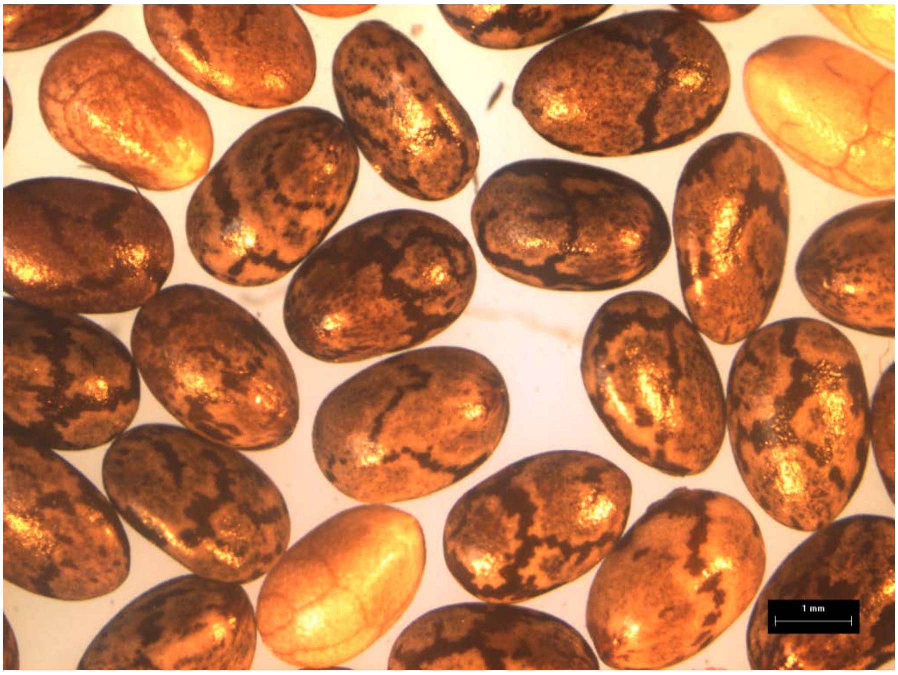
BOOK OF PROCEEDINGS

1st INTERNATIONAL CONFERENCE OF CHIA-LINK NETWORK

2015



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BOOK OF PROCEEDINGS 1st INTERNATIONAL CONFERENCE OF CHIA-LINK NETWORK

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José Ángel Pérez Álvarez

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ORGANIC FARMING OF CHÍA, *Salvia hispanica* L. (Lamiaceae)

<u>Amanda Di Fabio</u>

Universidad Juan Agustín Maza, Mendoza – Argentina

ABSTRACT

Growing Chia under organic conditions results in an environmentally sustainable production of healthy food, avoiding toxic chemical contaminants. Conventional agriculture uses agrochemicals, which consequently cause food contamination. The results of recent research have revealed the potential risk of contamination either by drift during pesticide application or leaching flows, thus generating risks for the aquatic biota and humans. Global problems of environmental pollution and ecological imbalance are linked to conventional agriculture. Surface and ground waters have been contaminated with nitrates and pesticides, and numerous pests have become resistant to pesticides. Ecological agriculture offers benefits that are sustainable over a long term; it aims to produce food while establishing an ecological balance to prevent soil fertility or pest or disease problems. Accordingly, high quality nutritious food products, free from agrochemical residues, are obtained, and the organic yields may reach a satisfactory level for farmers, working in a healthier labor atmosphere. Biotic factors, that include microorganisms, flora and fauna in soil, and plants and animals, should be considered in the farming system, trying to interact in the ecosystem, increasing soil fertility over a long term and preventing its degradation.Chia grows in a tropical and subtropical climate, and does not tolerate frosts. It is developed in well drained loamy soils. It is a short-day plant; growth rate and fruiting period depend on the latitude where it is implanted. It is important to establish the proper cycle of crop rotation. Monocultures increase the population of soil-borne pathogens. Crop rotation favors biological control and constitutes the most effective nonchemical means of managing pathogen populations in the soil. Weed management is an integral part of disease management. The pathogens that are spread through these herbs can infect a wide range of hosts; therefore control of weeds helps to reduce the risk of diseases and viruses transmitted by insects from infected weeds and affecting the plants.

Abbreviated *Curriculum vitae*

Amanda Di Fabio is an Agricultural Engineer graduated from the National University of Cuyo, School of Agrarian Sciences. Specialist in production, development, and industrialization of aromatic and medicinal plants, and non-traditional crops. Head of the Chair of Pha macobotany at University Maza. From 2006 to 2012, Coordinator of the Research Area. Has participated in Technical Missions on production, and marketing of aromatic products organized by the EU in France, and Italy. She has been a consultant for national and international companies, acted as a peer reviewer, and managed several research projects. She participated in the Latin American Science & Technology Development Program; the Subprogram IV on Biomass; the Project IV 5: "Obtaining Capsicum Derivatives with Commercial Interest", and represented Argentina in a) Cochabamba, 1996**,** 1999 and 2000; b) Lisbon, *Universidade Nova de Lisboa*, 1998; c) Montevideo, U. of the Republic, 2000; d) U.of Buenos Aires, 2002. She contributed to the Arequipa Strategic Plan, Peru, 2006**.** She was also present at the Forum on Argentine Oregano in 2003, 2004, 2005 and 2008, and at the Federal Forum on Aromatic Herbs and Spices. She was involved in the formulation of the Argentine National Strategic Plan for the production of aromatic and medicinal plants in 2006, 2007 and 2008. She provided consultancy services to Potasio Rio Colorado in 2006. b. "El Taller", Peru, Arequipa, in 2007. She has been a third-party consultant for the Federal Investment Board since 2003. She acted as a peer reviewer in the licensing of Pharmacy and Biochemistry Undergraduate Degrees in 2006 and 2010-2011. She participated as a third-party reviewer of INTA Projects, 2009, Technological Platform projects for innovation and development of productive processes and quality in aromatic plants, 2010; and in the Development of innovative technologies for aromatic plant diversification, intensification and differentiation, 2010. Project Management: San Luis, 1996. CFI. Production of aromatic plants. Mendoza: 7 regional projects on Capsicum for paprika, selection of oregano and basil for the frozen food market, organic chamomile, rosemary and thyme, for the domestic market. Argentine Federal Projects for Productive Innovation 2004-1.Secretariat of Science, Technology and Innovation. Comparative study of the yield of different ecotypes of "*Origanum spp"* in Neuquén, Argentina. Medicinal plants, financed by the IFAD/UNOPS in 2007. She managed 5 Projects financed by UMaza. Development of technology package for the production of 46 medicinal and aromatic species. Obtained "Adzet" chamomile cultivar, included in the National Registry of Cultivars. Taught courses: 50, to producers and professionals in 12 provinces in Argentina and in Santa Cruz de la Sierra, Bolivia; in Talca, Chile, and in Arequipa, Peru, 2007. She has attended and submitted the relevant papers for 22 training courses and 30 Conferences and Research Sessions. Technical Supervision and Consultancy to Latin American companies. Perú: NGO El Taller; Cerx Arequipa, Prompex Perú and Prosur. Consultant for companies in the industry in the provinces of Mendoza, San Juan, Jujuy, Salta, Catamarca, Río Negro, Chubut, Neuquén and Entre Ríos. Publications. In UMaza magazines: 5. CYTED Program, 2 chapters in Yearbook 2001: Capsicum and Its Derivatives. 2007 INTA. Assessment of the Therapeutic Activity of the S-allyl Cysteine Sulfoxide Derivative Compounds. 2008. INTA. Formulation of Aged Garlic Extract. 2008. Influence of Aromatic Species in Wine's Bouquet. International Winemaking Magazine ISSN: 1668-3889, 2008. Chamomile Management, Diseases and Plagues Regional Program to Support the Development of Medicinal Plants in Mercosur.

EFFECT OF CHIA BY-PRODUCTS AS BREAD-MAKING INGREDIENT ON NUTRITIONAL QUALITY, MINERAL AVAILABILITY AND GLYCAEMIC INDEX OF BAKERY PRODUCTS

Claudia Mónika Haros[1*], Moisés Laparra[1,2], Francisco Millán[3]

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ABSTRACT

Chia (*Salvia hispanica* L.) has been cultivated for centuries by Aztec Indians. At present it is commonly consumed throughout the world, whereas is practically unknown in Europe. Due the European Food Safety Authority opinion about the safety of chia as a food ingredient, in European Union, chia seed and grounded chia seed may be placed on the market in the Community as a novel food ingredient to be used in bread. Therefore the purpose of the present investigation was to provide further information on how replacing wheat flour by chia seeds and their by-products (whole chia flour, semi-defatted chia flour and low-fat chia flour) affects bakeries products to assess their functionality as nutritious and functional ingredients. Glycaemic index and mineral bioavailability were studied using *in vivo* and *in vitro* methodologies, respectively. The greater levels of proteins, lipids, and minerals registered in raw chia flours (proteins: 22.7-30.2 g/100g, lipids: 13.5-41.5 g/100g; ω-3: 46.8-95.2 mg/100g; ω–6: 19.3-38.1 mg/100g; ash: 2.2-4.9 g/100g in dry basis) with regard to the wheat flour (proteins: 10.1 g/100 g, lipids: 0.79 g/100g; ω-3: 0.38 mg/100g; ω–6: 7.51 mg/100g; ash: 0.64 g/100g in dry basis) directly affected the increase of these nutrients, as expected. However, high levels of phytates were found in chia ingredients (5.10-6.63 μmol/g in dry basis) and this contributed to similarly high phytate levels in bread containing chia by-products, which affect the mineral bioavailability of Zn and Fe, as was predicted by phytate/mineral molar ratios. All the samples with 5% of chia by-products presented as the main fatty acid the linoleic acid. It highlights the high linolenic acid content of samples containing chia seeds primarily due to exercising protection of the seed integrity during baking. Chia seed and its flours had higher amino acids content, as

a result of the higher protein wealth, being glutamic/glutamine acid, arginine and aspartic acid/asparagine acid the most abundant. It is also to remark high sulfur amino acid content as lysine, essential amino acids from a nutritional standpoint and deficient in cereals. Glycaemic index was lower in bread with whole chia flour (77±5 %) and whole wheat bread (89±4 %) compared to white bread (97±4%). Thus, suggesting beneficial effects on glucose metabolism and, potentially a number of components of the metabolic syndrome. Chia could be used as a replacement for wheat flour in bread formulations, increasing the product's nutritional and functional value, with higher bread quality when used in proportions 5g/100g, therefore its inclusion is recommended, even at greater levels than 5%. The potential contribution of chia flours could have clinically relevant implications at controlling metabolic diseases prevention.

Abbreviated *Curriculum vitae*

Claudia M. Haros, graduated as a Bachelor of Chemistry from the School of Exact and Natural Sciences, University of Buenos Aires (UBA), Argentina in 1990. She obtained an MSc in Bromatology and Food Technology (1992); and an MSc in Biology Analysis (1997) from UBA. She is Ph.D in Chemistry (UBA-1999). From 1991-2003, she worked as university professor in the Organic Chemistry Department, Food Science and Technology Area of UBA. From 1991-1999 she was Research Assistant in the Cereals and Oilseeds Group, Department of Industrial Chemistry, UBA. Later, from 2000-2002 she worked in Spain as a visiting professor in the Cereal Group of the Institute of Agrochemistry and Food Technology (IATA) in Valencia. In 2003, she was a postdoc fellow at the Department of Food Microbiology, Institute of Animal Reproduction and Food Research (CENEXFOOD-EU), Polish Academy of Science, Olsztyn, Poland. From 2003-2004 she received an award for working with Prof. Sandberg of the Department of Biology and Biological Engineering, Food and Nutrition Science Division, University of Chalmers, Gothenburg, Sweden. In 2005 she became an Associate Researcher of the Spanish Council for Scientific Research (CSIC) in the framework of a Ramón y Cajal Programme. Since 2008 she is a Senior Scientist at CSIC and continues her investigation in the Cereal Group, Department of Food Science of IATA.

Since the early stages of Dr. Haros' career she has mainly been engaged in research in the Cereal Science and Technology field. The major theme in Dr. Haros. research is the utilization of different strategies to improve nutritional and/or functional value of cereal by-products or cereal ingredients. These strategies include the use of different physical, biochemical or biological treatments during milling cereal process; development of new cereal by-products by including novel ingredients; use of new starter phytase producers for regulating content and composition of lower *myo*-inositol phosphates in cereal by-products with clear nutritional and health benefits. In recent years she has focused on:

- Utility of Latin-American crops (quinoa, amaranth, and chia) for improving nutritional value and health benefits of bakery products;
- Develop new cereal wet milling process for obtaining starches and protein isolations with new nutritional and functional features;
- Use of phytases from *Bifidobacterium* for increasing mineral availability of vegetable foods (bakery-products, infant cereals, fermented soy milk).

DECLARACIONES DE PROPIEDADES SALUDABLES EN LA UE. EL CASO DE LA CHÍA

<u>José Vicente Gil</u>

Universidad de Valencia y Grupo de Actividad Biológica de Compuestos Alimentarios, IATA-CSIC, Valencia – Spain

ABSTRACT

In the last years the so-called functional foods, those that provide a health benefit beyond their nutritional value, have been one of the few food subsectors which have grown in sales, with an annual growth rate between 2003 and 2010 of 14% and a global market in 2013 of 43,000 million US dollars, 26.7% more than in 2009. An important part of this market belongs to sales in the EU, where nutrition and health claims in food and food supplements are regulated in EC Regulation No 1924/2006. Since the entry into force of the regulation many health claims have been submitted to the commission for approval but only 11.2% have been authorized on the basis of the criteria required by the European Food Safety Authority (EFSA). Chia seeds have ingredients that have been considered beneficial for health and could be the subject of nutritional or health claims. Alpha-linolenic and linoleic acids, abundant in chia seeds, are essential fatty acids that have health claims authorized in relation to the maintenance of normal cholesterol levels and for the normal development and growth of children. On the other hand there are numerous declarations adopted in relation to different types of fiber, especially from cereals, so that with appropriate studies might be feasible give support to health claims with respect to the fiber of chia seeds. For other health claims related to the antioxidant capacity exhibited by other components of chia, such as polyphenols, it would be necessary to accumulate more studies and scientific evidence supporting its potential effects.

Abbreviated *Curriculum vitae*

Dr. José Vicente Gil Ponce gained his PhD degree working at the Department of Microbiology in the Faculty of Biological Sciences of the University of Valencia (1994-1997). He made a postdoctoral stage at the Department of Biotechnology in the Institute of Agrochemistry and Food Technology of the Spanish Research Council (IATA-CSIC, Valencia, Spain) and in Max Planck Institute for Plant Breeding Research

(Cologne, Germany) where he gained expertise in molecular biology, food technology, chromatographic and mass-spectrometry techniques and enzyme technology. He is currently Associate Professor and docent coordinator of Food Technology knowledge area in the University of Valencia.

His main research interests are the use of *in vivo* methodologies using simple organisms for the screening of the biological activity of plant extracts and investigate about which genes or metabolic pathways are affected in model organisms as a result of exposure to studied ingredients, using high-throughput "omic" technologies and mutant strains.

LA CHÍA COMO UN AUXILIAR TECNOLÓGICO EN LA ELABORACIÓN DE ALIMENTOS DE ORIGEN ANIMAL

José Ángel Pérez-Álvarez, Juana Fernández-López

Industrialización de Productos de Origen Animal (IPOA). Escuela Politécnica Superior de Orihuela. Universidad Miguel Hernández, Orihuela. Spain.

ABSTRACT

En la industrialización de productos de origen animal, se tiene en cuenta distintos criterios técnicos, científicos, nutritivos y del bienestar que son de suma importancia para la elaboración de los mismos.

A nivel industrial se tiene muy en cuenta aspectos más tecnológicos, fundamentalmente basados en retener el agua y "estabilizarla". A nivel nutritivo su composición y que éste incluya la mayor cantidad de nutrientes y que estos estén bioaccesibles y biodisponibles y del bienestar, que el alimento nos nutra, nos satisfaga física, sensorial y "psicológicamente". Por ello se habla de los alimentos 5S (sanos, seguros, sabrosos, sostenibles y socialmente aceptados).

En la actualidad se buscan ingredientes que permitan desarrollar estos alimentos del bienestar y que puedan ser incorporados a los alimentos de origen animal. Recientemente, la industria se está interesando cada día más en una semilla prácticamente desconocida por los consumidores europeos, la Chia (*Salvia hispanica L*). Desde un punto de su composición, la Chía, está caracterizada totalmente, en cuanto a la aplicación de sus componentes (fibra, aceite, harina..) en los productos de origen animal, está de plena actualidad tanto por parte de la industria y los consumidores, ya que carece de gluten, abriendo una puerta muy importante para el desarrollo e innovación de muchos productos de origen animal. Desde un punto de vista tecnológico, son fundamentales aquellos ingredientes que permitan retener o incrementar la capacidad de retención de agua y que después de los tratamientos tecnológicos (secado o cocción, etc.) el agua quede retenida en las estructuras y que éstas (emulsiones geles, espumas fundamentalmente) no se desestabilicen por su incorporación, como ocurre con muchos compuestos de naturaleza polifenolica. El éxito o fracasó en el desarrollo o innovación de un alimento de origen animal adicionado con alguno de los componentes de la chía, dependerá de cómo podamos manejar

tecnológicamente sus propiedades sin que estos alimentos pierdan sus características físicas, químicas y sensoriales por lo cual son tan apreciados.

Abbreviated *Curriculum vitae*

Químico Farmacéutico Biólogo por la Universidad Nacional Autónoma de México homologado a Licenciado en Farmacia por el Ministerio de Educación y Ciencia, Máster en Ciencia e Ingeniería de Alimentos y Doctor Ingeniero Agrónomo por la Universidad Politécnica de Valencia. Catedrático de Universidad, en el área de Tecnología de Alimentos en el Departamento de Tecnología Agroalimentaria, Escuela Politécnica Superior de Orihuela de la Universidad Miguel Hernandez. En cuanto a su actividad investigadora ha publicado más de 100 artículos científicos en revistas indexadas dentro del Journal Citation Reports, algunos de los cuales están entre los más descargados o incluidos en el top 25 hottest articles de la base de datos Science Direct. También ha participado como autor/coautor de más de 40 capítulos de libros publicados prestigiosas editoriales internacionales y nacionales como CRC Press, Wiley, Limusa o Elsevier entre otras con temas relacionados con la Ciencia y Tecnología de Alimentos. Revisor de distintas revistas internacionales dentro del campo de la Ciencia y Tecnología de Alimentos. Director de la Red Temática del Entorno Social y Bienestar, del Campus de Excelencia Internacional CAMPUSHABITAT5U. Vicerrector Adjunto de Doctorado y CAMPUSHABITAT5U. Miembro del CYTED (Proyecto IBEROFUN), proyecto seleccionado entre las 12 redes destacadas por CYTED por sus impactos cualitativos entre más de 500 redes en diferentes áreas del conocimiento.

DEVELOPMENT OF FOOD SUPPLEMENTS USING PLANT PROTEINS (*Salvia hispanica*-CHIA)

Mª del Mar Yust Escobar, Mª del Carmen Millán Linares, Justo Javier Pedroche Jiménez and Francisco Millán Rodríguez

Plant Protein Group, Fat Institute – CSIC, Seville - Spain
Grupo Proteínas Vegetales, Instituto de la Grasa – CSIC, Sevilla, España

ABSTRACT

Scientific researches obtained in recent decades are giving proteins a more important role than a mere nutritional behaviour as a vehicle of essential amino acids for life. Today is more than accepted that certain peptides, from the human digestion of food protein or obtained from external and controlled assays show biological activities in biochemistry level, with cell line (*in vitro*) or animal models (*in vivo*) and every day there are new chapters of this paper in human clinical trials. In addition, limited protein hydrolysis involves an improvement of the rheological properties of these proteins, being more suitable for the food industry. These three aspects, nutritional, bioactivity and functional, are foundations that until recently lived separately and which currently have become a single topic to give foods to the consumer with a higher quality, better flavour and more healthy products.

This trifocal search has led to exploration of raw materials which already present a remarkable composition and with a potential for considerable improvement from a food point of view-at the consumer level and practical at industry scale. In this sense, Chia (*Salvia hispanica* L.) seems to far exceed the expectations of becoming a new ingredient with a wide acceptance and functionality at European and World level.

The work presented here within this project is aiming to show the revalorization of a by-product as Chia defatted meal, searching the obtention of protein hydrolysates with a greater functionality for the bakery industry and, at the same time, provides a healthy effect on the consumer. In this way, we have obtained protein isolates, exceeding 80% of protein content, from Chia defatted meal and we have used this new product for the production of hydrolysates with antioxidant activity through the study of these products as donors of electrons or recipients of protons in metabolic reactions of oxidation-reduction as well as the

ability to capture free radicals from DPPH. Hydrolysates with best activities will be selected to study its functional activities in subsequent trials.

Abbreviated *Curriculum vitae*

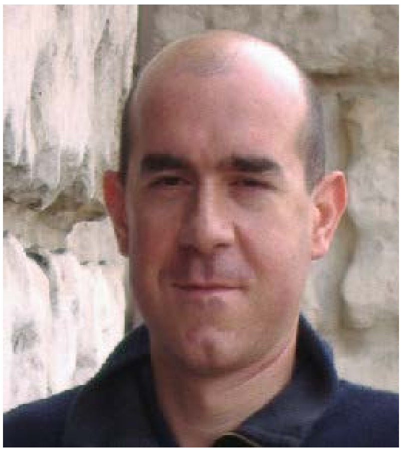
Justo Javier Pedroche Jiménez, Doctorate in Biological Sciences from the University of Seville, Tenured Scientific of the Research Group of Plant Proteins of the Instituto de la Grasa-CSIC, becoming Head of the Nutrition and Health Department of this institute during the years 2014-2015.

Responsible for various lines of research and innovation, both scientific and technical, his research career has been focused primarily in obtaining protein based-products of very high added value and great impact on health. He has published more than 78 articles in journals in the JCR. He has participated in nearly 21 national research projects, financed both by public and private entities. He has directed a thesis on properties anti-inflammatory proteins and author of 3 patents. He has participated in 20 Research & Development contracts with companies of the food industry and energy. It participates as teacher of University Master of “Science and Technology of Oils and Fermented Drinks” and he has participated in several invited conferences, training courses, national and international conferences on issues related to the revaluation of agro-industrial by-products, always seeking a unifying connection between food and health.

ADICIÓN DE CHÍA, COMO POSIBLE INGREDIENTE FUNCIONAL, EN PRODUCTOS CÁRNICOS

Manuel Viuda Martos

Industrialización de Productos de Origen Animal (IPOA). Escuela Politécnica Superior de Orihuela. Universidad Miguel Hernández, Orihuela – Spain



ABSTRACT

La carne y los productos cárnicos son un grupo de alimentos muy importante en la dieta de muchos consumidores, especialmente en el mundo desarrollado. Así, en Estados Unidos y Europa, por lo general se consumen dietas con alto contenido en carne y productos cárnicos y ricas en grasas saturadas; por el contrario el consumo de frutas, verduras y legumbres o granos es escaso. Este patrón en la dieta aumenta el riesgo de desarrollar diversas enfermedades que pueden llegar a ser crónicas en el mundo occidental como son el cáncer y fundamentalmente el cáncer de colon o las enfermedades cardiovascular. Actualmente, existen campañas promovidas por organizaciones o incluso gubernamentales para intentar cambiar los hábitos de la población, promoviendo dietas equilibradas, hacer ejercicio físico regular, y en general asumir hábitos saludables con un objetivo final el de promover la salud y el bienestar de las personas.

