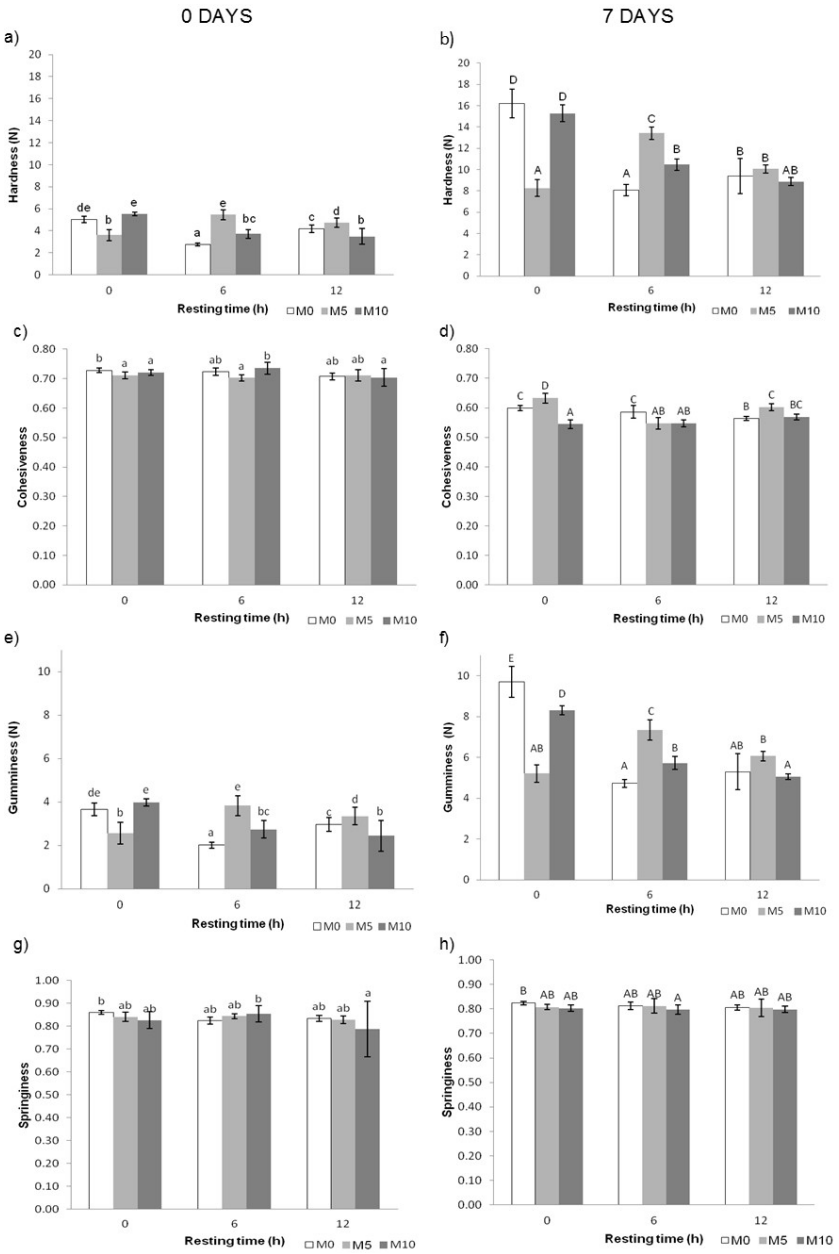

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Figure 2. The chromatic planes b*-a* (a and b) and L-a* (c and d), and the differences of colour (e and f) of the external colour of muffins formulated with different percentages of chia seeds (0, 5 and 10%) and depending on the resting time (0, 6 and 12 hours) of the batter before baking, initially (a, c and e) and after seven days of storage (b, d and f).

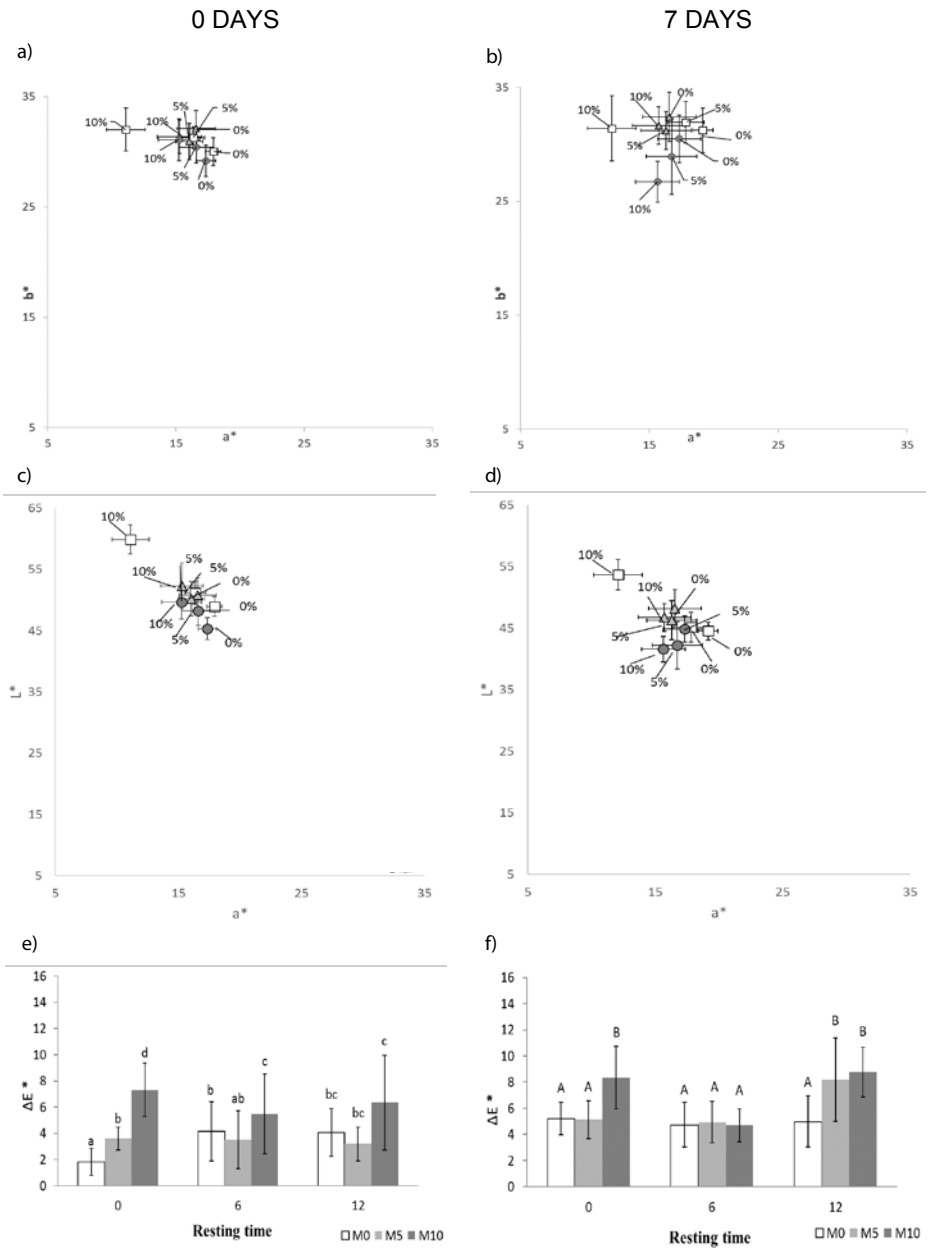

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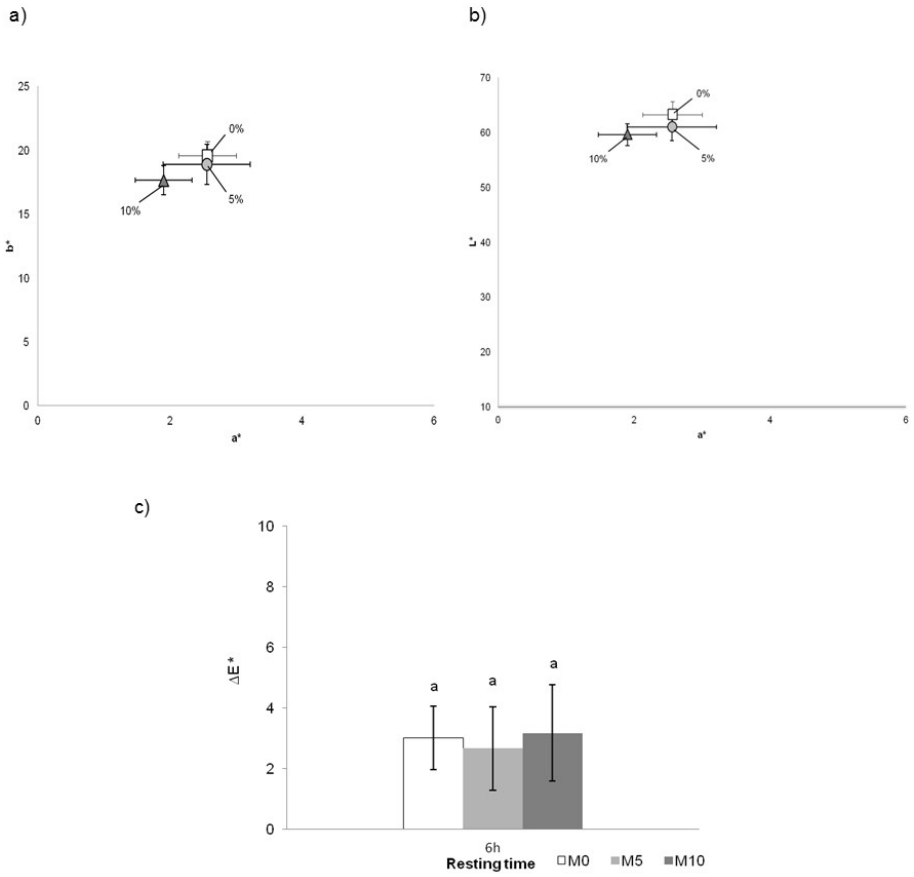
Figure 3. The chromatic planes b*-a*(a) and L-a* (b), and the differences of colour (c) of the internal colour of muffins formulated with different amount of chia seeds after baking with 6 hours of resting time.



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MASS LOSS, WATER ACTIVITY AND HEIGHT OF MUFFINS FORMULATED WITH CHIA SEEDS

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SUMMARY: Fat reduction and high quality of fat in foods are some of the major concerns of society. Chia might be an alternative to replace traditional fat since it has a good balance between omega-3 and omega-6 fatty acids. Thus, the aim of this work is to evaluate how the replacement of sunflower oil with 5 or 10% of chia seeds in muffins affects the mass loss during baking, the a_w and their height. Besides, different resting times of the batter were considered. Chia seeds could avoid the mass loss and they reduced a_w but muffin's height was lower.

Keywords: Chia, fat, height, mass loss, muffins, water activity.

RESUMEN: ***Pérdida de masa, actividad de agua y altura de magdalenas formuladas con semillas de chía***. La Chía puede ser una buena alternativa para reemplazar parcialmente la grasa tradicional ya que presenta un buen equilibrio entre los ácidos grasos omega-6 y omega-3. El objetivo de este trabajo consistió en evaluar como la sustitución de (5 -10 %) aceite de girasol por semillas de chía en magdalenas podría afectar a la pérdida de masa durante la cocción, la a_w y su altura. Además, se consideraron diferentes tiempos de reposo de la masa. La incorporación de chía en las magdalenas evita la pérdida de masa y reduce la a_w aunque la altura obtenida es menor.

Palabras clave: Chía, grasa, altura, pérdida de masa, magdalenas, actividad de agua.

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1. INTRODUCTION

Muffins are sweet, high-calorie baked products, which are highly appreciated by consumers due to their taste and soft texture. Fats improve tenderness, moistness and mouth feel in baked products (Brooker, 1996; Brooker, 1993). Fat reduction in food is one of the major concerns, as market demands increase for lower fat products. Because of this, substitution of fat by other ingredients is a great challenge for the food industry, especially to bakery products, which can contain elevated levels of fat. Chia seeds can contain up to 68% omega-3 alpha-linolenic acid and around 20% of omega-6 linoleic acid, providing a good balance between the two essential fatty acids (Ayerza & Coates, 2011). Moreover, chia seeds contain all of the essential amino acids, in particular leucine, lysine, valine and isoleucine (Sandoval-Oliveros & Paredes-Lopez, 2012). Despite this high content of proteins, there is no evidence of any adverse effects or allergenicity caused by whole or ground chia seeds (EFSA, 2009).

Moreover, chia seeds are rich in dietary fibre (up to 30% of the total weight) and expel a natural exudate in aqueous solution, which is a branched polysaccharide. This exudate can absorb up to 10 times its weight in water, allowing for a slower absorption of sugar into the body (Lin et al., 1994; Muñoz et al., 2012).

The objective of this study was to assess how the replacement of a part of the traditional fat in muffins with chia seeds (5 and 10%) could affect the percentage of mass loss, the water activity and also the height after baking considering also different resting times of the batter (0, 6 and 12 hours).

2. MATERIALS AND METHODS

2.1. Muffin formulation and manufacture conditions

Muffins were developed following a recipe provided by a traditional bakery (M0) and replacing part of the sunflower oil with 5% (M5) and 10% (M10) of chia seeds. Besides, batter was left different resting times before baking (0, 6 and 12 hours). For each formulation and resting time a sample of 15 muffins was analysed after baking.

2.2. Percentage of mass loss after baking

Muffin weights before and after baking were registered in order to evaluate the percentage of mass loss after baking.

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2.3. Muffin height

For each sample, the distance from the highest part of the muffin to the bottom part was measured with a digital calliper.

2.4. Measurement of water activity

It was determined using an AquaLab water activity meter (Decagon Devices, Inc., model 4TE, Pullman, Washington, USA) at 25 ºC.

3. RESULTS AND DISCUSSION

Fig. 1 represents the percentage of mass loss after baking of muffins formulated with different levels of chia seeds and different resting time of the batter. The results show that in directly baked samples the addition of chia significantly reduced the mass loss, which could be related with the high water holding capacity of chia (Inglet et al., 2014; A Vázquez-Ovando et al., 2009). However, when the batter was to rest, muffins with chia had a higher mass loss than without chia, which provides evidence that structure of chia seeds can evolve during time losing its capacity to retain water. On the contrary, the longer the resting time the lower the mass loss in muffins without chia, although in previous works no significant differences were found between 0 and 24 hours of resting time, but significant lower values were observed after 4 hours of resting time of the batter (Ripoll et al., 2015).

Fig. 2 shows the results of water activity of muffins with different percentages of chia seeds and resting time of the batter. As can be seen, the incorporation of chia seeds significantly reduced the water activity in directly baked muffin, which is coherent with the lowest mass loss aforementioned, although no differences with the amount of chia was detected. This proves the fact that chia seeds have high water holding retention. Application of dietary fibre in sweet bakery products is associated with an increase in batter viscosity and reduced product volume caused by the high water binding capacity of fibres (Struck et al., 2015).

Nevertheless, no significant differences of a_w values were observed in the other muffins, so resting time seems not to affect this parameter initially. After one week of storage, only samples without chia seed showed changes in a_w depending on resting time, its value being lower when batter was directly baked and higher after 6 or 12 hours or resting time. Therefore, addition of chia seeds would be advisable to preserve a_w and, consequently, for stability of the product.

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In all cases, the replacement of fat by chia seeds implied a reduction of height (Fig. 3), despite what was found by another author (Iglesias-Puig and Haros, 2013) in bread, which increased the specific volume when chia was added. It is possible that the combination of egg proteins, milk and sugar with chia seeds counteracts this effect or that the yeast used to grow the dough of bread enhanced this effect. No clear influence of the resting time was found in samples without chia, although there was an increase in height after 12 hours in muffins with chia seeds compared to directly baked samples after mixing the components.

4. CONCLUSIONS

Chia seeds could contribute to avoid the mass loss during baking and they also could extend the shelf life of bakery products due to the reduction of water activity. However, the final height is lower than in conventional muffins.

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The authors thank the Universitat Politècnica de València for providing their facilities.

REFERENCES

Brooker BE. 1993. The stabilisation of air in foods containing fat - *a review. Food Struct* **12**, 115-122.

Brooker BE. 1996. The role of fat in the stabilisation of gas cell in bread dough. *J Cereal Sci* **24**, 187-198. DOI: 10.1006/jcrs.1996.0052

Coates, W. 2011. Protein content, oil content and fatty acid profiles as potential criteria to determine the origin of commercially grown chia (*Salvia hispanica* L.). *Ind Crops Prod* **34**, 1366-1371. DOI: 10.1016/j.indcrop.2010.12.007.

Sandoval-Oliveros MR, Peredes-López O. 2012. Isolation and characterization of proteins from chia seeds. *J Agr Food Chem* **61**, 193–201. DOI: 10.1021/jf3034978.

EFSA, 2009. Scientific opinion of the panel on dietetic products nutrition and allergies on a request from the European Commission on the safety of 'chia seed (*Salvia hispanica*) and ground whole chia seed' as a food ingredient. *EFSA J* **996**, 1–2.

Inglett GE, Chen D, Liu SX, Lee S. 2014. Pasting and rheological properties of oat products dry-blended with ground chia seeds. *LWT-Food Sci Tech* **55**, 148-156. DOI: 10.1016/j.lwt.2013.07.011.

ÍNDICE >>>

Iglesias-Puig E, Haros M. 2013. Evaluation of performance of dough and bread incorporating chia (*Salvia hispanica* L.). *Eur Food Res Technol* **237,** 865-874. DOI: 10.1007/s00217-013-2067-x.

Alfredo VO, Gabriel RR, Luis CG, David BA. 2009. Physicochemical properties of a fibrous fraction from chia (*Salvia hispanica* L.). *LWT-Food Sci Technol* **42,** 168-173. DOI: 10.1016/j.lwt.2008.05.012.

Struck S, Gundel L, Zahn S, Rohm H. 2016. Fiber enriched reduced sugar muffins made from iso-viscous batters. *LWT-Food Sci Technol* **65**, 32-38. DOI: 10.1016/j.lwt.2015.07.053.

Muñoz LA, Cobos A, Diaz O, Aguilera JM. 2012. Chia seeds: Microstructure, mucilage extraction and hydration. *J Food Eng* **108,** 216-224. DOI: 10.1016/j.jfoodeng.2011.06.037.

Lin KY, Daniel JR, Whistler RL. 1994. Structure of chia seed polysaccharide exudate. *Carbohydr. Polym* **23**, 13-18. DOI: 10.1016/0144-8617(94)90085-x.

Ripoll E, Rubio-Arraez S, Castelló ML, Ortolá, MD. 2015 Influence of the resting time and freezing on the rheology properties of dough and characterization of cakes. *III National & II International Student Congress of Food Science & Technology, pp.* 59-60.

FIGURE CAPTIONS

Figure 1. Percentage of mass loss of muffins formulated with different amount of chia seeds and depending on the resting time of the batter before baking. Equal letter in bars refer to homogeneous groups (P<0.05).

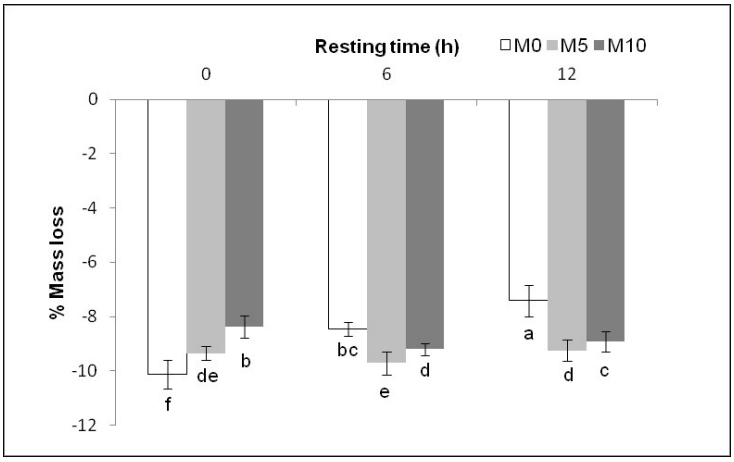


Figure 2. Water activity (a_w) of muffins formulated with different amount of chia seeds and depending on the resting time of the batter before baking. Equal letter in bars refer to homogeneous groups (P<0.05).

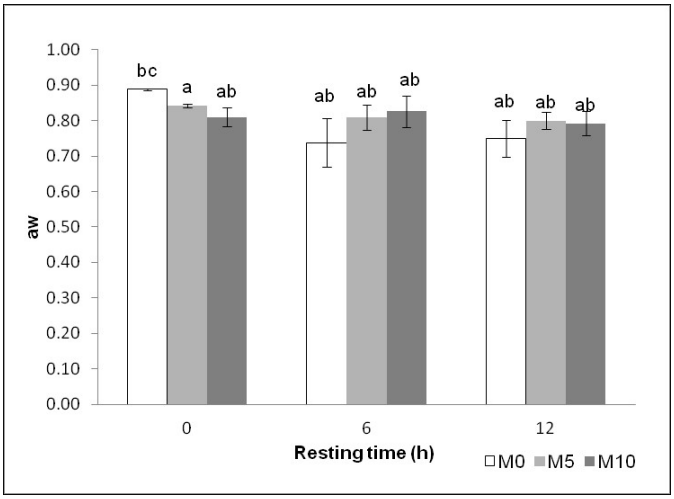
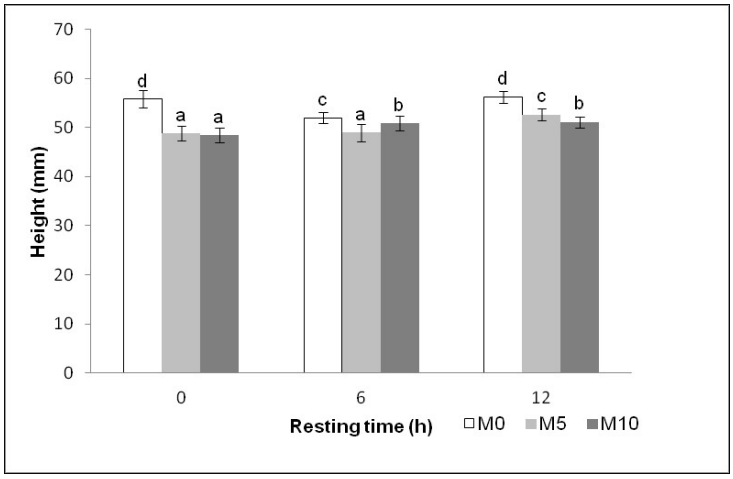


Figure 3. Height of muffins formulated with different amount of chia seeds and depending on the resting time of the batter before baking. Equal letter in bars refer to homogeneous groups (P<0.05).



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IN VITRO GLYCAEMIC INDEX AND MICROSTRUCTURE ANALYSIS OF BAKERY PRODUCTS WITH CHIA

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SUMMARY: It was evaluated the effect of the addition of chia by-products (seeds, whole and defatted chia flours) in bakery products on in vitro kinetics of starch hydrolysis. Micro-computed tomography scanner was used for microestructure determination of crumb bread. The higher lipid and fibre amounts in chia by-products could restrict enzymatic hydrolysis of starch in bakery products. They also impacted the microstructural organization inside of crumb, which could determine the bioaccessibility and/or bioavailability of glucose. The inclusion of chia ingredients in bread formulation could be an ideal strategy for reducing the glycaemic response in bakery products.

Keywords: Chia, bakery products, glycaemic index, µCT, food microstructure.

RESUMEN: ***índice glucémico in vitro y análisis de la microestructura de productos de panadería con chía.*** Se evaluó el efecto de la adición de subproductos de chía (semillas, harina integral y harinas desgrasadas) en productos de panadería sobre la cinética de la hidrólisis de almidón *in vitro*. Escáner de micro tomografía computarizada se utilizó para la determinación de la microestructura de miga de pan. Las mayor cantidad de lípidos y fibra en los subproductos de chía podrían restringir la hidrólisis enzimática del almidón en productos de panadería. Ellos también afectaron la organización microestructural en el interior de la miga, lo que podría determinar la bioaccesibilidad y/o biodisponibilidad de la glucosa. La inclusión de ingredientes de chía en la formulación de pan podría ser una estrategia ideal para la reducción de la respuesta glucémica en productos de panadería.

Palabras clave: Chía, productos de panadería, índice glucémico, µCT, microestructura de alimentos.

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1. INTRODUCTION

The last years the consumer demand for healthy, tasty and palatable food has been steadily growing. In this context, the introduction of new row materials and ingredients such as ancient Latin-American grains is a good alternative to offer at the consumer a variety in healthy meals.

The incorporation of chia seeds and its by-products in to the meals can be a good alternative to include a new source of proteins, lipids (mainly omega-3), fibre and antioxidants. One of the most important and interesting components is its dietary fibre; its consume can provide many health benefits such as regulation of intestinal transit, reduction of glycemic index and increased satiety, among others (Norlaily *et al.*, 2012, Muñoz; Cobos *et al.*, 2013). Some strategies for reducing the glycaemic response in bakery products are the use of whole grains as well as the addition of external parts of the kernel or sourdough fermentation (Steyn *et al.*, 2004). One of the most important aspects of chia seeds and flours is their high fibre content; its use has important benefits such as the regulation of intestinal transit, reduction in the glycaemic index and its corresponding insulin response, among others (Bushway *et al.*, 1981; Reyes-Caudillo *et al.*, 2008).

The food microstructure is connected with the bioaccessibility/bioavailability of nutrients. The use of non-destructive techniques is a helpful tool to know how the structure is correlated with the functionality of the food products, specifically bioaccessibility. In this sense, using X-ray micro-computed tomography (μCT) which provides a non-invasive, non-destructive and no sample preparation to the analysis, allows an evaluation of internal structural and microstructural features in food (Schoeman, Williams *et al.*, 2016).

The objective of this investigation was to assess the effect of the addition chia by-products in bread formulations on *in vitro* rate of starch digestion/GI and their connexion the bread microstructure.

2. MATERIALS AND METHODS

2.1. Materials

Commercial Spanish wheat flour was purchased from the local market. Chia seeds, whole chia flour, semi-defatted chia flour and low-fat chia flour products were purchased from the ChiaSA Company (Valencia, Spain). The characteristics of the raw materials were published by Iglesias-Puig and Haros (2013). Compressed yeast (*Saccharomyces cerevisiae*, Levamax, Spain) was used as a starter for the breadmaking process.

