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

EDITORIAL UNIVERSIDAD DE SEVILLA

ÍNDICE ›››
RESEÑA ››

Justo Javier Pedroche Jiménez
Francisco Millán Rodríguez
(coords.)

CHIA (*Salvia hispanica L.*)
THE OLD FOOD OF THE FUTURE
(CIRCHIA 2016)

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

Editorial Universidad de Sevilla

Sevilla 2018

Colección: Actas

C/ Porvenir, 27 - 41013 Sevilla.
Tlfs.: 954 487 447; 954 487 451; Fax: 954 487 443
Correo electrónico: eus4@us.es
Web: <http://www.editorial.us.es>

Impreso en papel ecológico
Impreso en España-Printed in Spain

ISBNe: 978-84-472-2103-5

D.O.I.: http://dx.doi.org/10.12795/9788447221035
Diseño de cubierta: santi@elmaquetador.es
Maquetación: santi@elmaquetador.es

Editors:
Mª Carmen Millán Linares. *Instituto de la Grasa, C.S.I.C., Sevilla*
Mª del Mar Yust Escobar. *Instituto de la Grasa, C.S.I.C., Sevilla*
Claudia Monika Haros. *Instituto de Agroquímica y Tecnología de Alimentos, C.S.I.C., Valencia*
Justo Javier Pedroche Jiménez. *Instituto de la Grasa, C.S.I.C., Sevilla*
Francisco Millán Rodríguez. *Instituto de la Grasa, C.S.I.C., Sevilla*

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

M.T. Fernández-Espinar[a*], J.V. Gil[a,b], M. Segura-Campos[a,c], C.M. Haros[a]

[a]Institute of Agrochemistry and Food Technology (IATA-CSIC), Valencia, Spain; [b]Department of Preventive Medicine and Public Health, Food Sciences, Toxicology and Forensic Medicine, University of Valencia (UV), Valencia, Spain; [c]Facultad de Ingeniería Química, Campus de Ciencias Exactas e Ingenierías, Universidad Autónoma de Yucatán (UADY), Mérida, Yucatán, México

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

* Corresponding author: tfer@iata.csic.es.

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.

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 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 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 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

This work was financially supported by grants I-Link0923 and QuiSalhis-Food (AGL2016-75687-C2-1-R) from the Ministry of Economy, Industry and Competitiveness (MEIC, Spain).

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.

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.

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.

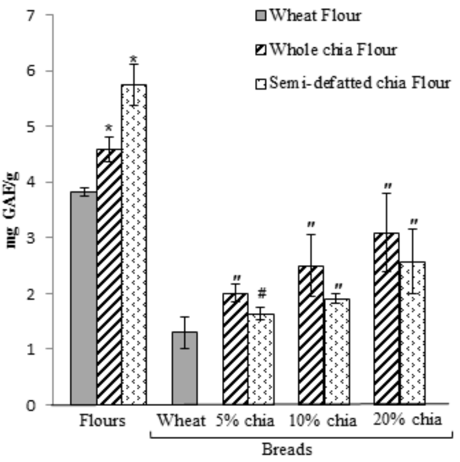

Figure 2. Antioxidant activity of the extracts of wheat and chia (whole and semi-defatted) flours and breads (control and 5, 10 and 20% whole or semi-defatted chia). Values are mean ± SD, *n* = 3. **P*<0.01 with regard to wheat flour; “*P*<0.05, “”*P*<0.01 with regard to wheat bread (control). Comparisons 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.

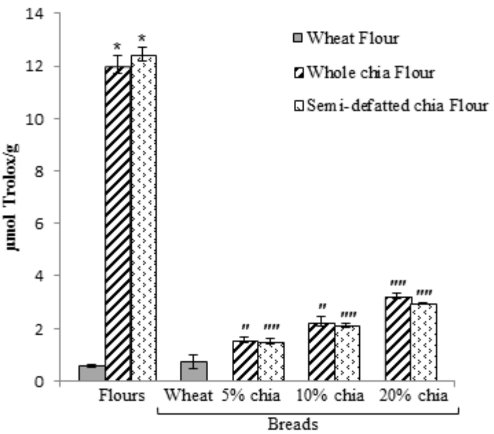

Figure 3. Dose-response curves of chia extract. Curves were constructed considering as effect value the greatest statistically significant effect detected in each of effect curves for BY4741 *S. cerevisiae* strain, after treatment with H_2O_2 0.5 mM (dotted line) or 4 mM (solid line).