Una estrategia que podría adoptar la industria cárnica para ofertar productos cárnicos mas “saludables” seria la sustitución de ingredientes potencialmente perjudiciales por otros con efectos beneficiosos o incorporar, directamente como ingrediente en sus formulaciones, compuestos que hayan demostrado ejercer un efecto beneficioso para el organismo. Uno de estos ingredientes, podría ser la Chia (*Salvia hispanica* L.). Esta semilla, en su composición, presenta una serie de compuestos bio-activos como son los ácidos grasos ω-3 y ω-6, la fibra dietética de ambos tipos, fibra dietética insoluble presente en la estructura de la semilla y fibra dietética soluble formada por el mucilago así como compuestos de naturaleza polifenólica que potencialmente podrían ser empleados como ingredientes en la elaboración de productos cárnicos disminuyendo así los posibles efectos perjudiciales de un alto consumo de este tipo de productos.

Abbreviated *Curriculum vitae*

Ingeniero Técnico Agrícola por la universidad Politécnica de Valencia, Ingeniero Agrónomo por la Universidad Miguel Hernández y Licenciado en Ciencia y Tecnología de Alimentos también por la universidad Miguel Hernandez. En el 2010 obtiene el grado de Doctor, con mención Europea, por la Universidad Miguel Hernández. Actualmente es Profesor Ayudante Doctor en el Departamento de Tecnología Agroalimentaria de la Universidad Miguel Hernández. En cuanto a su actividad investigadora ha publicado más de 60 artículos científicos en revistas indexadas dentro del Journal Citation Reports, algunos de los cuales están entre los más descargados o incluidos en el top 25 hottest articles de la base de datos Science Direct. También ha participado como autor/coautor de más de 25 capítulos de libros publicados prestigiosas editoriales internacionales y nacionales como CRC Press, Elsevier o Nova Publisher con temas relacionados con la Ciencia y Tecnología de alimentos. Además de más de 60 trabajos aceptados en distintos congresos nacionales e internacionales. Desde el año 2012 es miembro de la Editorial Board de la revista "Food Research International" así como revisor de distintas revistas internacionales dentro del campo de la Ciencia y Tecnología de Alimentos.

Salvia hispanica: UNA ALTERNATIVA PARA EL DESARROLLO DE MICROCAPSULAS Y PELÍCULAS COMESTIBLES

Maira Rubí Segura Campos

Facultad de Ingeniería Química, Universidad Autónoma de Yucatán, Merida, Yucatan – Mexico

ABSTRACT

En los últimos años ha sido relevante la percepción de los consumidores en la relación existente entre los alimentos que componen la dieta y el impacto de sus constituyentes mayoritarios y/o minoritarios sobre la salud. Simultáneamente, se ha intensificado la búsqueda y revalorización de sustratos provenientes de fuentes naturales cuyos componentes permitan el desarrollo de alimentos funcionales, aquéllos que además del aporte básico de sus nutrientes contribuyan sustancialmente con efectos benéficos para la salud, al ser promotores de la misma, del bienestar y de la reducción del riesgo de ocurrencia de ciertas enfermedades crónicas. Así, se ha incrementado la tendencia a una mayor utilización de subproductos; implementando diversas metodologías que permitan la obtención de alimentos de mayor calidad y estabilidad, con una adecuada caracterización y evaluación de su funcionalidad. En este contexto se encuentra la chía y subproductos, tales como el aceite, la semilla triturada y la harina residual, los cuales constituyen una buena fuente de fibra, proteínas y compuestos bioactivos, con excelentes propiedades aplicables a la formulación de alimentos funcionales. La proteína y el mucílago se pueden utilizar como materiales para la elaboración de películas comestibles y mejorar la calidad general de muchos alimentos, extender su tiempo de vida de anaquel, mejorar sus propiedades mecánicas y de manejo y sus características nutritivas. El uso de proteínas como materiales para la formación de películas podría conferir acción como barrera a la humedad y restringir el transporte de O_2 y CO_2. Polisacáridos como el mucílago, podrían forman películas con buenas propiedades de barrera al vapor de agua, adecuada resistencia y flexibilidad, así como adecuadas características mecánicas. La proteína y el mucilago podrían también ser empleados como materiales para proteger y aislar diversos compuestos sensibles a la influencia adversa del entorno químico mediante microencapsulación. Esta tecnología provee un medio para envasar, separar y almacenar materiales a microescala y luego liberarlos en condiciones controladas. En aceites vegetales con alto contenido de PUFAs como el de chía, el principal aporte de la microencapsulación es su protección frente a la oxidación durante el almacenamiento y/o condiciones de procesamiento. La elección del proceso de encapsulación depende de las propiedades del producto final,

la estabilidad del material microencapsulado durante el almacenamiento, el mecanismo de liberación, la interacción con otros componentes del alimento y el costo. Por lo anterior, el objetivo de la ponencia titulada "*Salvia hispanica*: Una alternativa para el desarrollo de microcapsulas y películas comestibles" es abordar la importancia de los componentes nutritivos de la semilla de chía como alternativa para la elaboración de microcápsulas y películas comestibles con uso potencial en la industria alimentaria.

Abbreviated *Curriculum vitae*

Químico Biólogo Bromatólogo (2004) de la Facultad de Química de la Universidad Autónoma de Yucatán (UADY) y ganadora del Premio ICT MEXICANA a la mejor tesis de licenciatura del período de Noviembre 2004-Octubre 2005. Maestría en Ciencia y Tecnología de Alimentos (2007) de la Facultad de Ingeniería Química de la UADY y Doctorado en Ciencias Agropecuarias del Doctorado Institucional del Campus de Ciencias Biológicas y Agropecuarias (UADY) (2010). Profesor investigador titular A y Responsable del Laboratorio de Ciencia de Alimentos de la Facultad de Ingeniería Química de la UADY. Investigador Nacional Nivel I (2012-2014; 2015-2018) del Sistema Nacional de Investigadores (No. expediente 53694), Perfil PROMEP-SEP; autor de 46 publicaciones en revistas nacionales e internacionales, 22 capítulos de libro, 5 libros y 5 memorias en extenso. Directora de tesis a nivel licenciatura, maestría y doctorado, participante activa en congresos nacionales e internacionales; miembro de la red "Estudio Físico-Químico, Nutricional y Tecnológico de la Contribución de Subproductos de Chía (*Salvia hispanica* L.) como Nuevos Ingredientes Alimentarios en Europa" (i-LINK+ 2014, CSIC-España) y "Bioactividad de Péptidos e Hidrolizados", Proyecto "Purificación y caracterización de péptidos bioactivos obtenidos por hidrólisis enzimática de proteínas de fuentes vegetales subutilizadas" (PROMEP-SEP). Actualmente, es responsable del proyecto titulado "Investigación científica dirigida al desarrollo de derivados proteínicos de *Mucuna pruriens* con potencial actividad biológica para la prevención y/o tratamiento de enfermedades crónicas asociadas al sobrepeso y la obesidad financiado por CONACYT-Ciencia básica 2013-2015 y del proyecto Aspectos tecnológico-funcionales de subproductos de chia (*Salvia hispanica* L.) aplicables al desarrollo de alimentos, CONACYT-CONICET, 2013-2015. Participa activamente como colaboradora de proyectos con investigadores de instituciones nacionales e internacionales como FIQ-UADY, ITM, CICY, UAEM, CIDCA-La Plata, Argentina; CSIC-España. Línea de generación y aplicación del conocimiento: Desarrollo de productos, ingredientes y aditivos alimenticios; Generación, desarrollo y caracterización de alimentos funcionales para problemas específicos.

STUDY OF THE ANTIOXIDANT CAPACITY OF WHITE BREADS ENRICHED WITH CHIA (*Salvia hispanica* L)

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ABSTRACT

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. In humans, oxidative stress may be associated with various pathologies including cancer, cardiovascular disease, autoimmune diseases, the aging process and diseases associated with it. OMS recommended daily intake of antioxidants through diet to prevent or mitigate diseases associated with cellular oxidative stress. Bread is one of the main components of the human diet so it is highly interesting its fortification with natural components. The use of chia as an ingredient might be a good strategy in this regard due to its nutritional and functional properties. So far, this oilseed has been little exploited in Food Science and Technology.

The aim of this work is to study the antioxidant potential of breads made in the laboratory by replacing 5, 10 and 20% of conventional wheat flour by chia flour. *In vitro* methods (Folin-Ciocalteu and DPPH) were used to characterize the flours used and the breads obtained. The total polyphenol concentration was higher in the case of breads with chia compared to the control bread and an increment in the total phenolic content and in the DPPH radical scavenging activity were observed as the percentage of chia increased. The phenolic acid compounds were identified by HPLC. The main peaks observed in cromatograms of breads with chia corresponded to rosmarinic acid (known for its antioxidant and preservatives properties) and to an unknown compound. Additionally, ferulic and caffeic acid were detected. None of these peaks were obtained in control bread. Increasing peak areas obtained for fortified breads were positively correlated with the percentage addition of chia.

Moreover, we have addressed the antioxidant activity using a multiwell assay for screening the ability of chia to induce antioxidant response in *Saccharomyces cerevisiae* cells. The use of invertebrates as model organisms is advancing a rapid pace and constitutes a very interesting strategy to understand the potential health benefits of plant polyphenols. To date, the ability of the wheat and chia flour to promote antioxidant response in *S. cerevisiae* has been evaluated in yeast cells stressed with hydrogen peroxide. As a result, the antioxidant protection was consistent only in the case of the wheat flour.

Abbreviated *Curriculum vitae*

Dr. Mª Teresa Fernández-Espinar García gained her PhD degree working at the Department of Biotechnology in the Institute of Agrochemistry and Food Technology of the Spanish Research Council (IATA-CSIC, Valencia, Spain) (1990-1994). She made a postdoctoral stage at the National Institute of Agronomic Research (INRA) of Bordeaux (France) (1995-1996) where she gained expertise in molecular microbiology techniques. At the present time she is Research Scientist at the IATA-CSIC.
Her main research interests are: i) molecular microbiology of industrial microorganisms ii) the use of *in vivo* methodologies using simple organisms for the screening of the biological activity of plant extracts iii) the molecular mechanisms responsible for the response to various stresses mediated by these compounds.

FUNCTIONAL CHARACTERIZATION OF BY-PRODUCTS FROM CHIA (*Salvia hispanica* L) SEEDS OF ARGENTINA

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ABSTRACT

The objective of this study was to characterize the physicochemical and functional properties of meals (M) and fibrous fractions (FRF) of chia seeds (*Salvia hispanica* L.), and to compare the effect of oil extraction methods (pressing -p- and solvent extraction -s-) and sieving process on these properties.
Both processes affect the physicochemical and functional properties of residual meals and their corresponding fibrous fractions. Mp and FRFp showed a significantly higher residual oil content than Ms and FRFs (11.39, 10.85, 0.21 and 0.21 g/100 dry base, respectively). The sieving process of both meals allowed to obtain fibrous fractions with a significant increase of crude fiber (27.57, 32.84, 23.81 and 28.35 g/100 g in Ms, FRFs, Mp and FRFp, respectively), and a marked decrease of protein content (41.36, 35.32, 35.00 and 33.74 g/100 g in Ms, FRFs, Mp and FRFp, respectively). Total dietary fiber and their respective components (soluble and insoluble dietary fiber) were significantly higher in FRF. All the samples exhibited a high antioxidant activity due to the presence of phenolic compounds and tocopherols in the case of Mp and FRFp. Ms and FRFs presented a better oil-holding capacity, organic molecule absorption capacity, emulsifying activity and emulsion stability than Mp and FRFp, and allowed to achieve more stable emulsions. FRFs showed the highest values of water absorption and adsorption capacity.

Abbreviated *Curriculum vitae*

Dra. en Ciencias Químicas (UNLP) (1988) e Investigador Principal de CONICET. Desarrolla actividades de investigación y desarrollo en “Propiedades funcionales de lípidos en alimentos” en el Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA) - CONICET, (FCE - UNLP) desde 1984.

Es Profesor Adjunto de Toxicología de los Alimentos en la Facultad de Ciencias Exactas de la UNLP y ha colaborado como profesor invitado en la coordinación y el dictado de diversas asignaturas relacionadas con Propiedades Físico - Químicas y Funcionales de los Alimentos y Cursos de Posgrado en varias Universidades de Argentina, Uruguay, AOCS, ILPS.

Ha publicado 1 libro, capítulos de libro, artículos en revistas con referato internacional, participado en numerosos cursos de perfeccionamiento en Tecnología de Alimentos, Congresos Nacionales e Internacionales en calidad de disertante.

Ha dirigido becarios, tesistas de Doctorado y de Maestría así como diversos proyectos de investigación a nivel nacional e internacional.

Es Presidente saliente de la ISF (International Society for Fat Research) (2013-2015), miembro de la Phospholipid Division de la American Oil Chemists' Society (AOCS) desde 1999, Presidente del Comité Científico, miembro del Comité Organizador, coordinadora de las sesiones Estructura, funcionalidad y aplicaciones en los alimentos y Oxidación de lípidos y antioxidantes en alimentos y disertante en el World Congress on Oils and Fats and 31st ISF Lectureship Series, Rosario octubre de 2015.

THE INFLUENCE OF BREAD-CONTAINING CHIA ON IRON ABSORPTION AND BIOMARKERS OF IMMUNONUTRITION

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ABSTRACT

Chia (*Salvia hispanica* L.) represents a good source of minerals and essential micronutrients such as iron and bread, as staple food for large groups of population and high consumption frequency, can serve as vehicle to combine natural ingredients and improve its nutritional value. Previous of our research demonstrates that consumption of chia is able to restore hemoglobin concentration in iron-deficient animals at a same level than an inorganic supplement of the micronutrient. Notably, feeding chia did not increased the plasmatic concentration of hepcidin - a key regulator of iron metabolism associated to acute phase inflammatory processes within the gut-liver axis - as the supplement did without affecting interleukin (IL)-6 levels. Elevated hepcidin levels, and increased gene expression of IL-6, have been associated in patients with diabetes, obesity, or both to exacerbate and perpetuate the insulin resistance and inflammatory state. In this context, peroxisome proliferator-activated receptors (PPAR) elicits metabolically active brown adipocytes and weight loss in diet-induced obese mice. Further of our experiments confirmed chia-containing bread formulations as effective increasing the expression (mRNA) of PPARγ that were associated to a significant decrease in the glycemic index in comparison to animals fed a white bread. Future research should be directed to estimate how far can the risk and consequences of metabolic and liver-related disorders can be reduced via targeted dietary intervention based on the consumption of novel bread formulations.

Abbreviated *Curriculum vitae*

Dr. Laparra's research is focused on human nutrition, health and the cross-talk within the gut-liver axis. His studies made major contributions in the field of food safety being used by the European Food Safety Agency (EFSA) as well as the development of patents and innovative foods to control immunological and metabolic disorders that were performed in close collaboration with multidisciplinary research teams (ie, universities, pharma and food companies). His research provides the ability to reduce risk, severity and consequences of serious diseases that have an intestinal origin, and allow to develop novel nutritional intervention strategies for treatment and prevention of various diseases as well as the development of new, more effective and safe products. Dr. Laparra has published more than 60 internationally reviewed papers and participated in several different international talks concerning food safety, nutritional deficiencies, immune and metabolic disorders. As reference of his research capacity, he has participated as principal investigator and as member of the research team in both national and international projects of the EU-FP7 and H2020 program. His research run in parallel to active academic roles participating in Masters and courses of specialization from different Universities in Spain. Remarkable achievements in his research include 1) the demonstration that defined components of gut microbiota can modify immunogenic peptide generation, 2) development of novel strategies for the improvement of the nutritional quality of foods that fructified in novel inventions, 3) development of innovative physiologically-based tools for food safety and biological activity evaluation, 4) development of reliable innovative experimental animal models for the study of diet-induced diseases.

CHIA, AN ANCESTRAL SEED AND NEW FUNCTIONAL INGREDIENT

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ABSTRACT

Salvia hispanica L., commonly known as chia seed was a traditional food and one of the main crops used by Mayas and Aztecs in the pre-Columbian times. This oilseed contains high proportions of essential fatty acid, natural antioxidants, and significant amount of dietary fiber. In addition, the seed possesses more protein than other grains, is gluten free, and contains other important nutrients such as vitamins and minerals. One of the most interesting components of this seed is its mucilage. This biopolymer is a natural exudate from the seed, obtained immediately after seeds are exposed to water. Instantaneously, small filaments that correspond to mucilage appeared on the surface that began to stretch slowly until they became fully extended reaching its maximum after 2 h of hydration, forming a continuous and transparent capsule surrounding the seed. Mucilage is composed mainly by polysaccharides and has the capacity to absorb 30 times its weight in water.

In this study the mucilage was extracted by using an aqueous system in proportion 1:40. Then, the aqueous system was dried and the mucilage was separated from seeds. Subsequently, the mucilage was hydrated at different concentration 0.3; 0.5 and 0.8% and subjected to in vitro digestion. The in vitro model was designed to simulate and visualize the digestion stages: oral, gastric and intestinal. Three different digestions with three stages of different lengths each one, were carried out. The dynamic viscosity was measured and digestion progression of each sample was record by video microscopy.

Mucilage yield was 15.47%. The mucilage hydrated in water formed a highly viscous solution, even at lower concentrations. The dynamic viscosity in the three digestion decreases slightly when the digestion time increases.

The images show clear differences between the samples with and without digestion. In the mucilage without digestion, a homogeneous and porous network is observed. While after any digestion, porosity and porous size increased. Less but bigger porous are observed when the concentration of mucilage and

length of digestion increased. This phenomenon may be attributed to some molecular aggregation, which is produced by drastic changes of pH and the effect of digestive enzymes.

Is presumed that these changes in viscosity and molecular aggregation may occur inside the stomach and increase the satiety index when foods containing this mucilage are consumed.

Therefore, the mucilage has numerous potential applications and can contribute to regulate and manage some stages in the digestive process.

Abbreviated *Curriculum vitae*

Doctor en Ciencias de la Ingeniería, Pontificia Universidad Católica de Chile; Doctor en Ciencias e Ingeniería de los Alimentos, Universidad de Santiago de Compostela, Magister en Ciencias de los Alimentos, Universidad de Chile.

Profesor Asistente Adjunto del Departamento de Ingeniería Química y Bioprocesos y Facultad de Medicina- Carrera de Nutrición, Pontificia Universidad Católica de Chile.

Área de Investigación: Microestructura y Propiedades físicas de Alimentos, Ingredientes funcionales, matrices alimentarias e Ingeniería Gastronómica.

Premio BIMBO Panamericano 2012 por su investigación acerca de la semilla de chia.

OBTENCIÓN, CARACTERIZACIÓN Y ALTERNATIVAS PARA LA CONSERVACIÓN DEL ACEITE DE CHÍA (*Salvia hispanica* L).

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ABSTRACT

Chia (*Salvia hispanica* L.) seed oil is a very interesting source to provide a good equilibrium between two essential fatty acids (linoleic and α-linolenic acid). Currently, chia seed oil is not widely used commercially even though its characteristics are well-suited for industrial applications, and contribute to healthy human diets. One of the main objectives of chia oil production involves the appropriate selection of the extraction process. The yield and the quality of oil are very important to determine the feasibility of commercial production. Chia seed oil was obtained by different extraction processes, some of them commonly used by the oil industry (solid-liquid extraction and cold pressing) or by alternative technologies with supercritical CO_2 (SC-CO_2). The aim of this work was to perform a comparative study on the oil yield, fatty acid composition and the physicochemical and quality characteristics of chia seed oils obtained by different processes. The highest oil yield was 33.6% by solvent extraction (hexane). It was also possible to achieve similar values by adjusting the operating conditions (pressure, temperature and time of extraction) of the SC-CO_2 process. However, the oil yield reached by pressing was about 30% lower than those obtained by solvent (hexane) and SC-CO_2. The fatty acid composition of oils was similar for the different processes, highlighting the α-linolenic (~65%) and linoleic (~20%) acids content and a low level of saturated acids (~9%). Furthermore, the presence of a moderate amount of bioactive agents such as tocopherols, polyphenols, carotenoids and phospholipids was recorded. The quantitative composition of these compounds was influenced by the extraction process. Thus, chia seed oil could be used to improve

the ω-6:ω-3 ratio imbalance which currently characterizes Western diets, in addition to provide other nutritional compounds of interest.

Abbreviated *Curriculum vitae*

Vanesa Y. IXTAINA se graduó como Ingeniera Agrónoma de la Universidad Nacional de La Plata (UNLP, Argentina) en 1999 y obtuvo el grado de Doctor de la Facultad de Ciencias Exactas (UNLP, Argentina) en el año 2010. Desde 1999 se ha desempeñado como docente de la Facultad de Ciencias Agrarias y Forestales (UNLP) y recientemente como Profesora de la Maestría en Tecnología e Higiene de los Alimentos (UNLP). Desde el año 2012 es Investigador del Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET, Argentina), llevando a cabo sus actividades en el Centro de Investigación y Desarrollo en Criotecnología de Alimentos- CIDCA (CONICET-UNLP). Autora de diversos trabajos publicados en revistas científicas internacionales, capítulos de libro y numerosas comunicaciones presentadas en reuniones científicas y técnicas tanto nacionales como internacionales.

COMPOSICION LIPIDICA DE SEMILLAS DE CHÍA (*Salvia hispanica* L.) Y SU POTENCIAL EMPLEO EN UNA ALIMENTACION SALUDABLE

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ABSTRACT

There is scientific evidence that chia (*Salvia hispanica* L.) is native to Mexico and was part of the prehispanic diet alongside maize, beans, and amaranth. Upon arrival of the Spanish colonizers, its use was suppressed from Aztec and Mayan customs and traditions. It is not until the end of the twentieth century that chia seeds attracted great interest due to their high alpha-linoleic acid content and its relationship to human nutrition and health. The aim of this study was to determine the chemical composition and fatty acid profile in chia seeds grown in various areas of Mexico. Five lots of chia seeds were obtained, which they were chemically analyzed according to methods proposed by international organizations (AOAC, 1995). The oil was extracted with soxhlet equipment and petroleum ether. The fatty acid profile was determined by gas chromatography with a flame ionization detector. In the chemical composition of the five samples of chia seeds, despite coming from different areas of Mexico and likely conditions of cultivation, harvesting and different storage only significant differences ($p < 0.05$) were found in the contents of protein, neutral detergent fiber and hemicellulose. Chromatographic analysis permitted identification and quantification of nine fatty acids in the chia oil samples: palmitic (C16:0) and palmitoleic acid (C16:1), stearic (C18:0), cis-9 oleic (C18:1 c9), cis-11 vaccenic (C18:1 c11), linoleic (C18:2 c9c12), arachidic (C20:0), gamma-linolenic (C18:3 c6c9c12) and alpha-linolenic (C18:3 c9c12c15). Alpha-linolenic acid had the greatest concentration (62.67%). The content of saturated and unsaturated fatty acids in oil from chia seeds grown in various regions of Mexico is within the range reported by other countries. Given its nourishing properties, there is a great chance, to introduce and promote the use of chia seeds or chia oil for a healthy human nutrition.

Keywords: chemical composition, chia, fatty acids, Mexico, *Salvia hispanica* L.

Abbreviated *Curriculum vitae*

Dr. en Bioquímica por la Universidad Autónoma de Madrid (UAM) y actualmente Investigador Científico del Consejo Superior de Investigaciones Científicas (CSIC) y Jefe del Grupo Lípidos del Instituto de Investigación en Alimentos (CIAL CSIC-UAM), Madrid, España. Entre sus líneas de Investigación destaca la Química y Bioquímica de lípidos bioactivos en alimentos y, en especial, en productos y derivados lácteos así como la biodisponibilidad y bioaccesibilidad de los lípidos de la dieta. Entre sus contribuciones científicas, destacan más de 90 publicaciones en revistas científicas de alto impacto (SCI), así como capítulos de libros, patentes y otros trabajos de divulgación. Ha dirigido 6 Tesis Doctorales. Forma parte de comités nacionales e internacionales (miembro del grupo de expertos de la FIL-IDF). Dirige y participa en Proyectos de Investigación Nacionales e Internacionales así como en contratos de I+D con empresas.