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2.2. Breadmaking process

The control bread dough formula consisted of wheat flour (500 g), compressed yeast (2.5% flour basis), sodium salt (1.6% flour basis) and distiller water (up to optimum absorption, 500 Brabender Units). The ingredients were mixed for 4 min, rested for 10 min, divided (100 g), kneaded and then rested (15 min). Doughs were manually sheeted and rolled, proofed (up to optimum volume increase, at 28 °C, 85% relative humidity) and baked at between 170-190 °C during 18-23 min, according to the formulation (Sanz-Penella *et al.*, 2009). The chia ingredients were added at 10% on flour basis to the bread dough formula, providing the following samples: bread with 10% of chia seeds (PS), bread with 10% of whole chia flour (PWF), bread with 10% of chia semi-defatted flour (PSD), bread with 10% of low-fat chia flour (PLF). Fermentation was monitored by measuring pH, temperature and volume increase of the dough at regular intervals. After the fermentation step, the doughs were baked in an electric oven and cooled at room temperature for 75 min for subsequent analysis (Sanz-Penella *et al.*, 2009).

2.3. *In vitro* starch digestion and GI estimation

To evaluate the *in vitro* rate of starch hydrolysis was employed the method described by Goñi *et al.* (1997) with slight modifications (Sanz-Penella *et al.*, 2014). The rate of starch digestion was expressed as the percentage of total starch hydrolysed at 0, 20, 40, 60, 90, 120 and 180 min. The total starch content was determined by the AOAC official method (1996). Finally, the area under the curve (AUC) from 0 to 120 min and total digestible starch was used to calculate an *in vitro* glycaemic index value normalised against white bread (SigmaPlot software, Version 12.0) expressed as a percentage.

2.4. Micro tomography analysis

The samples were scanned by using a SkyScan 1272 desktop µCT System (Bruker, Belgium). Power setting was selected at 50 kV and 100 µA obtaining a good contrast. The samples were fixed a specimen stage using ortho wax and a set of flat cross section 2-D projected images were acquired rotating 180°. The x-ray projected images were acquired using a digital CCD-camera cooled 11 Mp detector and reconstructed with Nrecon® software and later visualized and processed by CTVox® and CT Analyser® software to quantify internal microstructural details.

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3. RESULTS AND DISCUSSION

3.1. *In vitro* glycaemic index

In general, the glycaemic effect of foods depends on the food texture and particle size, type of starch, degree of starch gelatinization, physical entrapment of starch molecules within food, food processing and other ingredients (Pérez *et al.*, 2013). The samples formulated with chia seeds and whole chia flour showed the lowest rate of starch hydrolysis and provided a significant decrease ($P<0.05$) in the total hydrolysable starch amount of bread (Fig. 1, Table 1). Chia by-products supplementation in bread formulations produced a significant decrease ($P<0.05$) in GI, compared to the reference (Table 1). The GI largely depends on the starch granules' accessibility to starch-splitting enzymes. Non-starch polysaccharides and proteins bind to starch granules' surface layers; these lower the starch granules' vulnerability to enzymes (Schuchardt *et al.*, 2016). There are studies reporting that lipids delayed the appearance of exogenous glucose in blood (Englyst *et al.*, 2003). The possibility could be that starch–lipid complexes may have formed during the processing of cereal products, and this could restrict enzymatic hydrolysis (Biliaderis, 1991).

3.2. Micro CT analysis

Food microstructure impacts many physical properties such as textural, rheological and sensorial, and the structural organization inside the foods determine how will be the bioaccessibility and bioavailability of each one of its nutritional components. Fig. 2 represents the 3D images reconstruction of each sample. Fig. 3A shows the structure thickness distribution of the sample made with 10% of whole chia flour and Fig. 3B corresponds to the sample made with 10% of low fat chia flour. In both graphs a clear trend in porous distribution is observed. More and small porous were observed in the crumb bread made with chia seeds, while less porous between 10 and 1000 μm of diameter were observed in the sample made with whole chia flour (Table 2).

Table 2 shows the range structure thickness for all the samples. All the breads show the higher proportion in porous distribution between 10 and 1000 μm of diameter. The control and sample made with low fat flour show similar behaviour in porous distribution, while the sample made with whole chia flour presents the higher dispersion in porous size compared with the other samples showing a 30.9% of porous over 1000 mm of diameter.

4. CONCLUSIONS

The chia ingredients impact the microstructural organization inside the food, which could determine the bioaccessibility and/or bioavailability of nutrients. It

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seems that the higher lipids content in chia by-products and the higher amount of fibre could restrict enzymatic hydrolysis of starch. Further studies and human trials are needed to gain a better understanding of the potential influence of chia by-product as strategy for reducing the glycaemic response in bakery products.

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REFERENCES

AOAC Official Method 996.11. 1996. Starch (Total) in Cereal Products, Amyloglucosidase-α-Amylase Method. First Action.

Biliaderis CG. 1991. The structure and interactions of starch with food constituents. *Can J Physiol Pharmacol* **69**, 60–78. PMID: 2036603.

Bushway AA, Belyea PR, Bushway RJ. 1981. Chia seed as a source of oil, polysaccharide and protein. *J Food Sci* **46**, 1349–1350. DOI: 10.1111/j.1365-2621.1981.tb04171.x.

Englyst KN, Vinoy S, Englyst HN, Lang V. 2003. Glycaemic index of cereal products explained by their content of rapidly and slowly available glucose. *Brit J Nutr* **89**, 329–339. DOI: 10.1079/BJN2002786.

Goñi I, Garcia-Alonso A, Saura-Calixto F. 1997. A starch hydrolysis procedure to estimate glycemic index. *Nutr Res* **17**, 427-437. DOI: 10.1016/S0271-5317(97)00010-9.

Iglesias-Puig E, Haros M. 2013, Evaluation of dough and bread performance incorporating chia (*Salvia hispanica* L.). Eur Food Res Technol 237, 865-874. DOI: 10.1007/s00217-013-2067-x.

Norlaily MA, Swee KY, Wan YH, Boon KB, Sheau WT, Soon GT. 2012. The Promising Future of Chia, *Salvia hispanica* L. *J Biomed Biotechnol* **2012**, 1-9. DOI: 10.1155/2012/171956.

Muñoz LA, Cobos A, Diaz O, Aguilera JM. 2013. Chia Seed (*Salvia hispanica*): An Ancient Grain and a New Functional Food. *Food Rev Int* **29,** 394-408. DOI: 10.1080/87559129.2013.818014.

Pérez FR, Salazar-García MG, Romero-Baranzini AL, Rubio AR, Ramírez-Wong B. 2013. Estimated Glycemic Index and Dietary Fiber Content of Cookies Elaborated with Extruded Wheat Bran. *Plant Foods Hum Nutr* **68**, 52-56. DOI: 10.1007/s11130-013-0338-0.

Reyes-Caudillo E, Tecante A, Valdivia-Lopez MA. 2008. Dietary fibre content and antioxidant activity of phenolic compounds present in Mexican chia (*Salvia hispanica* L.) seeds. *Food Chem* **107**, 656–663. DOI: 10.1016/j.foodchem.2007.08.062.

Sanz-Penella JM, Tamayo-Ramos JA, Sanz Y, Haros M. 2009. Phytate reduction in bran-enriched bread by phytase-producing bifidobacteria. *J Agr Food Chem* **57**, 10239-10244. DOI: 10.1021/jf9023678.

ÍNDICE >>>

Sanz-Penella JM, Laparra M, Haros M. 2014. Impact of alpha-amylase during breadmaking in *in vitro* kinetics of starch hydrolysis and glycaemic index of enriched bread with bran. *Plant Foods Hum Nutr* **69**, 216-221. DOI: 10.1007/s11130-014-0436-7.

Schoeman L, Williams P, du Plessis A, Manley M. 2016. X-ray micro-computed tomography (μCT) for non-destructive characterisation of food microstructure. *Trends Food Sci Tech*, **47**, 10-24.

Schuchardt JP, Wonik J, Bindrich U, Heinemann M, Kohrs H, Schneider I, Möller K, Hahn A. 2016. Glycemic index and microstructure analysis of a newly developed fiber enriched cookie. *Food Funct* **7**, 464-474. DOI: 10.1039/C5FO01137J.

Steyn NP, Mann J, Bennett PH, Temple N, Zimmet P, Tuomilehto J, Lindström J, Louheranta A. 2004. Diet, nutrition and the prevention of type 2 diabetes. *Public Health Nutr* **7**, 147–165. DOI: 10.1079/PHN2003586.

van Dalen, G, Nootenboomp P, Van Vliet LJ, VoortmanL, Esveld E. 2007. 3-D imaging, analysis and modelling of porous cereal products using x-ray microtomography. *Image Anal Stereol* **26**, 169-177. DOI: 10.5566/ias.v26.p169-177.

TABLES

Table 1. Effect of chia by-products on glycaemic index.

Formulation	Total starch %	GI
Control	76.6 ± 0.7^{b}	100 ± 2^{c}
Seeds	70.1 ± 0.9^{a}	75 ± 2^{a}
Whole Flour	70.6 ± 0.7^{a}	79 ± 5^{ab}
Semi-Defatted Flour	79.9 ± 1.0^{c}	88 ± 4^{b}
Low Fat Flour	78.8 ± 1.2^{bc}	92 ± 5^{b}

Mean ± SD, $n = 3$. Values followed by the same letter in the same column are not significantly different at 95 % confidence level; GI glycaemic index.

Table 2. Range structure thickness, %.

Bakery products With 10% of chia	10<1000	1000<2000	Diameter μm 2000<3000	3000<4000	4000<5000
Control	91.50	8.50	0.00	0.00	0.00
Seeds	96.68	2.99	0.33	0.00	0.00
Whole flour	65.98	16.47	6.80	6.21	1.42
Semi-defatted flour	89.66	8.52	1.51	0.31	0.00
Low fat flour	91.37	8.63	0.00	0.00	0.00

FIGURE CAPTIONS

Figure 1. Kinetics of starch hydrolysis in bread samples. Bread formulations: 10% of chia seeds (PS), bread with 10% of whole chia flour (PWF), bread with 10% of chia semi-defatted flour (PSD), bread with 10% of low-fat chia flour (PLF).

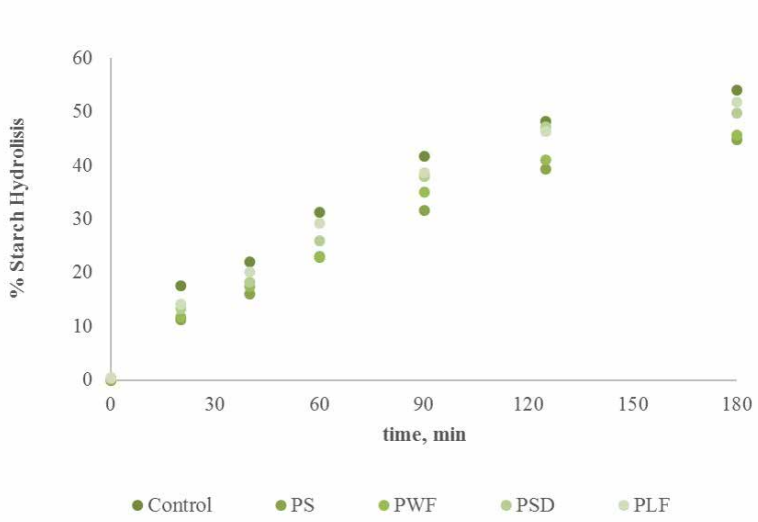
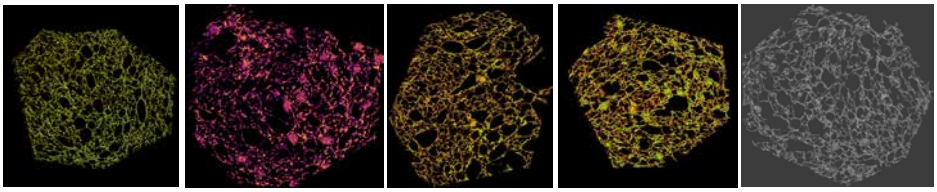


Figure 2. Effect chia by product on bread structure. Bread formulations: 100% wheat flour (Control), 10% of chia seeds (PS10), bread with 10% of whole chia flour (PWF10), bread with 10% of chia semi-defatted flour (PSD10), bread with 10% of low-fat chia flour (PLF10).



Control PS10 PWF10 PSD10 PLF10

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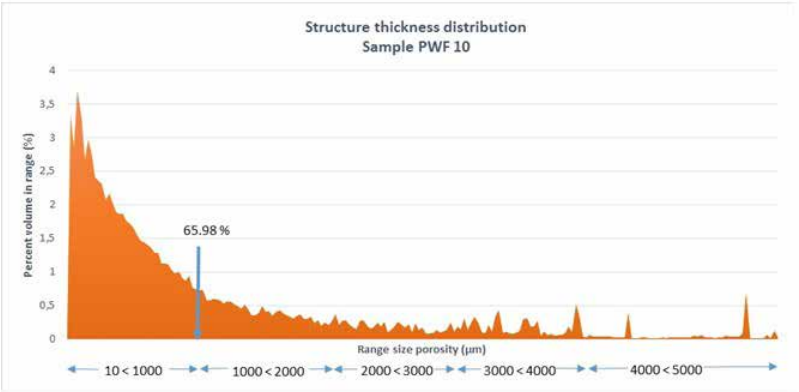
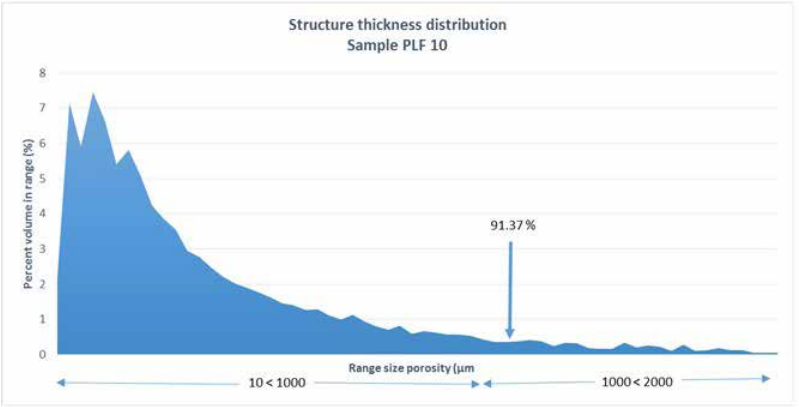
Figure 3. Structure thickness distribution A. bread with 10% of whole chia flour (PWF10); B. bread with 10% of low-fat chia flour (PLF10).

A



B



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CHIA, A PROMISING PRODUCTIVE ALTERNATIVE

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SUMMARY: The aim was the addition of whole chia flour in wheat flour and in corn grits and to evaluate its influence on the process of making loaves, cakes and extruded products. In all cases, response surface methodology was used with a Central Composed Rotational Design (2^2). Results showed it is technologically possible to obtain products with better nutritional characteristics, enhanced mainly by the fatty acids n-3/n-6 ratio and protein content with this mixed flours. The obtained products showed high acceptability by consumers.

Keywords: Whole chia flour, loaves, cake, extruded product.

RESUMEN: ***Chía, una prometedora alternativa productiva.*** El objetivo fue adicionar harina integral de chía en harina de trigo y sémola de maíz y evaluar su influencia en el proceso de fabricación de panes, pasteles y productos extruidos. En todos los casos, se utilizó la metodología de superficie de respuesta con un CCRD (2^2). Los resultados mostraron que es posible obtener productos con características nutricionales mejoradas principalmente por el incremento de la relación de ácidos grasos n-3/n-6 y contenido de proteína con esas harinas mezcla. Los productos obtenidos mostraron alta aceptabilidad por consumidores.

Palabras clave: Harina integral de chía, pan, budín, productos extruidos.

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1. INTRODUCTION

Chia (*Salvia hispanica* L.) is an ancient seed revived by a group of scientists and farmers the last decade of the twentieth century due to its nutritional and functional characteristics (Ayerza & Coates, 2011; Jiménez *et al.*, 2010). In Argentina it dates back to the mid-90s, the cultivation is mainly located in the North West, where the agro-ecological conditions allow obtaining high quality products. Currently there are more than 60,000 ha planted. Chia seeds are an important source of α-linolenic acid (60%) and dietary fibre (approximately 40%). Several kinds of flour have been mixed with wheat flour in order to obtain different products for the baking industry. The nutritional benefits resulting from a combination of cereals with other flour seeds have been previously studied (Tireki, 2008). Extruded processed foods offer the possibility to be vehicle of important nutrients (Obradović *et al.,* 2014; Ndagire *et al.,* 2015). The objective of this work was developing common consumer products that incorporate chia in its formulation in order to introduce it into the regional diet and adding value to the productive chain.

2. MATERIALS AND METHODS

2.1. Materials

Chia seeds grown in Yuto, Jujuy, Argentina (Northwest) were used. Whole chia flour (WCF) was obtained by milling chia seeds in a laboratory scale mill Quadrumat Senior (Brabender GmbH & Co. KG, Duisburg, Germany). The extruded products were processed in a single-screw extruder (GNF1014/2 model Brabender OHG, Duisburg, Germany).

2.2. Methods

Loaf bread formulation: wheat flour, WCF (0-20%) and vital gluten (VG) (0-4%), added water (50-60%), instant baker's yeast (2%), salt (2%), sugar (5%), hydrogenated vegetable fat (6%), egg (1%), powdered milk (4%) and bread improver (1%).

Cakes formulation: flour mixture (wheat flour and WCF) (100 g), sugar (100 g), egg (40 g), baking powder (3.3 g) and whole milk powder (11.2 g). The amount of WCF added ranged between 0 and 30 g/100 g flour mixture and the amount of hydrogenated vegetable fat (HVF) between 12 and 20 g/100 g flour mixture.

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Snacks: elaborated with corn grits and WCF (0-25%); a preconditioning of the corn grits was made by adding distilled water under stirring to reach 16% moisture.

The amounts of WCF and VG for preparing loaf bread; amounts of WCF and HVF for cakes and amount WCF and extrusion temperature for snacks (124-166 °C), were adjusted according to a Central Composed Rotational Design (2^2) with a total of 11 trials each one (Table 1).

The centesimal composition was determined by methods AACC (2010). Fatty acid profile was obtained according to method UNE 55-037-73 (AENOR, 1991) followed by capillary gas chromatography (CGC 6890 System Plus; capillary column was DB–225 J&W 122-2232- 50% cyanopropylphenyl-dimethylpolysiloxane, Agilent Technologies, Mississauga, Canada).

For sensory evaluation, acceptance tests and purchase intention were performed by 40 untrained consumers. The attributes colour, flavour and texture were evaluated using a 9-point hedonic scale where 1 = dislike extremely and 9 = like extremely. The purchase intention was measured on a five point scale where 5 = certainly would buy to 1 = certainly would not buy.

3. RESULTS

3.1. Nutritional and sensory characteristics

Fig. 1 shows the photograph of the best products obtained according to the model RCCD. The results indicated that products with the best technological parameters for loaves and cakes (specific volume, colour, moisture, firmness) were: chia loaf (CHL) with 10 g WCF and 2 g VG/100 g mixture; chia cake (CHC) with 15 g WCF and 20 g HVF/100 g flour mixture. For the snacks with best technological parameters (expansion index, hardness, extrusion temperature) two formulations were selected, CHS1 and CHS2, elaborated with 12.5 g WCF/100 g flour mixture and processed to 145 °C and 166 °C barrel temperature, respectively. In Table 2 it can be seen that incorporation of WCF improved the nutritional value of these products. All the best products with WCF added, presented significant increase in protein, lipids and ash contents. In relation to lipids, the fatty acid profile has presented an important increase in polyunsaturated fatty acid (60%) due to the major content of α-linolenic acid. However it should be noted a slight decrease in the content of n-3 acid in CHS2.

These products also showed good sensory acceptance (Table 3); although the loaf, cake and snack presented lower scores for the attribute colour acceptance in relation to the respective reference products, the scores of flavour, taste and texture were similar. Results showed that 83% of the consumers possibly

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or certainly buy the CHL; 60% of the consumers would possibly or certainly buy the CHC, representing a positive purchase intention. Concerning snacks, only 40% of the consumers would possibly or certainly buy them.

4. DISCUSSION

The increase in protein, lipids and ash content are due to the high contents of these nutrients in the WCF (Table 2). The improvement of the fatty acid profile in the products with WCF is mainly due to the increase of α-linolenic acid content, which makes them a good source of n-3 fatty acid. Therefore, the ratio of n-3/n-6 observed in these products is consistent with the FAO recommended in order to prevent the development of chronic diseases (FAO, 2010). Also, increasing the n-3 fatty acids is essential for brain function and for the management of cardiovascular disease, arthritis and some types of cancer (Simopoulos, 2008; Yashodhara *et al*., 2009).

The products elaborated with WCF, selected by presenting the best technological features, also had good sensory acceptance**.** The addition of chia flour gives a darker brown colour to all products. Although bread loaf and snack presented lower scores for the attribute colour acceptance in relation to their respective references products, the scores of flavour, taste and texture were similar. In general, the three products were well accepted by consumers, with scores between 6.5–7.7 for loaf and cake and 6.5 for snacks. Results showed no statistical difference in consumer purchase intention between CHL and the respective reference. Consumers had similar behaviour for CHC although purchase intention was lower probably due to the WCF gives it a darker colour compared with the reference (0% WCF). In reference to CHS, although it presented lower scores than the control for the attributes of colour, the taste and hardness scores were similar for both. The results for purchasing intention did not present statistical difference between CHS1 and CHS2 and the reference. Only 40% of the consumers would possibly or certainly buy the snack, this represents a positive purchasing intention if it is considered that the snacks were sensorially evaluated without salt, sugar or flavourings added, as they would be prepared for sale.

5. CONCLUSIONS

It possible to add WCF to wheat flour or corn grits and get products with better nutritional characteristics, improved mainly due to higher content of n-3 fatty acids, fibre and protein. These products show high consumer acceptance.

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ACKNOWLEDGMENTS

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REFERENCES

AACC American Association of Cereal Chemists. (2010). International. Approved methods of analysis. St. Paul: Approved Methods Committee. Available at: <http://methods.aaccnet.org/>.

AENOR y Asociación Española de Normalización y Certificación. (1991). Catálogo de normas UNE. Norma UNE 55037-73. Madrid: AENOR.

Ayerza R, Coates W. 2011. Protein content, oil content and fatty acid profiles as potential criteria to determine the origin of commercially grown chia (*Salvia hispanica L.*). Ind Crops Prod **34**, 1366-1371. DOI: 10.1016/j.indcrop.2010.12.007.

FAO (2010). Fats and fatty acids in human nutrition: report of an expert consultation. FAO. Food and Nutrition Paper 91. Rome: Food and Agricultural Organization of the United Nations.

Jiménez FEG, Beltrán-Orozco MC, Martínez VMG. 2010. The antioxidant capacity and phenolic content of chia's (*Salvia hispanica L.*) integral seed and oil. *J Biotechnol* **150**, S315-S315. DOI: 10.1016/j.jbiotec.2010.09.295.

Ndagire C, Muyonga J, Manju R, Nakimbugwe D. 2015. Optimized formulation and processing protocol for supplementary bean-based composite flour. *Food Sci Nutr* **3**, 527-538. DOI: 10.1002/fsn3.244.

Obradović V, Babić J, Šubarić D, Ačkar D, Jozinović A. 2014. Improvement of nutritional and functional properties of extruded food products. *J Food Nutr Res* **53**, 189-206.

Simopoulos AP. 2008. The Importance of the Omega-6/Omega-3 Fatty Acid Ratio in Cardiovascular Disease and Other Chronic Diseases. Experimental Biology and Medicine (Maywood) 233:674-688.

Tireki S. 2008. Technology of cake production. In S.G. Sumnu, & S. Sahin (Eds.). Food engineering aspects of baking sweet goods (pp. 99-120). Boca Raton: CRC Press.

Yashodhara B, Umakanth S, Pappachan J, Bhat S, Kamath R, Choo B. 2009. Omega-3 fatty acids: a comprehensive review of their role in health and disease. *Postgrad Med J* 85, 84-90. DOI: 10.1136/pgmj.2008.073338.

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TABLES

Table 1. Central composed rotational design for preparation of bread loaf, cakes and snacks with whole chia flour (WCF).

Trials	Codified		Bread Loaf		Cakes		Snacks	
			Real		Real		Real	
	x_1	x_2	WCF X_1	VG X_2	WCF X_1	HVF X_2	WCF X_1	T X_2
1	- 1	- 1	3.0	0.6	4.4	13.2	3.7	130
2	+1	- 1	17.0	0.6	25.6	13.2	21.3	130
3	- 1	+ 1	3.0	3.4	4.4	18.8	3.7	160
4	+1	+ 1	17.0	3.4	25.6	18.8	21.3	160
5	-α	0	0	2.0	0	16.0	0	145
6	+α	0	20.0	2.0	30.0	16.0	25.0	145
7	0	-α	10.0	0	15.0	12.0	12.5	124
8	0	+α	10.0	4.0	15.0	20.0	12.5	166
9	0	0	10.0	2.0	15.0	16.0	12.5	145
10	0	0	10.0	2.0	15.0	16.0	12.5	145
11	0	0	10.0	2.0	15.0	16.0	12.5	145

± [α] = 1.4142; x_1, x_2 = codified values; X_1 e X_2 = independent variables: WCF and Vital Gluten contents for bread loaf; WCF and HVF contents for cakes; and WCF content and temperature (T) of process for snacks.

Table 2. Proximal and fatty acid composition of the selected products.