BIOLOGICAL PROPERTIES OF CHIA (*Salvia hispanica* L.) PROTEINS

Michele Silveira Coelho and Myriam de las Mercedes Salas-Mellado

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ABSTRACT

Native to southern Mexico, chia (Salvia hispanicaL.) was part of the mesoamerican culture, being consumed primarily by the Mayans and Aztecs to increase physical endurance. The protein content of approximately 19-23% is higher than most traditionally used grains, such as wheat (14%), corn (14%), rice (8.5%), oats (15,3%) and barley (9.2%). The major proteins of chia seeds are proteins of reserve, accounting for approximately 60-80% of the total protein, and its analysis is complicated by the heterogeneity of polypeptides and the different behaviors of solubility. The amino acid composition of chia seeds demonstrates that it is a good source of amino acids, especially sulfur, aspartic and glutamic amino acid. The procedures that are used for obtaining the protein-rich fraction of chia may affect the properties of the same and cause the denaturation of the proteins, formation of new complexes of proteins, amino acid degradation, Maillard reaction, and the reduction of the digestibility of amino acids, among other things. The production and identification of bioactive peptides have received attention in recent years. Bioactive peptides are protein fragments that have a positive impact on body functions and can influence health. To date, some biological activities have been evaluated in chia peptides, such as antimicrobial, antioxidant and angiotensin-converting enzyme inhibition. characteristics and biological properties of chia proteins.

Abbreviated *Curriculum vitae*

Myriam Salas Mellado was born in Valparaiso, Chile. She is graduated in Food Engineering from Catholic University of Valparaíso, Chile (1971), Master's in Food Technology (1991) and Ph. D. in Food Technology (2000) from State University of Campinas, Brazil (1991), and post-Doctoral stage at the

Institute of Agro-chemistry and Food Technology, in Valencia, Spain (2011). She is currently Full Professor at Federal University of Rio Grande, in Southern Brazil. Salas's scientific publications cover a wide range of topics related to food science and technology, and include a large number of peer-reviewed articles and book chapters. She has been teaching in a variety of subjects in undergraduate and graduate in food engineering, including: agriculture raw-materials, food hygiene and legislation, cereals technology. She has experience in Food Science and Technology, focusing on Technology of Products of Vegetable Origin, acting on the following subjects: bread, baking, processing, gluten-free products, and utilization of different by-products from vegetable industry to obtain bioactive compounds, in order to improve the nutritional and functional quality of baking products.

CONTRIBUTIONS

ORGANIC GROWING OF CHIA

Amanda Di Fabio

Universidad Juan Agustín Maza, Mendoza – Argentina

INTRODUCTION

The chia (*Salvia hispanica* L) is a plant native to southern Mexico, Guatemala, El Salvador, Belize, Honduras, Nicaragua and Costa Rica. Archaeological evidence indicates it was used 3500 years B.C. In pre-Hispanic times, it was used by the Maya and the Aztec civilizations for medicinal, nutritional, artistic and religious purposes (Cahill, 2003). The largest genetic diversity is found towards the Pacific Ocean. In pre-Columbian times it was a basic food item but it was later forbidden by the Spaniards, as they considered it a sacrilegious seed due to its use in ceremonies dedicated to the “Aztec gods”; this information was registered by Friar Bernardino de Sahagún. The religious persecution was the reason why this species was not cultivated for 500 years (Ayerza and Coates, 2005).

Chia means “seed that produces oil” (Watson, 1938). Aztec warriors subsisted on chia seeds during their battles and expeditions, until Spaniards introduced cereals and displaced chia cultivation. This species continued to exist in the mountainous areas of Mexico and Guatemala, and, by the end of last century, there was a renewed interest in it, as it was confirmed as a source of Omega-3 (Beltrán-Orozco and Romero, 2003; Cahill, 2004).

Priority is given to its biological production, which intensifies the biotic cycles that include the land flora and fauna microorganisms, thus helping to maintain and increase fertility in the long term and preserving the genetic diversity, with no pesticide remains in the production. In conventional agriculture, pesticides alter the ecosystem and the microorganisms, and also affect higher animals. It has been observed that herbicides 2, 4-D and 2, 4, 5-T register severe dermatitis in exposed populations. Dioxin is produced as a byproduct during the synthesis of the 2, 4, 5-T and it remains as a contaminant of the herbicide. It is very toxic for mammals, teratogenic, a cause for fetal toxicity and a tumor inducer (Villaamil Lepori, 2013).

When fertilizers with high levels of nitrates are incorporated to crops, they are stored in the cells, and when they are ingested or cooked, they become nitrites that can combine with amines and result in *nitrosamines,* which are carcinogenic. Fungicides are cytotoxic; mutagenicity tests were applied on many products in which mutagenic activity was detected. Their use implies a potential risk with teratogenic and carcinogenic effects on human health (Villaamil Lepori, 2013). The use of glyphosate, chlorpyrifos and pyrethroids during the development of crops contaminates during pesticide application or leaching flows, thus generating risks for the aquatic biota and humans (Villaamil Lepori, 2013). Glyphosate has cytotoxic and genotoxic effects; organophosphate compounds cause neurobehavioral disorders. Organochlorine insecticides remain in the environment and build up in biological and non-biological media. Carbamate insecticides such as aldicarb are very toxic in cases of oral intake and skin contact; carbaryl has less acute toxicity and it is teratogenic in some animals.

In organic farming, rotations are given priority, since they increase yield. The organic matter content in the soil is strengthened, fertility is increased, draining and aeration are improved, water and wind erosion is reduced, and the presence of weeds, plagues and diseases decreases.

CULTIVATION

It is a tropical or subtropical climate plant, it does not tolerate freezing temperatures and it needs direct sunlight to thrive. It requires temperatures between 20 °C and 30 °C; low temperatures affect its growth and blooming. It grows in well drained sandy-loamy and clayey-loamy soils. Light and not very moist soils are preferred. It tolerates acidity and drought.

It is a day length sensitive plant; it has a short-day photoperiod and it blooms when there are less than 12 sunlight hours per day. The sowing date is essential since it determines the duration of the vegetative growth. Late sowing, done on short days, results in poorly developed plants with lower yield. Very early sowing (long days) induces vegetative growth and plants reach a great height, with low seed yield.

Dry farming requires a mean annual rainfall of 900 mm; moist is necessary for germination. Once the plant is established, it requires a small amount of water. It is sensitive to flooded, heavy soils; it does not tolerate moist or waterlogging.

Fig. 1. Rotation with *Setaria italic*

Fig. 2. Crop residues to be added

Fig. 3. Soil preparation

A crop rotation cycle is required; monoculture increases the population of certain soil pathogens. Rotation favors the biological control of plagues and it is the most effective non-chemical means to limit the pathogen populations in the soil. Figure 1 presents the development in vegetal biomass of a rotation with a species suitable for grazing: *Setaria italica* (L.) In addition to being a good fodder, *P. Beauv.* helps to fight against nematodes in the soil, as it is not a host for those organisms. Figure 2 shows vegetable matter that has been crushed to be later added to the soil.

Weed management is an integral part of disease management. The pathogens that are spread through these herbs can infect a wide range of hosts; therefore control of weeds helps to reduce the risk of diseases and viruses transmitted by insects from the weeds and affecting the plants.

PREPARATION OF THE PLOT.

The previous crop residues or the planted green fertilizer is added to the soil with a disk harrow (Figure 3); then, if it is necessary, nutrients are added. The “humus”, formed by colloidal organic products and resulting from the decomposition of organic remains by benefic organisms and microorganisms, increases fertility, is highly soluble and fast-absorbing, and has a high percentage of N, P, K, 2.8 - 5.8 % humic acid and 14 - 30 % fulvic acid. It also has indoleacetic and gibberellic acid, which favors root development, disintegrates clay in heavy soils and gives cohesion to sandy soils. It increases permeability and porosity and has a great water retention capacity and colloidal action. Some teams perform simultaneous operations; they place the nutrient in the first place; then they prepare the seedling bed and finally the seeds are placed.

The furrows are located at a distance of 60 cm between lines and 2-3 kg of seeds per hectare are planted. Seeding is done manually or with fine grain drills which place 20 to 25 seeds per lineal meter. Emergence occurs after 5-7 days, when the first couple of leaves are formed and thinning is done leaving 50 - 60 plants per lineal meter.

Weeding is done when the plant reaches 20 cm high. Weeds are removed manually from the crop line, and animal traction or tractor plows are used between the furrows; after that, earthing up is done. These activities are done twice or three times, depending on the development of the plant. There is a time period of 130 to 150 days from sowing to harvesting. When 80% of the foliage gets discolored, the plant is cut at ground level and left on the furrows for the drying to finish and, after that, it is threshed. The cleaning is done with vibratory sieves provided with flat screens and absorption ventilators. Grains are classified according to their size, and separated from impurities. It is bagged and stored in areas that are not in direct sunlight, with relative moisture of 45 %, at 22 ºC. Bags are piled up on 15 to 20 cm high platforms. The yield is 1200 to 1500 kg/ha.

DISEASES

It is affected by Pythium, Rhizoctonia, *Peronospora farinosa* and *Spongophora*. The control is done by adding composted tree bark to the soil, as it reduces the incidence of Pythium and Rhizoctonia. Compost extract is used to control *Peronospora farinosa.* The glucosinolates that are present in the Cruciferous vegetable family control Spongophora when used as rotation crop.

PLAGUES

Slugs: they are found in crop residues and waste, and they affect sprouts. The summer and winter surface tasks expose them to dehydration and freezing temperatures.
Beetles: *Phyllophaga: l*arvae feed on roots and stems. Cultural practices reduce the populations; dry farming exposes the larvae to the sun. A rotation with leguminous plants helps to repel this plague.
Ants. *Atta cephalotes:* they cut and defoliate at night. Crushed *Yucca sp.* leaves are used to control them. When ants take those leaves to their caves, they get decomposed and act as a fungicide. *Spodoptera* frugiperda (Lepidopteran) larvae: these larvae are capable of masticating; to control them, the perimeters need to be cleared, thus avoiding their entrance into the crops.

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EFFECT OF CHIA BY-PRODUCTS AS BREADMAKING INGREDIENT ON NUTRITIONAL QUALITY, MINERAL AVAILABILITY AND GLYCAEMIC INDEX OF BREAD

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INTRODUCTION

Numerous epidemiological and experimental studies suggest that changes in the diet are important determinants in the prevention of various metabolic disorders included in the so-called metabolic syndrome, such as type-2 diabetes, insulin resistance, hypertension, obesity and cardiovascular disease. Furthermore, intake of food with high amounts of omega-3 leads to lower blood cholesterol and consequently lowers the risk of cardiovascular disease (Albert et al., 2005). In this sense, health and wellbeing are currently driving innovation in the bread sector. Bakers have responded to current trends in changing consumer tastes with the development of a wide choice of breads with added health benefits including prebiotics, n-3 fatty acids, wholegrains, high fibre and seeded breads (Iglesias and Haros, 2013). Therefore, chia seeds and their by-products could be ingredients of interest to enrich foods. Chia seeds are described as a core element of the diet of pre-Columbian civilizations, especially the Aztecs. Today is commonly consumed in several countries, including the USA, Canada and Australia, as well as in Latin American countries, whereas it is practically unknown in Europe. As a result of the opinion given by the European Food Safety Authority about the safety of chia as a food ingredient (EFSA, 2009), chia seed and ground chia seed may be placed on the market in the European Community as a novel food ingredient to be used in bread products, with a maximum content of 10% chia seeds (OJEU, 2013). Therefore the purpose of the present work was to provide further information on how replacing wheat flour by chia seeds and chia by-products at a 5% level to assess their functionality as ingredient with high nutritional value.

MATERIALS AND METHODS

Materials and bread-making procedure

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). Compressed yeast (*Saccharomyces cerevisiae*, Levamax, Spain) was used as a starter for the 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 distilled water (up to optimum absorption, 500 Brabender Units). The ingredients were mixed for 4.0 min, rested for 10 min, divided (100 g), kneaded and then rested (15 min).

The chia ingredients were added at 5% on flour basis to the bread dough formula. The breadmaking process was carried out according to previously described by Iglesias-Puig and Haros (2013).

Composition of raw materials and breads

Protein determination was carried out by the Kjeldahl technique, lipid content was extracted with hexane under reflux conditions by the Soxhlet equipment, whereas ash content was determined in a muffle by incineration. Minerals were measured by flame atomic absorption and phytates by HPLC in reverse phase according García-Mantrana et al. (2015). Amino acid profile was analysis according to Alaiz et al. (1992) and Yust et al. (2004). Linoleic and linolenic acids were determened by the methodology described by Garcés and Mancha (1993).

Experimental models

Animal experiments were performed according to the University of Valencia Ethics Committee Guidelines for Animal Experiments, (SCSIE, University of Valencia, Spain). Experimental animals were female Wistar rats. At day 21, animals were randomly distributed in different groups of treatment (n=5), fasted for 5h prior to blood sampling for glucose analysis, fed with the different bread formula (white bread, whole wheat bread and bread with 5% whole chia flour) in a single dose (500 mg), and blood samples were taken alternatively from each leg every 10 min. Serum glucose concentrations were determined by using a commercial glucometer (Accu-Chek, Roche).

RESULTS AND DISCUSSION

The greater levels of proteins, lipids, and minerals registered in raw chia flours with regard to the wheat flour directly affected the increase of these nutrients, as expected. However, high levels of phytates were found in chia ingredients (5.10-6.63 μmol/g in dry basis) and this contributed to similarly high phytate levels in bread containing chia flour, which affect the mineral bioavailability of Zn and Fe, as was predicted by phytate/mineral molar ratios (Table 1).

The amounts of lipids, proteins, and minerals were significantly higher in chia than in wheat flour. Cereal flours contain high proportions of starch, while chia seed is virtually devoid of it. The greater levels of proteins, lipids and ash registered in the chia seeds and by-products with regard to the wheat flour directly affected the increase of these parameters in the bread, as expected (Tables 1-3).

Wheat flour and the bakery products showed a content of lipids low, being oleic, palmitic and linoleic acids the most abundant. The chia seeds and chia whole flour containing high amount of lipids, where the linolenic acid was the most majority, followed by oleic and linoleic acids. As expected, samples semi defatted and low-fat samples presented lower amounts of lipids, being the linolenic acid the most abundant. All the samples with 5% of chia by-products presented as the main fatty acid the linoleic acid. It highlights the high linolenic acid content of samples containing chia seeds primarily due to exercising protection of the seed integrity during baking.

Table 1. Phytates/mineral molar ratio in raw materials and breads

Parameter	White Wheat Flour	Chia Ingredients			
		Seeds	Whole Flour	Semi-Deffated	Low-Fat
Ash, % in d.m.	0.64±0.01	2.3±0.4	2.2±0.5	3.53±0.04	4.90±0.09
InsP_6/Fe<1.0*	2.52	3.97	3.67	4.15	4.84
InsP_6/Ca<0.24*	0.04	0.06	0.06	0.06	0.05
InsP_6/Zn<5.0*	2.02	3.63	5.58	5.37	5.00
Parameter	**White Bread**	**Chia breads (5%)**			
		Seeds	**Whole Flour**	**Semi-Deffated**	**Low-Fat**
InsP_6/Fe<1.0*	--	4.47	2.45	2.62	2.55
InsP_6/Ca<0.24*	--	0.06	0.04	0.04	0.05
InsP_6/Zn<5.0*	--	3.80	2.05	2.85	2.96

*Threshold ratios (InsP_6/mineral) for mineral availability inhibition; mineral: Ca, Fe or Zn acording to Morris and Ellis (1985), Hallberg et al. (1989), and King (2000). InsP_6: phytic acid or phytates; d.m.: dry matter

Table 2. Linoleic and linolenic acids of raw materials and breads

mg fatty acid/g of ingredient, d.m.	White Wheat Flour	Chia Ingredients			
		Seeds	Whole Flour	Semi-Deffated	Low-Fat
Lipids, % d.m.	0.8±0.3	39.3±1.3	41.5±2.3	20.6±0.7	13.5±0.2
Linoleic (C18:2n6)	7.51±0.10	30.16±0.47	38.11±2.35	26.08±1.25	19.26±0.85
Linolenic (C18:3n3)	0.38±0.01	90.91±1.41	95.24±5.88	67.56±3.25	46.78±2.06
mg fatty acid/g of bread, d.m.	**Control Bread**	**Chia-Bread (5%)**			
		Seeds	**Whole Flour**	**Semi-Deffated**	**Low-Fat**
Linoleic (C18:2n6)	3.19±0.01	6.29±0.26	5.13±0.05	3.77±0.21	5.89±0.17
Linolenic (C18:3n3)	0.25±0.00	6.31±0.27	1.64±0.02	1.15±0.09	0.76±0.04

Values are expressed as mean ± standard deviation (n=3). d.m.: dry matter.

Chia seed had higher amino acids content, as a result of the higher protein wealth, being glutamic/glutamine acid, arginine and aspartic acid/asparagine acid the most abundant. It is also to remark high sulfur amino acid content as lysine, essential amino acids from a nutritional standpoint and deficient in cereals. The whole flour sample has a similar composition to the seed, while semi-defatted and low-fat samples presented higher amino acid contents. This is a direct consequence of degreasing process, removing fats concentrated the proteins (Table 3). The inclusion of chia-flours in bread formulations directly affected the increase of these nutrients, as expected.

Starch hydrolysis of bread samples was significantly affected (p<0.05) by the type and proportion of flour amount added in the bread formulation. Glycaemic index for chia-bread (77.5%) was lower to that calculated for whole wheat bread (88.9%).

Table 3. Essencial amino-acid composition of raw materials used in this study, mg/g of raw materials in dry matter

Amino-Acid	**White Wheat Flour**	**Chia Ingredients**			
		Seeds	**Whole Flour**	**Semi-Deffated**	**Low-Fat**
Proteins, % d.m.	10.1±0.1	22.7±0.2	23.3±0.1	26.2±0.5	30.2±0.1
His	2.56±0.12	5.24±0.24	5.81±0.15	6.73±0.05	6.11±0.06
Thr	3.23±0.03	8.27±0.25	8.28±0.10	9.74±0.44	10.02±0.07
Arg	4.21±0.21	19.59±0.74	22.03±0.36	26.27±0.52	23.44±0.99
Val	4.40±0.10	8.46±0.18	8.66±0.22	10.35±0.45	10.47±0.09
Met	1.93±0.05	3.23±0.46	4.58±0.28	3.36±0.24	4.05±0.17
Ile	4.03±0.13	6.83±0.11	6.84±0.05	8.65±0.27	8.43±0.17
Trp	0.88±0.01	1.34±0.08	1.49±0.00	1.63±0.00	1.75±0.01
Leu	8.23±0.18	14.15±0.19	14.31±0.15	16.88±0.78	17.36±0.17
Phe	5.70±0.14	10.86±0.01	11.07±0.15	13.40±0.33	13.14±0.14
Lys	2.47±0.02	10.89±0.79	10.20±0.23	12.40±0.35	12.08±0.05

Values are expressed as mean ± standard deviation (n=3). d.m.. dry matter

CONCLUSIONS

The incorporation of 5% chia by-products increased the nutritional value of bread products with regard to the concentrations of proteins of greatest biological value, lipids with a high proportion of omega fatty acids and minerals compared to control sample. The mineral bioavailability depends on the formulation and breadmaking process, basically because of the presence of phytates, as predicted by inhibitory threshold values for mineral absorption phytate/mineral molar ratios. Animals fed with whole wheat- and chia-bread showed significantly lower glycaemic index those fed with control bread. Thus, suggesting beneficial effects on glucose metabolism and, potentially a number of components of the metabolic syndrome.

Chia could be used as a replacement for wheat flour in bread formulations, increasing the product's nutritional and functional value, with higher bread quality when used in proportions 5g/100g, therefore its inclusion is recommended, even at greater levels than 5%. The potential contribution of chia flours could have clinically relevant implications at controlling metabolic diseases prevention.

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TECHNOLOGICAL STRATEGIES TO INTRODUCE CHIA (*Salvia hispanica* L) INTO ANIMAL BASED FOOD

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Chia (*Salvia hispanica* L.) is a great unknown to the European consumers, but not in Latin America, where chia has been used since ancient times. During Spanish Viceroyalty period, this seed was “punished” by religious and cultural issues. Its cultivation and consumption almost disappeared (Perez-Alvarez, 2015). In Mexico and Guatemala, chia takes an important role in social, cultural and gastronomic habits. For example, chia even becomes part of the name of one of the Mexican states, Chiapas (Chia River). For this reason, all anthropological information over how Mexicans or Guatemalans uses chia in their food can be very useful.

In Spanish, the word “chia” is an adaptation of the plural of chian. In Nahuatl (Mexican native language) chia means “oily”. For European consumers “oily” has not good notations. So this aspect must be taken into account. Ii is important to keep in mind the basic knowledge of the seed, and its technological implication in the elaboration process of animal based food. Most of European consumers consider chia as “unusual” ingredient, so they are reticent to accept this seed in their food and diets. So, European consumers must be informed about the health benefits of chia consumption. In addition, chia is a new ingredient for food that Europeans are beginning to discover.

Nowadays consumers are connected to social media and networks. If someone is looking for food, in internet can be found “Ah”, food ... who does not love food? It's essential to our survival, of course, but more importantly, it acts as a universal language. Every culture has their own cuisine and we can communicate a lot about ourselves in how we assemble raw ingredients into delicious meals. Consumers prefer some type of food more than others. So-called "foodies" are amateur gourmets who simply love food - eating it, talking about it, preparing it, and learning about it. And for foodies, the social web is an amazing playground (Catone, 2009).

As we have seen, “new” communication technologies and social media networks play a major role in how consumers view food brands and the way of these brands interact with their customers. So with this relationship is necessary “to spread” chia health benefits. However, the misinformation that many websites have on chia “benefits” could be prejudicing to use it in unappropriated way, so can cause that does not meet consumers expectations. We must to avoid that our consumers seek comments by “Dr. Google” (Scott-Thomas, 2015) or “Wikipedia” (Anonymous, 2015a), unfortunately, this attitude is very common. That's why you should be very carefully about publishing information and download it: such sources have a lot of references like to "superpower chia" (Anonymous, 2015b). Since you can’t avoid that, we can find very specific information in this regard, including: (i) Chia seeds have a high content of dietary fibre, so they are suitable for controlling the "craving"; (ii) They are easy to digest but create a feeling of fullness that helps us to control ourselves “not snack between meals”; (iii) It is advisable to take them at breakfast to keep fit throughout the day; (iv) can be added to milk or egg or (v) consume chia, No excuses!. Some of these "attributes" are justified, but others as acting as an antidepressant, and increases brain function, must remain clearly under "suspicion".

The industry of animal based food, have a great number of goal to be achieved: (i) to maintain, sensorial descriptors, (ii) to reduce additives in food formulation, (iii) to reduce or substitute fats and (iv) to reduce salt and sodium content. So this makes quite complicated accept a new “oily” ingredient in these type of food. That is also why European Food industries ask, Is it worth to "invest" in chia? Of course the benefits of chia are too large to dismiss this possibility, even more than being developed and innovating dairy and egg products where chia seed or its co-products (oil, dietary fibre protein-rich flours, etc.) could be directly incorporated. Since the dairy, meat, fish or egg products are of high economic value, chia incorporation should maintain or improve their sensory characteristics (flavour, appearance, texture, smell, etc.), and this can only be done through good awareness campaigns, by universities or research centres together with producers and processors, popular science magazines, radio and TV campaigns (Perez-Alvarez, 2015a). In Europe, unfortunately, chia is not permitted for use in animal based food products, but in other countries, is possible to use in food we have examples of this: Port-Salut type cheese. Unfortunately in these cases there is a lack of a scientific basis for knowing exactly its behaviour during the processing of these products.

For animal based food processing industries, chia raises a number of concerns when deciding to invest in R&D + innovation. The Industrial arise among other questions, the following: Is chia an attractive alternative for food of animal origin?, Do you give an added value to the product by the addition of chia?, Are food animal matrices compatible with chia? Considering that dairy, meat, fish or eggs food matrices are extremely complex, could the chia reduce its shelf life? By definition, the incorporation of a new ingredient should never reduce shelf-life of any food product, it should be at least equal or better. However, in addition to consider these questions, the food technologist must also consider the main trends in research, development and innovation. These trends are: (i) high-fibre products; (ii) low-sodium products; (iii) protein enrichment of food; (iv) food products with "antioxidant" properties (López-Marcos et al., 2015a). After considering these criteria, chia could be an excellent choice for all these trends.

Once you have decided to add this seed or its co-products (oil, fibre flour ..), in the dairy, meat, fish or egg based food, the following technological aspects must be taking into account: (i) scientific and technological characteristics of new ingredients (chia), food additives; (ii) food product formulation; (iii) food processing technology selection of the to apply (chopped, pre-emulsion, cooking, maturity ...); (iv) food packaging system (vacuum, modified atmosphere ...); (v) identification of the food product characteristics susceptible to degradation during processing and storage ; (vi) chia coproducts characteristics (chia oil: we must compete with olive oil "Everyone you know that olive oil is good”, or “extra virgin olive oil, a source of flavours”, “healthy choice” among others); and (vi) the applicable legal framework, for the moment, is unfavourable in the European Union, by limited scientific information.

Normally, ethically industry seeks consumer’s health benefits without neglecting economic benefits. By this reason they use technological criteria to maintain or improve health aspects, when develop, innovate or optimize processes and products. Chia has important techno-functional properties that can be very useful. But why are important techno-functional properties? and what are they? From a single view, techno-functional properties are used to generate "attractive“ features to animal based food. From a more concrete view, what would be sought in such products when chia has been incorporated? Below are listed the main features to consider in the new product development of animal based food with chia: (i) binding properties (water retention and oil); (ii) sensory characteristics (colour, taste, aroma, odour); (iii) antioxidant properties (mechanism oxidation inhibition); (iv) surface properties (formation and stability of emulsions and foams); (v) properties of hydration (solubility); (vi) ultrastructural properties (microstructure of gels, foams ...); (vii) textural properties (elasticity, cohesiveness, gumminess ...); (viii) rheological properties (viscosity, gelation, etc.) and (ix) transport properties (vehicle bioactive compounds or "carriers") (Quiles et al., 2003; Fernández-Ginés et al., 2005; Viuda-Martos et al., 2010; Sánchez-Zapata et al., 2013; López-Marcos et al., 2015b).

CHIA: A NEW INGREDIENT TO DISCOVER FOR THE SCIENTIFIC COMMUNITY, INDUSTRY AND CONSUMERS

Generally towards the development and innovation of animal based food products that incorporate chia, you need to generate prototypes of this type of food. In a prototype, all final parameters of the process and the formulation of the new food must be well established to assess the technological properties, sensory and nutritional quality. Thereby identifying possible nutrition claims.

Head to the implementation of chia in animal based food, we must establish how we will incorporate it, that is, if “fresh” or toasted seeds, dietary fibre, chia oil, etc. Or at what stage of the elaboration process must be added (kneading, fermentation, pressing ...). Taking into account that chia seed is high in fibre (30%), proteins, minerals (iron, calcium, magnesium, phosphorus, selenium) and omega-3 fatty acids, these properties making it a very attractive use, for both nutritional and functional points of views. An example of development of a new chia-dairy product, is the Greek yogurt with blueberries, chia and hemp.

However, from a legal point of view, the use of chia in animal based food products, in Europe, is not allowed. However, the adoption by the EU of chia oil, will allow a "limited" in dairy products development, similar to the use of olive oil in these products (Anonymous, 2015c).

CHIA: A NEW INGREDIENT FOR INDUSTRIAL AND DELI FOOD

From a gastronomic point of view, the chefs will be responsible for incorporating this seed in their menus and help to spread it. However, more and more chefs are part of the R&D teams helping to improve the sensory properties of processed food, in which chia can be useful.

Cooking has become a form of entertainment (the kitchen is fashionable) and the proliferation of programs related to cooking highlight its importance. As a result, the industry has more and more products on the market that address this need. But keep in mind, by the chefs and industry, that such consumers are well “documented” by culinary blogs or “tele-dining” and demands high quality products. It is here that chia may play a major role.

At "chef level", chia seeds are very versatile, giving a nutty flavour, they can be incorporated into several food products. In some cases it will be necessary to add a little more liquid to prevent food drying or thicken. Of course, European chefs need to "experience" more with chia to get a dish "outfitted" (proper consistency and / or concentration of appropriate seed). The chefs have a unique opportunity to spread the benefits of chia through healthy eating programs (for example, UMH saludable) or wellness networks (for example, Entorno Social y Bienestear, International Excellence Campus Campus Habitat5U) (Oficina de Comunicación, 2015) or through the new global agenda for United Nations sustainable development 2030, which will guarantee healthy food for all, promote sustainable food systems, educate population about healthy eating and reduce "waste" (FAO, 2015).

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DEVELOPMENT OF FOOD SUPPLEMENTS USING PLANT PROTEINS (*Salvia hispanica* L. - CHIA).

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ABSTRACT

The work presented here within this project is aiming to show the revalorization of a by-product as Chia defatted meal, searching the obtention of protein hydrolysates with a greater functionality for the bakery industry and, at the same time, provides a healthy effect on the consumer. In this way, we have obtained protein isolates, exceeding 80% of protein content, from Chia defatted meal and we have used this new product for the production of hydrolysates with antioxidant activity through the study of these products as donors of electrons or recipients of protons in metabolic reactions of oxidation-reduction as well as the ability to capture free radicals from DPPH. Hydrolysates with best activities will be selected to study its functional activities in subsequent trials.

INTRODUCTION

Scientific researches obtained in recent decades are giving proteins a more important role than a mere nutritional behaviour as a vehicle of essential amino acids for life. Today is more than accepted that certain peptides, from the human digestion of food protein or obtained from external and controlled assays show biological activities in biochemistry level, with cell line (*in vitro*) or animal models (*in vivo*) and every day there are new chapters of this paper in human clinical trials. In addition, limited protein hydrolysis involves a improvement of the rheological properties of this proteins, being more suitable for the food industry. These three aspects, nutritional, bioactivity and functional, are foundations that until recently lived separately and which currently have become a single topic to give foods to the consumer with a higher quality, better flavor and more healthy products.

This trifocal search has led to exploration of raw materials which already present a remarkable composition and with a potential for considerable improvement from a food point of view-at the consumer level and practical at industry scale. In this sense, Chía (*Salvia hispanica* L.) seems to far exceed the expectations of becoming a new cereal with a wide acceptance and functionality at European and World level.

The aim of this study has been the production of protein isolates from Chia defatted meal which were used as substrate for the obtention of different protein hydrolysates by means of Alcalase and Flavourzyme as proteases. Likewise, antioxidant activity of the protein isolate and hydrolysates were performed by the determination of the reducing power capacity and DPPH free radical scavenging activity.

MATERIALS AND METHODS

Materials

Chia seeds were donated by Primaria (Valencia, Spain). The seeds were ground and extracted with hexane in a soxhlet extractor for 9 h. The resulting defatted flour was used as the starting material for preparing the protein isolate. Alcalase 2.4 L and Flavourzyme 1000 MG were donated by Novozymes A/S (Bagsvaerd, Denmark) Amiloglucosidase and α-amylase were purchased from Sigma (St. Louis,MO, USA). All other chemicals were of analytical grade.

Analytical methods

Moisture, and ash were determined using AOAC, 1990 approved methods. Total fiber was determined according to the procedure described by Lee et al. (1992). The protein concentrations were determined by elemental microanalysis as % nitrogen content X 6.25 using a Leco CHNS- 932 analyser (St. Joseph, MI, USA).

Obtention of protein isolates

The Chia protein isolate was obtained using the method of Pedroche et al. (2004). Briefly, defatted lupine flour (5 g) was extracted in 100 mL of water with 0.25% Na_2SO_3 (w/v) at pH 11.0, for 1 h. After centrifuging the extract at 8000 rpm for 15 min, the supernatant was recovered, and the pellet was extracted again. Both of the supernatants were adjusted to the isoelectric point of chia proteins (pH 4.0). The resulting precipitate was washed with distilled water adjusted to pH 4.0 and centrifuged to remove residual salts and other non-protein compounds. Finally, the precipitated proteins were lyophilised and stored at room temperature.

Hydrolysis of chia protein isolates

Hydrolysis was conducted in a reactor under stirring at a controlled pH and temperature. The Chía protein isolate was suspended in distilled water (10% w/v), and two types of hydrolysis were performed: one with Alcalase and one with Flavourzyme. Alcalase and Flavourzyme are two food-grade enzymes with different activity. Alcalase is a endopeptidase and Flavourzyme is a aminopeptidase, that means, they break the peptide bonds in a different way, Alcalase by the inner of the protein and Flavourzyme releases one by one amino acid from amino extreme of the proteins. The following conditions were used. <u>Hydrolysis with Alcalase</u>: The Chía protein isolate was hydrolysed with Alcalase for 1 h at pH 8, 50 ºC, and E/S = 0.3 AU/g protein. <u>Hydrolysis with Flavourzyme</u>: The Chía protein isolate was hydrolysed with Flavourzyme for 2 h at pH 7, 50 ºC, and E/S = 50 LAPU/g protein. Samples were taken at different times, and enzymes were inactivated by heating at 85 ºC for 15 min. The supernatants obtained from centrifugation at 8000 rpm for 15 min constituted the Chía protein hydrolysates.

Reducing power capacity

To 0.5 mL of antioxidant solution (1 mg/mL) were added 0.25 mL of 0.2 M phosphate buffer (pH 6.6) and 0.25 mL of $K_3Fe(CN)^6$ solution (1% w/v); the mixture was incubated at 50 °C on a water bath for 20 min. The incubated mixture was left to cool to room temperature, and 0.25 mL of TCA (10% w/v) was added.

The solution was thoroughly mixed, an aliquot of 0.5 mL was withdrawn, and 0.5 mL water followed by 0.1 mL of $FeCl_3 \cdot 6H_2O$ solution (0.1% w/v) was added. The absorbance of the resulting Prussian blue solution at 700 nm was measured against a reagent blank. BHT (0.1 mg/mL) was used as positive control.

DPPH free radical scavenging activity

The scavenging effect of the hydrolysates on α,α-diphenyl-β-picrylhydrazyl (DPPH) free radical was measured by Wu et al. (2003) with some modifications. A volume of 1.5 ml of each sample (1 mg/ mL) was added to 1.5 ml of 0.1 mM DPPH in 95% ethanol. The mixture was shaken and left for 30 min at room temperature, and the absorbance of the resulting solution was measured at 517 nm. A lower absorbance represented a higher DPPH scavenging activity. The scavenging effect is expressed as [(Blank absorbance−Sample absorbance)/Blank absorbance] ×100%. BHT (1.0 mg/mL) was used as positive control.

RESULTS AND DISCUSSION

Chia protein isolate was obtained from defatted meal after hexane extraction. Chemical characterization of Chia protein isolate and defatted meal is shown in the Table 1. As it can be observed, the main differences are found in the protein and fibre levels. Protein content is improved from 34.6 % in the meal to 84.9 % in the protein isolate. In the case of the fibre, it has been reduced up to 80 %, from 49.6 % in the meal to 10.2 % in the protein isolate. Probably, this fibre content is associated to the protein fraction and it is recovered in conjunction with protein in the whole process of obtention of protein isolate.

Table 1. Chemical composition of chia defatted meal and protein isolate.

	Defatted meal	Protein Isolate
Protein	34.56±0.43	84.93±0.41
Moisture	7.73±0.07	1.39±0.01
Ash	6.61±0.05	1.65±0.08
Fibre	49.60±1.98	10.20±1.97

The amino acid content of chia protein isolate and defatted meal is shown in the Table 2. Both of them are very similar, deducing that the developed process is very accurate with the final quality of the amino acids, mainly in essential amino acids. As it can be seen, the aminoacidic composition of chia meal and protein isolate comply with the minimum essential amino acid composition required according to the requirements proposed by the FAO/WHO (2007) for a equilibrated diet, except for the amino acid lysine and tryptophan. In any case, this recommendation is included as part of an equilibrated and varied diet, that means, these amino acid compositions would be part of a broad range of foods where the limiting amino acids of chia products could be completed with other protein resources. Thus, in the worst cases, chia protein hydrolysates contribute more than 50% of the value required by the FAO in these limiting amino acids. In this sense, it can be affirmed that the amino acid contribution of chia protein products could be of a great interest as protein complement in an equilibrated diet.

Table 2. Amino acid composition of chia defatted meal and protein isolate.

	Defatted meal	Protein Isolate	FAO
Asp+Asn	9.03±0.10	8.83±0.15	
Glu+Gln	16.86±0.16	17.74±0.21	
Ser	7.83±0.20	7.19±0.26	
His	4.31±0.16	3.85±0.21	1.9
Gly	4.84±0.08	4.82±0.00	
Thr	3.65±0.11	3.84±0.05	3.4
Arg	9.88±0.15	10.11±0.15	
Ala	4.73±0.04	4.92±0.08	
Pro	2.40±0.03	2.27±0.13	
Tyr	8.27±0.38	6.42±0.15	
Val	4.40±0.07	4.64±0.00	3.5
Met	2.40±0.05	2.41±0.08	2.5[a]
Cys	2.01±0.13	1.81±0.14	
Ile	3.23±0.08	3.55±0.06	2.8
Trp	0.63±0.07	0.77±0.04	1.1
Leu	6.20±0.08	6.71±0.05	6.6
Phe	4.71±0.11	5.40±0.02	6.3[b]
Lys	4.64±0.07	4.68±0.04	5.8

Two types of protein hydrolysates were obtained from chia protein isolates. The degree of hydrolysis and the protein solubility of every hydrolytic process is shown in the Figure 1.

The hydrolysis with Alcalase was very fast at the beginning of the hydrolytic reaction (D.H.: 36.2 % at 15 minutes), maintaining these values of degrees of hydrolysis during all process (D.H.: 43.8 % at 60 minutes). Likewise, this hydrolysis involved a substantial improvement of the protein solubility (97.7 % at 60 minutes) respect to the initial solubility (36.1 % at 0 minutes). Alcalase produced low weight molecular peptides due to its activity endopeptidases improving very quickly these two parameters of hydrolysis and solubility. In contrast, the evolution of the degree of the hydrolysis with the enzyme Flavourzyme was more gradual (from 32.3 % at 15 minutes to 61.4 % at 120 minutes). At the same time, the protein solubility in Chia protein hydrolysates with Flavourzyme was a staged increasing. The releasing of single amino acids involved a slower enhancing of hydrolysis and protein solubility.

The antioxidant activity of the samples was tested by two methods: the reducing power capacity (Figure 2) and the DPPH free radical scavenging activity (Figure 3).

As it can be observed, the hydrolytic process did not involve an improvement of the reducing power capacity in both types of hydrolysates. In this case, the protein isolate showed the higher activity due probably to the presence of other non-protein compounds with antioxidant activity (e.g. polyphenols). In contrast, the hydrolysis by Alcalase and Flavourzyme implicated a better the DPPH free radical scavenging activity in the hydrolysates obtained in comparison with the protein isolate being more evident in the hydrolysates obtained with Flavourzyme.

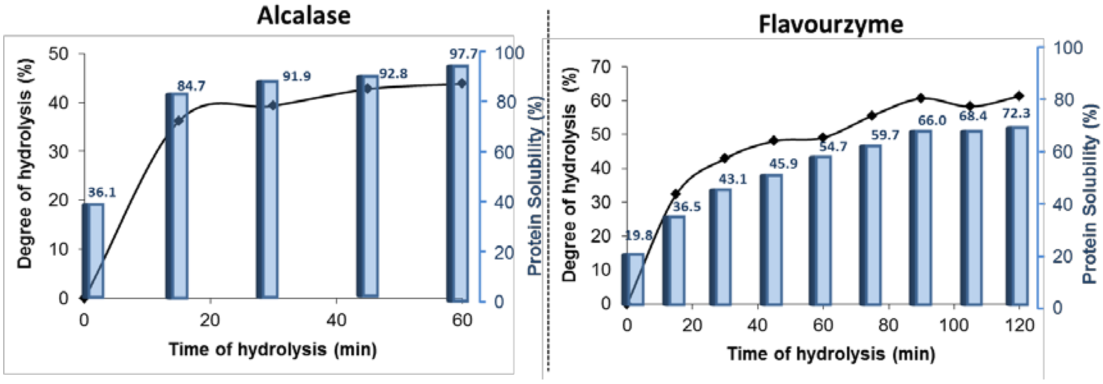

Fig. 1. Degree of hydrolysis and protein solubility of Chia protein hydrolysates using Alcalase and Flavourzyme as proteases.

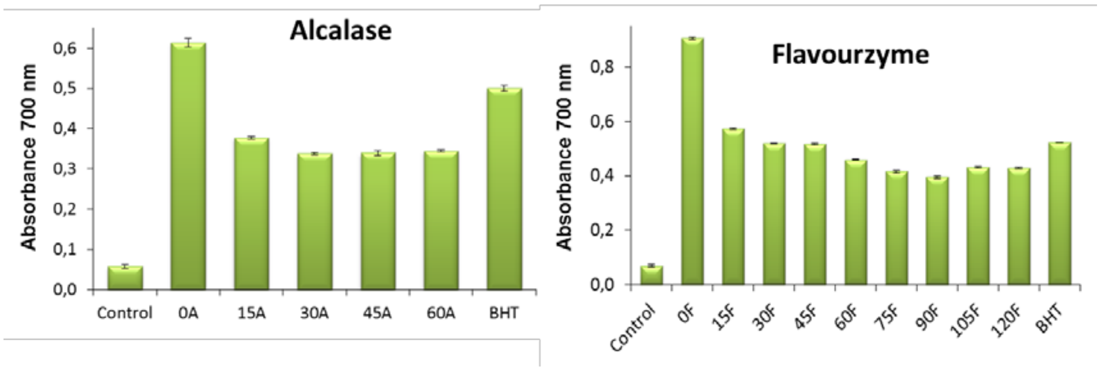

Fig. 2. Reducing power capacity of Chia protein isolate and hydrolysate using Alcalase and Flavourzyme as proteases. BHT was used as control positive.

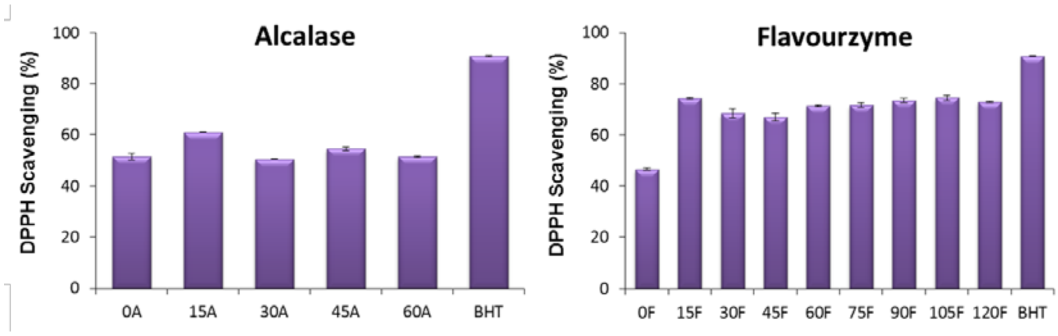

Fig. 3. DPPH free radical scavenging activity of Chia protein isolate and hydrolysate using Alcalase and Flavourzyme as proteases. BHT was used as control positive.

In conclusion, Chia defatted meals are rich in proteins. These protein values are similar to sunflower or soybean, which are worldwide used for human or animal feeding. From the present study, we have obtained protein isolates which presented higher protein content than the initial meals. According to the amino acid composition, the protein found in Chia defatted meal and protein isolate is of good nutritional quality, showing a composition in essential amino acids equilibrated with the recommended values by FAO. The molecular size of protein isolate could difficult its solubility and its absorption and assimilation potential by the organism, and for this reason, it has carried out the hydrolysis of the isolates to obtain protein hydrolysates, searching, at the same time, the obtention of antioxidant bioactive peptides. Therefore, from this research study, several protein products can be obtained from Chia seeds with different chemical and functional characteristics according to the food requirements or applications.

However, further studies about nutritional (digestibility, bioavailability,…) and bioactive (hypotensive, hypocholesterolemic, anti-inflammatory, chelating,…) characteristics of Chia protein products, and how these processes in the obtention of protein isolates and hydrolysates could affect to these qualities, are necessary before using them in several applications, particularly in food industry to be used as food supplement directly or as a replacement of the conventional protein in animal or human feed.

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Salvia hispanica: AN ALTERNATIVE TO THE DEVELOPMENT OF MICROCAPSULES AND EDIBLE FILMS

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The results of the National Council for evaluation of Social Development Policy (CONEVAL) established in Mexico, that between 2012 and 2014 the percentage of the population living in poverty rose from 45.5 to 46.2 % and extreme poverty fell from 9.8 to 9.5 %. One of the most important factors that shaped the measurements of poverty and extreme poverty were the social deprivation: educational backwardness, lack of access to health services, social security, quality and space in the housing, basic services in housing and access to food. In the period 2012-2014 all social shortcomings significantly reduced. However, the lack of access to the foods increases from 23.3 to 23.4 %, i.e. 27.4 to 28 million people (CONEVAL, 2014).

The technical committee for the measurement of poverty convened by the Social Development Secretary (SEDESOL), defined food poverty in accordance with the results of the National Survey of Household Income and Expenditure (ENIGH) 2002, such as the inability to obtain a basic food basket even if use is made of all the disposable income in the household to buy only the assets of this basket. To analyze the macroeconomic dynamics of Mexico during the period 1990-2010, with the aim of associate the evolution of the food-related problems under an aggregate perspective, it was noted that the evolution of the Gross Domestic Product (GDP) of Mexico presented three recessions. The first stage of crisis was presented between 1994-1995, the second between 2000-2001 and the third between 2006-2009 with reductions in GDP of 6.2, 0.2 and 13.1 %, respectively, the last two stages caused by the cycle of world economy. The move this dynamic of the economy to the analysis in per capita terms, it was possible to detect a significant effect on the evolution of income poverty in Mexico, which indicates that there is an inverse relationship to the expansion of the economic cycle with the reduction of poverty. In response to the behavior of the food prices represented by the evolution of the indexes constructed by the United Nations Organization for Food and Agriculture (FAO), it was observed a decrease in food prices between 1997-2002, however since there have been significant increases in the same reaching maximum accumulated in this study in the period 2010-2011. This behavior influenced growth in food prices in Mexico. From 1994 it launched an escalation in the levels of the national consumer price index. Thus, the negative effect of the economy as well as the international growth of the food price has had a significant impact on the evolution of the food-related problems of Mexico (SEDESOL, 2012).

According to FAO (2014), the hunger is a complex problem and there is no universal recipe to eradicate it. Each country must choose its own path. However, the positive experiences in Latin America and the Caribbean are to understand that there are a number of common factors that serve as a roadmap: (i) the political commitment of governments, (ii) the mobilization of the entire society, (iii) a holistic approach that combines the reinforcement of social protection systems with measures to support the production, especially of the family farming; and (iv) in the development and strengthening of legal frameworks to consolidate the progress and to provide a budgetary resources to the fight against hunger, the fruit of the incorporation of the legislators and parliamentarians in the region. The promotion programs for the family agriculture existing in Latin America and the Caribbean Respond to a greater extent to four areas of support: 1) technical assistance, agricultural extension and transfer of technology, 2) integration of markets and supply chains, 3) financing, insurance and loans, and 4) access to inputs, infrastructure and productive assets (FAO, 2014). In this sense, in Mexico has been encouraged the incorporation of the families to productive projects with the purpose of banishing poverty food from own family endeavor and economic autonomy through the granting of funds as the seeds.