Component (g/100 g)	Standard loaf	CHL	Standard cake	CHC	Standard snack	CHS1	CHS2
Moisture	29.72 ± 0.23[a]	28.95 ± 0.16[b]	24.64 ± 0.21[a]	24.69 ± 0.69[a]	3.02 ± 0.02[a]	2.80 ± 0.06[b]	2.83 ± 0.06[b]
Protein	11.24 ± 0.09[b]	13.32 ± 0.17[a]	7.98 ± 0.04[b]	8.55 ± 0.10[a]	7.68 ± 0.06[c]	9.76 ± 0.06[a]	9.41 ± 0.03[b]
Lipids	2.48 ± 0.04[b]	2.76 ± 0.04[a]	12.44 ± 0.15[b]	16.28 ± 0.25[a]	0.71 ± 0.02[b]	3.35 ± 0.04[a]	3.42 ± 0.03[a]
Ash	6.74 ± 0.06[b]	8.48 ± 0.04[a]	1.18 ± 0.02[b]	1.40 ± 0.02[a]	0.54 ± 0.04[b]	1.11 ± 0.02[a]	1.10 ± 0.04[a]
Carbohydrates total*	49.82 ± 0.26[a]	46.49 ± 0.34[b]	53.76 ± 0.26[a]	49.08 ± 0.74[b]	88.05 ± 0.07[a]	83.21 ± 0.11[b]	83.23 ± 0.06[b]
Fatty acid (g/100 g methyl esters)							
C 6:0	0.03 ± 0.01[a]	0.03 ± 0.01[a]	0.12 ± 0.05[a]	0.15 ± 0.02[a]	---	---	---
C 8:0	0.38 ± 0.02[b]	0.42 ± 0.01[a]	0.48 ± 0.18[a]	0.28 ± 0.07[a]	---	---	---

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Component (g/100 g)	Standard loaf	CHL	Standard cake	CHC	Standard snack	CHS1	CHS2
C 10:0	0.09 ± 0.01^{b}	0.12 ± 0.01^{a}	0.33 ± 0.07^{a}	0.26 ± 0.01^{a}	---	---	---
C 12:0	1.13 ± 0.02^{a}	1.22 ± 0.06^{a}	1.74 ± 0.23^{a}	1.31 ± 0.05^{b}	---	---	---
C 14:0	1.00 ± 0.02^{a}	0.89 ± 0.01^{b}	2.01 ± 0.19^{a}	1.78 ± 0.05^{a}	---	---	---
C 15:0	---	---	0.19 ± 0.06^{a}	0.13 ± 0.01^{a}	---	---	---
C 16:0	22.52 ± 0.09^{a}	19.08 ± 0.08^{b}	24.98 ± 0.21^{a}	23.11 ± 0.37^{b}	15.48 ± 0.71^{a}	9.23 ± 0.11^{c}	11.49 ±0.15^{b}
C 17:0	---	---	0.07 ± 0.02^{a}	0.16 ± 0.01^{a}	---	---	---
C 18:0	10.45 ± 0.02^{a}	9.00 ± 0.09^{b}	9.99 ± 0.29^{b}	10.76 ± 0.08^{a}	4.48 ± 0.26^{a}	3.66 ± 0.16^{b}	4.61 ± 0.06^{a}
C 20:0	0.37 ± 0.04^{a}	0.35 ± 0.05^{a}	0.28 ± 0.08^{a}	0.33 ± 0.05^{a}	0.34 ± 0.02^{a}	0.37 ± 0.07^{a}	0.50 ± 0.03^{a}
C 22:0	0.28 ± 0.01^{a}	0.09 ± 0.01^{b}	0.14 ± 0.05^{a}	0.19 ± 0.01^{a}	---	---	---
Total SFA	36.26	31.22	40.33	38.45	20.30	13.26	16.60
C 14:1	---	---	0.04 ± 0.01^{a}	0.08 ± 0.01^{a}	---	---	---
C 16:1	0.39 ± 0.06^{a}	0.12 ± 0.01^{b}	0.58 ± 0.16^{a}	0.54 ± 0.05^{a}	---	---	---
C 18:1	43.36 ± 0.06^{a}	36.89 ± 0.12^{b}	42.22 ± 0.85^{a}	38.49 ± 0.15^{b}	33.13 ± 0.55^{a}	13.85 ± 0.26^{b}	13.57± 0.25^{b}
C 20:1	0.29 ± 0.01^{a}	0.12 ± 0.06^{b}	0.24 ± 0.08^{a}	0.13 ± 0.06^{a}	0.01 ± 0.01^{b}	0.13 ± 0.01^{ba}	0.20 ± 0.03^{a}
Total MUFA	44.03	37.12	43.09	39.24	33.14	13.85	13.57
C 18:2ω-6	19.26 ± 0.02^{a}	18.72 ± 0.07^{b}	16.37 ± 0.38^{a}	15.30 ± 0.12^{b}	45.08 ± 0.82^{a}	26.89 ± 0.05^{b}	25.58 ±0.11^{c}
C 18:3ω-3	0.45 ± 0.03^{b}	12.94 ± 0.13^{a}	0.21 ± 0.01^{b}	7.01 ± 0.24^{a}	1.49 ± 0.60^{c}	46.00 ± 0.44^{a}	44.26 ± 0.36^{b}
Total PUFA	19.71	31.66	16.58	22.31	46.37	72.89	69.84
SFA:MUFA:PUFA Ratio	1:1.21:0.54	1:1.19:1.01	1:1.07:0.41	1:1.02:0.58	1:1.63:2.28	1:1.04:0.50	1:0.82:4.22
PUFA/SFA	0.54	1.10	0.41	0.58	2.28	5.50	4.21
ω -6/ω-3	42.68 ± 0.41^{a}	1.45 ± 0.01^{b}	77.11 ± 1.93^{a}	2.18 ± 0.06^{b}	39.1 ± 1.46^{a}	0.58 ± 0.01^{b}	0.58 ± 0.01^{b}

*Calculated by difference. Means for the same product followed by the same letter in the same line did not differ according to Tukey's test (P<0.05).

CHL: Chia loaf selected; CHC: Chia cake selected; CHS1: Chia snack (temperature of processed: 145 °C); CHS2: Chia snack (temperature of processed: 166 °C).

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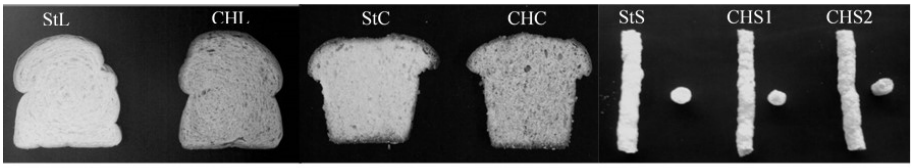
Table 3. Results of sensory acceptance of the selected products

Sensory acceptance[1]	Standard loaf	CHL	Standard cake	CHC	Standard snack	CHS1	CHS2
Colour	8.0 ± 0.7[a]	6.5 ± 1.4[b]	7.9 ± 0.9[a]	6.6 ± 1.8[b]	7.7 ± 0.9[a]	5.6 ± 1.9[b]	5.9 ± 1.6[b]
Flavour	7.7 ± 1.2[a]	7.0 ± 1.4[a]	---	---	---	---	---
Taste	7.9 ± 0.8[a]	7.5 ± 1.2[a]	7.6 ± 1.1[a]	6.8 ± 1.8[b]	6.6 ± 1.6[a]	5.7 ± 1.9[ba]	5.2 ± 1.7[b]
Texture	7.8 ± 1.2[a]	7.7 ± 1.0[a]	7.6 ± 1.1[a]	7.2 ± 1.4[a]	---	---	---
Hardness	---	---	---	---	7.0 ± 1.8[b]	7.9 ± 1.0[a]	7.6 ± 1.3[ba]
Purchase intention[2]	4.6 ± 0.5[a]	4.2 ± 0.9[b]	4.1 ± 0.9[a]	3.8 ± 1.0[a]	3.5 ± 1.2[a]	3.2 ± 1.2[a]	2.9 ± 1.3[a]
Positive purchase intention (%)[3]	97	83	77	60	55	50	40

[1] Hedonic scale ranging from 1 = "disliked extremely" to 9 = "liked extremely"; [2] Hedonic scale ranging from 1 = "would certainly not buy" to 5 = "would certainly buy"; [3] Consumer who attributed scores from 4 to 5 (in a scale from 1 = "would certainly not buy" to 5 = "would certainly buy") were considered.

FIGURE CAPTIONS

Figure 1. Photograph of the selected products. StL: Standard loaf, StC: Standard cake, StS: Standard snack, CHL: Chia loaf selected; CHC: Chia cake selected; CHS1: Chia snack (temperature of processed: 145 °C); CHS2: Chia snack (temperature of processed: 166 °C).



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2.
TECHNOLOGY AND OILS

2nd International Conference of Chia-Link Network

6-7 October, Seville - Spain

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EXTRACTION AND OXIDATIVE STABILITY OF CHIA OIL

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SUMMARY: The aims of this research were to: i) study the effect of screw pressing extraction parameters on chia oil yield and quality; ii) evaluate the effectiveness of natural antioxidants alone and/or in combination and microencapsulation process on the oxidative stability of chia oil. The maximum oil yield (82.2 g/100 g oil) was obtained under the following conditions: 10% seed moisture content, 6 mm restriction die, 20 rpm screw press speed and 30 °C barrel temperature. The screw pressing technology displayed oils with acceptable chemical quality. Microencapsulation procedure increased chia oil shelf life of about 30 to 48% during the storage period.

Keywords: Antioxidants, chia oil, freeze-drying, microcapsules, oxidative stability, shelf life, spray-drying.

RESUMEN: ***Extracción y estabilidad oxidativa del aceite de chía.*** Los objetivos del presente trabajo fueron: i) estudiar el efecto de las variables del proceso de extracción por prensado sobre el rendimiento y la calidad del aceite de chía; ii) evaluar la efectividad de antioxidantes naturales agregados en forma individual o combinados y del proceso de microencapsulado sobre la estabilidad oxidativa del aceite de chía. El mayor rendimiento en aceite (82.2 g/100 g aceite) se obtuvo bajo las siguientes condiciones: humedad de la semilla, 10 %; restricción, 6 mm; velocidad de prensado, 20 rpm y

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temperatura del barral, 30 °C. La calidad química del aceite de chía resultó aceptable bajo todas las condiciones extractivas analizadas. El proceso de microencapsulación incrementó entre un 30 y 48 % la vida útil del aceite de chía.

Palabras clave: Aceite de chía, antioxidantes, estabilidad oxidativa, liofilización, microcápsulas, secado por aspersión, vida útil.

1. INTRODUCTION

Chia seeds contains between 0.25 and 0.38 g oil/g seed, where the major constituents are triglycerides, in which α-linolenic and linoleic acids are present in high amounts (Ixtaina *et al.,* 2011).

The major goal in chia oil extraction is to find an appropriate method to isolate it from the seeds preserving oil quality (Martínez *et al.,* 2008).

Although such fatty acid composition is favourable from a nutritional point of view (Galli *et al*., 2006), a higher content of ω3 and ω6 fatty acids results in poorer oxidative stability. The incorporation of natural or synthetic antioxidants and microencapsulation of oils can be used in order to protect these fatty acids against oxidative degradation (Ixtaina *et al.,* 2012; Gallardo *et al*., 2013).

For the above exposed, the effect of screw pressing extraction parameters on chia oil yield and quality and the effectiveness of natural antioxidants alone and/or in combination and microencapsulation process on the oxidative stability of chia oil were studied.

2. MATERIALS AND METHODS

2.1. Screw press extraction

The oil extraction was carried out according to Martinez *et al.* (2012). The oil yield and quality were determined according to Martinez *et al.* (2008) and AOCS (2009).

2.2. Experimental design for storage stability test

Antioxidants as ascorbyl palmitate (AP), terbutylhydroquinone (TBHQ), tocopherols (TOC) or their mixtures were added to oil. The bottled oils were placed in a thermostatic chamber at 25 ± 1 °C kept in the dark during ten months.

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2.3. Microcapsules preparation

Dispersions and spray drying process were performed according to Roccia *et al*. (2014).

The microcapsule characterization: solid yield (SY), surface oil (SO), encapsulation efficiency (EE) and moisture content (MC) were determined according to Roccia *et al.* (2014).

3. RESULTS AND DISCUSSION

3.1. Effect of process variables on chia oil yield and quality

The decrease of seed moisture, restriction die and pressing speed resulted in an increase of oil yield (Figure 1). The combination of factor levels that suggested a maximum on oil yield (82.2 g/100 g oil) within the experimental values was seed moisture 10 % (0.113 g/g dry solid); 20 rpm pressing speed and 6 mm restriction die of the press (Martinez *et al.*, 2012). The oil chemical quality was not adversely affected by the extraction process (PV <0.70 meq O_2/kg oil).

3.2 Storage stability test of chia oil

Chia oil (CO) samples reached the end point for rancidity (Codex Alimentarius, 2001) at 105 days of storage. The combination of AP and TOC at 200 mg/kg oil each is more effective on CO stabilization than the addition of TBHQ at 200 mg/kg oil (0.66 and 2.35 meq O_2/kg oil at 300 days of storage, respectively).

3.3 Microencapsulation

In first instance, CO microencapsulation conditions were selected according to Roccia *et al.* (2014). In this research the authors used as wall material maltodextrin (MD) and hydroxypropylmethylcelulose (HPMC) and the wall material:oil ratio was 2:1. They observed that SY was improved principally according variations of air inlet temperature, atomization airflow and aspiration setting parameters (Table 1). Similar results were reported by Gallardo *et al.* (2013). It is important to note that at 130 °C with a low feed flow rate the solid yield increased according to Gallo *et al.* (2011). On the other hand, the MC, SO and EE parameters did not change with the process variable combination. The high oil amount retained into microcapsules was related to the nature of wall material and their mixtures and emulsion quality and stability (Davidov-Pardo *et al*., 2008).

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CO was dried under the optimum conditions defined: inlet temperature: 163 °C, atomization airflow rate: 279 L/h, feed flow rate: 10% and drying air flow rate: 100%. The SY, MC, SO and EE obtained were 39.7%, 2.13%, 27% and 73%, respectively. After 90-day storage, CO and M-CO achieved PV of 14.2 and 214 meq O_2/kg oil, respectively (Martinez *et al.*, 2015) (Figure 2). The chemical damage caused by the drying temperature (163 °C) in CO could not be countered by the wall matrix.

In order to maximize CO chemical quality new assays were conducted using different spray drying conditions and microcapsule formulations. Based on preliminary experiments, the spray drying conditions cited in Table 1 were selected to produce CO microcapsules using isolated soy protein (ISP) and MD. Microcapsules prepared with ISP and ISP/MD 1:1 presented higher SY than ISP/MD 1:3 and MD/HPMC (described previously). However, the SO and EE were affected negatively. No differences in EE values were observed among the wall component proportions (Table 1). In contrast with Liu *et al.* (2010), no significant effect of MD incorporation on EE was found. The bulk CO reached the end point for rancidity or the acceptability limit for virgin and cold-pressed vegetables oils (15 meq O_2/kg oil) around 20 to 30 days before than that of encapsulated oils (Figure 3). This result represented an increase in the time of the oil shelf life of between 30 and 48%. Ixtaina *et al.* (2015) reported PV values of unencapsulated and encapsulated CO showing a shelf-life of approximately 40 and higher than 55 days, respectively, considering a maximum PV of 10 meq O_2/kg oil, with CO microencapsulated with sodium caseinate and lactose. Our results revealed that the components of the microcapsule wall seemed to be an effective barrier, which reduced CO oxidative degradation (González *et al.*, 2016).

4. CONCLUSIONS

Oil yield from chia seeds by pressing can be enhanced by adjusting moisture content 0.113 g/g dry solids (10%), 6 mm restriction die, 20 rpm screw press speed and 30 °C barrel temperature to obtain an oil yield value of 82.2 g/100 g oil.

Microcapsules produced with isolated soy protein as wall material containing ~30% of chia oil were stored for 90 days showing peroxide values below the Codex limit (15 meq O_2/kg oil) and a ω3/ω6 ratio of 3:1.

Thus, the present results provided important information about the processing parameters to produce 90-days-stable food powders with ~30% of chia oil, which allows the handling of products rich in oils with high ω3/ω6 ratio as solid materials and facilitates its incorporation in certain foods such as bakery products.

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REFERENCES

AOCS 2009. Methods and recommended practices of the AOCS. USA: American Oil Chemist's Society.

C. Alimentarius, Fats, Oils and Related Products, second ed. FAO/WHO Food Standards Programme, Rome, Italy, 2001.

Codex 2001. Alimentarius commission. Available from http://www. codexalimentarius.net/web/index_es.jsp Accessed 02.08.11.

Davidov-Pardo G, Roccia P, Salgado D, León AE, Pedroza-Islas R. 2008. Evaluation of the different wall materials to microencapsulate fish oil and its behavior in bread products. *Am. J. Food Technol* **3**, 384–393. DOI: 10.3923/ajft.2008.384.393.

Gallardo G, Guida L, Martinez V, López MC, Bernhardt D, Blasco R, Pedroza-Islas R, Hermida LG. 2013. Microencapsulation of linseed oil by spray drying for functional food application, *Food Res. Int* **52**, 473–482. DOI:10.1016/j.foodres.2013.01.020.

Galli C, Marangoni F. 2006. ω−3 fatty acids in the Mediterranean diet. *P. L. E. F. A.* **75**, 129–133. DOI:10.1016/j.plefa.2006.05.007.

Gallo L, Llabot JM, Allemandi D, Bucalá V, Piña J. 2011. Influence of spray-drying operating conditions on Rhamnus purshiana (*Cáscara sagrada*) extract powder physical properties. *Powder Technol* **208**, 205–214. DOI:10.1016/j.powtec.2010.12.021.

González A, Martínez ML, Paredes AJ, León AE, Ribotta PD. 2016. Study of the preparation process and variation of wall components in chia (*Salvia hispanica* L.) oil microencapsulation. *Powder Technol* **301**, 868–875. DOI:10.1016/j.powtec.2016.07.026.

Ixtaina VY, Nolasco SM, Tomás SM. 2012. Oxidative stability of chia (*Salvia hispanica* L.) seed oil: Effect of antioxidants and storage conditions. *Journal of the American Oil Chemists´ Society* **89**, 1077-1090.

Ixtaina VY, Julio LM, Wagner JR, Nolasco SM, Tomás MC. 2015. Physicochemical characterization and stability of chia oil microencapsulated with sodium caseinate and lactose by spray-drying. Powder Technol **271**, 26–34. DOI:10.1016/j.powtec.2014.11.006.

Ixtaina VY, Martínez ML, Spotorno V, Mateo CM, Maestri DM, Diehl BWK., et al. 2011. Characterization of chia seed oils obtained by pressing and solvent extraction. *J Food Comp Anal* **24**, 166-174. DOI:10.1016/j.jfca.2010.08.006.

Liu S, C. Elmer NH, Nickerson MT. 2010. Effect of pH on the functional behaviour of pea protein isolate-gum Arabic complexes. *Food Res Int* **43**, 489–495. DOI:10.1016/j.foodres.2009.07.022.

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Martínez ML, Curti MI, Roccia P, Llabot JM, Penci MC, Bodoira RM, Ribotta PD. 2015. Oxidative stability of walnut (*Juglans regia* L.) and chia (*Salvia hispanica* L.) oils microencapsulated by spray drying. *Powder Technol* **270**, 271–277. DOI:10.1016/j.powtec.2014.10.031.

Martínez ML, Marín MA, Salgado Faller CM, Revol J, Penci MC, Ribotta PD. 2012. Chia (*Salvia hispanica* L.) oil extraction: Study of processing parameters. *Food Sci Technol* **47**, 78-82. DOI:10.1016/j.lwt.2011.12.032.

Martínez ML, Mattea MA, Maestri DM. 2008. Pressing and supercritical carbon dioxide extraction of walnut oil. *J Food Eng* **88**, 399-404. DOI:10.1016/j.jfoodeng.2008.02.026.

Martínez ML, Mattea MA., Maestri DM. 2006. Varietals and crop year effects on lipid composition of walnut (*Juglans regia* L.) genotypes. *J Am Oil Chem Soc* **83**, 791-796.

Roccia P, Martínez ML, Llabot JM, Ribotta PD. 2014. Influence of spray-drying operating conditions on sunflower oil powder qualities. *Powder Technol* **254**, 307–313. DOI:10.1016/j.powtec.2014.01.044.

TABLES

Table 1. Spray drying conditions and microcapsules characteristics.

Wall material	A	B	C	D	MC	SY	SO	EE	PV	Ref.
MD/HPMC	200	400	15	100	3.18	39.88	17.75	82.25	---	Roccia et al. 2014
MD/HPMC	200	400	5	100	2.96	39.34	22.13	77.87	---	Roccia et al. 2014
MD/HPMC	165	279	10	90	2.93	38.68	22.9	77.1	---	Roccia et al. 2014
MD/HPMC	135	400	5	100	2.35	38.95	18.18	81.82	---	Roccia et al. 2014
ISP	130	538	10	100	3.3	50.4	39.1	60.82	0.12	González et al. 2016
ISP/MD (1:1)	130	538	10	100	3.9	46.6	32.86	67.16	1.21	González et al. 2016
ISP/MD (1:3)	130	538	10	100	3.1	38.7	37.72	62.3	1.60	González et al. 2016

Abbreviations, A: Inlet temperature (°C); B: Atomization air flow rate (L/h); C: Feed flow rate (%); D: Drying air flow rate (%); MC: Moisture content (%); SY: Solid yield (%); SO: Surface oil (%); EE: Encapsulation efficiency (%).

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FIGURE CAPTIONS

Figure 1. Effects of seed moisture content, screw press speed and restriction die on oil yield (Martinez et al., 2012).

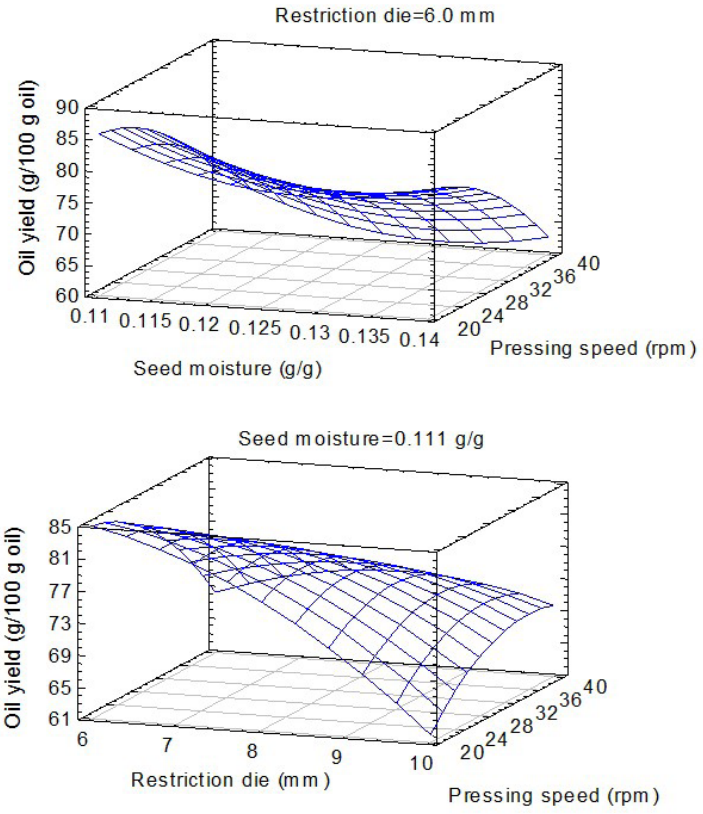

Figure 2. Peroxide value (PV) evolution during the storage time of CO with and without microencapsulation with HPMC and MD.

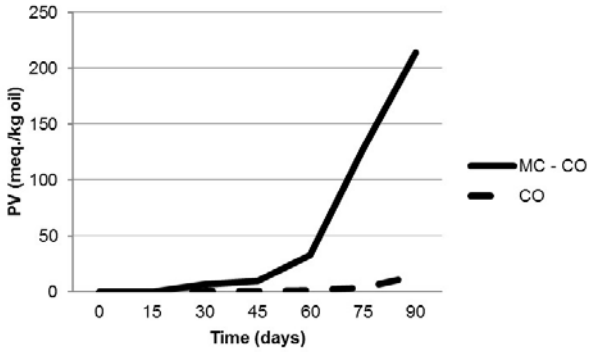

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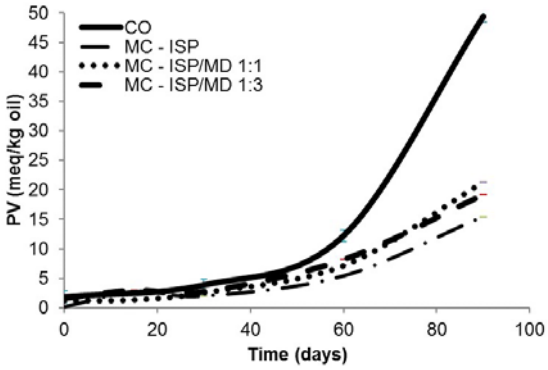
Figure 3. Peroxides values (PV) at different storage times for ISP, ISP/MD1:1 and ISP/MD1:3 microcapsules.



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DEVELOPMENT AND CHARACTERIZATION OF SUNFLOWER-CHIA OIL BLENDS

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SUMMARY: Sunflower-chia (90:10 and 80:20 wt/wt) oil blends with the addition of rosemary (ROS), ascorbyl palmitate (AP) and their blends (AP:ROS) were formulated to evaluate the oxidative stability during storage at two temperature levels (4 ± 1 and 20 ± 2 °C) for a period of 360 days. Temperature had a strong influence on oil oxidation. DSC study showed that ROS had the best antioxidant effect, whereas AP:ROS was the most effective antioxidant during storage at 4 ± 1 °C according to Rancimat tests, PV and *p*-AV values.

Keywords: Differential scanning calorimetry, oil blend, oxidative stability, Rancimat, storage condition.

RESUMEN: ***Desarrollo y caracterización de mezclas de aceites de chía y girasol***. Aceites mezclas girasol-chía (90:10, 80:20 p/p) con la adición de extracto de romero (ROS), ascorbil palmitato (AP) y sus mezclas (AP:ROS) fueron formulados para evaluar la estabilidad oxidativa durante el almacenamiento a dos niveles de temperatura (4 ± 1 y 20 ± 2 °C) durante 360 días. La temperatura presentó una fuerte influencia sobre la oxidación del aceite. ROS mostró el mejor efecto antioxidante en el estudio de termooxidación mediante DSC, mientras que AP:ROS fue el antioxidante más eficaz durante el almacenamiento a 4 ± 1 ° C de acuerdo con las pruebas de Rancimat, PV y *p*-AV.

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Palabras clave: Calorimetria diferencial de barrido, aceites mezcla, estabilidad oxidativa, Rancimat, condiciones de almacenamiento.

1. INTRODUCTION

Sunflower (*Helianthus annuus* L.) oil is one of the most consumed vegetable oils in Argentina. The oil obtained from traditional hybrids contains 65-70% of linoleic acid (ω-6) (Gunstone 2002). Chia (*Salvia hispanica* L.) seed oil is an interesting source of polyunsaturated fatty acids (PUFA), containing the highest content of α-linolenic acid (~60%) of any known vegetable source. This fatty acid (FA) belongs to the ω-3 family, which is essential for normal growth and development in the human body. PUFA oxidation generates volatile compounds that impart undesirable flavours and aromas, and compromise the nutritional quality of the oil limiting its shelf life (Ixtaina *et al.* 2012). FAO/WHO have recommended that the essential ω-6:ω-3 FA balance in the diet should be between 5:1 and 10:1. Individuals who consume a ratio in excess of 10:1 should be encouraged to eat more ω-3 rich foods.

Blending of vegetable oils has emerged as an economical way of modifying the physicochemical characteristics of vegetable oils and of enhancing their oxidative stability (Shiela *et al.* 2004).

The objective of this work was to study the oxidative deterioration of oil blends in order to evaluate the influence of temperature and time, and the effectiveness of antioxidants during the storage of sunflower-chia oil blends, determining the evolution of primary and secondary oxidation products.

2. MATERIALS AND METHODS

2.1. Materials

The oils used in this work were chia seed oil (Nutracéutica Sturla S.R.L., Argentina) obtained by cold pressing, and refined sunflower oil (Molinos Río de la Plata S.A, Argentina). Rosemary extract and ascorbyl palmitate were obtained from Danisco (Denmark). All the antioxidants used are classified as GRAS additives in the United States. All the chemicals and solvents used were of analytical grade.

The oil blends were formulated by blending sunflower with chia seed oil in proportions of 80:20 and 90:10 (wt/wt). Rosemary extract (ROS), ascorbyl palmitate (AP) and their blends AP:ROS (1:1) were added to the oil blends in the following concentrations: 5000, 2000 and 2000:20 ppm of the commercial products, respectively. Samples without antioxidants were used for the control systems.

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2.2. Analytical methods

Fatty acid composition was analysed by GC according to IUPAC 2.302 standard method; free fatty acid according to AOCS recommended practice Ca 5a-40; and the tocopherol content (HPLC Hewlett Packard 1050 Series, Germany) following the procedures described in IUPAC 2.432.

Thermal-oxidative decomposition of the vegetable oil blends was studied by differential scanning calorimetry method (DSC). Oil samples of 3–5 mg were placed in an aluminium pan and then heated at constant heating rates β = 2.5, 5.0, 10.0, 15.0 and 20.0 °C/min from 10 to 350 °C in an oxygen flow of 100 mL/min.

The oxidative stability of the oil blends during storage was monitored by measuring periodically the peroxide value (PV) and the *p*-anisidine value (*p*-AV) according to AOCS methods Cd 8-53 and Cd 18-90, respectively.

The oxidative stability of each sample during storage was evaluated by the Rancimat method Mod 743 (Metrohm AG, Herisau, Switzerland).

3. RESULTS AND DISCUSSION

3.1. Characterization of oils and their blends

The initial physicochemical characteristics of the sunflower and chia oils and their blends are given in Table 1. Refined sunflower oil has a profile consisting mainly of linoleic (C18:2, ω-6) and oleic (C18:1, ω-9) acids, whereas chia oil has a high content of α-linolenic acid (C18:3, ω-3), which represents ~65% of total fatty acids. The 80:20 and 90:10 wt/wt sunflower-chia blends contain approximately 17.4 and 9.0% of C18:3, corresponding to a ω-6:ω-3 ratio of 2.7:1 and 5.3:1, respectively. The total tocopherol concentration for the oils studied was in the range of 411-502 mg/kg.

Free fatty acid content, PV and *p*-AV were low, indicating the high quality of the starting oils used. Sunflower oil had the highest initial t_i value, while chia oil recorded the lowest due to its high PUFA content. The 80:20 and 90:10 wt/wt oil blends showed intermediate initial t_i values depending on the content of each oil.

3.2. Differential scanning calorimetry (DSC)

DSC oxidation curves obtained for sunflower oil, chia oil and the 80:20 and 90:10 sunflower-chia control blends were studied at the same heating rate (β = 10 °C/min) (Fig. 1). All thermograms presented two main peaks, which were more

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or less pronounced depending on the type of oil or blend studied. The oxidation curve obtained for chia oil showed the highest first peak, whereas sunflower oil had the lowest one. Oil blends presented intermediate heights.

3.3. Storage of sunflower-chia oil blends

The evolution of the PV and *p*-AV values for the different systems studied during storage at 4 ± 1 °C and 20 ± 2 °C are shown in Figs. 2 and 3, respectively.

After 360 days of storage at 4 ± 1 °C, none of the sunflower-chia oil blends (90:10 and 80:20 wt/wt) with the addition of different antioxidants exceeded the upper limit of PV (10.0 meq O_2/kg oil) established by the *Codex Alimentarius* (Fig. 2a). Both oil blends with AP:ROS had the lowest levels of PV, being significantly lower ($P<0.05$) than the control systems during all the storage time. In contrast, during storage at 20 ± 2 °C, the legal upper limit was reached between 120 and 240 days of storage (Fig. 3a).

The *p*-AV of oils was low during storage at 4 ± 1 °C (Fig. 2b), while at 20 ± 2 °C all the systems varied widely (Fig. 3b). Chia oil showed the highest formation of secondary oxidation products for both storage temperatures, which may be attributed to its high content of PUFA.

In spite of the differences in the fatty acid composition and tocopherol content between the 90:10 and 80:20 wt/wt sunflower-chia oil blends, few differences were found between the oxidative stability of these systems at the different conditions studied. This could be attributed to the natural tocopherol content, mainly γ-tocopherol, which has a high *in vitro* antioxidant activity.

3.4. Rancimat

The induction times showed a decrease with increasing PUFA content (mainly C18:3), temperature and storage time. The addition of antioxidants increased the t_i in both oil blends. The addition of AP:ROS was more effective, improving the oxidative stability of the 80:20 and 90:10 sunflower-chia oil blends during storage at 4 ± 1 °C after 90 and 180 days, respectively. Oil blends stored at 4 ± 1 °C exhibited higher t_i values than those stored at 20 ± 2 °C.

4. CONCLUSIONS

The fatty acid composition of the sunflower-chia oil blends studied in this work indicates that the essential ω-6/ω-3 fatty acid balance can be achieved with a low proportion of chia oil (10 and 20% wt/wt).

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Temperature had a strong influence on oxidation, oil blends stored at 4 ± 1 °C exhibited higher stability oxidative than those stored at 20 ± 2 °C.

DSC study showed that ROS had the best antioxidant effect, whereas AP:ROS was the most effective antioxidant during storage at 4 ± 1 °C according to Rancimat tests, PV and p-AV values.

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REFERENCES

AOCS 1998. Official Methods and Recommended Practices of the AOCS, 5th edn. AOCS Press, Champaign.

IUPAC 1992. Standard Methods for the Analysis of Oils, Fats and Derivates (7th ed.). Eds. Paquot C, Hautffenne A. International Union of Pure and Applied Chemistry, Blackwell Scientific Publications Inc., Oxford.

Gunstone FD, 2002. Vegetable Oils in Food Technology: Composition, Properties and Uses, Ed. Blackwell Publishing, CRC Press, Oxford.

Ixtaina VY, Nolasco SM, Tomás MC. 2012. Oxidative Stability of Chia (*Salvia hispanica* L.) Seed Oil: Effect of Antioxidants and Storage Conditions. *J Am Oil Chem Soc* **89**, 1077-1090.

Shiela PM, Sreerama YN, Gopala Krishna AG. 2004. Storage Stability Evaluation of Some Packed Vegetable Oil Blends. *J Am Oil Chem Soc* **81**, 1125-1129.

TABLE

Table 1. Physicochemical characteristics of sunflower, chia oil and their blends.

	Sunflower oil	Chia oil	Sunflower-chia 80:20 wt/wt	Sunflower-chia 90:10 wt/wt
Fatty acids (%)				
$C_{16:0}$	6.6 ± 0.1	7.1 ± 0.3	7.6 ± 1.5	7.3 ± 1.4
$C_{18:0}$	2.3 ± 0.1	2.1 ± 0.2	2.3 ± 0.1	1.1 ± 0.4
$C_{18:1}$	36.6 ± 0.1	6.3 ± 0.3	26.1 ± 0.3	34.6 ± 2.3
$C_{18:2}$	54.4 ± 0.1	19.4 ± 0.1	46.7 ± 1.4	48.0 ± 1.8
$C_{18:3}$	nd	65.2 ± 0.9	17.4 ± 0.1	9.0 ± 0.8

	Sunflower oil	Chia oil	Sunflower-chia 80:20 wt/wt	Sunflower-chia 90:10 wt/wt
Free fatty acids (% oleic acid)	0.06 ± 0.01	0.55± 0.02	0.18 ± 0.01	0.10 ± 0.01
Tocopherols (mg/kg)				
Total	502 ± 19	411 ± 19	454 ± 19	433 ± 19
α-	498 ± 19	nd	376 ± 1	422 ± 1
β-	4 ± 1	nd	6 ± 17	2 ± 17
γ-	nd	404 ± 3	72 ± 3	9 ± 3
δ-	nd	7 ± 2	nd	nd
Metal content (mg/kg)				
Cu	-	-	0.03 ± 0.01	0.01± 0.00
Fe	-	-	0.28 ± 0.06	0.61 ± 0.00
Peroxide value (meq O_2/kg)	1.5 ± 0.2	0.8 ± 0.1	1.5 ± 0.3	1.0 ± 0.2
***p*-anisidine value**	5.1 ± 0.2	0.5 ± 0.1	3.6 ± 0.1	4.5 ± 0.2
Induction time (h)	13.0 ± 0.3	3.0 ± 0.1	7.6 ± 0.3	9.2 ± 0.4

Mean values ± SD of two independent batches (n = 2).
nd: not detected.

FIGURE CAPTIONS

Figure 1. DSC curves of thermal oxidation of sunflower oil, chia oil and their blends at a heating rate of β = 10 °C/min.

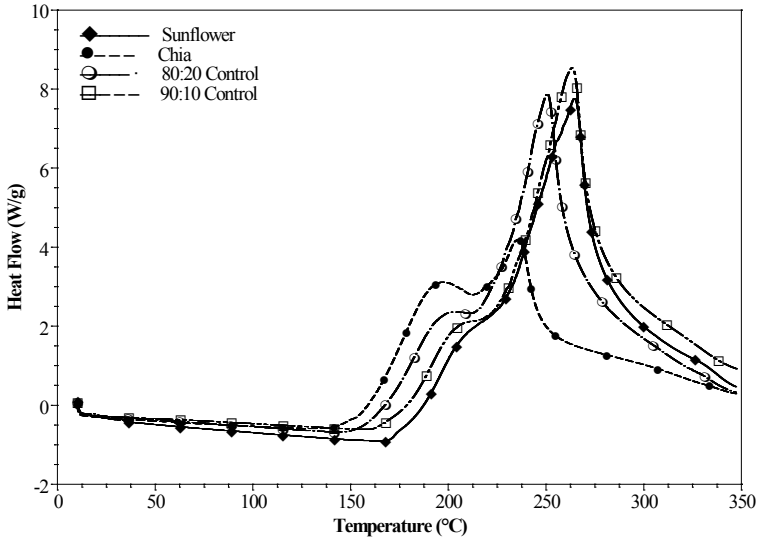

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Figure 2. Peroxide (a) and p-anisidine (b) values of sunflower oil, chia oil and their blends with and without the addition of antioxidants, stored at 4 ± 1 °C. Values are the mean of the two independent batches (n = 2) and vertical bars indicate SD.

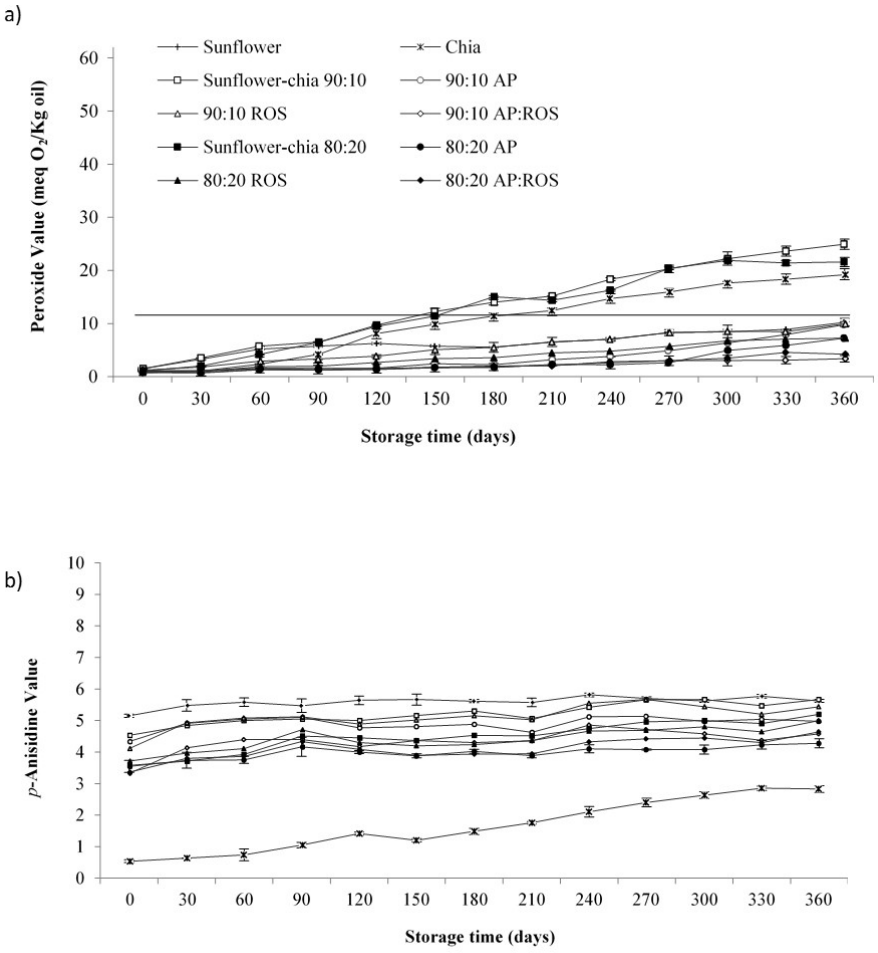

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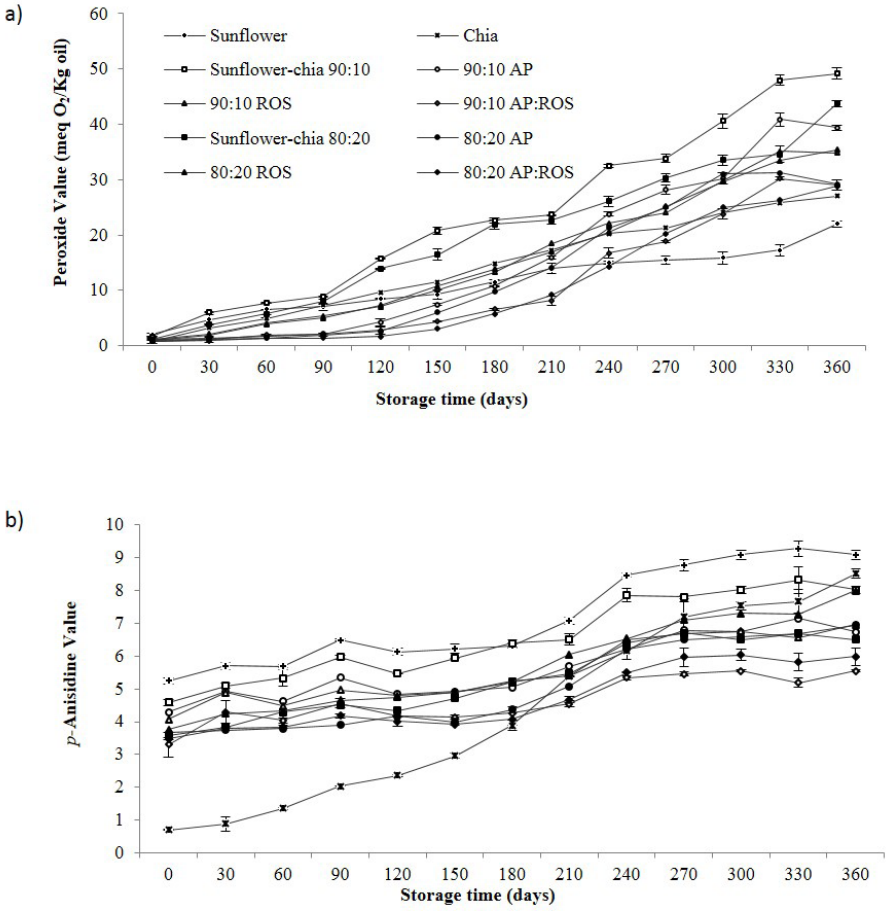
Figure 3. Peroxide (a) and p-anisidine (b) values of sunflower oil, chia oil and their blends with and without the addition of antioxidants, stored at 20 ± 2 °C. Values are the mean of the two independent batches (n = 2) and vertical bars indicate SD.



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COMPARISON OF GREEN PROCESSES BASED ON COMPRESSED FLUIDS FOR THE EXTRACTION OF CHIA OIL

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SUMMARY: Chia seed oil is rich in polyunsaturated fatty acids, particularly omega-3 (alfa-linolenic acid) and omega-6 (linoleic acid), which pose great benefits for human and animal health. Considering its high quality oil, and the development of the concepts of green chemistry (clean extraction processes which are less harmful to the environment and provide acceptable yields) the use of compressed fluids is encouraged. The main factors involved in supercritical fluid extraction (SFE) and pressurized liquid extraction (PLE) of chia seeds have been analysed. Different experimental designs considering the oil extraction yield value, as an effect of time, temperature and pressure were studied for SFE while effects of time, temperature and type of solvent were also evaluated for PLE.

Keywords: Supercritical extraction, pressurized liquid extraction, lipid analysis.

RESUMEN: *Comparación de procesos limpios basados en fluidos comprimidos para la extracción de aceite de chía.* Las semillas de chía son ricas en ácidos grasos poliinsaturados, especialmente omega-3 (ácido alfa-linolénico) y omega-6 (ácido linoleico), lo

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que supone grandes beneficios en la salud humana y animal. Teniendo en cuenta la gran calidad de su aceite y el desarrollo del concepto de química verde (procesos de extracción limpios que son menos perjudiciales para el medio ambiente y que proporcionan rendimientos aceptables), el uso de fluidos comprimidos es propicio.

Los principales factores que envuelven la extracción con fluidos supercríticos (SFE) y la extracción con líquidos presurizados (PLE) de semillas de chía han sido analizados. Diferentes diseños experimentales considerando el valor del rendimiento de extracción, por el efecto del tiempo, temperatura y tipo de disolvente han sido evaluados en PLE.

Palabras clave: Extracción supercrítica, extracción de líquidos presurizados, análisis lipídico.

1. INTRODUCTION

1.1. Green processes based on compressed fluids

1.1.1. Supercritical Fluid Extraction (SFE)

Supercritical fluid extraction (SFE) with carbon dioxide constitutes a green alternative to traditional extraction with hexane-type solvents for obtaining oil from seeds. SFE provides important advantages, such as the possibility of obtaining extracts free of solvents and the ability to modify the solvation power of the supercritical solvent altering the pressure and temperature conditions. Carbon dioxide ($SCCO_2$) is a non-toxic, non-flammable and non-corrosive solvent. In addition, due to its critical temperature and the absence of oxygen during extraction, extracts with low degradation and superior quality can be obtained. For this reason, SFE has been thoroughly studied for the extraction of oil from different seeds (Boutin and Badens, 2009; Sánchez-Vicente *et al.*, 2010; Passos *et al.*, 2010).

1.1.2. Pressurized liquid extraction (PLE)

The pressurized liquid extraction (PLE) or accelerated solid extraction (ASE) is a technique used in solid and semi-solid samples in order to increase the lipid extraction efficiency throughout the use of different solvents submitted to high pressures and temperatures during different times and number of extraction cycles. On a hand, this increment of temperature and pressure leads to a favourable kinetic, diffusion, solubility and other parameters, as well as the improvement of the extraction capabilities of other manual and/or traditional techniques (Castro-Gomez *et al.*, 2014). On the other hand, the combinations of all

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cited parameters result in a significant reduction of time and solvent consumption. Furthermore, additional advantages of this technique are the possibilities of automate the extraction process (Jansen *et al.*, 2006, Yao and Schaich, 2015) and the option of using food grade solvent making the extract suitable for consumption providing acceptable yields (Silva *et al.*, 2016).

2. RESULTS

2.1. Supercritical fluid extraction (SFE) for chia seeds oil

In regard to the SFE extraction of oil from chia seeds, Table 1 lists the studies reported to date. In general, authors identified pressure as the parameter of greatest influence for the extraction of oil. Additionally, a crossover point was observed, reaching the largest oil yields at high pressure and temperature, despite the lower density of CO_2 as a result of increasing temperature. Ixtaina *et al.* (2010) obtained the highest oil recovery (92.8%, expressed as mass of oil extracted/mass of oil in seeds x 100) at 45 MPa and 80 °C. The amounts of omega-6/omega-3 ratio (0.75-0.31) in the extracts varied depending on the SFE conditions. In another contribution, Ixtaina *et al.* (2011) recovered oil in the range from 82% (25 MPa, 40 °C) to 97% (45 MPa, 60 °C). In this case, the unsaturated fatty acid profile in the fractions was very similar to that corresponding to the overall extract.

Rocha Uribe *et al.* (2011) carried out extractions in two steps: an initial static extraction period, followed by a dynamic mode. The highest extraction yield (7.2%, expressed as mass of oil extracted/mass of seeds x 100) was obtained at 40.8 MPa and 80 °C after 10 min and 30 min of static and dynamic extraction time, respectively. Additionally, authors reported a kinetic extraction at 40.8 MPa and 80 °C, reaching around 20% yield after 390 min. In contrast to Ixtaina *et al.* (2010) at similar extraction conditions (45 MPa, 80 °C, and 300 min) who used 75 g/g of CO_2/seed mass ratio, Rocha Uribe *et al.* (2011) applied only 23 g/g and it could be the cause of the low yields reported. The fatty acid content and omega-6/omega-3 ratio (0.29-0.27) obtained hardly varied and no thermal degradation was observed at the different operation conditions.

Guindani *et al.* (2016) evaluated the oil extraction from chia seed cakes (the residue obtained from chia seed oil extraction by cold pressing) using pure CO_2 and CO_2 with ethanol or ethyl acetate as a co-solvents. The highest extraction yield (10.6%) with pure CO_2 was obtained at 30 MPa and 50 °C. As expected, the use of co-solvents (2.5% w/w) produced an appreciable increase in the extraction yield, especially when ethanol was used. Regarding major fatty acid composition (omega-6/omega-3 ratio), little variation at the different SFE operation conditions was reported.

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All these studies show the increasing interest that can be found nowadays relative to the commercial production of oil from chia seeds by SFE that has led to the application of a patent (Minatelli *et al*., 2013).

2.2. Pressurized liquid extraction for chia seeds oil

The chia seed has been submitted to different extraction methods in order to obtain its bioactive compounds including dietary fibre, antioxidants but especially the oil. Previous studies of our group, Tolentino *et al*. (2014), and others as Segura-Campos *et al*. (2014) and Amato *et al*. (2015) extracted chia seed oil using the Soxhlet procedure (IUPAC, 1992) with petroleum ether. Recently, de Mello *et al*. (2015) isolated the total oil using ultrasound-assisted extraction and ethyl acetate. Also Silva *et al*. (2016) isolated chia oil using an orbital shaker and ethyl acetate, isopropanol and n-hexane as extraction solvents. Although the PLE has been used in order to isolate oils from numerous seeds as grape (Chamorro *et al*., 2013), annatto seeds (Rodrigues *et al*., 2014) or oleaginous seed (Luque-Garcıa and De Castro, 2004), until now this methodology has not been applied to chia seeds, as far as we know. Therefore, in the present study the conditions of the PLE procedure as temperature (40, 60 and 80 °C), solvents as dichloromethane/methanol (2:1) or ethanol for the extraction of chia seed oil have been optimized.

ACKNOWLEDGMENTS

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REFERENCES

Amato M, Caruso MC, Guzzo F, Galgano F, Commisso M, Bochicchio R, Labella R, Favati F. 2015. Nutritional quality of seeds and leaf metabolites of Chia (*Salvia hispanica* L.) from Southern Italy. *Eur Food Res Technol* **241**, 615-625. DOI: 10.1007/s00217-015-2488-9.

Boutin O, Badens E. 2009. Extraction from oleaginous seeds using supercritical CO_2: experimental design and products quality. *J Food Eng* **92**, 396-402. DOI: 10.1016/j.jfoodeng.2008.12.007.

Castro-Gomez MP, Rodriguez-Alcala LM, Calvo MV, Romero J, Mendiola J, Ibanez E, Fontecha F. 2014. Total milk fat extraction and quantification of polar and neutral lipids of cow, goat, and ewe milk by using a pressurized liquid system and chromatographic techniques. *J Dairy Sci* **97**, 6719-6728. DOI: 10.3168/jds.2014-8128.

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Chamorro S, Viveros A, Centeno C, Romero C, Arija I, Brenes A. 2013. Effects of dietary grape seed extract on growth performance, amino acid digestibility and plasma lipids and mineral content in broiler chicks. *Animal* **7**, 555-561. DOI: 10.1017/S1751731112001851.

de Mello BTF, dos Santos Garcia VA, da Silva C. 2015. Ultrasound-Assisted Extraction of Oil from Chia (*Salvia hispanica* L.) Seeds: Optimization Extraction and Fatty Acid Profile. *J Food Process Eng* **40**, e12298. DOI: 10.1111/jfpe.12298.

Guindani C, Podestá R, Block JM, Rossi MJ, Mezzomo N, Ferreira SRS. 2016. Valorization of chia (*Salvia hispanica*) seed cake by means of supercritical fluid extraction. *J Supercrit Fluids* **112**, 67-75. DOI: 10.1016/j.supflu.2016.02.010.

IUPAC. 1992. Standard Methods for the Analysis of Oils, Fats and Derivatives, 7th ed.; International Union of Pure and Applied Chemistry. Blackwell Scientific: Oxford, U.K.

Ixtaina VY, Vega A, Nolasco SM, Tomás MC, Gimeno M, Bárzana E, Tecante A. 2010. Supercritical carbon dioxide extraction of oil from Mexican chia seed (*Salvia hispanica* L.): Characterization and process optimization. *J Supercrit Fluids* **55**, 192-199. DOI: 10.1016/j.supflu.2010.06.003.

Ixtaina VY, Mattea F, Cardarelli DA, Mattea MA, Nolasco SM, Tomás MC. 2011. Supercritical carbon dioxide extraction and characterization of Argentinean chia seed oil. *J Am Oil Chem Soc* **88**, 289-298. DOI: 10.1007/s11746-010-1670-2.

Jansen B, Nierop KGJ, Kotte MC, de Voogt P, Verstraten JM. 2006. The applicability of accelerated solvent extraction (ASE) to extract lipid biomarkers from soils. *Appl Geochem* **21**, 1006-1015. DOI: 10.1016/j.apgeochem.2006.02.021.

Luque-Garcıa J, De Castro ML. 2004. Ultrasound-assisted Soxhlet extraction: an expeditive approach for solid sample treatment: application to the extraction of total fat from oleaginous seeds. *J Chromatogr A* **1034**, 237-242. DOI: 10.1016/j.chroma.2004.02.020.

Minatelli JA, Hill WS, Moerck RE, Nguyen U. 2013. Plant derived seed extract rich in essentially fatty acids derived from Salvia hispanica L. seed: composition of matter, manufacturing process and use. U.S. Patent **8**, 586, 104.

Passos CP, Silva RM, Da Silva FA, Coimbra MA, Silva CM. 2010. Supercritical fluid extraction of grape seed (*Vitis vinifera* L.) oil. Effect of the operating conditions upon oil composition and antioxidant capacity. *Chem Eng J* **160**, 634-640. DOI: 10.1016/j.cej.2010.03.087.

Rocha Uribe JA, Novelo Pérez JI, Castillo Kauil H, Rosado Rubio G, Alcocer CG. 2011. Extraction of oil from chia seeds with supercritical CO_2. *J Supercrit. Fluids* **56**, 174-178. DOI: 10.1016/j.supflu.2010.12.007.