Salvia hispanica is an alternative to the programs of family farming by its nutritional and functional potential. Chia seeds were described as the main element of the pre-columbian civilizations diet, mainly in the case of the Aztecs, along with the corn, the amaranth and beans. With the emerging information on the chia as a natural source of fatty acids ω-6 and ω-3, antioxidants and dietary fiber, heightened the expectations surrounding its cultivation. The average yield of this species in commercial plantations is of about 500-600 kg/ha, although there have been up to 1260 kg/ha. Currently, the largest producers of chia in Mexico are located in Jalisco and Puebla. In Yucatan, the classified land with high potential for planting this seed due to the climate, soil type and annual precipitation are Tizimin, Calotmul, Temozon and Peto, among others. With regard to its nutrient value, the content of oil present in the chia seed is about 34.8 %. Due to the high content of ω-3 and its relation ω-6: ω-3, the chia oil is an alternative in the functional food. Fiber is the most abundant component in Chia seeds (35.8 %) (Segura et al., 2013). Craig and Sons (2004) reported that once the oil has been extracted from the seed, the remnant material contains a high content of fiber (33.9 %), of which 30.4 % corresponds to insoluble dietary fiber and the 3-5 %, approximately represents a fraction mucilaginous that behaves physiologically as soluble dietary fiber. The mucilage emerges from the seed when it enters in contact with water, covering it in the form of a transparent halo. By their functional properties, such as water affinity and capacity to dissolve or disperse in it, the mucilage has potential use in the development of biodegradable and/or edibles films. The chia, compared to other seeds also provides a high source of protein, representing 23.9 % of its weight, value that justifies the potential use of the seed as a source of protein in human diets. In addition to their functional properties chia seeds represent an alternative such as food additives and source of bioactive compounds with potential in functional food (Vázquez et al., 2015).

Fig. 1: Seeds, protein, and mucilage of *Salvia hispanica.*

Currently, the "globalization of the life styles" is an increasing factor in the mortality and morbidity, as reflected by the increasing rates of cancer, diabetes and cardiovascular and respiratory diseases. In the last few decades the lifestyle of the people has suffered major modifications, such as changes in feeding patterns, decrease of the physical labor and increase of a sedentary lifestyle. Since the late 80's the world lived an important epidemiological transition in which the contagious diseases were overtaken by non-communicable diseases. Thus, inadequate intake of food in quantity or quality, or the poor functioning of the metabolic process, causes a poor nutrition, which can be by deficit (malnutrition), or by excessive consumption (overweight and obesity). In Mexico, in accordance with the National Health and Nutrition Examination Survey (ENSANUT, 2012) children of school age (between 5 to 11 years of age), adolescents (between 12 and 19 years of age) and adults have a combined national prevalence of overweight and obesity of 34.4, 35 and 71 %, respectively which increases the risk of non-communicable diseases at younger ages.

For the foregoing reasons, in recent years has emphasized the importance of the consumption of food on health. The boom surprising of the functional foods industry suggested that people buy food with added value to the nutrient contribution, since their profits are improving the health status and quality of life, as well as reduce the risk of disease. Currently, two of the most studied topics in food science are, obtaining new ingredients from natural products, that can be used as additives and/or nutraceutical ingredients to develop functional foods as well as the production of these products from underutilized sources, through low-cost technologies and minimal environmental impact.

In addition to the functional potential of Chia seeds, the protein and the mucilage can be used as materials for the preparation of edible films and improve the overall quality of many foods, extend its time of shelf life, improve its mechanical properties and management as well as its nutritional characteristics. The proteins as films materials, are biodegradable, increase the nutritional value and confer properties such as a barrier to moisture and restriction of the transport of O_2 and CO_2; while the mucilage as film material confers barrier properties to water vapor, adequate strength and flexibility in the product obtained as well as mechanical characteristics. Today, the use of multi-component mixtures is an alternative to offset deficiencies inherent in each component and thus complement and enhance the properties of the resulting materials (Segura-Campos et al., 2016).

On the other hand, both by-products can also be used as materials to protect and isolate compounds sensitive to the adverse influence of the chemical environment using microencapsulation. This technology provides a means for packaging, separate and store materials to microscale for then releases under controlled conditions. In vegetable oils with high content of polyunsaturated fatty acids, such as chia, the main contribution of the microencapsulation is its protection against oxidation during storage and/or processing conditions. The process of encapsulation depends on the properties of the final product, the stability of the material microencapsulated during storage, the release mechanism, the interaction with other food components and the cost (Segura-Campos et al., 2016).

In the ionic gelation method, the gelation occurs as a result of the intermolecular crosslinking of polysaccharide chains to form a three dimensional network that traps or inmobilize great quantity of water within a rigid structure resistant to the flow thus increasing its viscosity. This technique allows obtaining particles of a size of approximately 50 µm. The intercrossing arises from polysaccharides with a negative charge (alginates, carrageenan's, agar) and some divalent cation (Ca^{+2}). The calcium ion in the union areas with the polysaccharide rearranges the molecule presenting a kind of egg box which makes it form bridges between two molecules chains in a parallel manner, acquiring rigidity and forming microcrystalline regions that allow gels (Acosta, 2012) (Figure 2a). The general procedure for obtaining this type of capsules is to disperse the material of coverage with the active ingredient and let it pass through an extruder by controlling the dripping of the emulsion side a bathroom of ions. In this way you get particle sizes that depend on the nozzle of the extruder. Well, the ionic gelation is a reproducible method at laboratory level and extensively used due to the fact that you can encapsulate any type of food either hydrophobic, hydrophilic, thermo sensitive, liquid or solid.

The spray-dried method is widely used in the food industry as a method for microencapsulate because it is cost effective in the protection of materials such as flavors, vitamins, essential oils, dyes, and lipids. By definition corresponds to the transformation of a fluid in solid material, cleanly deliver in the form of droplets in a drying medium hot (Re, 1998). The spray-dried is a viable alternative, as the use of encapsulates materials and short drying times allow in the final product, the protection of the susceptible constituents to the adverse effect of the medium that is exposed the product or any of the ingredients of the processed food. In this way, preserving their bioactive properties, avoiding its oxidation up to the time of its release to the environment in contact with the food and prolong the organoleptic properties desirable in the matrix where it is incorporated (Buffo and Reineccius et al., 2000). It should be borne in mind that the parameters that control are the inlet and outlet temperature of drying air, the power flow of the product to dry, the residence time and the packaging of the raw material. The advantages of microencapsulate using the method of spray-dried are the operation simplicity, the particle size of 5 to 5000 µm, the low costs of processing, the good volatile retention, the stability of the final product, the possibility of producing a large-scale, continuous mode as well as a encapsulation efficiency between 96 and 100% compared with other methods (Figure 2b).

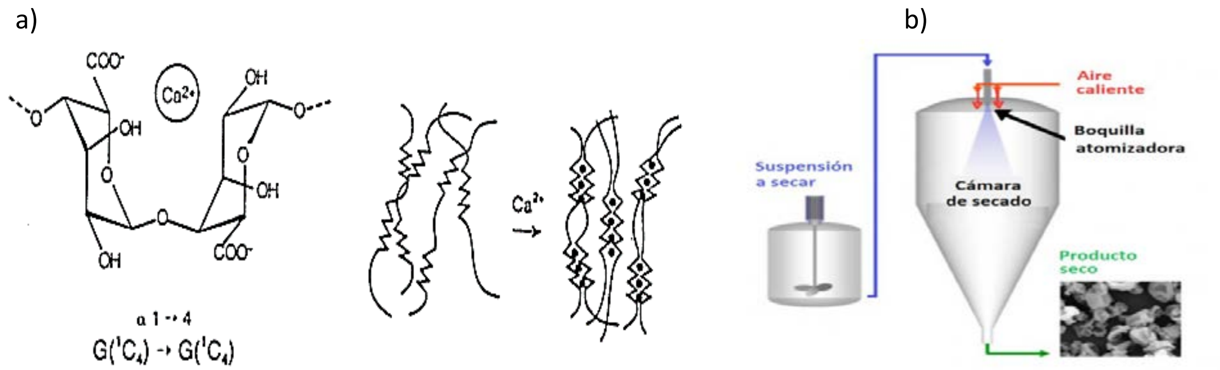

Fig. 2. a) Ionic gelation method, b) spray-dried method

CONCLUSION

The development of biodegradable and edible films with protein and mucilage of chia, are an alternative to improve the quality and properties of the food. On the other hand, these by-products are also an alternative as microcapsules forming agents that together to the nutraceutical potential of their oil once microencapsulated will allow new strategies in functional food. The foregoing will contribute not only to the prevention of no communicable diseases but also to the promotion of the chia cultivation in Mexico through programs such as "family farming" in pro to eradicate the food poverty.

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STUDY OF THE ANTIOXIDANT CAPACITY OF WHITE BREAD ENRICHED WITH CHIA (*Salvia hispanica* L)

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INTRODUCTION

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. In humans, oxidative stress may be associated with various pathologies including cancer, cardiovascular disease, autoimmune diseases, the aging process and diseases associated with it. WHO recommended daily intake of antioxidants through diet to prevent or mitigate diseases associated with cellular oxidative stress. Bread is one of the main components of the human diet so it is highly interesting its fortification with natural components. The use of chia (*Salvia hispanica* L) as an ingredient might be a good strategy in this regard due to its nutritional and functional properties. The incorporation of chia flour and seeds in bread has been evaluated and the positive effect on the technological and sensory values has been shown (Iglesias-Puig and Haros, 2013; Silveira Coelho and Salas-Mellado, 2015). Some scientific papers have reported the presence of antioxidants in chia seeds (Reyes-Caudillo et al*.,* 2008; Martínez-Cruz and Paredes-López, 2014; Porras-Loaiza et al*.*, 2014; Marineli et al*.*, 2014; Amato et al*.*, 2015) and in chia by-products (Capitani et al*.*, 2012; Marineli et al*.*, 2014) but the antioxidant capacity has never been evaluated in chia-based bakery products.

The ultimate goal of the present work is to study the antioxidant potential of breads made in the laboratory by replacing 5, 10 and 20% of conventional wheat flour by chia flour. To date, the phenolic compounds and antioxidant activity of the raw materials (wheat and chia flours) and breads were determined using *in vitro* methods. Moreover, preliminary results about the ability of wheat and chia flours to induce antioxidant response in *Saccharomyces cerevisiae* cells have been obtained. *In vivo* studies are needed as complement of *in vitro* determinations to understand the potential health benefits of plant polyphenols. The yeast *S. cerevisiae* has been widely used to study natural antioxidants because it is easy to handle in the laboratory and cheap, about 30% of known human disease-associated genes have yeast orthologues (Dani et al*.*, 2008) and yeast genes can be easily manipulated. Another advantage in using *S. cerevisiae* is the possibility of studying the effect of ingredients on survival since the yeast constitutes a complete organism. Therefore, *S. cerevisiae* is an interesting tool to evaluate the biological oxidative stress protection in mammalian upon exposure to food rich in natural antioxidants.

MATERIALS AND METHODS

Bread production

The control bread dough formula consisted on wheat flour (450 gr), compressed yeast (2.5% flor basis), sodium salt (1.6% flor basis) and water (261 ml). The whole chia flour was added at 5, 10 and 15 % on flour basis to the bread dough formula. Breads production was performed in a bread-maker Severin 3989. Breads were obtained by duplicate.

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 rotavapor at 35 ºC until methanol elimination.

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.

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 gallic acid equivalents. Experiments were carried out at least in triplicate.

Determination of phenolic compounds

Quantification and identification of phenolics were carried out using a Dionex Summit HPLC system with an Ultrabase C18 2.5 µm column. The binary mobile phase consisted of 0.05% trifluoracetic acid in water (solvent A) and 0.05% trifluoracetic acid in acetonitrile (solvent B).

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

The yeast *S. cerevisiae* was inoculated in liquid YPD medium containing the antioxidant ingredient for 18 h at 28 ºC. 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 minutes with 0.5 mM and 4 mM H_2O_2 and then growth was monitored by reading the $O.D_{600}$ using a 96-well plate spectrophotometer reader (BMG Labtech Omega Spectrostar) for 18 h.

RESULTS AND DISCUSSION

Polyphenol analysis and antioxidant activity

The amount of polyphenols obtained in chia flour was 5.44 ± 0.27 mg GAE / g (Figure. 1A). This result is higher than the values reported by the literature for other chia seeds (Table 1). Specifically 9.2, 8.7, 6, 5.8 and 3.3-fold higher than the concentration obtained by Amato et al. (2015), Porras-Loaiza et al. (2014), Reyes-Caudillo et al. (2008), Marineli et al. (2014) and Martínez-Cruz and Paredes-López (2014), respectively. These differences may occur due to the geographic origin of seeds as pointed by Marineli et al. (2014) and Amato et al. (2015). The methodology used for the extraction of phenolic compounds can also influence the performance of the extraction (Kim et al., 2006).

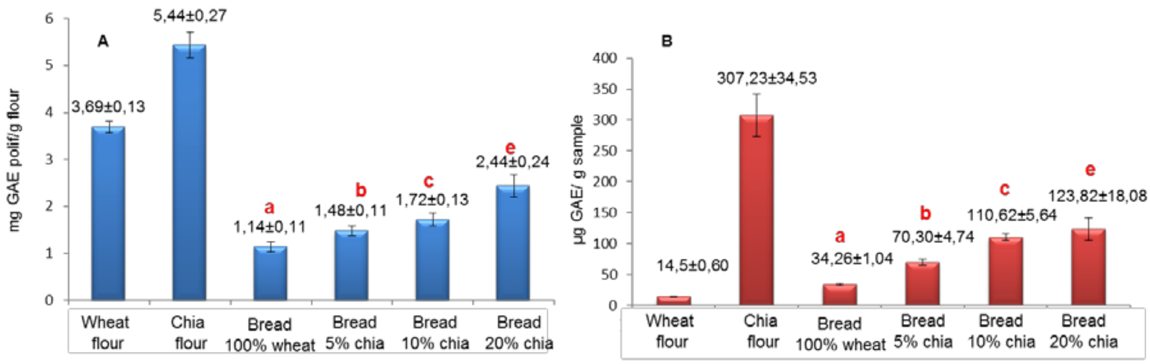

Figure 1. Total phenolic content (A) and antioxidant activity (B) of the extracts of wheat and chia flours and breads (control and 5, 10, 20 % chia). Values with different letters are significantly different from each other (values are mean ± SD, n=3, p≤0,05).

Table 1. Polyphenol content and antioxidant activity obtained in this work for chia seeds in comparison with values reported in others scientific papers.

Total polyphenols	Antioxidant activity		Seed origin	Reference[c]
Quantification [a] (mg GAE/g)	Quantification	Methodology		
0.56	222.05	$ABTS^{+}$ (µm TE/g)	Perú	Amato et al. (2015)
0.68	213.6	$ABTS^{+}$ (µm TE/g)	Australia	Amato et al. (2015)
0.53	275.05	$ABTS^{+}$ (µm TE/g)	Italy	Amato et al. (2015)
1.64	68.8	DPPH (inhibition %)	Mexico[b]	Martínez-Cruz and Paredes-López (2014)
0.53-0.72	ND	--	Mexico[b]	Porras-Loaiza et al. (2014)
0.94	436.61	DPPH (µm TE/g)	Chile	Marineli et al. (2014)
0.88-0.92	87.84-95.7	$ABTS^{+}$ (% scavenging)	Mexico[b]	Reyes-Caudillo et al. (2008)
5.44	307.23	DPPH (µg GAE/g)	Mexico[b]	This work

[a] Determination of total polyphenols using Folin-Ciocalteu.
[b] Different regions from Mexico.
[c] Only they are taken into account literature values concerning the whole seeds.
ND: Non determined

Inter-laboratory antioxidant activity comparisons are difficult because of the different methodologies used in its determination as is shown in Table 1. What is clear is that the extract from chia flour displayed higher antioxidant activity than that found in the wheat flour (Fig. 1B). This behavior was also observed for the amount of total polyphenols (Figure 1A).

The total polyphenol concentration was significantly higher in the case of breads with chia (5% chia: 1.48±0.11; 10% chia: 1.72±0.13; 20% chia: 2.44±0.24) compared to the control bread (1.14±0.11 mg GAE/g of chia flour) and as expected, an increment in the total phenolic content was observed as the percentage of chia increased (Fig. 1A). Likewise, the inclusion of chia flours produced breads with increasing DPPH radical scavenging activity (5% chia: 70.30±4.74; 10% chia: 110.62±5.64; 20% chia: 123.82±18.08) and in all cases significantly higher compared to the control (34.26±1.04 µg GAE/g) (Fig. 1B).

The phenolic acid compounds were identified by HPLC (Fig. 2).. The main peaks observed in cromatograms of chia flour and breads with chia corresponded to rosmarinic acid, known for its antioxidant and preservatives properties, and to an unknown compound. Additionally, ferulic and caffeic acid were detected but in low amounts. These peaks were obtained neither in wheat flour nor in control bread. Increasing peak areas obtained for fortified breads were positively correlated with the percentage addition of chia in the case of major phenolic compounds (data not shown).

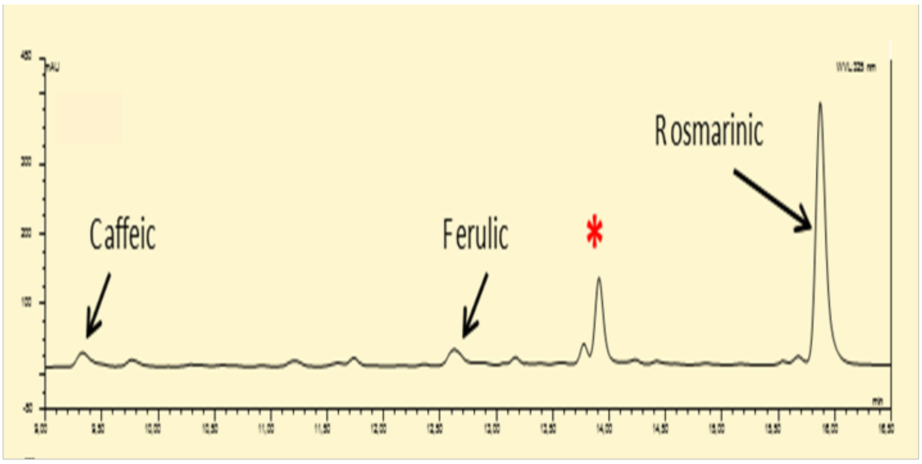

Figure 2. HPLC chromatograms of phenolic compounds in chia flour extract and breads with chia extracts. Interval between 9 and 17 minutes of retention times. (*) Unknown compound.

Antioxidant response in *S. cerevisiae* cells

Two concentrations of oxidant (0.5 and 4 mM of H_2O_2) were used to lead a moderate and a severe oxidative stress conditions respectively (Fig. 3).

Polyphenol extracts from chia and wheat flour were tested at two different doses (chia: 27 mg/L and 54 mg/L, wheat: 19 mg/L and 39 mg/L). A cocoa extract with proved antioxidant activity (Martorell et al., 2011) was used as positive control at two final concentrations in the culture medium (350 mg/L and 700 mg/L). Preliminary results about the ability of wheat and chia flours to induce antioxidant response in the yeast S. *cerevisiae* have been obtained (Fig. 4). The pre-incubation with wheat flour showed a growth recovery for the higher concentration (39 mg/L) at both H_2O_2 concentrations and for the lower concentration (19 mg/L) at 4 mM H_2O_2 (Fig. 4A, B). Therefore, an antioxidant protection seems to be probable in the case of the wheat flour. In the case of chia, only the higher concentration showed a slight protective effect to 4 mM hydrogen peroxide. This result draws attention because the higher protective effect was expected for chia. Due to the fact that the oxidant and pro-oxidant effects depend upon the dose, further studies should be undertaken to test a wider range of concentrations. Moreover, the antioxidant protective effect of breads with chia should be addressed.

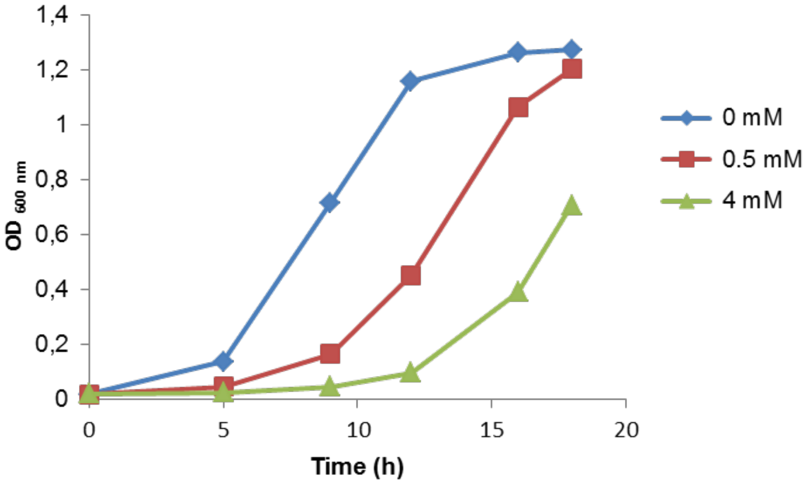

Figure 3. Growth response of wild-type strain BY4741 after 1 h of exposure to two concentrations of hydrogen peroxide (weak oxidative stress: 0.5 mM and severe oxidative stress: 4 mM).

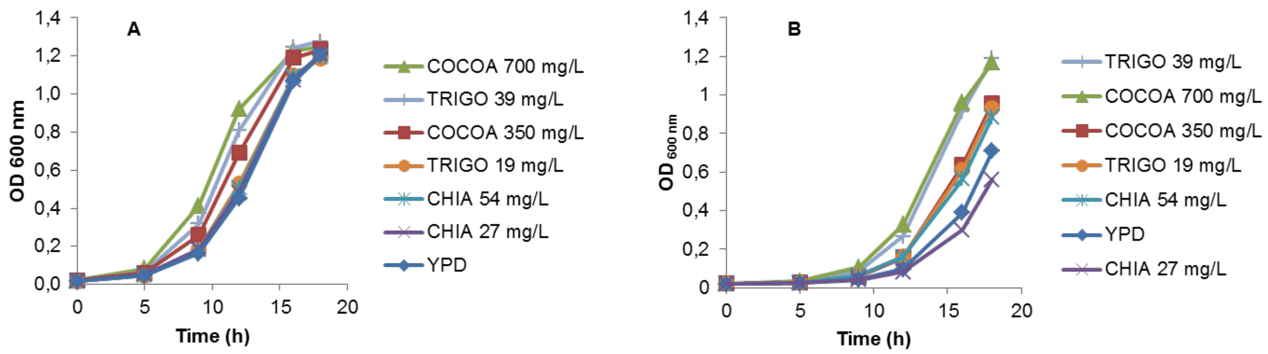

Figure 4. *S. cerevisiae* growth response to oxidative stress (**A**: 0,5 mM H_2O_2, **B**: 4 mM H2O2) after pre-incubation with chia and wheat flour polyphenol extracts. Two concentrations of chia extract (27 and 54 mg/L) and wheat extract (19 and 39 mg/L) were tested. A cocoa extract was used as positive control.