Sánchez-Vicente Y, Cabañas A, Renuncio JAR, Pando C. 2009. Supercritical fluid extraction of peach (*Prunus persica*) seed oil using carbon dioxide and ethanol. *J Supercrit Fluids* **49**, 167-173. DOI: 10.1016/j.supflu.2009.01.001.

Segura-Campos MR, Ciau-Solís N, Rosado-Rubio G, Chel-Guerrero L, Betancur-Ancona D. 2014. Physicochemical characterization of chia (*Salvia hispanica*) seed oil from Yucatán, México. *Agric Sci* **5**, 220-226. DOI: 10.4236/as.2014.53025.

Silva C, Garcia VAS, Zanette CM. 2016. Chia (*Salvia hispanica* L.) oil extraction using different organic solvents: oil yield, fatty acids profile and technological analysis of defatted meal. *Int Food Res J* **23**, 998-1004.

Tolentino RG, Vega MLR, Vega y León S, Fontecha J, Rodríguez LM, Medina AE. 2014. Fatty acid content in chia (*Salvia hispanica* L.) seeds grown in four Mexican states. *Rev Cubana Plant Med* **19**, 199-207.

Yao L, Schaich KM. 2015. Accelerated Solvent Extraction Improves Efficiency of Lipid Removal from Dry Pet Food While Limiting Lipid Oxidation. *J Amer Oil Chem Soc* **92**, 141-151. DOI: 10.1007/s11746-014-2568-1.

TABLE

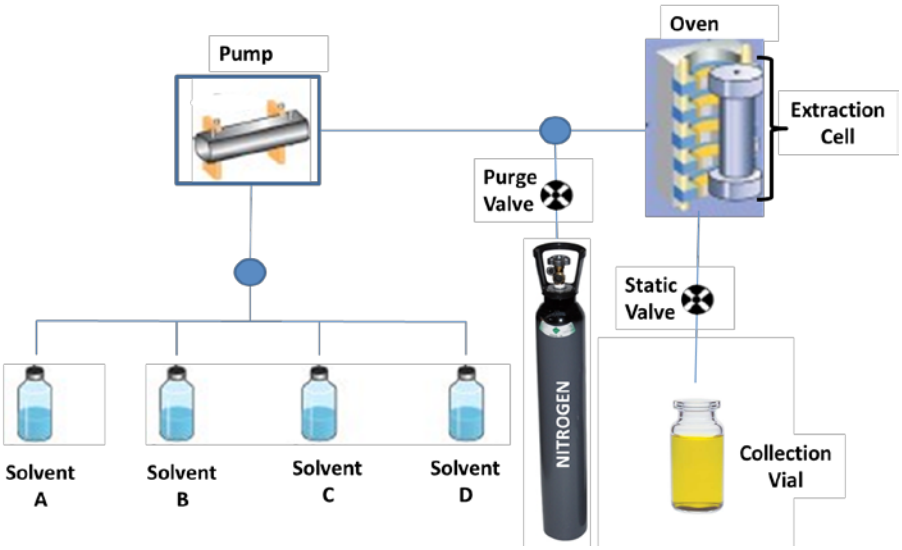
Table 1. Operation conditions and oil yield for the supercritical fluid extraction of chia seed oil.

Extraction conditions	Oil yield (%)	Reference
25-45 MPa; 40-80 °C; 60-240 min	88.1[a]	Ixtaina *et al.* (2010)
25-45 MPa; 40 and 60 °C; 138 min	97.0[a]	Ixtaina *et al.* (2011)
13.6-40.8 MPa; 40-80 °C; static extraction (10 min) followed by dynamic extraction (30 min)	7.2[b]	Rocha Uribe *et al.* (2011)
15-30 MPa; 40 and 50 °C; co-solvents: ethanol and ethyl acetate (0% and 2.5-7.5% w/w)	10.6[b]	Guindani *et al.* (2016)

[a]Expressed as mass of oil extracted/mass of oil in seeds x 100.
[b]Expressed as mass of oil extracted/mass of seeds x 100.

FIGURE CAPTION

Figure 1. Scheme of the pressurized liquid extraction (PLE) process.



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MICROBIOLOGICAL ANALYSIS OF CHIA SEEDS SOLD IN MENDOZA, ARGENTINA

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SUMMARY: In view of the episodes of salmonella food poisoning from the consumption of organic chia seeds, it was important to carry out the present study of the microbiological properties of chia. The samples analysed were apt for human consumption, Nº 2 having the highest count as a result of impurities in the sample. It would be advisable to set the levels of microorganisms for marketing purposes, as chia seeds are being consumed without prior processing.

Keywords: Chia seeds, microbiological analyses, safety.

RESUMEN: ***Análisis microbiológico de semillas de chía producidas en Mendoza, Argentina.*** Dado que existen antecedentes de intoxicación por salmonella tras el consumo de semillas de chía orgánica, se llevó a cabo la presente investigación sobre la calidad microbiológica de la chía. Las muestras analizadas resultaron aptas para su consumo, siendo la M2 la que presentó mayores recuentos, coincidente con una deficiente limpieza. Sería conveniente fijar los niveles de microorganismos con los cuales se comercializan, porque las semillas de chía se ingieren sin tratamientos previos.

Palabras clave: Semillas de chía, análisis microbiológico, seguridad.

1. INTRODUCTION

Argentine consumers of chia (*Salvia hispanica* L.) eat the seeds raw. They add them to yogurt, milk or fruit juice, or soak them in water to form a

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gel before they eat them. In both cases, it is convenient to evaluate the safety of the chia seeds that are sold in our stores through a microbiological analysis, taking into account that there is a history of salmonella poisoning produced by the consumption of organic chia seed registered in the United States and Canada in 2014.

The Argentine Food Code, section 918, sets forth the rules for their marketing as follows: "They shall not contain more than 0.5% of damaged seeds. They will be free from live insects. They shall not show more than 1% of foreign material, of which not more than 0.25% shall be mineral material, and not more than 0.10% shall be dead insects, fragments or remains of insects and/or other impurities of animal origin. Foreign material means mineral or organic matter (dust, twigs, seedcoats, seeds of other species, dead insects, fragments or remains of insects and other impurities of animal origin)." Nothing is specified in relation to microbiological safety (Código Alimentario Argentino, 2016).

The objective of this study was to determine the microbiological quality of chia seeds being consumed in the Great Mendoza, Argentina.

2. MATERIALS AND METHODS

To obtain a cross section of chia seeds, a two-stage, non-random sampling was carried out. In the first stage, a health food store was selected in each of the districts of the Great Mendoza, obtaining five samples in all. In the second stage, sampling of seeds carried out had the exclusive condition that they be packaged; so five samples in triplicate and from different brands were obtained, taking into account that they belong to the same batch. Sealed packages were opened at the time of the microbiological analysis, which allowed the following determinations:

a) Aerobic mesophilic microorganisms. PCA, 35 °C, 48 hours. Method: As per procedure Specific 09 - Bacteriology Analytical Manual online - FDA, January 2001.
b) Total coliform count VRB lactose, 35 °C, 24 hours. Method: ISO 4832:2016.
c) Yeast count. YGC, 25 °C, 5 days. Method: Bacteriology Analytical Manual.
d) Fungi count. YGC, 25 °C, 5 days. Method: Bacteriology Analytical Manual.
e) *Escherichia coli*. TBX, 44 °C, 24 hours. Method: ISO 16649-2:2001.
f) Detection of Salmonella sp. In 25 g Agar XLD; Agar HK 35 °C, 24 hours; and Agar BS 35 °C, 24-48 hours. Method: Bacteriology Analytical Manual online - FDA, December 2015.

The data were analysed using the software package for statistical analysis SPSS®, version 15.0. A one-way ANOVA was performed to compare mean differences. A significance level of $P<0.05$ was used in all cases.

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Identification of the samples**:**

Sample Nº 1: A. Chia seeds "El Peoncito" - Batch 160118. Expiration date: July 2017.

Sample Nº 2: B. Chía Paraguay 2016. Without expiration date.

Sample Nº 3: C. STURLA - Chia seeds. Expiration date: July 2017 - Batch C17C28 - 10:28 am.

Sample Nº 4: D. STURLA - Chia seeds. Expiration date: July 2017 - Batch Y4446 - 10:20 am.

Sample Nº 5: E. STURLA - Chia seeds. Expiration date: July 2017 - Batch Z71A11 - 6:44 am.

3. RESULTS

Out of the five analysed samples. Sample identified as Nº 2 had some foreign elements such as remains of stems, dust and spotted seeds. Fig. 1 reveals remains of stems and other impurities. This is confirmed in the results of the microbiological analyses detailed below. In the rest of the samples, as shown in sample Nº 3, no foreign elements were observed.

3.1. Microbiological analysis

As shown in Table 1, aerobic mesophilic microorganisms were present within normal limits, while Sample Nº 2 showed the highest count, followed by sample Nº 5. The statistical analysis indicated that there is no significant difference between samples Nº 2 and Nº 5, being different from the rest, and there are no differences between these two.

As regards total coliforms and contaminant indicators, Table 2 shows the results obtained. The result of increased contamination is observed again in sample Nº 2, followed by sample Nº 5. The other samples are free of contamination. The statistical analysis showed similar results to what was expressed before: there are no differences between samples Nº 2 and Nº 5.

The statistical analysis shows that there are significant differences between samples Nº 2 and Nº 1, and with the rest of the samples, but there are no differences among the samples Nº 3, Nº 4 and Nº 5.

Pathogenic bacteria: *Escherichia coli* and Salmonella were absent in all cases, as shown in Table 3.

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4. DISCUSSION

Although salmonella was not detected in the results, it is important to highlight that the analysed samples are packaged seeds coming from conventional, non-organic crops. It is worth mentioning that sample Nº 2, which showed the highest count, consisted of seeds with impurities easily observed as shown in Fig. 1. Sample Nº 5 also gave evidence of some high count, but no visible impurities were found. Parameters of the other three samples range within appropriate limits.

On the other hand, the fact that the seeds may be hydrated and consumed as gel promotes microbiological replication, for which it would be advisable to set limits and incorporate good agricultural practices at the time of their planting and harvesting.

5. CONCLUSIONS

The analysed samples were apt for consumption. The Sample Nº 2 showed the highest count as a result of poor cleaning. It would be advisable to set the levels of microorganisms for marketing purposes, as chia seeds are being consumed without processing.

ACKNOWLEDGMENTS

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REFERENCES

AOAC. *Official Methods of Analysis of the Association of Official Analytical Chemist International*. 2005. 18th Ed. Maryland, U.S.A.

Bacteriological Analytical Manual (BAM). Fecha de consulta junio 2016. http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm2006949.htm.

Código Alimentario Argentino. Fecha de consulta junio 2016. http://www.anmat.gov.ar/alimentos/normativas_alimentos_caa.asp.

EFSA, European Food Safety Authority. 2009. Opinion on the safety of "Chia seeds *(Salvia hispanica L.)* and ground whole Chia seeds" as a food ingredient. *EFSA J* **99**, 1- 26. DOI: 10.2903/j.efsa.2009.996.

Haros M. 2013. La chía en Europa sigue siendo una gran desconocida, y por ende sus propiedades. *Grasas Aceites* **92**, 384-385.

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Peiretti P, Gai F. 2009. Fatty acid and nutritive quality of chia (*Salvia hispanica L.*) seeds and plant during growth. *Anim Feed Sci Tech* **148**, 267-75. DOI: 10.1016/j.anifeedsci.2008.04.006.

TABLES

Table 1. Total count of aerobic mesophilic microorganisms

Determination	Nº 1	Nº 2	Nº 3	Nº 4 Nº 5
Aerobic mesophilic microorganisms UFC/g PCA, 35 °C, 48 hours	2 x 10^2 RE	6 x 10^3	2.1 x 10^2	7.5 x 10^2 3.1 x 10^3

RE: Out of range estimated count 25-250 CFU/plate range.

Table 2. Total coliform count.

Determination	Nº 1 Nº 2 Nº 3	Nº 4	Nº 5
Total coliform count CFU/g VRB lactose, 35 °C, 24 hours	Absence 5 x 10^1 Absence	Absence	2 x 10^1

Table 3. Detection of *Escherichia coli* and detection of Salmonella.

Determination	Nº 1	Nº 2	Nº 3	Nº 4	Nº 5
Escherichia coli CFU/g TBX, 44 °C, 24 hours	Absence	Absence	Absence	Absence	Absence
Detection of Salmonella sp./25 g Agar XLD; Agar HK 35 °C, 24 hours; and Agar BS 35 °C, 24-48 hours	Absence	Absence	Absence	Absence	Absence

FIGURE CAPTION

Figure 1. A. Sample Nº 2 chia from Paraguay; B. Sample Nº 3 chia from Sturla.

PHYSICOCHEMICAL CHARACTERIZATION OF CHIA SEED FLOUR AND THEIR COPRODUCTS, SPAIN

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SUMMARY: Chia (*Salvia hispanica* L.) is an annual plant of the family Lamiaceae, native to central and southern Mexico and Guatemala. The word "chia" is derived from the Nahuatl word *chian*, meaning oily. Production of chia is commercially growing for its seeds, which are composed of proteins, lipids, ash, and carbohydrates, and have high fibre content. Preliminary researches indicate potential health benefits from consuming chia seeds. Chia seed flour and their coproducts may present significant variations in their nutrient content, which highlights the importance of knowing the physicochemical properties of each one and thus can be exploited.

Keywords: Chia seed flour, physicochemical properties, fatty acids, fibre, protein isolate.

RESUMEN: *Caracterización fisicoquímica de la harina de semillas de chía y sus coproductos, España.* Chía (*Salvia hispanica* L.) es una planta anual perteneciente a la familia Lamiaceae, la cual es nativa del centro y sureste de México y Guatemala. La palabra "chía" se deriva del Náhuatl *chian*, que significa aceitoso. La producción de chía está creciendo comercialmente por los beneficios que aporta el alto contenido de fibra presente en sus semillas, las cuales están compuestas además por proteínas, lípidos, hidratos de carbono y cenizas, entre otros. Investigaciones preliminares indican los beneficios para la salud

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que conlleva el consumir chía. La harina obtenida a partir de las semillas de chia y sus coproductos presentan variaciones significativas en cuanto al contenido de sus nutrientes, lo cual resalta la importancia de conocer sus propiedades fisicoquímicas y de este modo darle un aprovechamiento óptimo a este recurso vegetal.

Palabras clave: Harina de semillas de chía, propiedades fisicoquímicas, ácidos grasos, fibra, aislado proteico.

1. INTRODUCTION

The number of consumers of chia (*Salvia hispanica* L.) has increased considerably in recent times so it has become a popular commercial product in Central and South America. The most common form of consumption is usually as aggregate in smoothies, yogurts, fruit juices, milk, etc. It has been found that consumption of chia may be useful in cases of gastrointestinal problems, atherosclerosis, obesity and diabetes, among other diseases. Recent researches highlight the importance of consuming chia because of their fibre content, being quite useful in case of appetite, facilitating digestion and intestinal transit, and on the other hand their protein content, which is a source of high biological value because it has all the essential amino acids, making it a good quality product to be used as a staple food. Moreover, fatty acid content of chia seeds makes it an important source of omega-3, which must be ingested in the diet because it can´t be synthesized by the organism.

Several studies detailed chia seed nutritional content, which is about 30 grams of fibre per 100 grams of flour, between 19 and 23% protein content which is greater than other grains and also approximately 25 and 38% of fatty acids. Usually these studies are based only on the use of the starting material and the resulting coproducts in the production of protein enriched products such as concentrate and isolate protein are discarded. For this reason it is appropriate to evaluate the nutrient content of not only the chia flour and defatted meal, but also these final protein products obtained, as well as their coproducts from the whole process such as the precipitation supernatant and the residual material.

The aim of this work was to physicochemically characterize the flour obtained from chia seeds and their coproducts in order to determine their nutrient content and which of them are the better balanced.

2. MATERIALS AND METHODS

Chia seed of this research comes from Mexico. Firstly, seeds were ground on a Retsch GM 200 (10000 rpm/15 sec) and then the flour obtained was defatted in a 20 L Solvent Extraction Unit with hexane for 16 h. Chia protein

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isolates were obtained from defatted meal by alkaline solubilisation of proteins and subsequent precipitation at their isoelectric point (Pedroche *et al.*, 2007). The chia flour, defatted meal, protein isolate and the coproducts obtained (precipitation supernatant and residual material) were stored under refrigeration (4 °C) for subsequently performing the following determinations:

a) Moisture content. Based on the methods published by the AOAC (2005).
b) Fatty acid content. Soxhlet extraction with hexane based on the methods published by the AOAC (2005).
c) Fibre and ash content. This method is based on the digestion of the samples by thermostable alpha amylase, protease and amyloglucosidase, and subsequent determination of the resulting residue by gravimetry according to Burkitt *et al.* (1974).
d) Protein content. The determination of total nitrogen is based on the method by combustion according to Dumas (1831). The nitrogen content is converted to crude protein considering that all the nitrogen is in raw form using a conversion factor. The equipment used is an elementary microanalyser LECO CHNS-932.
e) Soluble sugars content. The methodology consists of ethanol extraction of soluble sugars and subsequent quantification by the method of Dubois *et al.* (1956).
f) Polyphenols content. The methodology consists of ethanol extraction of polyphenols and subsequent quantification by the method of Moores *et al.* (1948).
g) Amino acids quantification. Samples were hydrolysed with 4 mL of 6N HCl in tubes and incubated in an oven at 110 °C for 24 h. Amino acid composition was determined according to the method of Alaiz *et al.* (1992). Tryptophan was determined by basic hydrolysis with 4 N NaOH at 110 °C for 4 h according to the method of Yust *et al.* (2004).
h) Fatty acids methyl esters composition. Based on the methods published by the AOAC (2005).

3. RESULTS

During defatting, meal obtained from chia seeds could not be pressed to obtain pellets due to the large amount of oil it contains (Figure 1). Also, during the process of obtaining of protein isolate from chia defatted meal, a mucilaginous layer was formed, which complicated the precipitation of proteins.

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3.1. Physicochemical analysis

As shown in Table 1, the lipid content was significantly higher in the starting flour (not defatted) respect to the other samples. Moreover, the fibre content was higher in the final coproduct obtained (residual material) and lower in the protein isolate, which is relevant because this residual material is considered a waste by-product. Also, the protein content was higher in the protein isolate followed by defatted meal, the sample obtained from the supernatant of precipitation, chia flour and the residual material obtained, respectively. Other complementary analyses were the determination of moisture, ash, soluble sugars and polyphenols content.

3.2. Amino acid quantification

Derivatization of amino acids was determined in order to know the content of certain amino acids that are important to add to the diet. The amino acid ratio of all chia samples is shown in Table 2.

3.3. Fatty acid methyl esters composition

Oil extracted from chia seed flour contains palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2) and alpha-linolenic acid (C18:3). From these results, it can be seen that chia seeds are quite rich in omega 3 as shown in Table 3.

4. DISCUSSION

The most valuable thing in the high content of fatty acids of chia seeds (~38%) is the presence of omega-3 alpha-linolenic acid (~55%), a component with an important role in health associated to foods. Also, chia oil has higher yields than other oils commonly used in food, therefore it can be used as a functional substitute because of the benefits on cardiovascular diseases. However, these results are lower than values reported by Peiretti and Gai (2009), which may be due mainly to factors such as seed variety and environmental conditions of the site where it was collected.

Meanwhile, the amino acid quantification shows that the content of some important amino acids in human nutrition, such as cysteine, arginine and lysine, are contained in good amount in the products and coproducts from chia seeds. The protein isolate (~75% protein content) may represent a rich

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source of protein quality and an excellent food supplement due to the content of lysine and cysteine, normally present in very low amounts in cereals (FAO/WHO, 1991).

On the other hand, the fibre content was higher in the final coproduct (residual material), which highlights the importance of its use in formulations for elaborated foods where a high fibre supplement is need. Digestibility studies should be performed to sustain this application.

5. CONCLUSIONS

Chia oil has high yield during the extraction process and is an important source of omega-3. The coproduct of chia (residual material) contains a high percentage of fibre, which could be exploited in the livestock industry. The physicochemical properties of the chia seed flour and their coproducts obtained are influenced by the initial defatted treatment as well as by the isolation process of their proteins. However, the choice of the sample with the most balanced nutrient content depends on the purpose for which it is sought to be used.

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REFERENCES

Alaiz M, Navarro JL, Girón J, Vioque E. 1992. Amino acid analysis by highperformance chromatography alters derivatization with dietil ethoxymethylenemalonate. *J Chromatogr A* **591**, 181-186. DOI: 10.1016/0021-9673(92)80236-N.

AOAC. *Official Methods of Analysis of the Association of Official Analytical Chemist International*. 2005. 18th Ed. Maryland, U.S.A.

Burkitt DP, Walker ARP, Painter NS. 1974. Dietary fiber and disease. *J Am Med Assoc* **229**, 1068-1074. DOI: 10.1001/jama.1974.03230460018013.

Dubois M, Gilles K, Hamilton J, Rebers P, Smith F. 1956. Colorimetric method for determination of sugars and related substances. *Anal Chem* **28**, 350-356. DOI: 10.1021/ac60111a017.

Dumas JBA. 1831. Procedes de l´analyse organic, *Ann Chim Phys* **247**, 198-213.

FAO/WHO. 1991. Protein Quality Evaluation. Report of a Joint FAO/WHO Expert Consultation. Food Nutr Paper **51**, FAO, Geneva, 23-24.

Moores RG, McDermott DL, Wood TR. 1948. Determination of chlorogenic acid in coffee. *Anal Chem* **20**, 620-624. DOI: 10.1021/ac60019a007.

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Pedroche J, Yust MM, Lqari H, Megías C, Girón-Calle J, Alaiz M, Vioque J, Millán F. 2007. Obtaining of *Brassica carinata* protein hydrolysates enriched in bioactive peptides using immobilized digestive proteases. *Food Res Int* **40**, 931-938. DOI: 10.1016/j.foodres.2007.04.001.

Peiretti P, Gai F. 2009. Fatty acid and nutritive quality of chia (*Salvia hispanica* L.) seeds and plant during growth. *Anim Feed Sci Tech* **148**, 267-75. DOI: 10.1016/j.anifeedsci.2008.04.006.

Yust MM, Pedroche J, Giron-Calle J, Vioque J, Millán F, Alaiz M. 2004. Determination of tryptophan by high-performance liquid chromatography of alkaline hydrolysates with spectrophotometric detection. *Food Chem* **85**, 317-320. DOI: 10.1016/j.foodchem.2003.07.026.

TABLES

Table 1. Physicochemical analysis.

Determination	Chia flour	Chia defatted meal	Precipitation supernatant	Protein isolate	Residual material
Moisture (%)	5.99 ± 0.02	1.32 ± 0.03	6.75 ± 0.05	3.90 ± 0.08	4.06 ± 0.02
Fatty acids (%)	37.82 ± 0.09	5.1 ± 0.15	1.75 ± 0.01	4.1 ± 0.00	2.79 ± 0.01
Fibre (%)	25.40 ± 0.01	49.39 ± 0.46	17.57 ± 0.01	11.54 ± 0.01	57.69 ± 0.04
Ash (%)	5.24 ± 0.04	7.22 ± 0.08	6.04 ± 0.13	2.91 ± 0.03	6.42 ± 0.04
Protein (%)	19.64 ± 0.05	31.76 ± 0.09	24.22 ± 0.02	75.58 ± 0.01	17.50 ± 0.05
Soluble sugars (%)	5.86 ± 0.01	5.12 ± 0.05	7.94 ± 0.01	1.92 ± 0.03	1.59 ± 0.05
Polyphenols (%)	0.0167 ± 0.00	0.0399 ± 0.00	0.0077 ± 0.00	0.0123 ± 0.00	0.0118 ± 0.00

Results are expressed as media ± SD of three independent assays.

Table 2. Amino acid quantification.

Amino acid	Chia flour	Chia defatted meal	Precipitation supernatant	Protein isolate	Residual material (coproduct)
Aspartic acid	7.238 ± 0.076	8.555 ± 0.118	9.044 ± 0.030	9.391 ± 0.196	10.323 ± 0.009
Glutamic acid	17.467 ± 0.133	18.038 ± 0.048	19.920 ± 0.173	19.367 ± 0.130	18.879 ± 0.061
Serine	6.237 ± 0.032	6.639 ± 0.029	6.361 ± 0.039	6.505 ± 0.135	6.317 ± 0.063
Histidine	3.085 ± 0.091	3.314 ± 0.092	1.791 ± 0.037	4.076 ± 0.102	3.059 ± 0.055
Glycine	4.881 ± 0.091	5.789 ± 0.085	4.242 ± 0.037	4.950 ± 0.103	5.597 ± 0.046
Threonine	3.848 ± 0.065	4.366 ± 0.045	3.867 ± 0.012	3.726 ± 0.114	4.327 ± 0.054

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Amino acid	Chia flour	Chia defatted meal	Precipitation supernatant	Protein isolate	Residual material (coproduct)
Arginine	9.808 ± 0.003	10.465 ± 0.006	10.430 ± 0.046	9.775 ± 0.169	8.650 ± 0.055
Alanine	4.783 ± 0.010	5.582 ± 0.034	4.825 ± 0.049	4.962 ± 0.040	5.288 ± 0.012
Proline	0.625 ± 0.004	2.237 ± 0.000	2.715 ± 0.000	2.698 ± 0.000	2.439 ± 0.000
Tyrosine	6.043 ± 0.094	5.531 ± 0.094	7.577 ± 0.064	8.735 ± 0.000	6.388 ± 0.075
Valine	4.540 ± 0.092	4.403 ± 0.078	4.071 ± 0.128	4.015 ± 0.158	5.951 ± 0.000
Methionine	0.904 ± 0.039	1.516 ± 0.000	2.925 ± 0.014	0.761 ± 0.021	0.621 ± 0.035
Cysteine	2.712 ± 0.003	2.376 ± 0.034	1.351 ± 0.007	2.467 ± 0.068	1.255 ± 0.074
Isoleucine	4.099 ± 0.073	3.359 ± 0.052	2.611 ± 0.084	2.424 ± 0.084	3.401 ± 0.052
Tryptophan	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Leucine	7.868 ± 0.070	7.261 ± 0.135	6.371 ± 0.097	6.639 ± 0.015	7.347 ± 0.044
Phenylalanine	6.166 ± 0.022	5.505 ± 0.097	6.313 ± 0.024	5.222 ± 0.003	6.283 ± 0.204
Lysine	5.519 ± 0.060	5.166 ± 0.094	5.110 ± 0.137	4.716 ± 0.000	4.063 ± 0.013

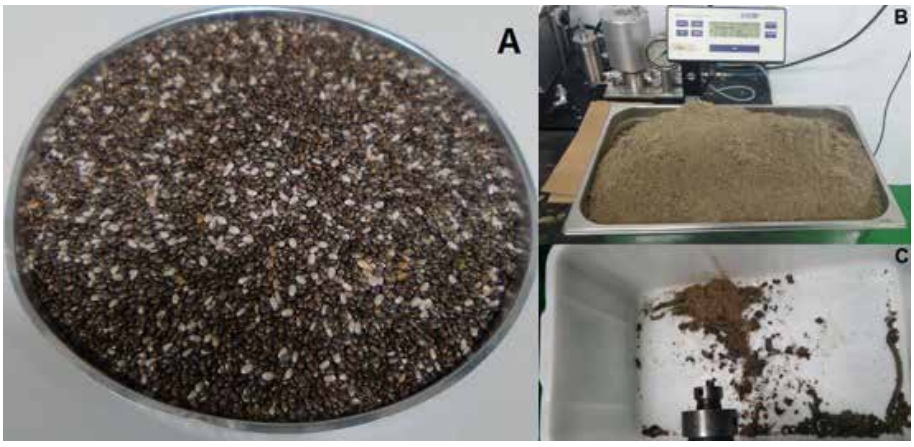
Results are expressed as media ± SD of three independent assays.

Table 3. Chia seed flour fatty acid composition.

Name	%
Palmitic acid (C16:0)	6.88
Stearic acid (C18:0)	4.13
Oleic acid (C18:1)	8.22
Linoleic acid (C18:2)	19.40
Alpha-linolenic acid (C18:3)	54.66

FIGURE CAPTION

Figure 1. A. Chia seeds; B. Chia seed flour; C. Flour could not be pressed.



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EXTRACTION AND QUANTIFICATION OF PHTHALATE ESTERS IN CHIA SEED OIL

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SUMMARY:

1. INTRODUCTION

Worldwide production of phthalic acid esters (PAEs) and their common application in different products of everyday use has resulted in their widespread presence in environment and foods. PAEs are used extensively as plasticizers in different materials, especially polyvinyl chloride, polyvinyl acetate, polystyrene, polyamide and polyester materials. Bis-(2-ethylhexyl) phthalate (DEHP) and benzylbutylphthalate (BBP) are the most abundant phthalate esters due to their wide use in many industrial sectors like cosmetics and paints. As plasticizers are not chemically bound to the polymer, migration of PAEs in significant amounts is possible. Phthalate extraction from chia or sacha inchi seed oils is particularly difficult due to its high content in omega-3 and omega-6 polyunsaturated fatty acids. Those fatty acids hamper the isolation of PAEs by classical methods as liquid-liquid extraction (LLE) or solid-phase microextraction (SPME).

The aim of this work is the isolation and clean-up of PAEs from these characteristic seed oils using solid-phase extraction (SPE) with a specific adsorbent and a method developed in our laboratory. Subsequently, phthalate

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esters are analyzed and quantified by gas chromatography coupled to ion trap tandem mass spectrometry (GC-ion trap MS-MS).

Keywords: Phthalates, solid-phase extraction, gas chromatography ion trap-mass spectrometry, chia seed oil.

2. MATERIALS AND METHODS

Chia seed oils were obtained from different packaging companies located in Peru and used as oil matrix for experiments. To avoid external phthalate contamination, all glassware used was soaked and washed in acetone for at least 30 min, rinsed with hexane, and dried at 200 °C for at least 4 h. Oil samples were extracted with ACN:acetone and processed by SPE. Full scan and MS/MS (MS^2) were used alternately and consecutively for MS analysis. Maximum excitation energy (Q) and CID excitation voltage were optimized previously for each individual standard compound and precursor ion in order to get the best isolation efficiencies and ensure maximum production or transmission of collision fragments.

3. RESULTS

The developed method was applied to three real samples of chia oils packed in glass containers. The samples were analyzed for PAEs and only few of them studied in this work were detected. Analyses were done in duplicate and, if any, levels in blank were subtracted. Only significant amounts of BBP and DEHP were detected in the samples kept in glass containers.

4. CONCLUSIONS

This method is suitable for the analysis and quantification of PAEs in oils for human consumption or cosmetics where the allowed limit is very low and especially for oils with high levels of omega-3 and omega-6 fatty acids. The oil matrix effects are greatly reduced with high specificity, being possible to reach a high detection level up to 0.1 ppm.

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3.
FOOD AND HEALTH

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IMMUNONUTRITIONAL INFLUENCE OF CHIA ON THE HEPATIC METABOLIC DYSFUNCTION

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SUMMARY: The study approaches immunonutritional effects of a chia-containing (*Salvia hispanica* L., 5%) bread formulation (ChB) on biomarkers that are important determinants of intestinal and liver metabolic health. ChB was compared to whole wheat and white bread in relation to the glycaemic index, and the hepatic expression of transferrin receptor 2 (TfR2) as systemic iron sensor and peroxisome proliferator-activated receptor gamma (PPARγ) as key regulator of nutrients distribution. Chia flour as breadmaking ingredient promoted beneficial changes in immunonutritional biomarkers with potential relevant implications for insulin resistance and an inflammatory state.

Keywords: Chia bread, glycaemic index, PPARγ.

RESUMEN: *Influencia inmunonutricional de la chía en la disfunción metabólica hepática.* El estudio evalúa la influencia de una formulación panaria con chía sobre parámetros inmunonutricionales importantes para la salud intestinal y metabolismo hepático. La formulación panaria con harina de chía (*Salvia hispanica* L., 5%) se comparó con panes de trigo integral y blanco en relación al índice glucémico y la expresión hepática del receptor de transferrina 2 debido a su papel como sensor sistémico de hierro y de PPARγ como regulador crítico de la distribución metabólica de nutrientes. La inclusión de harina de chía como nuevo ingrediente en panificación favoreció variaciones beneficiosas

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en los biomarcadores inmunonutricionales con una potencial implicación relevante en la prevención de la resistencia a insulina y un estado inflamatorio.

Palabras clave: Pan con chía, índice glucémico, PPARγ.

1. INTRODUCTION

Western diet commonly favors overnutrition with an altered food supply and a particular high intake, among others, of refined grains. Type and amount of dietary carbohydrates are important determinants of postprandial glucose and insulin responses. Currently, it has been demonstrated that high-glycaemic index (GI) diets are associated with developing metabolic dysfunction and predispose to type 2 diabetes (T2D) and overweight/obesity and associated risk factors in children and adolescents.

To tackle this worldwide spread pandemic several different immunonutritional strategies are currently being used. In this context, clarification of the influence of innovative bread formulations is needed to facilitate the development of effective nutritional intervention strategies.

2. MATERIALS AND METHODS

2.1. Breadmaking

Compressed yeast (*Saccharomyces cerevisiae*) was used as a starter in identical breadmaking processes to prepare chia-containing bread formulation at 5% according to previously established processes (Iglesias-Puig &Haros, 2013).

2.2. Animals

For the experiments there were used Wistar rats in strict accordance with the recommendations included in the Guide for the Care and Use of Laboratory Animals of University of Valencia (SCSIE, University of Valencia, Spain).

2.3. Glucose quantification

Blood glucose was determined using a commercial glucometer (Accu-Chek®, Roche). The data obtained were used to plot time-course curves to calculate the area under the curve (AUC) for each treatment group (SigmaPlot

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v10.0, Systat Soft. Inc, UK). From the AUC values there were calculated apparent hydrolysis indexes (HI) in relation to a reference sample (white bread) as HI (%) = ($AUC_{Bread\ formulation}/AUC_{White\ bread}$) x 100. The estimated glycemic indexes (GI) were calculated as previously described (GI = 39.71 + 0.549 (HI)) (Mardiana & Noor, 2009).

2.4. Hematological parameters

Hemoglobin (Hb) concentration was determined according to the International Council for Standardization in Hematology (ICSH) (Laparra *et al.*, 2014).

2.5. Gene expression of hepatic biomarkers

Total RNA was extracted from liver tissue samples using an RNeasy mini kit (Qiagen, USA) and the transcripts of TfR2, PPARγ and β-actin, used as a housekeeping gene, were analyzed by reverse transcription-real time PCR (Laparra *et al.*, 2014).

2.6. Statistical analysis

The analyses were performed using SPSS v.15 software (SPSS Inc., Chicago, IL, USA) and statistical significance was established at $P<0.05$ for all comparisons.

3. RESULTS

3.1. Glycaemic index

Time course of glucose concentration in animals fed with the different bread formulations is shown in Fig. 1. Overall, maximum glucose concentrations were quantified after 20 min as follows: ChB < WWB < WB. The slopes that can be calculated from plotting blood glucose concentrations during this period of time reveal different kinetics for glucose absorption that result clearly advantageous when feeding ChB in comparison to WB.

The AUC values, HI and estimated GI for the different bread formulations are shown in Fig. 2. Feeding WWB or ChB significantly decreased ($P<0.05$) AUC values in relation to WB. Notably, HI values calculated for WWB and ChB were about 10.3% and 31.1% lower than values calculated for WB, respectively.

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Significant decreases of HI values in bread samples were reflected in significantly ($P< 0.05$) decreased GI values.

3.2. Hepatic biomarkers

Changes in the gene expression (mRNA) of TfR2 and PPARγ in animals fed with the different bread formulations are shown in Fig. 3. Only ChB had a significant influence on Fe bioavailability at the investigated level of substitution that was reflected in a down-regulated expression of TfR2. There were quantified changes in the expression of PPARγ according to the following gradation: ChB > WWB = WB.

4. DISCUSSION

Several different technological factors have been shown to influence GI of food, but also the effect attributable to other ingredients is of physiological relevance. Notably, differences in lipid profiles and their associations with glycemic outcomes have been identified in obese, nondiabetic patients during weight-management interventions (Valsesia *et al.*, 2016). An aim of weight loss is to reduce the risk of T2D; however, the relation of lipid metabolism and long-term effects on glucose homeostasis remains unsolved. In this context, prior research has largely focused on total calorie intake and consumption, with a continuous positive balance promoting obesity and/or the metabolic syndrome and finally fatty liver. Recent data suggest that the composition of the food, irrespective of calorie count, and its influence on and interaction with the gut microbiota, and finally their crosstalk with the host's intestinal immune system may be even more important determinants of liver metabolic health.

Impaired glucose tolerance is accompanied by increased measures of oxidative stress as well as increased systemic iron (i.e., serum ferritin) because the decreased iron transport due to impaired insulin signalling. Ferritin is found in most tissues as a cytosolic protein, but small amounts are secreted into the serum where it functions as an iron carrier. Importantly, this alteration on iron status appears associated to the metabolic syndrome and progression of non-alcoholic fatty liver disease (Jin *et al.*, 2015). Moreover, there is increasing evidence from mouse models that the macrophage iron depletion seen in iron deficiency may have pro-inflammatory effects (Pagani *et al.*, 2011). Systemic iron homeostasis is predominantly regulated by the liver where the expression of TfR2 constitutes an important systemic iron sensor regulating the production of pro-inflammatory hormones

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such as hepcidin. The modulation of pro-inflammatory profiles in hepatocytes and macrophages has been suggested as effective approaches for intervention in obesity (Kheder *et al*., 2016). Increased understanding of these dynamics may allow us to target potentially harmful populations whilst promoting anti-inflammatory or restorative populations to ultimately guide the development of effective treatment strategies.

PPARγ plays a key role in maintaining, among other, insulin-mediated signalling and glucose homeostasis. Thus, the important role attributed to PPARγ in substrate fractionation towards energy expenditure or accumulation is crucial to promote insulin resistance and overweight/obesity development. As such, ChB-induced PPARγ overexpression can be associated to increased energy expenditure that together with the decreased GI allow to hypothesize a normalized insulin sensitivity and down-regulated lipogenesis, but improved fat partitioning and metabolism.

5. CONCLUSIONS

The inclusion of flour from chia (*Salvia hispanica* L.) at 5% exerts beneficial effects decreasing glycaemic index and promoting immunonutritional changes with a preventive role in glucose tolerance and a number of components of the metabolic syndrome.

ACKNOWLEDGMENTS

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REFERENCES

Iglesias-Puig E, Haros M. 2013. Evaluation of dough and bread performance incorporating chia (*Salvia hispanica* L.). *Eur Food Res Tech* **237**, 865-874. DOI: 10.1007/s00217-013-2067-x.

Jin Y, He L, Chen Y, Fang Y, Yao Y. 2015. Association between serum ferritin levels and metabolic syndrome: an updated meta-analysis. *Int J Clin Exp Med* **8**, 13317-13322.

Kheder RK, Hobkirk J, Stover CM. 2016. *In vitro* Modulation of the LPS-Induced Proinflammatory Profile of Hepatocytes and Macrophages- Approaches for Intervention in Obesity?. *Front Cell Dev Biol* **4,** Article 61. DOI: 10.3389/fcell.2016.00061.

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Laparra JM, Díez-Municio M, Herrero M, Moreno FJ. 2014. Structural differences of prebiotic oligosaccharides influence their capability to enhance iron absorption in deficient rats. *Food & Funct* **5**: 2430-2437. DOI: 10.1039/c4fo00504j.

Mardiana A, Noor Aziah AA. 2009. *In vitro* starch hydrolysis and estimated glycaemic index of bread substituted with different percentage of chempedak (*Artocarpus integer*) seed flour. *Food Chem* **117**, 64-68. DOI: 10.1016/j.foodchem.2009.03.077.

Pagani A, Nai A, Corna G, Bosurgi L, Rovere-Querini P, Camaschella C, Silvestri L. 2011. Low hepcidin accounts for the proinflammatory status associated with iron deficiency. *Blood* **118**, 736–746. DOI: 10.1182/blood-2011-02-337212.

Valsesia A, Saris WH, Astrup A, Hager J, Masoodi M. 2016. Distinct lipid profiles predict improved glycemic control in obese, nondiabetic patients after a low-caloric diet intervention: the Diet, Obesity and Genes randomized trial. *Am J Clin Nutr* **104**, 1-10. DOI: 10.3945/ajcn.116.137646.

FIGURE CAPTIONS

Figure 1. Mean (n = 5) typical profile of blood glucose concentrations in rats fed with bread formulations prepared by inclusion of flour from chia (5%, ChB), whole wheat (WWB) or white wheat (WB).

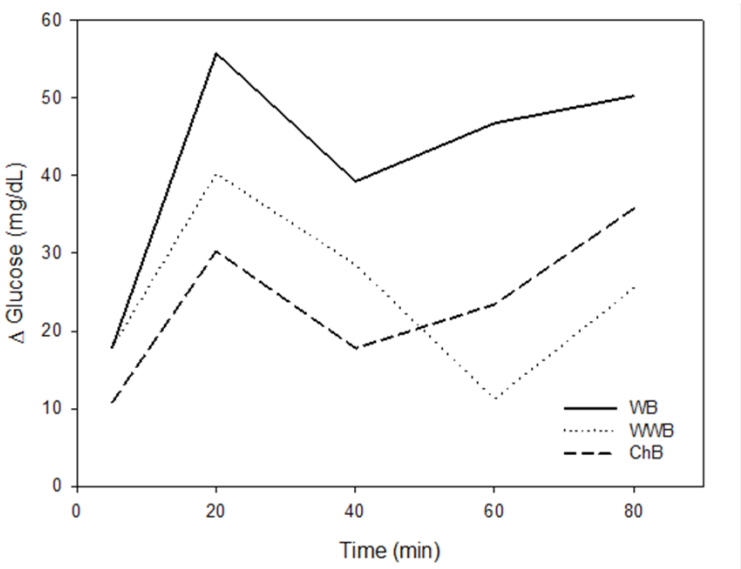

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Figure 2. Area under the curve (AUC), hydrolysis index (HI) and estimated glycemic index (GI) of chia-containing bread formulation in comparison to whole wheat (WWB) and white bread (WB). Results are expressed as mean ± SD (n = 5). Different superscript letters indicate statistical (P<0.05) differences.

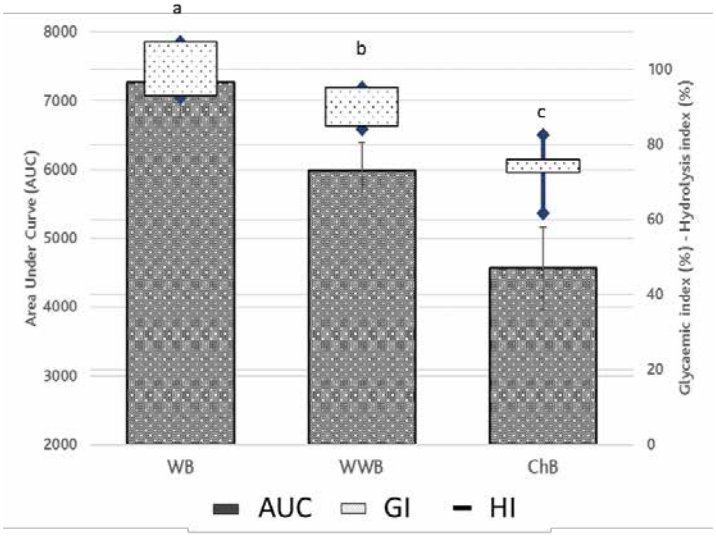
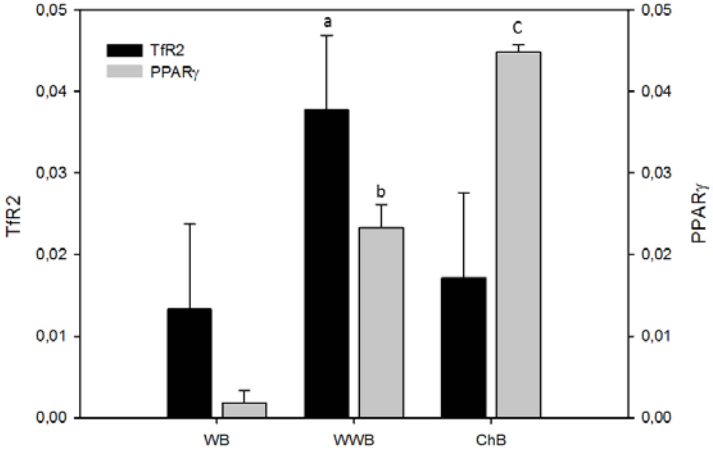


Figure 3. Fold change in the hepatic expression (mRNA) of transferrin receptor 2 (TfR2) and the peroxisome proliferator-activated receptor gamma (PPARγ) in animals fed with a chia-containing bread formulation (ChB), a whole wheat (WWB) or white bread (WB). Results are expressed as mean ± SD (n = 5). Different superscript letters indicate statistical (P<0.05) differences for each individual parameter in relation to WB.



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CHIA SEED AND HUMAN HEALTH

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SUMMARY: Chia seeds can be considered as "functional food" because besides of its contribution to human nutrition and health such a good source of omega-3 fatty acids, fibre, proteins, antioxidants, etc., the consume of this seed helps to increase satiety index, prevents cardiovascular diseases, inflammatory and nervous system disorders and diabetes, among others.

The objective of this work is the evaluation of the mucilage from chia seed at low, medium and high initial viscosity on the digestion process by using *in vitro* model system to simulate the digestive stages (oral, gastric and intestinal).

The use of chia seed and its mucilage increase the viscosity of the meals. During the digestive process the viscosity decreases slightly, probably due to changes in pH, but the changes in viscosity are also associated with slightly structural changes in the fibre. It is evident that structure and function of mucilage are directly related.

The "viscous effect" of the meals added with chia seed and mucilage can produce a physiological response induced by dietary fibre consumption. Then, the consume of chia seed or this mucilage may help to lead to weight loss by delaying gastric emptying helping to promote satiety, between others healthy effects.

ACKNOWLEDGMENTS
The authors acknowledge the financial support of FONDECYT Project 11150307.

Keywords: Chia, functional properties, *in vitro* digestion.

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COOKING HEALTH AND PLEASURE WITH OIL SEED AND CHIA (*SALVIA HISPANICA*)

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SUMMARY: Introduction: Chia (*Salvia hispanica*) is an ingredient not yet introduced in the current cuisine. Its unique characteristics inspire us to a large number of recipes to surprise and delight diners. Its nutritional value makes it even more interesting to use.

Objective: Study the organoleptic and technological characteristics of the seeds of chia and their derivatives. Introduce the use of the seeds and derivatives in the kitchen taking advantage of characteristics and properties.

Materials and Methods: Current contemporary cuisine together procedures, with local materials available in southern Spain.

Results: Different organoleptically valuable culinary creations in which the beneficial nutritional properties is enriched by the use of chia and its by-products.

Conclusions: Due to the work of the various groups participating in the Chia-Link Network, chefs have valuable information to develop new recipes with these ingredients so little used in actual cuisine. Direct relationship science and gastronomy and media coverage can make possible the availability of the results of investigations for most of the population.

ACKNOWLEDGMENTS
Special thanks to Instituto de la Grasa (IG-CSIC) to share knowledge and give us the possibility of participation in this conference.

Keywords: Healthy cooking, innovation, new products, science and gastronomy.

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BIOACTIVE ACTIVITIES OF PRODUCTS FROM CHIA SEEDS

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SUMMARY: Chia protein hydrolysates (CPHs) were obtained from chia protein isolate by hydrolysis with Alcalase or Flavourzyme. Some bioactive activities of these protein hydrolysates were tested, specifically antioxidant activity, angiotensin converting enzyme (ACE) inhibition and thrombin activity inhibition. All hydrolysates produced using Alcalase inhibited more than 80% of ACE activity, whereas the inhibition were lower than 40% in hydrolysates obtained with Flavourzyme. Antioxidant activity was also increased after hydrolysis; in this case, CPHs produced with Flavourzyme showed better results. Regarding thrombin activity, only CPHs obtained after 60 and 120 min of hydrolysis with Flavourzyme inhibited this enzyme.

Keywords: Bioactive peptides, chia defatted flour, protein hydrolysis.

RESUMEN: *Actividades biológicas de productos derivados de semillas de chía.* Se han obtenido hidrolizados proteicos de chía mediante la hidrólisis con Alcalase o Flavourzyme del aislado proteico. Se estudiaron algunas propiedades bioactivas de estos hidrolizados, en concreto la actividad antioxidante, la inhibición de la enzima convertidora de angiotensina (ACE) y la inhibición de trombina. Todos los hidrolizados obtenidos con Alcalase inhibieron más del 80% de la actividad ACE, mientras que los hidrolizados obtenidos con Flavourzyme no superaron el 40% de inhibición. La hidrólisis también mejoró la actividad antioxidante; en este caso, los hidrolizados obtenidos con Flavourzyme

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presentaron mejores resultados. Solo los hidrolizados obtenidos tras 60 y 120 min de hidrólisis con Flavourzyme mostraron inhibición de trombina.

Palabras clave: Harina desgrasada de chía, hidrólisis de proteínas, péptidos bioactivos.

1. INTRODUCTION

Nutritionally, proteins are a source of energy and amino acids, which are essential for growth and maintenance. Functionally, proteins contribute to the physicochemical and sensory properties of various protein-rich foods. Furthermore, many dietary proteins possess biological properties, which make them potential ingredients of functional or health-promoting foods. Many of these properties are attributed to biologically active peptides encrypted in protein molecules that can be released during gastrointestinal digestion or by controlled hydrolytic processes using exogenous proteases (Pedroche *et al*., 2007).

In this sense, plant protein hydrolysates obtained by treatment with proteases have been reported to carry specific bioactivities, and so constitute a group of under-exploited functional food ingredients. Much research has focused on hydrolysates with angiotensin converting enzyme (ACE) inhibitory and antioxidant activities whereas other bioactive properties such as the anti-inflammatory effects are less studied (Yust *et al*., 2012).

Salvia hispanica, commonly known as chia, is a species of flowering plant in the mint family, Lamiaceae, native to Mexico and with special significance in Latin America due to the fact that it has been consumed since ancient times. The culinary uses of chia have been as whole seed, seed flour, seed mucilage and seed oil (Valdivia-López and Tecante, 2015). Chia seeds were allowed to be commercialized in Europe in 2009. One of the main uses of chia seeds is the production of chia oil, rich in omega 3 fatty acids. A protein-rich flour is also obtained in this process, which could be a good raw material for the production of bioactive peptides.

The objective of this work was to determine some bioactive properties of CPHs: antioxidant activity, ACE inhibition and thrombin inhibition. CPHs were obtained by hydrolysis of chia protein isolate (CPI) with Alcalase and Flavourzyme, two food-grade proteases produced by Novozymes that have been previously used for the generation of bioactive peptides (Korhonen and Pihlanto, 2006; Yust *et al*., 2012).

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2. MATERIALS AND METHODS

2.1. Materials

Linoleic acid, β-carotene, ACE from rabbit lung, thrombin from bovine plasma, N-(p-tosyl)-Gly-Pro-Arg p-nitroanilide acetate salt were purchased from Sigma-Aldrich (St. Louis, MO, USA). Alcalase 2.4L and Flavourzyme 1000L were a gift from Novozymes Spain, S.A. (Madrid, Spain). *O*-aminobenzoylglycyl-p-nitro-L-phenylalanyl-L-proline was from Bachem S.A. (Budendorf, Switzerland). All other reagents were of analytical grade.

2.2. Methods

Preparation of chia protein isolate

Chia protein isolate (CPI) was prepared according to Yust *et al.* (2010) with slight modifications. Chia defatted flour (50 g) was suspended in 1 L of 0.25% Na_2SO_3 (w/v) and extracted at pH 10.5 for 1 h at room temperature and under stirring. After centrifuging at 8500 rpm for 15 min, an additional extraction was carried out with half of the volume. The supernatants were pooled, and pH was adjusted to isoelectric point (4.3). The precipitated was recovered by centrifuging and spray-dried.

Enzymatic hydrolysis of chia protein isolate

Hydrolysis of CPI was performed by treatment with Alcalase (for 1 h) or Flavourzyme (for 2 h) in a reactor with stirring and controlled pH and temperature. The following hydrolysis parameters were used:

Alcalase hydrolysis: substrate concentration 50 g L^{-1}, enzyme concentration 4.6 mL L^{-1}, temperature 50 °C, pH 8.

Flavourzyme hydrolysis: substrate concentration 50 g L^{-1}, enzyme concentration 1.8 mL L^{-1}, temperature 50 °C, pH 7.

Samples were withdrawn at different times, and enzymes were inactivated by heating at 85 °C for 15 min. The supernatants obtained after centrifuged at 8000 rpm for 15 min constituted the CPHs. The LPHs obtained using Alcalase were designated 0A, 15A, 30A, 45A and 60A. The CPHs obtained using Flavourzyme were designated 0F, 15F, 30F, 45F, 60F, 75F, 90F, 105F and 120F. The number indicates the time of hydrolysis in minutes.

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Antioxidant activity of chia protein hydrolysates

It was determined by measuring the oxidative losses of β-carotene in a β-carotene-linoleic acid emulsion as described by Yust *et al.* (2012).