Acknowledgements

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FUNCTIONAL CHARACTERIZATION OF BY-PRODUCTS FROM CHIA (*Salvia hispanica* L.) SEEDS OF ARGENTINA

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INTRODUCTION

Chia (*Salvia hispanica* L.) is an annual herbaceous plant Lamiaceae family, native from southern Mexico and northern Guatemala. Chia seed together with corn, beans, and amaranth, were important crops for pre-Columbian civilizations in America, including the Mayan and Aztec populations. The plant produces numerous small white and dark seeds that mature in autumn. The seed has about 30 g oil/100 g seed weight, and it is mainly composed of unsaturated fatty acids. Chia seeds from Argentina exhibited 30.0-38.6 g oil/100 g,with 60.7-67.8 g/100 g of linolenic.The chia seed is a good source of protein (19-27 g/100 g). The protein content is higher than that of other traditional crops such as wheat, corn, rice, oat, barley and amararanth. Although chia is not commercially grown as a protein source, its amino acid profile has no limiting factors in the adult diet but threonine, lysine and leucine were the limiting aminoacids in a preschool child's diet. The residual meal from the oil-extracting process of chia seeds is a good source of dietary fiber and polyphenolic compounds with antioxidant activity and could be used as a source of important natural antioxidants with commercial applications. The consumption of chia dietary fiber can be an important alternative to improve people's health. The physicochemical and physiological properties of a fibrous fraction obtained from Mexican chia seeds defatted by solvent extraction were evaluated for some researchers suggesting its use as an ingredient in health and diet food products. Dietary fiber (DF) includes cellulose, hemicellulose, lignin, pectins, gums, mucilage and other polysaccharides and oligosaccharides associated with plants. It is resistant to digestion and absorption in the human small intestine, with complete or partial fermentation in the large intestine. Fibers have been classified as soluble (SDF), such as viscous or fermentable fibers which are fermented in the colon, and insoluble fibers (IDF), that have bulking action with limited extent fermentation in the colon. The addition of dietary fiber can affect food texture, playing a role as texturing and stabilizing agent. SDF contributes to the stabilization of food structure (dispersions, emulsions, etc.) through gel formation or thickening of the continuous phase. IDF increases the firmness of the products and provides a high fat absorption capacity. The knowledge about functional properties such as water holding, absorption and adsorption capacity, as well as those linked to the affinity for lipid components, is useful for the food industry, from manufacture to final destination of the product and marketing conditions. Fibers are added to cooked meat products in order to increase the cooking yield due to their water and fat retention properties. On the other hand, in fried food products, the addition of fiber reduces lipid retention and increases moisture content). The properties of DF may be affected by its chemical form and by the conditions of the processes for obtaining food. Thus, the milling process can be modulated to obtain dietary fiber-rich cereal byproducts and also to increase the SDF/IDF ratio. Heat treatments have a strong impact on the chemical composition and physical properties of cereal dietary fiber. Heat treatments could affect the properties of DF in various foods such as apples, oat bran and corn, among others. The effect of extrusion on the DF and phytic acid in cereal brans was investigated observing a decrease in the IDF content during extrusion cooking, and an increase in the amount of SDF. The hydration properties of DF might be modified by grinding, increasing the ability to retain water within the matrix of the fiber. The preparation of the raw materials and the different processes of oil extraction from the seeds (pressing and solvent extraction) could affect differently the cell structure and the residual oil content of the meal. In addition, the milling process can influence the physicochemical, functional and physiological properties. The extraction methods affected significantly the oil yield, and the quality and composition of some minor constituents of chia seed oil. The fractionation of meals by dry sieving is a method that allows to obtain fractions with different chemical compositions, and byproducts rich in fiber, protein and starch. The purpose of this study was to characterize the physicochemical and functional properties of meals and fibrous fractions of chia seeds, and to compare the effect of oil extraction methods (pressing and solvent extraction) and sieving processes on these properties.

MATERIALS AND METHODS

Seeds

Commercial chia seeds used in this study were obtained from Salta (25°south and 65.5°west), Argentina (10 kg). Seeds were packed in hermetic plastic vessels and stored at 5°C until use.

Meals Meals were obtained as by-products of two oil extraction methods:

Solvent extraction

Meal (Ms) was obtained after oil solvent extraction (n-hexane) by thermal cycles at 80 C for 8 h, IUPAC Standard Method (1992), of chia seed previously ground in a laboratory mill (Moulinex, horizontal blade grinder, Buenos Aires Argentina) at 424 mm.

Pressing extraction

Seeds were pressed at 25°C using a Komet screw press (Germany), with a 5-mm restriction dye and a screw speed of 20 rpm. The screw press was first run for 15 min without seed material, but with heating via an electrical resistance-heating ring attached around the press barrel to raise the screw press barrel temperature to the desired temperature (25±2°C). The meal obtained by pressing (Mp) was ground with a laboratory mill (Moulinex, horizontal blade grinder, Buenos Aires, Argentina) at 498 mm. Meals from both extraction methods (Ms and Mp) were homogenized and stored in plastic vessels at 5 C until use.

Fibrous fractions

The fibrous fractions were obtained by dry fractionation of defatted meals (Vázquez-Ovando et al., 2010). The meals (Ms and Mp) were sieved in a Zonitest agitator (Buenos Aires, Argentina), using a 100 ASTM mesh (149 mm) for 20 min. The material retained by the sieve was considered rich in fiber (FRFs and FRFp, respectively). Samples were submitted to granulometry determination in a Zonytest agitator (Buenos Aires, Argentina) (with 10,14, 20, 35, 60, 100, 140, 200 and 325 ASTM meshes) for 60 min. The material retained by each sieve was weighed and the percentage of each fraction calculated. The following fraction representing a different size particle: A (<44 mm), B (44-74 mm), C (74-105 mm), D (105-149 mm), E (149-250 mm), F (250-500 mm), G (500-840 mm), H (840-1410 mm), I (1410-2000 mm), J (>2000 mm).

Proximate composition

AOCS (1998) procedures were used to determine moisture and ash. Oil (IUPAC 1.122, 1992) and nitrogen content (N2) (AOAC, 1997). Protein content was calculated as nitrogen X 6.25. Carbohydrate content was estimated as nitrogen-free extract (NFE) and these were calculated by difference using NFE = 100 – (oil + protein + crude fiber + ash)

Total (TDF), soluble (SDF) and insoluble (IDF) dietary fiber

Total dietary fiber (TDF), soluble dietary fiber (SDF) and insoluble dietary fiber (IDF) were determined according the gravimetric enzymatic method (Prosky et al., 1988).

Minerals

Ca, Mg, Fe, Cu and Zn were measured by flame atomic absorption spectrophotometry. Results were expressed as mg/kg meals or fibrous fractions. Phosphorus content was determined following AOCS method Ca 12e55 (1998).

Functional properties

Water-holding (WHC) and oil-holding capacity (OHC) were determined according to Chau,Cheung, and Wong (1997). Water adsorption capacity (WAdC) was determined by the method of Chen, Piva, and Labuza (1984). Water absorption capacity (WAbC) according to the AACC (1984). Average water absorbed was calculated and the WAbC calculated, and expressed as g water absorbed per g sample. Organic molecule absorption capacity (OMAC). was determined according to the method of Zambrano et al. (2001), expressed as the absorbed hydrophobic component and calculated in terms of sample weight gain (g oil/sample g). Also, Emulsion activity (EA) and stability (ES) were evaluated.

RESULTS AND DISCUSSION

The seed oil extraction process produced 80.8 ± 0.3 (g/100g d.b.) residual meal by solvent (Ms) and 75.2 ± 0.5 (g/100g d.b.) residual meal by pressing (Mp), whereas the sieving procedure yielded 79.9± 0.8 g/100 g and 85.8 ± 0.5g/100 g fibrous fractions (FRFs and FRFp, respectively).

Experimental results showed that the oil extraction methods (solvent and pressing) from chia seeds (*S. hispanica* L.) affect the physicochemical and functional properties of residual meals and their corresponding fibrous fractions. Meals and fibrous fractions of chia showed a high content of total dietary fiber (TDF), consisting mostly of insoluble dietary fiber (IDF). The concentration of TDF and its corresponding components (SDF and IDF) were significantly higher in the chia fiber fractions. The meal obtained by the solvent extraction method (Ms) evidenced significantly higher functional properties (OHC, OMAC, EA and ES) than the meal obtained by pressing extraction (Mp),whereas among the chia fibrous fractions (FRFs and FRFp), all the functional properties tested (WHC, OHC, WAbC, WAdC, OMAC, EA and ES) were statistically significant. Also, the emulsions formulated with by-products of chia obtained by solvent extraction were more stable than those obtained from pressing. These results could be attributed to the lower percentage of residual lipids and the higher protein content present in meals and fibrous fractions obtained by solvent extraction. They could also be explained in terms of the influence of certain experimental parameters that can alter the physical structure of the fiber, leading to important changes in these properties. The physicochemical and functional properties turned chia byproducts into important ingredients for the manufacture of products such as desserts, drinks, breads, jellies, emulsions, cookies, among others.

Table 1. Proximate composition of chia (*Salvia hispanica* L.) meals and fibrous fractions from different extraction methods (g/100g d.b.)

Component	Ms	FRFs	Mp	FRFp
Moisture	10.47 ± 0.16	10.34 ± 0.03	10.84 ± 0.21	10.00 ± 0.16
Protein *	41.36 ± 0.28 ^{c}	35.32 ± 0.17 ^{b}	35.00 ± 0.35 ^{b}	33.74 ± 0.05 ^{a}
Crude fiber	27.57 ± 0.07 ^{b}	32.84 ± 0.34 ^{c}	23.81 ± 0.34 ^{a}	28.35 ± 0.80 ^{b}
Oil	0.21 ± 0.08 ^{a}	0.21 ± 0.05 ^{a}	11.39 ± 0.59 ^{b}	10.85 ± 0.13 ^{b}
Ash	7.24 ± 0.15 ^{c}	6.64 ± 0.03 ^{b}	6.27 ± 0.08 ^{a}	6.04 ± 0.01 ^{a}
NFE	23.62 ± 0.94	24.99 ± 0.56	23.53 ± 0.87	21.02 ± 0.79

Values followed by different letters differ at $p < 0.05$, according to Tukey test (n =3).**Ms**: solvent meal; **FRFs**: solvent fibre-rich fraction; **Mp**: pressing meal; **FRFp**: pressing fibre-rich fraction

Table 2. Mineral content (mg/kg) of chia (*Salvia hispanica* L.) meals and fibrous fractions of both extraction methods (d.b.)

Element	Ms	FRFs	Mp	FRFp
Ca	8060 ± 0.05 ^{d}	6150 ± 0.05 ^{c}	5615 ± 0.05 ^{a}	6110 ± 0.05 ^{b}
Mg	3460 ± 0.03 ^{b}	3220 ± 0.03 ^{a}	4624 ± 0.02 ^{d}	3690 ± 0.02 ^{c}
Fe	117.3 ± 0.001 ^{a}	121.0 ± 0.001 ^{c}	117.7 ± 0.001 ^{b}	142.7 ± 0.001 ^{d}
Zn	100 ± 0.001 ^{d}	96 ± 0.001 ^{b}	99.6 ± 0.001 ^{c}	92.8 ± 0.001 ^{a}
Cu	24 ± 0.002 ^{c}	22.6 ± 0.002 ^{b}	18.7 ± 0.002 ^{a}	24.2 ± 0.02 ^{d}
P	10205 ± 1141 ^{a}	9012.5 ± 697 ^{a}	9988.5 ± 335 ^{a}	12476 ± 1505 ^{a}

Values followed by different letters differ at $p < 0.05$, according to Tukey test. (n =3).**Ms**: solvent meal; **FRFs**: solvent fibre-rich fraction; **Mp**: pressing meal; **FRFp**: pressing fibre-rich fraction

Table 3. Functional properties of chia (*Salvia hispanica* L.) meals and fibrous fractions of both extraction methods

Property	Ms	FRFs	Mp	FRFp
WHC (g/g)	10.64 ± 0.60 [b]	9.19 ± 0.29 [a]	10.58 ± 0.55 [b]	11.88 ± 0.33 [c]
OHC (g/g)	2.03 ± 0.08 [b]	2.06 ± 0.03 [b]	1.26 ± 0,03 [a]	1.40 ± 0.18 [a]
WA_bC (g/g)	6.45 ± 0.41 [a]	10.46 ± 0.87 [b]	6.81± 0.30 [a]	6.13± 0.37 [a]
WA_dC (g/g)	0.37 ± 0.02 [a]	0.51 ± 0.05 [b]	0.31± 0.04 [a]	0.31 ± 0.03 [a]
OMAC (g/g)	1.64 ± 0.02 [b]	1.73 ± 0.05 [c]	0.83± 0.01 [a]	0.82 ± 0.01 [a]
EA (mL/100mL)	56.00 ± 0.77 [d]	53.33 ± 0.00 [c]	51.00 ± 1.15 [b]	44.33 ± 1.15 [a]
ES (mL/100mL)	60.00 ± 0.00 [d]	57.67 ± 1.15 [c]	47.17 ± 1.00 [b]	34.33 ± 1.15 [a]

Values followed by different letters differ at $p < 0.05$, according to Tukey test.(n =3).**Ms**: solvent meal; **FRFs**: solvent fibre-rich fraction; **Mp**: pressing meal; **FRFp**: pressing fibre-rich fraction **WHC**: water-holding capacity; **OHC**: oil-holding capacity; **WA_bC**: water absorption capacity; **WA_dC**: water adsorption capacity; **OMAC**: organic molecule absorption capacity; **AA**: antioxidant activity; **EA**: emulsifying activity and **ES**: emulsion stability. Source Capitani et al. (2012).

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THE INFLUENCE OF CHIA-CONTAINING BREAD ON IRON ABSORPTION AND BIOMARKERS OF IMMUNONUTRITION

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INTRODUCTION

Iron (Fe) deficiency is a worldwide health problem causing detrimental health effects in children and women of reproductive age. Yet effective prevention strategies have barely been developed, urging the need to tackle this global health concern. Prior research demonstrated the important role of endogenous, dietary and environmental factors in Fe uptake influencing the risk of suffering iron deficiency. While the endogenous factors are difficult to influence, the environmental factors are predominant and addressable in a preventive or therapeutic intention. Most research efforts have largely focused on total Fe intake and consumption, but recent data suggest that the composition of the food and its influence on and interaction with inflammatory processes and the host's intestinal immune system, and finally their crosstalk within the gut-liver axis could be even more important determinants of the micronutrient absorption. Our current investigation evaluates the influence of the inclusion of chia (*Salvia hispanica*) in bread formulation on Fe absorption and the secretion of hepatic pro-inflammatory hepcidin peptide as well as the expression of hepatic peroxisome proliferator-activated receptor that together exert a critical control on intestinal Fe absorption and hepatic metabolism.

MATERIALS AND METHODS

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 and Haros, 2013).

Determination of iron and phytates

Total Fe concentration in bread formulation was determined at the Servei Central de Suport a la Investigació Experimental from the Universitat de València (SCSIE, University of Valencia, Spain). Phytates (InsP_6) present in the bread were purified by ion-exchange chromatography and measured as described elsewhere (Sanz-Penella et al., 2012).

Animals

For the experiments there was used a diet-induced iron-deficient animal model [3] 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). A 0.1 g aliquot of the different samples was administered three times per day during two consecutive days. Blood and liver tissue samples were preserved until analysis as previously described (Laparra et al., 2014).

Hematological parameters

Hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin concentration and the number of erythrocytes as well as the globular sedimentation speed were determined according to the International Council for Standardization in Hematology (ICSH) (Alférez et al., 2011).

Quantification of hepcidin

Plasmatic concentrations of bioactive hepcidin peptide secretion were performed according to a previously LC-Ms/Ms adapted method for murine samples (Laparra et al., 2014).

Expression of hepatic biomarker

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

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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.

RESULTS AND DISCUSSION

Total iron content and phytates (InsP_6) concentration in bread formulations and major hematological parameters as well as hepatic expression of the PPARγ are summarized in Table 1. According to the European Food Safety Authority oilseeds such as chia can be used to obtain flours and supplement bread formulations up to a maximum proportion of 5%. The latter appears not to be sufficient to significantly improve the nutritional contribution of the micronutrient. Besides, the inclusion of chia flour increases the molar ratio $InsP_6$/Fe to a value of 2.5, markedly higher than the critical value (>1) established as inhibitory for Fe uptake (Hurrell, 2004). Importantly, animals fed with the chia-containing bread formulation showed a 1.4-fold higher Hb concentration than those fed with the reference white bread. The increased Hb levels are supported by the positive trend increasing (1.2-fold) MCH values in comparison to white bread.

In this scenario, the increased mean plasmatic levels of hepcidin – a key regulator of Fe homeostasis associated to acute phase inflammatory processes within the gut-liver axis (Laparra et al., 2014) – quantified in animals fed with the chia-containing bread formulation cannot be straightforward associated to an inflammatory response. Systemic Fe homeostasis is finely regulated by the liver through synthesis of the peptide hormone hepcidin that is associated to the inflammatory stimuli-mediated inhibition of the expression of proliferator-activated receptor-γ coactivator-1α (PGC1α) (Qian et al., 2016) a transcriptional coactivator of PPARs pathway that participates in the regulation of metabolic, but also immune activation. Notably, animals fed with the chia-containing bread formulation had increased transcripts of PPARγ – a key regulator of energy expenditure associated to exacerbated immune responses if it is found repressed. Thus, suggesting innate immune-mediated alleviating effects of pathophysiological changes of Fe homeostasis dysregulation similar to anemia of inflammation. In the same line, hepatic overexpression of PGC1α has been directly linked to antagonizing effects of the innate immune Toll-like receptor 4 agonist (bacterial lipopolysaccharide)-induced hepcidin secretion inhibiting duodenal Fe absorption (Qian et al., 2013).

Table 1. Nutritional parameters of white bread and chia-containing bread formulation, haematological parameters and hepatic expression of peroxisome proliferator-activated receptor-γ (PPAR γ) in animals fed with the different bread samples.

Parameter		White bread	Chia-containing bread formulation
Nutritional	Iron (μg/g)	0.42 ± 0.03	0.48 ± 0.04
	phytates (InsP_6, μmol/g)	n.d	0.8
	$InsP_6$/Fe	--	2.5
Hematological	Haemoglobin (Hb, g/dL)	12.3 ± 0.2	17.4 ± 2.8*
	Mean corpuscular volumen (MCV) (x10^{-4})	1.69 ± 0.04	1.97 ± 0.04
	Mean corpuscular hemoglobin concentration (MCH) (pg)	25.3 ± 5.9	31.2 ± 5.0
	Hepcidin (bioactive peptide)	21.1 ± 0.6	23.4 ± 2.4
Hepatic	PPAR expression (fold-change in relation to controls)	0.016 ± 0.004	0,048 ± 0.006*

Mean±SD

CONCLUSIONS

These research efforts provide new information concerning the positive beneficial effects derived from the inclusion of chia (*Salvia hispanica*) in bread formulations favoring duodenal Fe absorption. The observed effects highlight an important innate immune-mediated regulation of metabolic processes. Thus, studies in this line are advisable to better understand the nutritional implications of the use of chia in bread formulations given the growing suggestions about it as a healthier alternative to white bread and other fiber-containing bread formulations.

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MUCILAGE FROM CHIA SEED, SOLUBLE FIBER AS NEW FUNCTIONAL INGREDIENT.

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ABSTRACT

Salvia hispanica L., commonly known as chia seed was a traditional food and one of the main crops used by Mayas and Aztecs in the pre-Columbian times. This oilseed contains high proportions of essential fatty acid, natural antioxidants, and significant amount of dietary fiber. In addition, the seed possesses more protein than other grains, is gluten free, and contains other important nutrients such as vitamins and minerals. One of the most interesting and less studied components of the seed is the mucilage; this natural biopolymer has the capacity to absorb 30 times its weight in water forming viscous dispersions even at low concentration. Is presumed that this viscosity may occur inside the stomach, producing an increasing the satiety index when foods containing this mucilage are consumed. In this study an in vitro digestion model was designed to simulate the different stages of the digestion of the mucilage at three different concentrations to evaluate microstructural and rheological changes.

INTRODUCTION

Salvia hispanica L., correspond an oilseed known as chia. This small seed contains high proportions of essential fatty acid α-linolenic [ALA; 18:3(n-3)] associated with some physiological functions; has important concentrations of primary and synergistic natural antioxidants such as chlorogenic and cafffeic acid, myricetin, quercetin and kaemferol and significant amount of dietary fiber (soluble and insoluble) with recognized functional properties (Meester et al., 2008; Muñoz et al., 2013; Taga et al., 1984). In addition, the seed possess more protein than other grains, such as oat, wheat and barley for example, is gluten free, compriseS nontoxic components, and contains other important nutrients such as vitamins and minerals (Ayerza and Coates, 2005). Chia seed can be considered as functional food due to its contribution to human nutrition, prevention of cardiovascular diseases, prevent inflammatory and nervous system disorders, diabetes and can helps to increase satiety index. One of the most interesting components of this seed is its dietary fiber, the seed contains between 30 and 40% per 100 g, equivalent to 100% of the daily recommendation for the adult population. The defatted flour contain 40% of fiber, 5-15% of which is soluble and form parts of the mucilage (Bushway et al., 1981; Reyes-Caudillo et al., 2008). The mucilage is a natural exudate from the seed, obtained immediately after seeds are exposed to water. Instantaneously, small filaments that correspond to mucilage appeared on the surface that began to stretch slowly until they became fully extended reaching its maximum after 2 h of hydration, forming a continuous and transparent capsule surrounding the seed (Muñoz et al., 2012). The aim of this study was extract and characterize the mucilage, to subsequently design an in vitro digestion system to reproduce oral, gastric and intestinal stages of digestion to evaluate microstructural and rheological changes during the process.

MATERIALS AND METHODS

Materials

The mucilage from chia seed was extracted by using the methodology proposed by Muñoz et al. (2012). The extracted mucilage was analyzed by using AOAC methods (AOAC, 1995).

In vitro static digestion

A model system was designed to simulate and display the different stages of the digestion according to Ekmekcioglu (2002). Figure 1 shows the *in vitro* digestion system micro-video controlled.

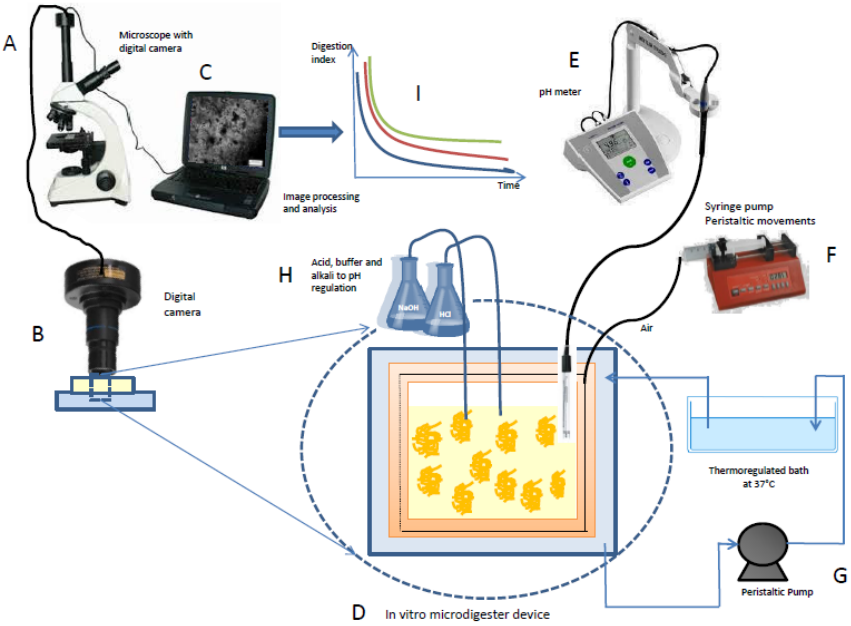

Fig. 1. *In vitro* digestion device. A: microscope connected to camera; B: digital camera; C: computer; D: in vitro system; E:pH Control; F: Syringe pump, produces air pulses simulating peristaltic contractions; G: peristaltic pump to temperature control; H: solutions to regulate pH; I: Digestion indexes.

Three different digestion were tested to simulate the process according to Table 1. The times for each digestion were selected according to previous research done by Hur et al. (2011) related with the type of food to be digested.

Each sample was placed in a glass capsule and located in a digestion chamber (Figure 1). Subsequently artificial saliva, gastric juice and intestinal juice was added. This procedure was repeated according to different digestion times (Table 1). After the different digestions, the samples were refrigerated to subsequent analysis.

Table 1. Digestion times

Digestion	Oral stage (seconds)	Stomach stage (minutes)	Intestinal stage (minutes)
1	20	30	60
2	30	40	90
3	40	60	120

Characterization of *in vitro* stages

The oral stage was performed to mimic of mouth conditions, for this purpose artificial saliva was prepared according to Kong and Singh (2008). To simulate the stomach conditions, gastric juice was prepared according to the methodology proposed by Sanz and Luyten (2006). Finally, to simulate the intestinal conditions, intestinal juice was prepared by using the method suggested by Sanz and Luyten (2006).