Inhibition of angiotensin converting enzyme activity

The method described by Sentandreu and Toldrá (2006) was used. The method relies on the ability of ACE to hydrolyse the internally quenched fluorescent substrate *o*-aminobenzoylglycyl-p-nitro-L-phenylalanyl-L-proline. Samples concentration was 0.17 mg/mL.

Thrombin activity assay

The thrombin activity was determined by following the increase in absorbance at 405 nm that accompanies hydrolysis of the chromogenic substrate N-(p-tosyl)-Gly-Pro-Arg p-nitroanilide (Ialenti *et al.*, 2001). CPHs were tested at 0.02 mg/mL.

Statistical analysis

The data are presented as the mean ± SD of three independent determinations. Group-wise statistical comparisons were performed by a one-way ANOVA with a *post hoc* Bonferroni test. Differences were considered to be significant at $P<0.05$.

3. RESULTS AND DISCUSSION

3.1. Antioxidant activity of chia protein hydrolysates

Antioxidant activity of CPHs was evaluated by measuring decolouration of β-carotene due to oxidation products of linoleic acid. This assay is widely used to measure the antioxidant activity of bioactive compounds because β-carotene is extremely susceptible to free radical mediated oxidation of linoleic acid. All samples tested, included non-hydrolysed proteins (CPHs 0A and 0F), showed protection against oxidation of β-carotene (Figures 1 and 2). Among CPHs obtained using Alcalase, only CPH 15A increased antioxidant activity of intact proteins (Figure 1). In contrast, all CPHs produced with Flavourzyme improved antioxidant activity, with the exception of CPH 15F (Figure 2). In general, hydrolysis with Flavourzyme was more effective to increase antioxidant activity of chia proteins. Increased antioxidant activity of proteins

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after hydrolysis has been reported for a great number of materials such as soy, chickpea, rapeseed, potato, gelatine, etc. This increase may be due to an increased exposure of antioxidants amino acids to solvent (Segura-Campos *et al*., 2013).

Antioxidant activities of CPHs have been previously described, although the raw material and hydrolysis conditions were different to the ones used in this paper. So, Orona-Tamayo *et al*. (2015) reported that hydrolysis of different fractions of chia proteins (albumin, globulin, prolamin and glutelin) with pepsin, trypsin and pancreatin resulted in peptides showing antioxidant activity. Moreover, hydrolysis of a chia protein-rich fraction with an Alcalase-Flavourzyme sequential system increased antioxidant activity too (Segura-Campos *et al*., 2013).

3.2. Angiotensin converting enzyme inhibition by chia protein hydrolysates

ACE activity leads to an increase in blood pressure by producing the vasoconstrictor peptide angiotensin II and by degrading the vasodilator peptide bradyquinin. Inhibitors of ACE (captopril, benazepril, enalapril, lisinopril, etc.) are used in therapy against hypertension (Pedroche *et al*., 2007). ACE inhibitory peptides have been produced by hydrolysis of a wide variety of animal and plant proteins (Halim *et al*., 2016; Maestri *et al*., 2016; Ryder *et al*., 2016).

ACE inhibitory activity of CPHs was tested at 0.17 mg/mL. At this concentration, CPHs obtained using Alcalase inhibited more than 80% ACE activity (Table 1). There were small differences among CPHs and it was not possible to correlate ACE inhibition with time of hydrolysis. CPHs produced with Flavourzyme showed increased ACE inhibition if compared with intact chia proteins, although values were much lower than with Alcalase, not reaching 40%. Again, there was no correlation between ACE inhibition and time of hydrolysis.

Considering these results CPHs 15A, 30A, 45A and 60A were tested at several concentrations to calculate IC_{50} (peptide concentration required to produce 50% ACE inhibition). All CPHs showed IC_{50} lower than 80 µg/mL (Table 3). These values are slightly higher than that reported by Segura-Campos *et al*. (2013) in CPHs produced by Alcalase followed by Flavourzyme, but much better that IC_{50} of CPHs produced by hydrolysis of several fractions of chia proteins with pepsin and pancreatin (Orona-Tamayo *et al*. 2015), where IC50 of globulin peptides was 339 µg/mL. However, in both cases ACE inhibition was measured by another method, so results may be not directly compared. Moreover, IC_{50} decreased as hydrolysis time increases; there was not significant difference between CPHs 45 A and 60A (Table 3).

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3.3. Effect of chia protein hydrolysates on thrombin activity

Thrombin has been found to exert a number of pro-inflammatory and mitogenic effects, and has been linked to the development of vascular disease (Morris *et al.* 1994). In this sense, it has been reported that thrombin stimulates the production of pro-inflammatory cytokines and interleukins, such as TNF-α, IL-1 and IL-6, and the production of monocyte chemotactic protein (MCP-1) by endothelial cells (Strande and Phillips, 2009). It has been described that lupine protein hydrolysates may act as anti-inflammatory agents due, partially, to their capacity to inhibit thrombin (Millán-Linares *et al.*, 2014).

CPHs obtained with Alcalase did not significantly inhibit thrombin activity (Figure 3), whereas 20% thrombin inhibition was observed in CPHs 60F and 120F (Figure 4), so they could regulate inflammatory response. As chronic inflammation is related to the development of numerous chronic diseases, such as cardiovascular diseases, cancer, diabetes, arthritis, neurological diseases, pulmonary and autoimmune diseases (Millán-Linares *et al.*, 2014), those CPHs could be a good ingredient in the formulation of functional foods that reduce the inflammatory risk factors of these pathologies.

4. CONCLUSIONS

Hydrolysates of a chia protein isolate obtained using commercial proteases showed some bioactive properties such as antioxidant, ACE and thrombin inhibitory activities. Therefore, the results of our study show the potential use of chia hydrolysates in the development of functional foods for the treatment of several pathologies. Additionally, the use of CPHs as ingredients may reduce the need of adding synthetic antioxidants to preserve food from rancidity. However, further work should be done to isolate and identify the specific peptides that are responsible for those beneficial effects.

REFERENCES

Halim NRA, Yusof HM, Sarbon NM. 2016. Functional and bioactive properties of fish protein hydrolysates and peptides: A comprehensive review. *Trends Food Sci Tech* 51, 24-33.

Ialenti A, Santagada V, Caliendo G, Severino B, Fiorino F, Maffia P, Ianaro A, Morelli F, Di Micco B, Cartenì M, Stiuso P, Metafora V, Metafora S. 2001. Synthesis of novel anti-inflammatory peptides derived from the amino-acid sequence of the bioactive protein SV-IV. *Eur J Biochem* **268**, 3399-3406. DOI 10.1046/j.1432-1327.2001.02236.x.

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Korhonen H, Pihlanto A. 2006. Bioactive peptides: Production and functionality. *Int Dairy J* **16**, 945-960. DOI 10.1016/j.dairyj.2005.10.012.

Maestri E, Marmiroli M, Marmiroli N. 2016. Bioactive peptides in plant-derived foodstuffs. *J Proteomics* **147**, 140-155. DOI 10.1016/j.jprot.2016.03.048.

Millán-Linares MC, Yust MM, Alcaide-Hidalgo JM, Millán F, Pedroche J. 2014. Lupine protein hydrolysates inhibit enzymes involved in the inflammatory pathway. *Food Chem* **151**, 141-147. DOI 10.1016/j.foodchem.2013.11.053.

Morris R, Winyard PG, Blake DR, Morris CJ. 1994. Thrombin in inflammation and healing: Relevance to rheumatoid arthritis. *Ann Rheum Dis* **53**, 72-79.

Orona-Tamayo D, Valverde ME, Nieto-Rendón B, Paredes-López O. 2015. Inhibitory activity of chia (*Salvia hispanica* L.) protein fractions against angiotensin I-converting enzyme and antioxidant capacity. *LWT-Food Sci Technol* **64**, 236-242. DOI 10.1016/j.lwt.2015.05.033.

Pedroche J, Yust MM, Lqari H, Megías C, Girón-Calle J, Alaiz M, Vioque J, Millán F. 2007. Obtaining of *Brassica carinata* protein hydrolysates enriched in bioactive peptides using immobilised digestive proteases. *Food Res Int* **40**, 931-938. DOI 10.1016/j.foodres.2007.04.001.

Ryder K, Bekhit AED, McConell M, Carne A. 2016. Towards generation of bioactive peptides from meat industry waste proteins: Generation of peptides using commercial microbial proteases. *Food Chem* **208**, 42-50. DOI 10.1016/j.foodchem.2016.03.121.

Sentandreu MA, Toldrá F. 2006. A fluorescence-based protocol for quantifying angiotensin-converting enzyme activity. *Nature Protocols* **1**, 2423-2427. DOI 10.1038/nprot.2006.349.

Segura-Campos MR, Salazar-Vega Im, Chel-Guerrero L, Betancur-Ancona DA. 2013. Biological potential of chia (*Salvia hispanica* L.) protein hydrolysates and their incorporation into functional foods. *LWT-Food Sci Technol* **50**, 723-731. DOI 10.1016/j.lwt.2012.07.017.

Strande JL, Phillips SA. 2009. Thrombin increases inflammatory cytokine and angiogenic growth factor secretion in human adipose cells *in vitro*. *J Inflamm* **6**, 4. DOI 10.1186/1476-9255-6-4.

Valdivia-López MA, Tecante A. 2015. Chia (*Salvia hispanica*): A review of native Mexican seed and its nutritional and functional properties. *Adv Food Nutr Res* **75**, 53-75. DOI 10.1016/bs.afnr.2015.06.002.

Yust MM, Millán-Linares MC, Alcaide-Hidalgo, JM, Millán, F., Pedroche, J. 2012. Hypocholesterolaemic and antioxidant activities of chickpea (*Cicer arietinum* L.) protein hydrolysates. *J Sci Food Agric* **92**, 1994-2001. DOI 10.1002/jsfa.5573.

Yust MM, Pedroche J, Millán-Linares MC, Alcaide-Hidalgo, JM, Millán, F. 2010. Improvement of functional properties of chickpea proteins by hydrolysis with immobilised Alcalase. *Food Chem* **122**, 1212-1217. DOI 10.1016/j.foodchem.2010.03.121.

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TABLES

Table 1. Angiotensin converting enzyme inhibition (%) by chia protein hydrolysates produced with Alcalase. Values marked with different letters are significantly different (P<0.05).

Hydrolysis time (min)	Inhibition
0	12.33 ± 0.54[a]
15	82.85 ± 0.42[b]
30	80.13 ± 0.40[c]
45	82.16 ± 0.25[b]
60	82.84 ± 0.13[b]

Table 2. Angiotensin converting enzyme inhibition (%) by chia protein hydrolysates produced with Flavourzyme. Values marked with different letters are significantly different (P<0.05).

Hydrolysis time (min)	Inhibition
0	15.92 ± 0.27[a]
15	42.19 ± 0.53[b]
30	36.17 ± 0.83[c]
45	40.50 ± 0.40[d]
60	36.66 ± 0.62[c]
75	28.79 ± 0.74[e]
90	34.33 ± 0.84[c]
105	30.36 ± 0.61[e]
120	31.83 ± 2.04[e]

Table 3. IC_{50} (μ/mL) of chia protein hydrolysates produced using Alcalase. Values marked with different letters are significantly different (P<0.05).

Hydrolysis time (min)	IC_{50}
15	78.84 ± 1.21[a]
30	74.63 ± 0.53[b]
45	67.50 ± 0.44[c]
60	68.06 ± 0.67[c]

FIGURE CAPTIONS

Figure 1. Antioxidant activity of chia protein hydrolysates produced using Alcalase.

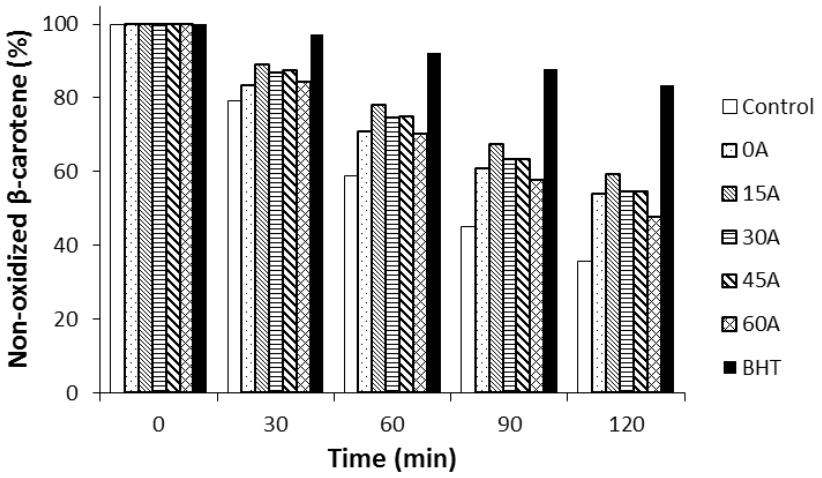

Figure 2. Antioxidant activity of chia protein hydrolysates produced using Flavourzyme.

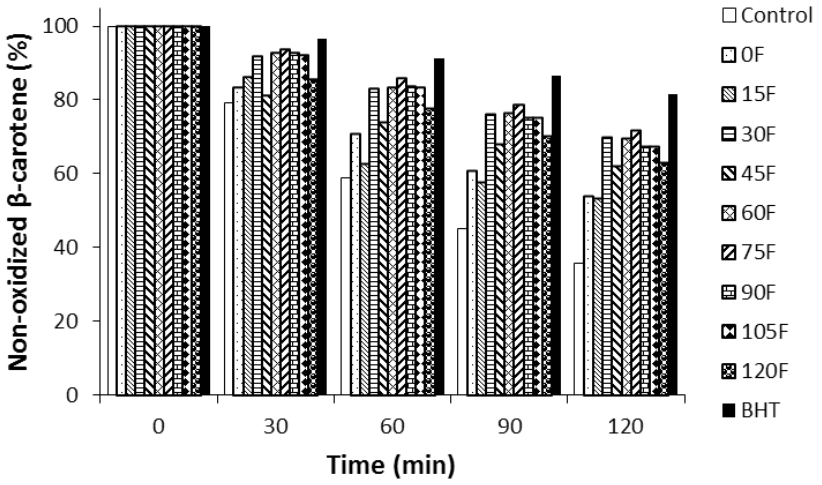

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Figure 3. Thrombin activity in presence of chia protein hydrolysates produced using Alcalase.

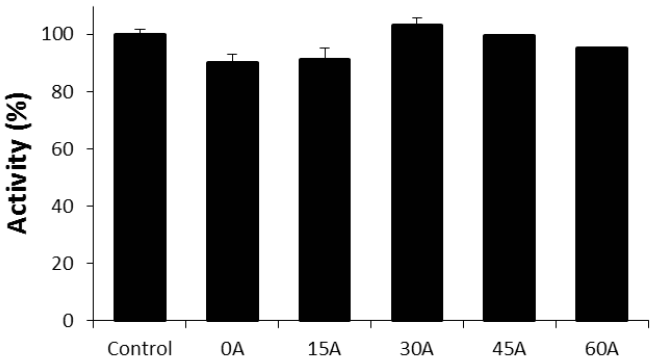
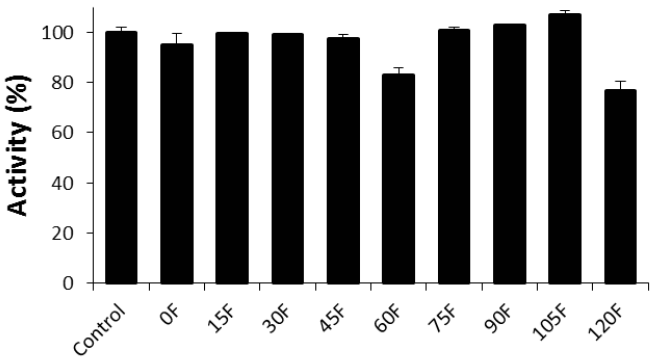


Figure 4. Thrombin activity in presence of chia protein hydrolysates produced using Flavourzyme.



TECHNO-FUNCTIONAL PROPERTIES AND ANTIOXIDANT ACTIVITY OF CHIA SEEDS

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SUMMARY: Ingredient characteristics such as techno-functional properties (water and oil holding capacity, emulsifying capacity and stability) and antioxidant capacity are very useful. Its determination can contribute to a better food application. The results showed that chia seeds have excellent techno-functional properties that make them desirable for its use as food ingredient. The best solvent (methanol:acetone; ethanol and ethylacetate) for polyphenols and flavonoids extraction was methanol:acetone. Chia seeds have antioxidant activity (ABTS, DPPH, FRAP, FIC, TBARs) independent of the extractive solvent used. Results showed a high capacity to reduce the ferrous ion, compared with a low capacity for hydrogen donating ability.

Keywords: Antioxidant activity, chia, polyphenols, methanol, techno-functional properties.

RESUMEN: *Propiedades tecno-funcionales y actividad antioxidante de semillas de chía.* Las propiedades tecno-funcionales (capacidad de retención de agua y aceite, capacidad y estabilidad de emulsión) y las propiedades antioxidantes son muy útiles para las industrias de los alimentos. La chía presenta unas excelentes propiedades tecno-funcionales que la hace apropiada para su uso como ingrediente alimentario. El mejor solvente a utilizar para la determinación de las propiedades antioxidantes (ABTS, DPPH, FRAP, FIC, TBARs) es la mezcla metanol:acetona (C_{MA}). Los resultados demuestran que los extractos de chía tienen una elevada capacidad de reducir el ión ferroso si se compara con los métodos que determinan la capacidad de donar iones de hidrógeno.

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Palabras clave: Actividad antioxidante, chía, polifenoles, metanol, propiedades tecno-funcionales.

1. INTRODUCTION

In spite of food industry efforts to create a more exciting, interesting food culture, and new food experiences, there seem to be ever-longer periods between great innovations in the food industry, by these reasons, chia seeds and its co-products can be very useful. The success of a new product can be viewed under two scopes: Industrial and health. In new products development, ingredient characteristics such as techno-functional properties (water and oil holding capacity, swelling capacity, emulsion capacity and stability) and antioxidant capacity are very useful. Its determination can contribute, to a chia seed and its co-products, for a better food application.

2. MATERIALS AND METHODS

2.1. Techno-functional properties

The water-holding capacity (WHC) and oil holding capacity (OHC), emulsifying capacity (EC) and emulsifying stability (ES) were also evaluated according to Viuda-Martos *et al.* (2015) recommendations. Each assay was carried out by triplicate.

2.2. Extraction of bioactive compounds with antioxidant properties

The total phenolic content (TPC) of each extract was performed using the Folin-Ciocalteu's reagent (Singleton and Rossi, 1965). For the total flavonoid content (TFC), the method based on Blasa *et al.* (2005) was used. As extractive solvents, methanol-acetone (C_{MA}), ethanol (C_E) and ethylacetate (C_{EA}) were used. Each assay was carried out by triplicate.

2.3. Antioxidant activity

To determine antioxidant activity of chia seed samples extracted with C_{MA}, C_E and C_{EA}, the following analytical methods were used: (i) DPPH (2,2´-diphenyl-1-picrylhydrazyl) radical scavenging (Brand-Williams *et al.*, 1995); (ii) ferric reducing antioxidant power (FRAP) (Oyaizu, 1986); (iii) TBARSs or

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thiobarbituric acid reactive species (Daker *et al.* 2008); and (iv) Carter (1971) method for ferrous ion-chelating ability assay (FIC).

3. RESULTS AND DISCUSSION

In Table 1 can be observed the result obtained for bioactive compounds with antioxidant properties and antioxidant activity from chia seeds.

3.1 Techno-functional properties

WHC is a great technological property for functional foods and expresses the amount of water that can be retained in the ingredient "structure". Chia seeds exhibited a WHC of 7.48 g H_2O/g sample, these values were lower than those reported by Coorey *et al.* (2014) and Vazquez-Ovando *et al.* (2009) for Australian (12.42 g H_2O/g) and Mexican (15.41 g H_2O/g) chia seeds, respectively. This variation in WHC is influenced by many factors that are related to fibres. These factors can be the fibre microstructure (particle size, porosity) and processing conditions (Nelson, 2001). OHC expresses the amount of oil that can be retained by the fibres. OHC depends on the fibre porosity, affinity of the fibre molecules (Nelson, 2001), number of lipophilic sites and overall hydrophobicity and capillary attraction. Furthermore, the pre-processing of the fibre with water (for washing) and reducing particle size (during grinding) result in reduced OHC values. Chia samples present an OHC 2.51 g oil/g sample; this value is lower than those reported by Coorey *et al.* (2014) for Australian chia seeds (19.82 g oil/g) or by Segura-Campos *et al.* (2014) for Mexican chia seeds (25.7 g oil/g). Vazquez-Ovando *et al.* (2009) reported that the OHC for Mexican chia seeds was 2.03 g oil/g sample, similar to our values. EC indicates the amount of oil emulsified by the fibre before the collapse of the emulsion occurs and also the oil left retained by the fibre after the collapse occurs. Chia seeds showed an emulsifying capacity of 75%. This value is higher than data reported by Vázquez-Ovando *et al.* (2009), who reported that the EC of Mexican chia seeds was 53%. As regards of ES is the ability of an emulsion to preserve the original structure of the sample analysed it has a chia is 60%. From this data, chia can be used as an emulsifier in food, both for its high capacity to form the emulsion and its ability to maintain the emulsion.

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3.2. Extraction of bioactive compounds with antioxidant properties (total phenolics and total flavonoid content)

The extraction process of phenolic compounds in different matrices is influenced by its chemical nature, the method used and the presence of substances that may interfere with the bioactive compounds (Sotelo *et al.*, 2010). For these reasons, in the present study three extraction systems were used different for the determination of total phenols content (TPC) and total flavonoid content (TFC) of chia seeds. In Table 1 can be observed that there are differences (P<0.05) between all solvent used, showing more TPC when C_{MA} was used. This same behaviour was observed in content in TFC, the sample extracted with C_{MA} showed the highest values with differences (P<0.05) with the other samples. Suhaj (2006) reported that methanol is effective for extracting polyphenolics in fibrous matrices, due to its high degree polarity. While mixtures of acetone are useful for polyphenol extraction (Bors *et al.*, 1990).

3.3. Antioxidant activity

All values can be observed in Table 1. The values obtained for the DPPH and ABTS methods showed large variations in function, the extractive solvent used in this experiment. Samples extracted with C_{MA}, showed the highest values (P<0.05) followed by samples extracted with C_{EA} and the last was the samples extracted with C_E (P<0.05). Regarding the FRAP method, again the extraction performed with C_{MA} showed the highest values for ferric ion reduction (P<0.05) and samples extracted with C_{EA} the lowest values (P<0.05). For TBARs no difference between C_{MA} and C_E were detected. The C_{EA} sample showed the lowest values (P<0.05). For FIC method, C_E extraction showed the highest values (P<0.05) closely followed by C_{MA}, although differences among them were detected (P<0.05). Again, the sample extracted with C_{EA} is the one with the lowest values. This antioxidant activity is mainly due to phenols and flavonoids content in chia seed. In literature there are numerous studies linking the content of these compounds with the antioxidant activity (Amensour *et al.,* 2010). Results shows that depending on the method used to determine the antioxidant capacity of samples are extracts or others who showed greater or lesser capacity. That is why in the capacity study antioxidant is necessary to use two or more methods to determine *in vitro* antioxidant capacity. It was observed that chia seeds have a high capacity to reduce the ferrous ion.

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4. CONCLUSIONS

Chia seeds have excellent techno-functional properties that make them very recommended for use as an ingredient in various foods. The best extraction of chia seed polyphenols and flavonoids was obtained when methanol:acetone as solvent was used. With all the antioxidant methods used, chia seeds have antioxidant activity regardless of solvent used in the extraction.

ACKNOWLEDGMENTS

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REFERENCES

Amensour M, Sendra E, Abrini J, Pérez-Alvarez JA, Fernández-López J. 2010. Antioxidant activity and total phenolic compounds of myrtle extracts. *CyTA J Food Tech* **2**, 95-101. DOI: 10.1080/19476330903161335.

Blasa M, Candiracci M, Accorsi A, Piacentini PM, Albertini MC, Piatti E. 2005. Raw Millefiori honey is packed full of antioxidants. *Food Chem* **97**, 217-222. DOI: 10.1016/j.foodchem.2005.03.039.

Bors W, Werner H, Michel C, Saran M. 1990. Flavonoids as antioxidants: determination of radical scavenging efficiencies. *Methods Enzymol* **186**, 343-355. DOI: 10.1016/0076-6879(90)86128-I.

Brand-Williams W, Cuvelier ME, Berset C. 1995. Use of free radical method to evaluate antioxidant activity. *LWT-Food Sci Tech* **28**, 25–30. DOI: 10.1016/S0023-6438(95)80008-5.

Carter P. 1971. Spectrophotometric determination of serum iron at the submicrogram level with a new reagent (ferrozine). *Anal Biochem* **40**,450–458.

Coorey R, Tjoe A, Jayasen V. 2014. Gelling Properties of Chia Seed and Flour. *J Food Sci* **79**, 859-866. DOI: 10.1111/1750-3841.12444.

Daker M, Abdullah N, Vikineswary S, Goh PC, Kuppusamy UR. 2008. Antioxidant from maize and maize fermented by *Marasmiellus* sp. as stabilizer of lipid-rich foods. *Food Chem* **107**, 1092–1098. DOI:10.1016/j.foodchem.2007.09.027.

Nelson A. 2001. Defining high-fiber ingredient terminology. High Fiber Ingredients: 1- 83.

Oyaizu M. 1986. Studies on products of browning reaction: antioxidative activity of products of browning reaction prepared from glucosamine. *Jpn J Nutr* **44**, 307-315.

Segura-Campos MR, Ciau-Solís N, Rosado-Rubio G, Chel-Guerrero L, Betancur-Ancona D. 2014. Chemical and Functional Properties of Chia Seed (*Salvia hispanica L.*) Gum. *Int J Food Sci* **2014**, 1-5. DOI:10.1155/2014/241053.

Singleton VL, Rossi JA. 1965. Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. *Am J Enol Vitic* **16**, 144-158.

Sotelo DI, Casas FN, Camelo MG. 2010. Borojó (*Borojoa Patinoi*): fuente de polifenoles con actividad antimicrobiana. *Vitae* **17**, 329-336.

Suhaj M. 2006. Spice antioxidants isolation and their antiradical activity: a review. *J Food Comp Anal* **19**, 531-537. DOI: 10.1016/j.jfca.2004.11.005.

Vazquez-Ovando A, Rosado-Rubio G, Chel-Guerrero L, Betancur-Ancona D. 2009. Physicochemical properties of a fibrous fraction from chia (*Salvia hispanica L.*). *LWT - Food Sci Tech* **42**, 168-173. DOI:10.1016/j.lwt.2008.05.012.

Viuda-Martos M, Barber X, Pérez-Alvarez JA, Fernández-López J. 2015. Assessment of chemical, physico-chemical, techno-functional and antioxidant properties of fig (*Ficus carica L.*) powder co-products. *Ind Crops Prod* **69**, 472–479. DOI: 10.1016/j.indcrop.2015.03.005.

TABLES

Table 1. Antioxidant activity measured by DPPH, FRAP, TBARs, ABTS and FIC, and total phenolics and total flavonoid content in chia seed (Salvia hispanica L.) extracts obtained by using solvents such as methanol-acetone: C_{MA}, ethanol: C_E and ethylacetate: C_{EA}.

Antioxidant method	Methanol-acetone (C_{MA})	Ethanol (C_E)	Ethylacetate (C_{EA})
DPPH	0.98 ± 0.00^{a}	0.37 ± 0.00^{c}	0.40 ± 0.00^{b}
FRAP	3.88 ± 0.08^{a}	1.13 ± 0.04^{b}	0.92 ± 0.02^{c}
TBARs	1.33 ± 0.02^{a}	1.41 ± 0.09^{a}	0.57 ± 0.03^{b}
ABTS	2.60 ± 0.02^{a}	0.34 ± 0.00^{c}	0.34 ± 0.00^{b}
FIC	0.14 ± 0.00^{b}	2.44 ± 0.01^{a}	2.44 ± 0.01^{c}
Bioactive compounds with antioxidant properties (total phenolics and total flavonoid content)			
Total phenolic content (mg GAE/g sample)	2.44 ± 0.01^{a}	0.34 ± 0.00^{c}	1.72 ± 0.01^{b}
Total flavonoid content (mg RE/g sample)	10.47 ± 0.01^{a}	3.55 ± 0.14^{c}	6.75 ± 0.07^{b}

[a-c]Values followed by different letters within the same line are significantly different ($P<0.05$) according to Tukey's multiple range test.

NUTRITIONAL AND FUNCTIONAL ASSESSMENT OF CONTRIBUTION OF CHIA BY-PRODUCTS AS FOOD INGREDIENT IN BAKERY PRODUCTS. PART I: NUTRIENT COMPOSITION AND ANTIOXIDANT ACTIVITY

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SUMMARY: The inclusion of chia flour in bread increased polyphenols content and DPPH radical scavenging activity in significantly higher levels compared to the control. Chia by-products had the ability to promote an antioxidant response on *in vivo* model *Saccharomyces cerevisiae*. Breads containing chia showed significantly lower starch content and higher levels of proteins, lipids and minerals than white bread, even at low levels of substitution. Antioxidant activity and chemical composition of chia ingredients showed that they can represent a healthier alternative to the frequently used wheat flour in bread formulations by helping in fortification and in a positive effect on the antioxidant properties.

Keywords: *Salvia hispanica*, chia, bread, phenolic compounds, oxidative stress, antioxidants.

RESUMEN: *Evaluación nutritiva y funcional de los subproductos de la chía como ingredientes alimentarios en productos de panadería. Parte I: Composición nutricional y actividad antioxidante.* La inclusión de harina de chía en pan incrementó el contenido de polifenoles y la actividad de eliminación de radicales DPPH en niveles significativamente más altos comparados con la muestra control. Los subproductos de chía tuvieron la capacidad de promover respuesta antioxidante en un modelo *in vivo* de *Saccharomyces cerevisiae*. Los panes con chía presentaron significativamente menor contenido de almidón y niveles más altos

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de proteínas, lípidos y minerales que el pan blanco, incluso a niveles bajos de sustitución. La actividad antioxidante y la composición química de los ingredientes de chía mostraron que pueden representar una alternativa saludable a la harina de trigo utilizada con frecuencia en las formulaciones de pan, ayudando en la fortificación y en un efecto positivo sobre las propiedades antioxidantes.

Palabras clave: *Salvia hispanica*, chía, pan, compuestos fenólicos, estrés oxidativo, antioxidantes.

1. INTRODUCTION

Health and well-being are currently driving innovation in the bread sector because bakery products are one of the main components of the human diet. In fact, bakers have responded to current trends in changing consumer tastes with the development of a wide choice of breads with added health benefits including antioxidant properties, omega-3 fatty acids, salt reduction, high fibre, vitamins and minerals, or wholegrain/seeded breads (Iglesias-Puig and Haros, 2013). In this sense, chia can be utilised as bakery ingredients because meets all these requirements (Iglesias-Puig and Haros, 2013).

Oxidative stress is caused by an imbalance between the production of reactive oxygen species (ROS) or reactive nitrogen species (RNS) and antioxidant mechanisms of the cell (Lobo *et al.*, 2010). In humans, oxidative stress may be associated with various pathologies including cancer, cardiovascular disease, autoimmune diseases, the aging process and diseases associated with it (Lamuela-Raventós *et al.*, 2005). WHO recommended daily intake of antioxidants through diet to prevent or mitigate diseases associated with cellular oxidative stress.

The main goal of this investigation was to study the nutritional and functional potential of whole and semi-defatted chia flours as bakery ingredients by assessing their antioxidant ability *in vitro* and in the *in vivo* model organism *Saccharomyces cerevisiae*.

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). The characteristics of the raw materials are shown in Table 1. Compressed yeast (*Saccharomyces cerevisiae*, Levital, Spain) was used as a starter for the breadmaking process.

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2.2. Composition of raw materials and bread

Protein determination was carried out by the Kjeldahl technique and starch content by means of enzymatic procedures. Lipids were extracted with hexane under reflux conditions by the Soxhlet technique, whereas ash content was determined in a muffle by incineration at 910 °C.

2.3. Bread production

The control bread dough formula consisted on wheat flour (450 g), compressed yeast (2.5% flour basis), sodium salt (1.6% flour 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 breadmaker Severin 3989. Breads were obtained by duplicate.

2.4. Extraction of phenolic compounds

Phenol compounds were obtained using 70% v/v methanol following the methodology proposed by Martínez-Cruz and Paredes-López (2014). The extracts were concentrated under reduced pressure in a rotatory evaporator at 35 °C until methanol elimination before using in the *S. cerevisiae* model.

2.5. Total polyphenols determination

Total polyphenols content of chia and wheat flour and of breads was determined by the Folin-Ciocalteu method described by Singleton and Rossi (1965) with some modifications. Briefly, 50 µL of sample were added to 500 µL of aqueous sodium carbonate solution. After 15 min at room temperature, 50 µL of Folin reagent were added and the mixture was incubated at room temperature for 30 min. The results were expressed as gallic acid equivalents and experiments were carried out in triplicate.

2.6. Free radical DPPH scavenging assay

Total antioxidant activity of samples was determined by the reduction of the stable free radical DPPH. The assay was carried out using a modified version of the method described by Schinella *et al.* (2010). Samples (7.5 µL) were added to 292.5 µL of DPPH 60 µM in methanol 80%, mixed and incubated

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for 30 min at room temperature in the dark. Results were expressed as Trolox equivalents. Experiments were carried out in triplicate.

2.7. Induction of intracellular antioxidant response assay in *S. cerevisiae*

The yeast *S. cerevisiae* (strain BY4741) was inoculated in liquid YPD medium containing the antioxidant ingredient (extracts from flours and breads) for 18 h at 28 °C. A Cocoa extract was used as a positive control and cultures without ingredient were used as a negative control. To induce a non-lethal oxidative stress, cells were incubated for 60 min with 0.5 mM and 4 mM H_2O_2 and then growth was monitored by reading the OD_{600} using a 96-well plate spectrophotometer reader (BMG Labtech Omega Spectrostar) for 18 h.

2.8. Statistical analysis

Statistically significant differences were calculated by the Student's t-test using Excel.

3. RESULTS AND DISCUSSION

3.1. Raw material and bread chemical composition

As was expected, the whole chia flour showed a high amount of lipids. The semi-defatted chia flour clearly showed a significant decrease in fat content, which corresponded to a 77% reduction of lipids. This reduction promoted a significant increase in the protein fraction and ash (Table 1). With the exception of moisture, the amounts of lipids, proteins, and minerals were significantly higher in chia flours than in wheat flour. Cereal flours contain high proportions of starch, while chia by-products are negligible or low of it. The greater levels of proteins, lipids and ash registered in the chia flours with regard to the wheat flour directly affected the increase of these parameters in the bread, as expected (data not shown).

3.2. Total phenolic content

Total phenolic content of the extracts of flours and breads is shown in Fig. 1. Polyphenol concentration was significantly higher in the case of whole and semi-defatted chia flours compared to the wheat flour. Moreover, all breads

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displayed polyphenol content significantly higher than that of the control bread (wheat bread) except that made with 5% semi-defatted chia.

An increment in the total phenolic content was observed in the breads as the percentage of chia increased; nevertheless, these differences between chia breads were not statistically significant except in the case of bread with 10% of semi-defatted chia that displayed content significantly higher than that with 5% semi-defatted chia. When whole chia breads and semi-defatted chia breads were compared, significant differences were only found between breads with 5% of chia flours.

3.3. Antioxidant activity

Extracts of chia flours showed an *in vitro* antioxidant activity remarkably significant compared to wheat flour (Fig. 2). Noteworthy is the low activity of wheat flour taking into account its polyphenol content. Chia breads showed an activity statistically higher than the control bread as it was expected by the polyphenol contents found for each of them. The increment observed in the activity of breads as the percentage of chia increased was statistically significant when semi-defatted chia was used. Again, no significant differences were found between breads made with the two-chia flour.

3.4. Evaluation of the ability of polyphenol extracts of chia to promote an antioxidant response in *S. cerevisiae*

In vitro tests do not consider fundamental aspects of the antioxidant activity in living organisms such as bioavailability and metabolism or provide information about the *in vivo* mechanisms of antioxidant cellular response. Here *S. cerevisiae* has been used as a model organism to study the capacity of chia extracts to trigger an antioxidant response. A methodology adapted to microtiter plates has been used to monitor yeast growth after the culture was pre-incubated with the polyphenols extracts and then exposed to oxidative stress by hydrogen peroxide at two concentrations to lead a moderate and a severe oxidative stress conditions (0.5 and 4 mM of H_2O_2, respectively).

Polyphenol extracts from chia were tested at different doses (10, 24, 97, 150, 600 and 1200 mg/L). A cocoa extract with proved antioxidant activity (Martorell *et al.*, 2011) was used as positive control (final concentration: 600 mg/L).

To evaluate the oxidant effect growth-ratio curves were first calculated as the quotient between the growth curve of the culture exposed to oxidant and the growth curve of the non-exposed culture. Subsequently, to evaluate whether the chia extracts provided protective antioxidant activity, 'effect

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curves' were constructed. To do so, the previously calculated growth-ratio curve for the culture pre-incubated with the ingredient was divided by the growth-ratio curve for the culture pre-incubated without the ingredient, both at the same oxidant dose. Fig. 3 shows dose-response curves of chia extracts constructed considering as effect value the greatest statistically significant effect detected in each of 'effect curves'. Dose-response studies are useful to gain a better understanding of the health effects of dietary polyphenols extracts. Fig. 3 shows a clear promoter antioxidant capacity of chia polyphenolic extracts at both concentrations of H_2O_2. This effect was less obvious under conditions of moderate stress (H_2O_2 0.5 mM) compared to the effect observed in severe stress conditions (H_2O_2 4 mM). It is possible to appreciate that at the both concentrations of H_2O_2 used as stressor, the effect reached a maximum at the higher concentration of chia (1200 mg/L).

4. CONCLUSIONS

Bread fortification with chia flour had a positive effect on the antioxidant properties and phenolic contents and therefore could improve the antioxidant potential of the final product. No differences were found when whole and defatted flours were compared. Nutritional and chemical composition analysis of chia ingredients showed that they could represent a healthier alternative to the frequently used wheat grain in bread formulations.

ACKNOWLEDGMENTS

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REFERENCES

Iglesias-Puig E, Haros M. 2013, Evaluation of dough and bread performance incorporating chia (*Salvia hispanica* L.). *Eur Food Res Technol* **237**, 865-874. DOI: 10.1007/s00217-013-2067-x.

Lamuela-Raventós RM, Romero-Perez A, Andres-Lacueva C, Tornero A. 2005. Review: health effects of cocoa flavonoids. *Food Sci Technol Int* **11**, 159-176. DOI: 10.1177/1082013205054498.

Lobo V, Patil A, Phatak A, Chandra N. 2010. Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacogn Rev* **4**, 118–126. DOI: 10.4103/0973-7847.70902.

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Martínez-Cruz O, Paredes-López O. 2014. Phytochemical profile and nutraceutical potential of chia seeds (*Salvia hispanica* L.) by ultrahigh performance liquid chromatography. *J Chromatogr* A **1346**, 43-48. DOI: 10.1016/j.chroma.2014.04.007.

Martorell P, Forment J V, de Llanos R, Montón F, Llopis S, González N, Genovés S, Cienfuegos E, Monzó H, Ramón D. 2011. Use of *Saccharomyces cerevisiae* and *Caenorhabditis elegans* as model organisms to study the effect of cocoa polyphenols in the resistance to oxidative stress. *J Agric Food Chem* **59**, 2077-2085. DOI: 10.1021/jf104217g.

Schinella G, Mosca S, Cienfuegos-Jovellanos E, Pasamar MA, Muguerza B, Ramón D, Ríos JL. 2010. Antioxidant properties of polyphenol-rich cocoa products industrially processed. *Food Res Int* **43**, 1614-1623. DOI: 10.1016/j.foodres.2010.04.032.

Singleton VL, Rossi JA. 1965. Colorimetry of total phenolics with phosphomolybdic–phosphotungstic acid reagents. *Am J Enol Vitic* **16**, 144–158.

TABLE

Table 1. **Chemical composition of raw materials**[a]

Main components g/100 g d.m.[b]	Wheat flour	Whole chia flour	Defatted chia flour
Moisture[c]	11.39 ± 0.03	7.35 ± 0.01	3.38 ± 0.04
Starch	70 ± 0.8	2.0 ± 0.8	1.8 ± 0.2
Proteins	12.4 ± 0.1	19.9 ± 0.6	29.4 ± 0.2
Lipids	n.d.[d]	34.7 ± 0.6	7.80 ± 0.03
Ash	0.58 ± 0.01	4.77 ± 0.04	6.29 ± 0.02

[a]Mean ± SD, $n \geq 3$.
[b]d.m., dry matter.
[c]Wet basis.
[d]n.d., no determined.

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FIGURE CAPTIONS

Figure 1. Total phenolic content of the extracts of wheat and chia (whole and semi-defatted) flours and breads (control and 5, 10 and 20% whole or semi-defatted chia). Values are mean ± SD, $n = 3$. *$P<0.01$ with regard to wheat flour; "$P<0.05$ with regard to wheat bread (control); #$P<0.05$ with regard to the corresponding whole chia bread (comparisons between breads with same percentage of whole and semi-defatted chia flour). Student's t test was used to compare samples.

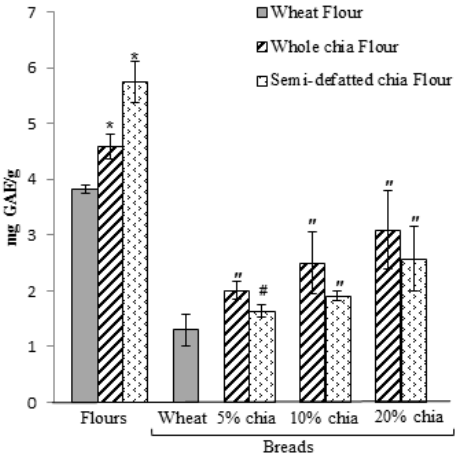

Figure 2. Antioxidant activity of the extracts of wheat and chia (whole and semi-defatted) flours and breads (control and 5, 10 and 20% whole or semi-defatted chia). Values are mean ± SD, $n = 3$. *$P<0.01$ with regard to wheat flour; "$P<0.05$, ""$P<0.01$ with regard to wheat bread (control). Comparisons between breads with the same percentage of whole and semi-defatted chia flour are statistically not significant. Student's t test was used to compare samples.

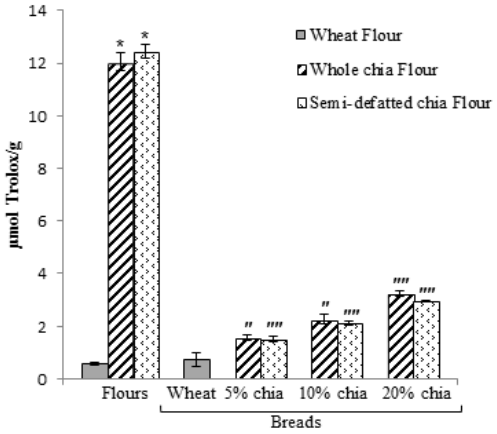

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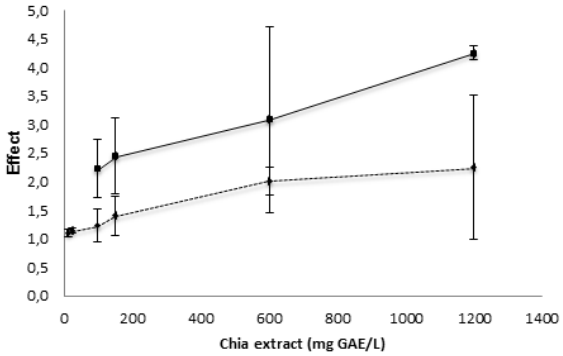
Figure 3. Dose-response curves of chia extract. Curves were constructed considering as effect value the greatest statistically significant effect detected in each of effect curves for BY4741 *S. cerevisiae* strain, after treatment with H_2O_2 0.5 mM (dotted line) or 4 mM (solid line).



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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.

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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.

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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

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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

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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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REFERENCES

AOAC, Association of Official Agricultural Chemists, 1991. Method. Total dietary fibre assay procedure. Megazyme. Based on AACC method 32-05. 985, 29.

Dineshkumar B, Mitra A, Manjunatha M. 2010. Studies on the anti-diabetic and hypolipidemic potentials of mangiferin (Xanthone glucoside) in streptozotocin-induced type 1 and type 2 diabetic model rats. *Int J Adv Pharmac Sci* **1**, 75-85. DOI: dx.doi.org/10.5138/154.

Fernández-Espinar MT, Gil JV, Segura-Campos M, Haros CM. 2016. Nutritional and functional assessment of contribution of chia by-products as food ingredient in bakery products. Part I: Nutrient composition and antioxidant activity. *Book of Proceeding of International Conference of Chia-Link Network*, October 6 and 7, Instituto de la Grasa - Seville, Spain.

Haros M, Iglesias E, Laparra M. 2014. Use of ancient Latin-American crops in bread. Effect on mineral availability and glycemic index. *J Nutr Food Sci* **4**, 69. DOI: 10.4172/2155-9600.S1.011.

Hayakari M, Kondo Y. 1978. A rapid and simple spectrophotometric assay of angiotensin-converting enzyme. *Anal Biochem* **84**: 361-369. PMID: 204217.

Herrera F, Betancur D, Segura M. 2014. Compuestos bioactivos de la dieta con potencial en la prevención de patologías relacionadas con sobrepeso y obesidad: péptidos biológicamente activos. *Nutr Hosp* **29**, 10- 20. DOI: 10.3305/nh.2014.29.1.6990.

Hui T, Lei C. 2016. α-Glucosidase and α-amylase inhibitors from seed oil: a review of liposoluble substance to treat diabetes. *Crit Rev Food Sci Nutr*. DOI: 10.1080/10408398.2015.1129309.

Iglesias-Puig E, Haros M. 2013, Evaluation of dough and bread performance incorporating chia (*Salvia hispanica* L.). *Eur Food Res Technol* **237**, 865-874. DOI: 10.1007/s00217-013-2067-x.

NAS - National Academy of Sciences. Dietary. 2005. Functional, and Total Fiber, in Institute of Medicine Food and Nutrition Board, *Dietary References Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein and Amino Acids*, The National Academies Press, Washington D.C., 339-421.

Orona D, Valverde M, Nieto B, Paredez O. 2015. Inhibitory activity of chia (*Salvia hispanica* L.) protein fractions against angiotensin I-converting enzyme and antioxidant capacity. *LWT-Food Sci Technol* **64**, 236- 242. DOI: 10.1016/j.lwt.2015.05.033.

Salas-Salvadó J, Bulló M, Pérez-Heras A, Ros E. 2007. Dietary fibre, nuts and cardiovascular diseases. *Brit J Nutr* **96**, 46-51. DOI: 10.1017/BJN20061863.

Sanz-Penella JM, Tamayo-Ramos JA, Sanz Y, Haros M. 2009. Phytate reduction in bran-enriched bread by phytase-producing bifidobacteria. *J Agr Food Chem* **57**, 10239-10244. DOI: DOI: 10.1021/jf9023678.

Segura M, Salazar I, Chel L, Betancur D. 2013. Biological potential of chia (*Salvia hispanica* L.) protein hydrolysates and their incorporation into functional foods. *LWT-Food Sci Technol* **50**, 723- 731. DOI: 10.1016/j.lwt.2012.07.017.

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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±2b	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[c]							
*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).

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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.

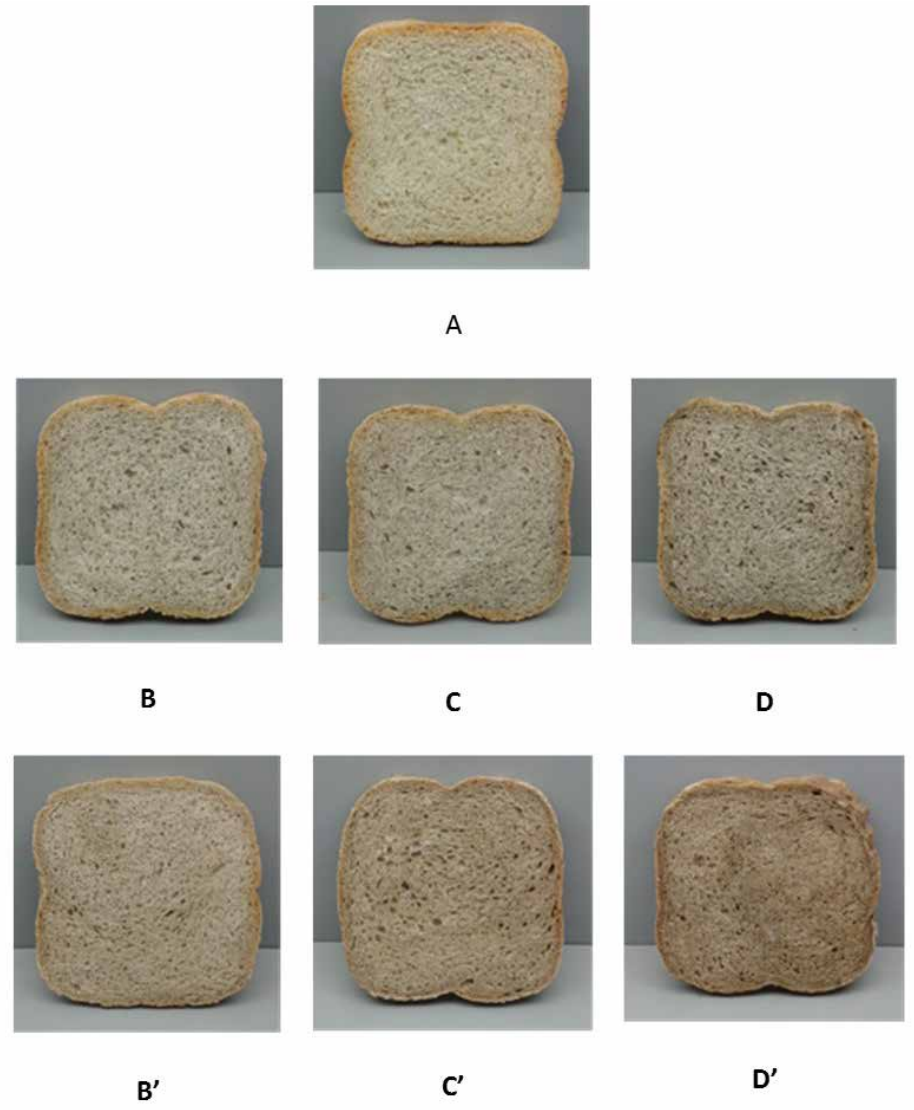

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Figure 2. ACE inhibition of chia breads. Data are mean ± SD of three determinations. [a-d]Different superscript letters indicate statistical difference ($P< 0.05$).

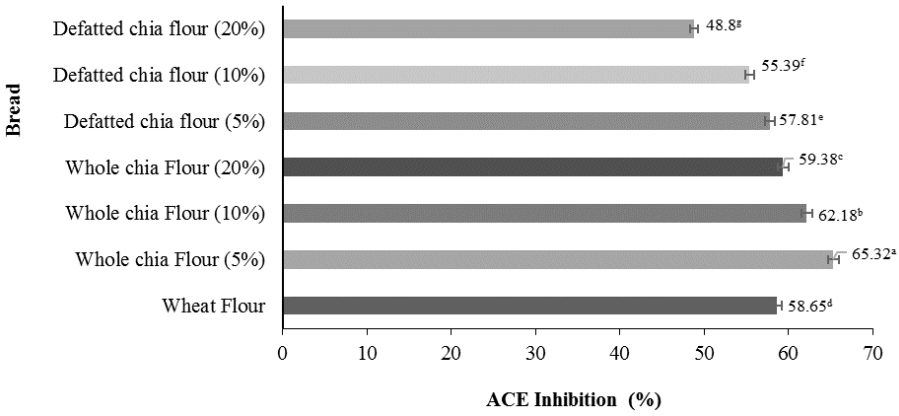
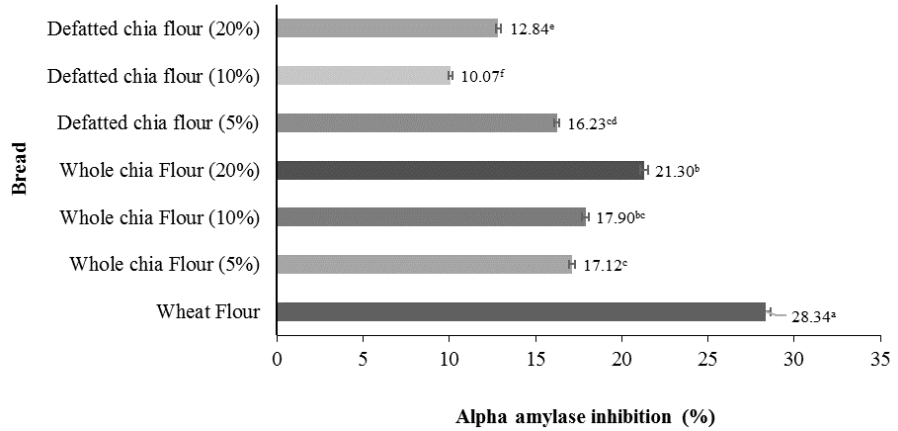


Figure 3. Inhibitory effect of breads on alpha-amylase activity. Data are mean ± SD of three determinations. [a-g]Different letters indicate statistical difference ($P<0.05$).



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