Steady shear flow behavior

Viscosities for each stage and sample were measured at 37±1°C (human body temperature) over shear rate range of 0 – 300 s^{-1} using a Hybrid rheometer (Discovery HR-2, TA Instruments, USA) and the software TA Instruments Trios Version: 3.1.0.3538 equipped with a 40 mm 2° cone-plate geometry and using a truncation gap of 50 µm. The rheological model that showed the best fit was selected and K (consistency index) and n (flow behavior index) was estimated.

Microscopy analysis

Microstructural changes were registered during the progression of digestion through acquisition of images by a system composed of a stereomicroscope (Olympus Optical CO, LTD, SZX7, Japan) and a digital camera (Olympus SC30) using the software SC30 for Windows. Then the images were analyzed by the software Image ProPlus 6.0 (Media Cybernetics, Silver Spring, USA).

RESULTS AND DISCUSSION

Mucilage is composed mainly by complex polysaccharides with high molecular weight (Capitani et al., 2013) and has the capacity to absorb 30 times its weight in water (Muñoz et al., 2012). The proximal analysis showed a content of 22.04 % proteins, 30.74 % total fat and 42.2% carbohydrates. In this study the mucilage was extracted by using an aqueous system in proportion 1:40. Then, the aqueous system was dried and the mucilage was separated from seeds. The yield of the extraction was 15.47% from the seed, more than earlier reported (Bushway et al., 1981; Reyes-Caudillo et al., 2008). Subsequently, the dried mucilage was hydrated at different concentrations 0.3; 0.5 and 0.8% and subjected to in vitro digestion.

The micro-digestion model was used to simulate, control and visualize the digestion stages: oral, gastric and intestinal. Three different digestions with three stages of different lengths each one were carried out according with Table 1. The progression of digestion of each sample was record by video microscopy (Figure 2).

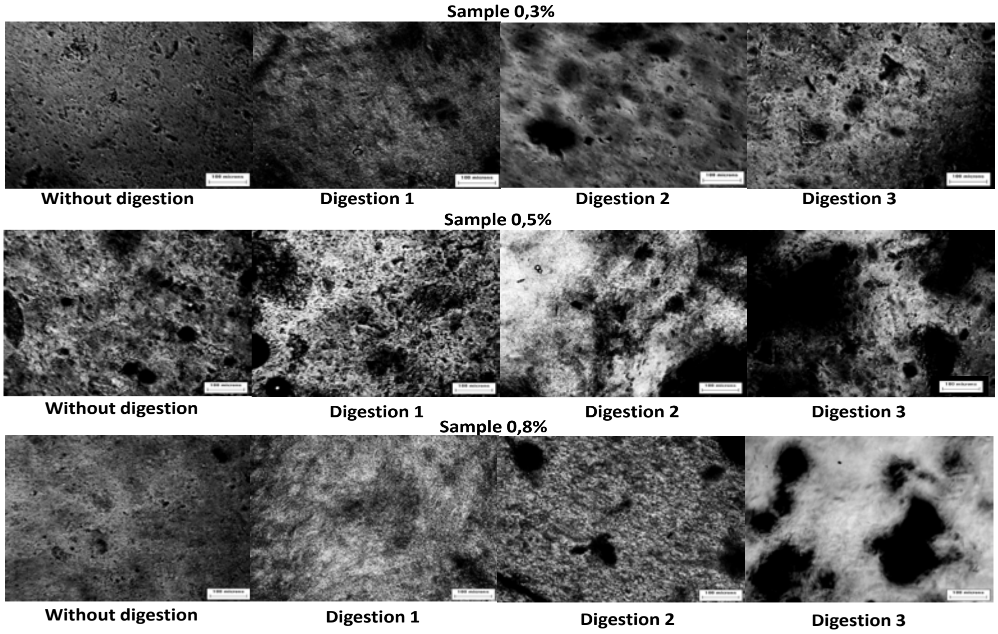

Fig. 2. Images of different digestion

The images show clear differences between the samples with and without digestion. The mucilage at the three concentrations without digestion shows a homogeneous and porous network. In oral stages, no microstructural changes were observed; probably the enzymes and artificial saliva do not have big influence on the structure of mucilage. Although, at the end of gastric stages for the three digestions the porosity starts to increase slightly. The figure 2, the digestion 1, 2 and 3 in any mucilage concentration corresponds at end of intestinal stage. At the end of this stage, porosity and porous size increased, more open network and some empty spaces and bigger porous are observed. This behavior is clear when the concentration of mucilage and length of digestion increased (concentration 0.8 - digestion 3). In the digestions, stages longer have more influence on porosity and aggregates formation. In addition, at the end of intestinal stage at the three concentrations, small white masses and gas bubbles were observed. This phenomenon may be attributed to some molecular aggregation, which is produced by drastic changes of pH, simulated peristaltic movements and the effect of digestive enzymes.

The dynamic viscosity was evaluated in each stage of the three digestions (Figure 3). The mucilage hydrated in water formed a highly viscous solution, even at lower concentrations. No significant changes between oral and gastric stages were observed, even a slight increase in viscosity occurred in the gastric stage. But, at the end of the last stage of digestion the viscosity slightly decrease. Similar behavior was observed in a study done to several hydrocolloids (Fabrek, 2011). According with Valentine et al. (1995), some hydrocolloids with relative high molecular weight, such as mucilage from chia seed, can provide more resistance to structural degradation caused by the changes in pH and enzymatic action.

The dynamic viscosity at the end of the intestinal stage in the three digestion decreases slightly when the digestion time increases. According with Vuksan et al. (2007; 2010) is presumed that these changes in viscosity and molecular aggregation may occur inside the stomach, producing a reduction in postprandial glucose response and increasing the satiety index when foods containing this mucilage are consumed.

Therefore, the mucilage has numerous potential applications and can contribute to regulate and manage some stages in the digestive process.

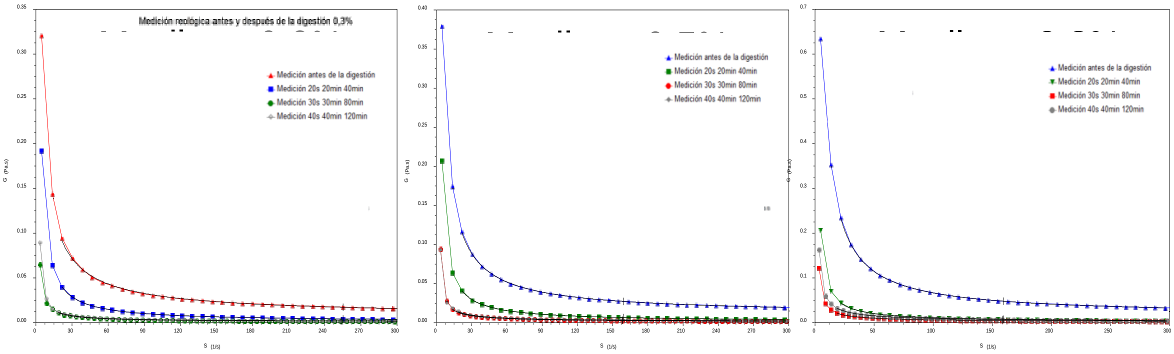

Fig. 3. Dynamic viscosity

CONCLUSION

Chia seed and specifically its dietary fiber has an enormous potential as functional ingredient. The mucilage could be used in different foods contributing and managing the viscosity of them helping to increase the satiety index between other probable health benefits.

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OBTENCIÓN, CARACTERIZACIÓN Y ALTERNATIVAS PARA LA CONSERVACIÓN DEL ACEITE DE CHÍA (*Salvia hispanica* L).

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INTRODUCTION

Chia seeds (*Salvia hispanica* L.) have a long history in the plant-human interaction. In pre-Columbian Mesoamerica the crop species was a major commodity and its seeds were valued for food, medicine and oil (Ayerza, 1995). Today, *S. hispanica* is mostly grown in Argentina, Mexico, Bolivia, Guatemala, Perú, Paraguay and Australia, exhibiting a great potential as a future crop plant (Coates and Ayerza, 1996). Chia seeds contain about 32-39% of oil which presents the greatest α-linolenic acid (C 18:3) content known up today (61-70%). Nowadays, chia seed oil is receiving increased attention, since it can improve human nutrition by providing a natural, plant-based source of ω-3 FA and antioxidants.

One of the main objectives of oil production is the proper selection of the extraction method. The extraction yield and the quality of the oil are very important to determine the feasibility of commercial production.

Liquid-solid extraction, mainly using hexane as solvent, is one of the more traditional processes employed in the production of seed oils. In spite of the high yield obtained by this process, organic solvents such as hexane pose safety risks and health and environmental hazards and its replacement is being sought by the oil industry.

In recent years, there is increased interest in the production of oils by cold-pressing technologies. For obtaining nontraditional vegetable oils this process provides an easy way to get oil from small seed lots (Wiesenborn et al., 2001; Zheng et al., 2003). Although oil yields obtained by pressing are lower than those achieved using solid-liquid extraction, this technology is suitable for materials with high oil content, requires less expensive equipment and involves safer operation and lower risk for the environment.

Nowadays, the extraction of vegetable oils with solvents under supercritical conditions has been proposed as an alternative to replace conventional process (pressing, solvent extraction). This process ensures the absence of traces of solvent in the extracted oil, and allows more efficiently preserving its chemical and organoleptic properties (Norulaini et al., 2009). CO_2 is the most commonly supercritical fluid used for the extraction of food because it has a number of advantages: inexpensive, non-toxic, non-flammable, easily removed from extracts, high interpenetration in solid matrices. In processing terms, carbon dioxide has a low critical temperature and pressure (31.1ºC and 73.8 atm, respectively), which make it the ideal solvent for natural products, since they do not suffer thermal degradation reactions during the process (Follegatti-Romero et al., 2009).

By other hand, protection from lipid oxidation is a critical factor in oil quality. Even though the fatty acid (FA) profile of chia seed oil is nutritionally favorable, it may result in poor oxidative stability. For this reason, the use of natural antioxidants is very important in order to extend the shelf life of this oil.

In this work, the oil yield and the physicochemical characteristics of chia seed oils obtained by hexane, pressing and CO_2 supercritical extraction (CO_2-SE) were examined. Also, the oxidative stability of chia oil was evaluated measuring the effectiveness of the addition of rosemary (ROS) and green tea (GT) extracts, tocopherols (TOC), ascorbyl palmitate (AP) and their blends, and studying the influence of storage conditions.

MATERIAL AND METHODS

Seeds

Commercial chia seeds were purchased from Argentina and Guatemala. They were manually cleaned, homogenized and packed in hermetic plastic vessels and stored at 5ºC until further use.

Liquid-solid extraction (solvent extraction)

The extraction was made from seed samples previously grinded using *n*-hexane in a Soxhlet apparatus by thermal cycles at 80ºC for 8 h, following the IUPAC Standard Method (IUPAC, 1992). The solvent was removed using a rotary vacuum evaporator at 40ºC (Büchi, Flawil, Switzerland), under nitrogen stream.

Pressing

The extraction was carried out in one step at 25-30 °C using a pilot scale Komet screw press (Model CA 59 G, IBG Monforts, Mönchengladbach, Germany). The restriction dye and the screw speed were 5-mm and 20 rpm, respectively.

Supercritical CO_2 extraction (CO_2-SE)

The extraction was carried out on a pilot plant system (extractor volume: 1.5 L) with a single step separation and solvent recycle capacity. Extraction experiments were done at two pressures (250 and 450 bar) and temperatures (40 and 60 °C) with a CO_2 mass flow rate of 8 kg/h. The kinetic curves of extraction for each operative condition were obtained.

Storage of oils

Oils obtained by the different processes were stored in dark vessels with a nitrogen atmosphere at 4ºC until their use.

Oil analytical determinations

The FA composition was determined as methyl esters by GC analysis (Christie, 2003). while triacylglycerol composition were determined by HPLC/APCI-MS.

Iodine and saponified values, refractive index, unsaponifiable matter and free fatty acid contents were determined according to AOCS recommended practices (AOCS, 1998). Total phosphorus content was measured by the method IRAM 5597 (IRAM, 1970).

Fe and Cu contents in chia seed oils were measured by flame atomic absorption spectrometry using a GBC 902 AA spectrometer.

Oil tocopherol content was determined by normal phase HPLC following the procedures described in IUPAC 2.432 (IUPAC, 1992) and AOCS Ce8-89 (AOCS, 1998).

Total polyphenol content was analyzed by HPLC/APCI-MS according to Ixtaina et al. (2011).

Effect of antioxidants and storage conditions on the oxidative stability of chia seed oil Rancimat Analysis

Rosemary (ROS) and green tea (GT) extracts, tocopherols (TOC), ascorbyl palmitate (AP) and blends containing rosemary extract:tocopherols (1:1) (ROS+TOC), rosemary extract:green tea extract (1:1) (ROS+GT) and green tea extract:tocopherols (1:1) (GT+TOC) were added separately to chia oil aliquots at concentrations of 250, 500, 1000, 1500, 2500 and 5000 ppm of commercial products.

Oil oxidative stability was evaluated by the Rancimat Mod 679 (Metrohm AG, Herisau, Switzerland) method, using 5 g of oil sample warmed at 98 ºC with an air flow of 20 L/h.

Storage experiments

Storage experiments of each pure chia seed oil, and chia seed oils with different antioxidants were carried out at two temperature levels usually used by consumers: room temperature (T=20±2ºC) and cooling (T=4±1ºC).

Primary oxidation products were determined by peroxide value (PV) expressed as milliequivalents of peroxides per kilogram of oil (meq peroxide/kg). Formation of secondary oxidation products was measured by *p*-Anisidine values (*p*-AV) [14]. Totox values were calculated from the PV and *p*-AV values of the samples using the Totox value equation= 2PV + *p*-AV (. Also free fatty acid contents were determined according to AOCS recommended practice.

RESULTS AND DISCUSSION

The highest oil yield was 0.34 g/g seed (d.b.) by solvent extraction (hexane). It was also possible to achieve similar values by adjusting the operating conditions (pressure, temperature and time of extraction) of the SC-CO_2 process. However, the oil yield reached by pressing was about 30% lower than those obtained by solvent (hexane) and CO_2-SE.

FA profiles are presented in Table 1. The variability observed in FA composition was within the normal range found in chia seed oil (Ayerza, 1995; AOCS, 1998; Ixtaina et al., 2010; 2011). α- linolenic acid was the main FA in chia seed oils, ranging from 64.5 to 65.6%. Linoleic acid was the second most prevalent FA (19.7-20.3%), followed by palmitic (6.2-6.7%) and oleic (5.0-5.5%) acids. The concentration of stearic acid was the lowest. The obtained data suggest the potential value-added use of these seed oils as dietary sources of essential fatty acids. The ω-6/ ω-3 ratio of chia seed oils was about 0.3, being this value markedly lower than that of most vegetable oils, e.g. canola oil (2.2), olive oil (7.7), soybean oil (6.7) and walnut oil (5.0) (Belitz and Grosch, 1999). Therefore, the incorporation of chia seed oil into the diet would be very beneficial for human health.

Table 1. Fatty acid composition (% of total FAs) determined by GC of chia seed oil obtaining by different processes

Extraction process		**Fatty acid**				
		Palmitic	**Stearic**	**Oleic**	**Linoleic**	**α-linolenic**
Solvent extraction		6.2	3.0	5.3	19.7	65.6
Pressing		6.6	3.1	5.4	20.3	64.5
	40ºC - 250 bar	6.6	2.7	5.2	20.0	65.5
CO_2-SE	**60ºC - 250 bar**	6.6	2.8	5.5	20.2	64.9
	40ºC - 450 bar	6.7	3.0	5.2	20.1	64.9
	60ºC - 450 bar	6.7	3.0	5.0	20.3	65.0

Mean values (n = 3). CO_2-SE, supercritical extraction

The main triacylglycerols found in chia seed oil were: αLnαLnαLn > αLnαLnL > αLnLL > αLnαLnP > αLnLO ~ αLnLP, which represent about 87-95% of the total content of these compounds.

The average FFA values ranged between 0.70 and 2.05 mg KOH/g oil, being higher in solvent than in pressure system.

Regarding genuineness indices, iodine values (208.5-215.0) were somewhat higher than those published elsewhere, whereas saponified values (193.0-193.1) were similar to those previously reported (AOCS, 1998; Velasco Varga et al., 2004). Unsaponifiable matter content was 0.68 - 1.27%, including this range the data published (1.2%) by AOCS (AOCS, 1998). No significant differences ($p > 0.05$) were found between both extraction systems for these physicochemical indices.

Refractive index ranged from 1.4763 to 1.4798, recording significant differences ($p \leq 0.05$) between the oils obtained by both extraction systems.

Trace of metals, particularly copper and iron ions, are known to be effective prooxidants in lipid oxidation, so they are undesirable in oils. Both metal contents in chia seed oils were lower than the maximum level accepted for virgin vegetable oils (Codex Alimentarius Commission, 1999),

The total amount of tocopherol showed a wide variation depending on the extraction process. Oils extracted by CO_2-SE showed low amount of these compounds. The total level of polyphenolic compounds was in the range of 5.30 10^{-5}-1.4x10^{-4} mol/kg.

The accelerated stability test using Rancimat showed that chia oils have a low oxidative stability. The induction times ranged from 1.12 to 2.75 h, being the lowest values, those of oils extracted by CO_2-SE. In spite of the presence of antioxidant compounds, the high content of PUFAs makes chia seed oil very instable. For this reason, the addition of natural antioxidants, such as green tea and rosemary extract, ascrobyl palmitate and tocopherols, and the storage conditions have been studied (Ixtaina et al., 2012).

The effectiveness of these natural antioxidants to improve the oxidative stability of chia seed oil was analyzed by using an accelerated test (Rancimat). Results showed that the addition of the different antioxidants increased the induction time of chia seed oil. This increase was related to the type and concentration of the antioxidant and their blends. The best effects were recorded in chia seed oil with the addition of ascorbyl palmitate (2500, 5000 ppm), rosemary extract (5000 ppm) and its blend (1:1) with green tea extract (2500, 5000 ppm) (Figure 1).

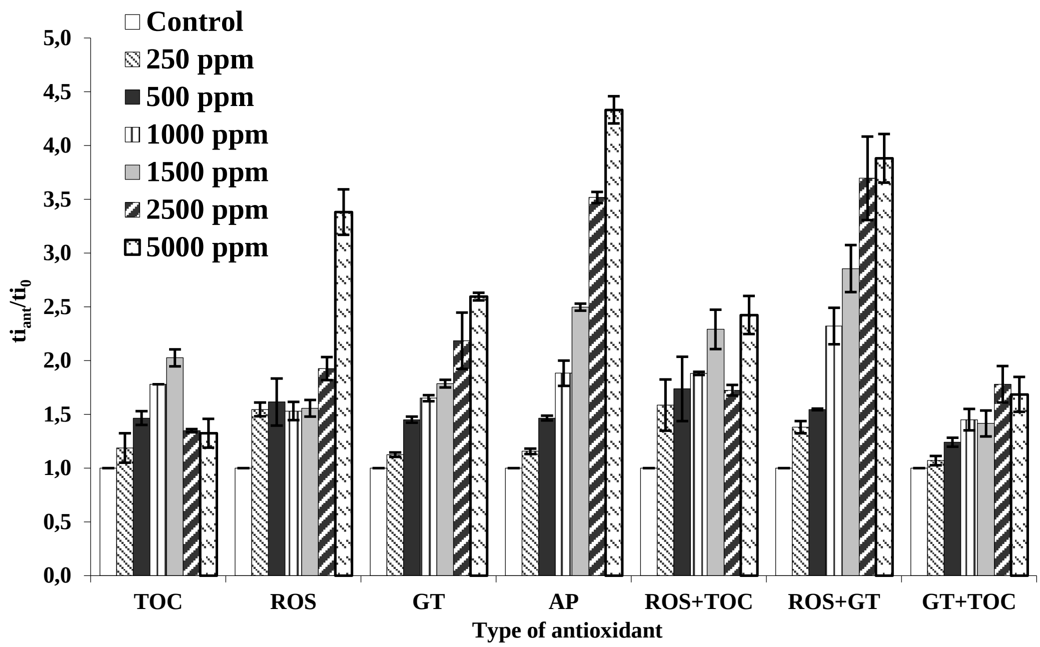

Fig. 1. Antioxidant effects on chia seed oil measured by Rancimat, under 98 ºC temperature and 20 L/h. Vertical bars indicate standard deviation

Storage of chia seed oil allowed evaluation of the influence of different treatments assayed on its adequate conservation. Temperature had a strong influence on oil oxidation. PV of oils stored at 4±1ºC with and without the addition of antioxidants recorded lower PV than the legal limit of 10 meq peroxide/kg oil, indicating that relatively low oxidation occurred at low temperature. In contrast, most samples stored at 20±2ºC achieved the legal limit between 60-120 days, except the oil with the addition of ascorbyl palmitate, whose PV remained relatively steady during the experiment.

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CHEMICAL COMPOSITION AND FATTY ACID PROFILE OF CHIA SEED (*Salvia hispanica* L.) GROWN IN DIFFERENT AREAS OF MEXICO AND ITS POTENTIAL TO PROMOTE A HEALTHY HUMAN NUTRITION

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INTRODUCTION

Chia (*Salvia hispanica* L.) belonging to the Lamiaceae family, is an annual herb 1-5 m tall, with branching quadrangular stems, with short and white pubescence. Leaves are 8 to 10 cm and 4 to 6 cm wide, they are opposed with serrated edges and bright green. With hermaphrodite flowers in violet-blue or white tone, stalked and gathered in groups of six or more, in whorls on the rachis of the inflorescence. Fruit is typically a schizocarp consistent in indehiscent cores, which separate to form four partial mericarps (nutlets), commonly known as "seeds", being oval, smooth and shiny, gray-brown with irregular spots, mostly brown and some white 1.5 to 2.0 mm length (Ayerza and Coates, 2005; Ivana, 2013).

Documented evidence shows that chia being native to mountainous areas of Mexico. Its origin though is likely to be spread to other Mesoamerican countries like El Salvador and Guatemala. Since 3500 BC, the prehispanic population used it as food, over time, it was cultivated and used as raw material for drug and paints production. Aztecs and Mayans had it as one of the main components of their diet, along with amaranth, corn, and beans (Ayerza and Coates, 2005; Muñoz et al., 2013).

The Spanish invasion suppressed traditions and customs of Aztecs and Mayans, so chia and amaranth almost disappeared from their diet since they were banned. However, few ethnic groups, located at inaccessible mountainous areas, retained some customs.

Chia interest resurfaced during the last century since it is considered a good source of dietary fiber, protein, antioxidants and bioactive peptides and lipids (Taga et al., 1984; Ayerza, 2011; Vázquez et al., 2013). In recent years, chia seeds have become very important due to their high content of alpha-linolenic acid (68%) and their relationship to human health and nutrition (Nazim et al., 2012; Porras et al., 2014). It has also been associated with beneficial properties in cardiovascular disease, but its effect has not been demonstrated (De Souza et al., 2015). The aim of this study was to determine the chemical composition and fatty acid profile in chia seeds grown in various areas of Mexico.

MATERIALS AND METHODS

Five lots of chia seeds (Figure 1) of one kilogram each were purchased in vegetable and fruit markets at the states of Jalisco (lots 1 and 2), Morelos (lot 3), Puebla (lot 4) and Mexico City (lot 5).

Fig. 1. Chia seeds (*Salvia hispanic*a L.) from Jalisco, Morelos, Puebla and Mexico City.

Chemical analyzes performed were: moisture and dry matter, crude ether extract, crude fiber, acid detergent fiber, neutral detergent fiber, hemicellulose, cellulose, protein and ash by methods of the AOAC (1995), lignin (Van Soest, 1963; AOAC, 1995) and nitrogen free extract (Weende method).

Oil extraction from chia seeds was performed by soxhlet method with organic solvent drag. The oil obtained was stored at -18˚C in sealed dark glass vials under nitrogen atmosphere until gas chromatography.

The fatty acid profile of chia seed oil was determined through their methyl esters, in order to do that samples were prepared by transesterification with potassium hydroxide in methanol (2N KOH in methanol), as described in the international standard ISO-IDF (ISO, 2002). Esterified fatty acids were analyzed on a gas chromatograph (Agilent 6890N model) equipped with a flame ionization detector. Fatty acids were separated using a fused silica capillary column CP-Sil 88 (100 m × 0.25 mm internal diameter × 0.2 microns film thickness). Injection volume was 0.5 µL and each determination was performed in duplicate. The identification and quantification of fatty acids from chia oil were performed by the standard external method using a standard mixture of 37 fatty acids in their methyl esters form from SUPELCO (Supelco™ 37 Component FAME Mix, Catalog No. 47885-U). Retention times and peak areas from chia oil were compared with standard fatty acid.

Data exploration was performed to evaluate atypical cases. Descriptive statistics and analysis of variance one-way followed by Tukey's test for means equality. We used the statistical package SPSS® release 20.0 for Windows.

RESULTS AND DISCUSSION

Chemical composition

In Table 1 results of chemical analysis of chia seeds are presented. The analysis of variance followed by Tukey´s test showed significant difference ($p<0.05$) only in the protein content of the samples.

Table 1. Composition of chia seed (*Salvia hispanica* L.) from different areas of Mexico (g/100 g).

	Chia samples				
	Lot 1	Lot 2	Lot 3	Lot 4	Lot 5
Moisture	5.65[a]	5.88[a]	5.47[a]	5.85[a]	5.80[a]
Protein (N x 5.7)	19.89[a]	17.58[bc]	17.24[c]	19.31[ab]	19.36[ab]
Fat	27.90[a]	29.21[a]	27.99[a]	27.55[a]	32.63[a]
Fiber	18.94[a]	17.97[a]	20.22[a]	18.68[a]	18.52[a]
Ash	4.10[a]	3.34[a]	3.99[a]	3.60[a]	3.66[a]
NFE	29.17[a]	31.89[a]	30.55[a]	30.86[a]	25.83[a]

NFE: Nitrogen free extract. Means with different letter in a row are statistically different ($p < 0.05$).

The moisture was maintained in a range of 5.5-5.9%, below those 6.8% reported by Segura et al. (2014). Compared to other oilseeds such as cotton, canola or flax (Yúfera, 1982), chia seeds have a lower moisture content, which favors its handling during storage and prevents the growth of fungal species as *Aspergillus flavus.*

Protein values found in the samples ranged between 17.2 and 19.9% lower than reported by Ayerza (1996) and Segura et al. (2014), which was between 19-24%, respectively. Chia seeds can consider them as an important source of protein when compared with other grains such as wheat (14%), corn (12%) and rice (8.5%) (Beltran and Romero, 2003).

The fat content was between 27.6 to 32.6%, with samples of lots 1, 3 and 4 being slightly below reported by Ayerza (2002), Bautista et al. (2007) and Segura et al. (2014) (29 to 35%). Compared to other oilseeds such as soybeans (18%) and sunflower (24%), the studied chia seeds showed higher fat content.

Crude fiber showed no significant differences ($p < 0.05$) between samples, but was less than 34.5% found by Segura et al. (2014) and 24.9% by Bautista et al., (2007). Chia seeds have higher fiber content (17.9-20.2%) than sunflower seeds (7.7%), sesame (6.3%), cereals such as maize (12.2%) and oilseeds such as soybeans (12%), which the it makes an important source for human consumption (Beltran et al., 2008).

Currently there are discrepancies about the veracity of Weende method for the determination of crude fiber in foods. This technique measures a crude fiber cell wall portion of food that survives acid and alkaline digestion, where a portion of cellulose and lignin is recovered, but has the disadvantage that it does not determine the hemicellulose content. To complete and properly define the content of the various components of the fiber we proceeded to determine the neutral detergent fiber (NDF) which measures the fraction of fully indigestible food, acid detergent fiber (ADF) which determine a portion of the cell walls and it includes cellulose, lignin and, other components (Table 2) (Colombatto, 2003; Segura et al., 2007).

Significant differences ($p < 0.05$) in NDF content and the hemicellulose but not FDA percentages, cellulose and lignin were found. NDF rates ranged from 45.9 to 57.7, with the sample from Jalisco lot 1 the least NDF presented and which had the highest value was shown in Mexico City (lot 5).

Also in the case of hemicellulose the sample of lot 5 had the highest percentage (22.3), while the lowest value was for lot 2 (13.3 %). Some percentages reported found in different chia seeds, are: oatmeal (ADF: 38.7%; NDF: 49.4%), wheat (ADF: 37.5%; NDF: 48.4%) and barley (ADF: 35 5%, NDF: 47.8%) (Elizalde and Menéndez, 2004).

Table 2. Fiber fractions in chia seeds (*Salvia hispanica* L.).

	Chia samples				
	Lot 1	Lot 2	Lot 3	Lot 4	Lot 5
NDF (%)	45.91[b]	46.47[b]	47.05[b]	47.23[b]	57.67[a]
ADF (%)	36.2[a]	33.20[a]	34.55[a]	33.47[a]	35.33[a]
Hemicellulose (%)	9.70[c]	13.28[bc]	15.82[b]	13.76[bc]	22.35[a]
Cellulose (%)	20.63[a]	18.66[a]	20.41[a]	20.04[a]	20.44[a]
Lignin (%)	15.80[a]	16.64[a]	16.31[a]	16.28[a]	17.73[a]

Means with different letter in a row are statistically different ($p<0.05$).

NDF: Neutral detergent fiber; ADF: Acid detergent fiber.

In the chemical composition of the five lot samples studied of chia seeds, despite coming from different areas of Mexico and likely conditions of cultivation, harvesting and storage different only significant differences in the content of protein, neutral detergent fiber and hemicellulose were found.

Fatty acid content

Oils derived from chia seeds grown in different areas of Mexico were characterized to obtain a similar profile of fatty acids (FA) (Figure 2). We had nine registered fatty acids in each sample of chia oil, identifying as palmitic acid (C16:0), palmitoleic (C16:1), stearic (C18:0), oleic cis-9 (C18:1 c9), vacenic cis-11 (C18:1 c11), linoleic (C18:2 c9c12), arachidic (C20:0), gamma-linolenic (C18:3 c6c9c12) and alpha-linolenic (C18:3 c9c12c15) acids. The absence of FA short and medium hydrocarbon chains was showed.

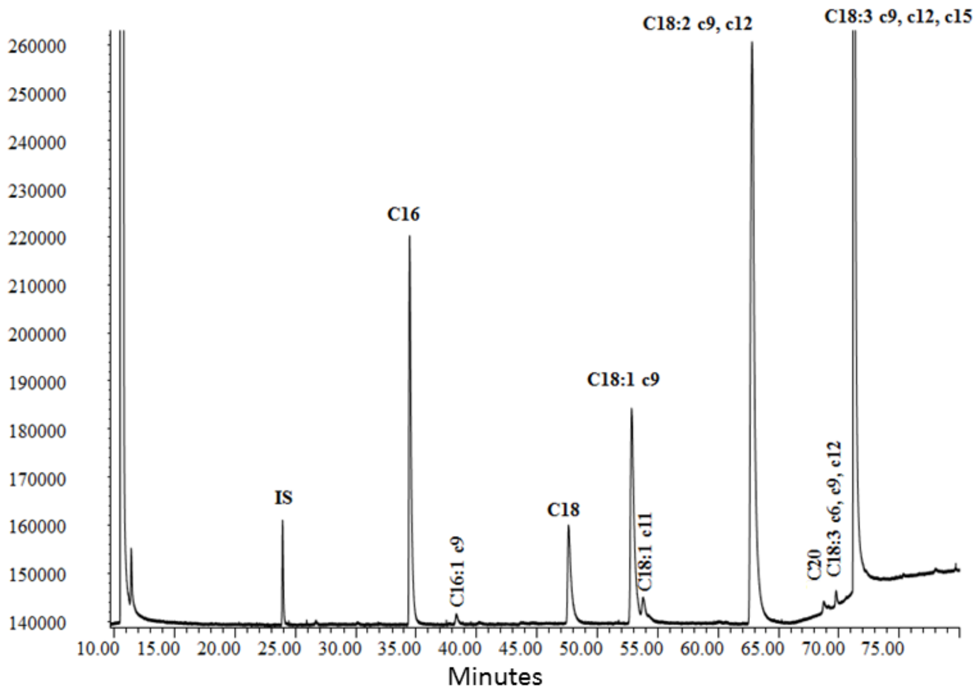

Fig. 2. Chromatographic profile for the fatty acids found in chia seed (*Salvia hispanic*a L.) oil.

Content values for FA in the oils extracted from the five chia seeds lots tested are reported in Table 3. No significant differences ($p \geq 0.05$) were identified among the values distribution of FA from the five lots. However, lot 4 showed the highest values for the FA with the higher relative content of C18:0 (3.7%), C18:1 c9 (7.6%) and C18:2 c9c12 (18.5%); lot 2 was highest in C16:0 (7.7%) and the lot 5 was the highest in the alpha-linolenic acid (62.5%). While the lowest levels were obtained in lot 1 for C16:0 AG (6.8%) and C18:0 (3.2%); lot 5 in the C18:1 c9 (6.8%); lot 2 in C18:2 c9c12 (16.7%); and lot 4 in alpha-linolenic (60.9%). Lot 3 kept showing concentrations of FA between the values of the remaining lots.

Other studies have identified different FA profiles in chia seed oil. For instance, research from the United States, Italy, Argentina, Canada and Cuba have reported between 5 and 16 FA, with different concentrations (Taga et al., 1984; Peiretti et al., 2009; Bueno et al., 2010; Nazim et al., 2012; Vicente et al., 2013; Porras et al., 2014). Nevertheless, the most important FA for presenting high content are the long chain FA as palmitic (C16:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2) and alpha-linolenic (alpha C18:3). Other FA usually show trace amounts.

Table 3. Content of fatty acids (g FA/100 g fat) in five lots of chia seed oil (*Salvia hispanic* L.).

	Chia samples				
	Lot 1	Lot 2	Lot 3	Lot 4	Lot 5
C16:0	6.84	7.32	7.01	7.31	7.32
C16:1 c9	0.24	0.29	0.28	0.27	0.29
C18:0	3.18	3.66	3.34	3.69	3.30
C18:1 c9	7.37	6.86	6.97	7.61	6.82
C18:1 c11	0.691	0.89	0.85	0.88	0.89
C18:2 c9c12 (n-6)	17.61	16.68	17.59	18.50	18.02
C20:0	0.18	0.18	0.16	0.16	0.17
C18:3 c6c9c12 (n-6)	0.22	0.24	0.25	0.23	0.24
C18:3 c9c12 c15 (n-3)	63.28	63.43	63.22	60.89	62.51

The Table 4 shows the mean values and standard deviations for FA contents in the analyzed chia oils from the five lots studied. Please note the sum of saturated FA was 10.76% and the remaining 88.82% corresponded to the sum of unsaturated FA. Omega 3 and omega 6 FA levels were 62.67, 17.92% respectively, which could give added value to these oils, as it has been widely reported to be essential for human consumption and to have potential effectiveness in reducing cardiovascular disease markers.

Contents variability of FA in the oil samples studied, which is likely due to their origin, especially since researchers have shown the effect of other factors such as soil quality and climatic conditions on the chemical composition of chia oil. Generally, it is documented that land elevation is associated with decreased temperature, and the strong relationship between the increasing seasonal temperature, and the number of basic metabolic and physiological processes of plants. This phenomena has been confirmed on plants oils such as corn, jojoba and chia, reports indicate, that, an increase in temperature relates to a decrease in oil content while composition of saturated FA increase, and unsaturated FA decrease (Ayerza, 2011).

Table 4. Fatty acid content (g FA/100 g fat) in five lots of chia seed oil (*Salvia hispanica* L.).

	Mean	SD*	RSD**
C16:0	7.16	0.225	3.137
C16:1 c9	0.27	0.021	7.904
C18:0	3.43	0.229	6.666
C18:1 c9	7.12	0.345	4.846
C18:1 c11	0.84	0.087	10.309
C18:2 c9c12 (n-6)	17.68	0.673	3.807
C20:0	0.17	0.012	7.208
C18:3 c6c9c12 (n-6)	0.24	0.010	4.380
C18:3 c9c12c15 (n-3)	62.67	1.053	1.680
Saturated (SFA)	10.76	0.161	5,67
Unsaturated (UFA)	88.82	0.133	5,71
Omega-6	17.92	0.128	3,90
Omega-6/Omega-3 ratio	0.29	1.417	2,33

*Standard deviation; ** Relative standard deviation

Scientific literature has boarded repeatedly the benefits of unsaturated FA for human nutrition; special attention has been given to the group of the omega 3 and 6 FA (linolenic and linoleic acids), which are important constituents of the cell membranes structure, have metabolic and reserve energy functions, and form the basic structure of some hormones and bile salts. These unsaturated FA have been called essential since they cannot be synthesized from precursor molecules in the human body (Valenzuela et al., 2003). Omega 3 FA may have effect on numerous factors involved in the development of atherosclerosis, which initially could influence a slower progression of the disease. It has been reported that omega 3 FA reduce concentration of chemoattractants, growth factors, and adhesion molecules products, which may favor a reduction in leukocyte migration and vascular smooth muscle cells on the inner vessel wall slowing the atherosclerotic process (López-Farré et al., 2006). Three main mechanisms seem to be involved in the cardiovascular protective effect of omega 3 FA: their anti-inflammatory and antithrombotic effects; together with antiarrhythmic action; and the increase of bleeding time preventing adhesion of platelets in the arteries. They prevent atherosclerosis by lowering cholesterol levels in plasma, they are useful in hypertensive patients, helping to lower blood pressure and reduce triacylglycerols (TAG) concentration in plasma, decreasing total cholesterol and VLDL-C (Very Low-Density Lipoprotein Cholesterol) (Simopoulos, 1999; Castro-González, 2002; Benatti et al., 2004; López-Farré et al., 2006). Previously exposed aspects provide a window of opportunity to boost use of chia for human food; considering life stage, physiological or pathological condition of individuals the estimated dietary energy requirement has been calculated 1 and 4% of omega 3 and omega 6 FA, respectively. In this paper, the average value of the omega-6/Omega-3 ratio was 0.3, comparable to that reported by other researchers (Taga et al., 1984; Pieretti and Gai, 2009; Bueno et al., 2010; Ayerza, 2011; Nazim et al., 2012; Vicente et al., 2013). It is evident that western diets are deficient in omega-3 and excessive in omega-6, and therefore balancing of this ratio would confer numerous health benefits. Given its nourishing properties, there is a great chance, to introduce and promote the use of chia seeds or chia oil for a healthy human nutrition.

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BIOLOGICAL PROPERTIES OF CHIA (*Salvia hispanica* L.) PROTEINS

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CHARACTERISTICS OF CHIA PROTEINS

The structural and functional differences in the proteins are the result of the sequence in which the amino acids are connected, their size and type and the size of the peptide chain. Therefore, in theory, this is a limitless number of proteins with unique properties (Rodrigues et al., 2012).

The amino acid composition of defatted flour showed that chia seeds are a good source of sulfur, aspartic, and glutamic amino acids (Table 1, Sandoval-Oliveros and Paredes-Lopez, 2013). Ayerza and Coates (2011) also determined that the amino acid profile of chia seeds is similar to that studied in 2013.

In general, the protein quality of chia has been demonstrated to be higher than that of some cereals and oil seeds, which may represent an important nutraceutical contribution to foods that contain chia seeds and isolated globulins as ingredients (Derbyshire et al., 1976). Several factors may be related to variations in the concentrations of the active compounds present in chia seeds, such as climate change, area, year and soil conditions for plant cultivation, which are connected with differences in environment and nutrient availability (Dubois et al., 2007; Ayerza and Coates, 2009). Ambient temperature largely contributes to a decrease in the protein content (Ayerza and Coates, 2011).

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Although chia is not currently cultivated for its protein content, following oil extraction, the meal that remains could be used as a high-quality protein source for animal or human consumption (Pallaro et al., 2004). In thermal characterization of the protein fraction of the chia, denaturation temperature (Td) is a measure of the thermal stability of the proteins (Ma and Harwalkar, 1991). According to Sandoval-Oliveros and Paredes-Lopez (2013), the denaturation temperatures of crude albumins, globulins, prolamins, and glutelins were 103, 105, 85.6, and 91°C, respectively, showing that albumins and globulins had relatively good thermal stability. These authors also concluded that relatively low enthalpy values and high denaturation temperatures found for chia proteins deserve further studies. Due to the thermostability of the proteins found in chia seeds, they may be used in food systems undergoing high heat treatments.

ISOLATION OF CHIA PROTEINS

The denomination of protein concentrates or isolates is assigned to the obtained extracts, depending on their protein concentration on a dry basis. They are protein concentrates or isolates when their concentration level reaches 65% or 90%, respectively (Kirk-Othmer, 1997; Moure et al., 2006). The isolation of protein is basically an extraction process that aims to get a product free of interferents and, therefore, more concentrated, so it has other properties and characteristics of conservation and use (Martins, 2009). Figure 1 presents the flowchart of obtaining a chia protein concentrate (69% of protein) obtained by variation of pH (pH 10 of solubilization and pH 3 of precipitation). Producing protein isolates from vegetal sources is of interest due to their increasing use as ingredients with functional properties that can also improve the nutritive quality of foods (Lqari et al., 2002).

Table 1. Amino acid compositions of chia defatted flour, globulin fraction and the contribution of essential amino acids with respect to the requirement patterns for two age groups*

Amino acid content ($mg.g^{-1}$ raw protein)			Contribution (%) of essential amino acids	
			Infants (6 months-1 year)	**Adults (>18 years)**
Amino acid	**Seed flour**	**Globulins**	**Requirement patterns**	**Requirement patterns**
Asp	47.3 ± 0.9	72.9 ± 0.4	--	--
Glu	70.8 ± 1.1	243.0 ± 1.3	--	--
Ser	26.2 ± 0.3	69.3 ± 0.7	--	--
Gly	22.8 ± 0.7	73.6 ± 0.6	--	--
Arg	42.3 ± 0.4	94.2 ± 1.6	--	--
Ala	26.8 ± 0.3	39.4 ± 0.5	--	--
Pro	19.9 ± 0.7	106.4 ± 1.0	--	--
His	13.7 ± 0.1	40.0 ±0.6	20	15
Thr	18.0 ± 0.2	62.3 ± 0.7	31	23
Val	28.5 ± 0.4	35.9 ± 0.6	43	39
Met + Cys	27.8 ± 0.5	57.5 ± 0.4	28	22
Ile	24.2 ± 0.4	30.1 ± 1.2	32	30
Leu	41.5 ± 0.6	44.4 ± 1.7	66	59
Phe + Tyr	38.8 ± 0.5	109.3 ± 0.8	52	38
Lys	29.9 ± 0.5	15.4 ± 0.6	57	45

*Sandoval-Oliveros and Paredes-López (2013).

PROTEIN HYDROLYSATES OF CHIA

The consumption of chia seeds provides numerous health benefits, but they are also a potential source of biologically-active peptides. Enzymatic hydrolysis is natural and safe and effectively produces bioactive peptides from a variety of protein sources, including chia seeds. Chia protein hydrolysates with enhanced biological activity could prove to be an effective functional ingredient in a wide range of foods (Segura-Campos et al., 2013a).

The protein hydrolysates are products intended for use by individuals with nutritional and/or physiological needs not covered by conventional feed, known as dietotherapy (Clemente, 2000). These can be defined as products consisting of free amino acids and peptides that exhibit a wide range of molecular masses resulting from the greater or lesser degree of hydrolysis of proteins. Hydrolysates can be obtained by chemical hydrolysis (acid or alkaline) or enzymatic process of the proteins (Batista, 2011).

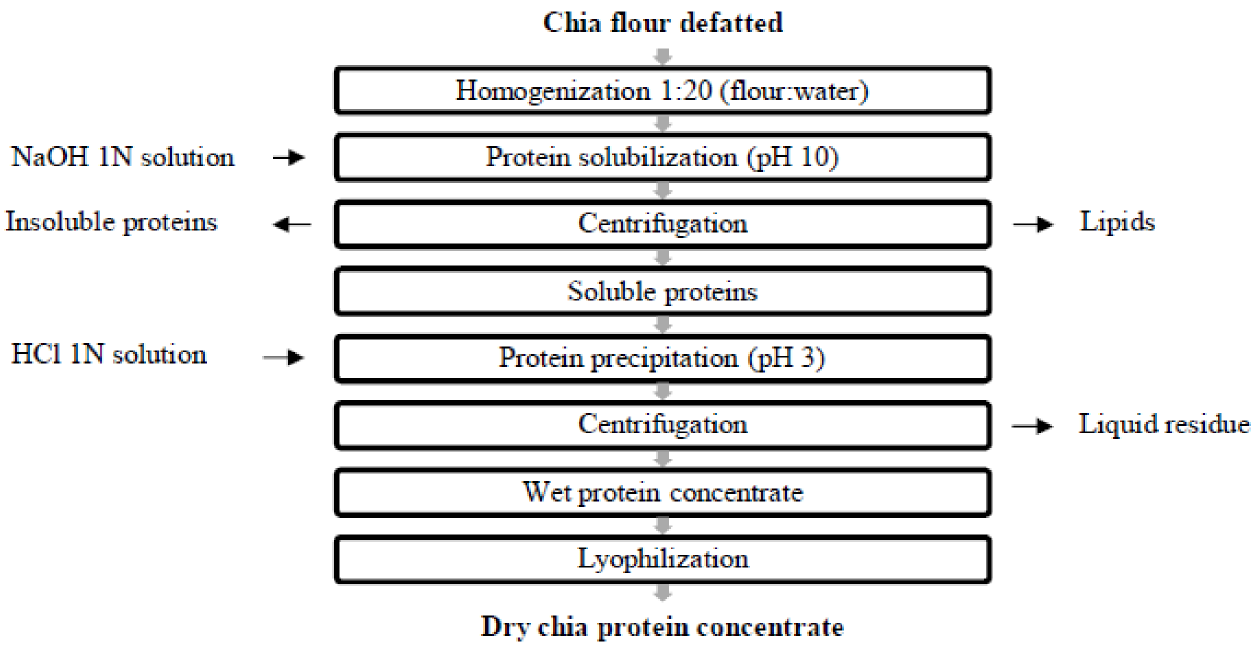

Fig. 1. Flowchart of obtaining the protein concentrate of chia.

Enzymatic hydrolysis is a method based on the addition of enzymes for the cleaving of proteins, a process used to refine or modify the chemical, functional and sensory properties of a protein without sacrificing its nutritional value (Abert and Kneifeli, 1993). The enzymatic process occurs under mild conditions, without producing the degradation products observed in acidic and alkaline hydrolysis. This type of hydrolysis offers advantages because it allows a good control of the process and, consequently, of the properties of the resulting products (Fonkwe and Singh, 1996; Holanda, 2004). The degree of hydrolysis (DH) is closely linked to the specificity of the enzyme, and this changes the solubility, the emulsifying and the gelling properties. Control of the size of the peptides is essential for use of the hydrolysates for dietary purposes (Fitzgerald and O'cuinn, 2006; Sinha et al., 2007).

BIOACTIVE PEPTIDES PRESENT IN CHIA SEED

Chia seed consumption provides numerous health benefits, and proteins can be made available as biologically active peptides. In general, peptides can perform various activities on the basis of its composition and the amino acid sequence, such as immunomodulatory (Gauthier et al., 2006), antimicrobial (McCann et al., 2006), antithrombotic (Shimizu et al., 2008), hypocholesterolemic (Zhong et al., 2007), antihypertensive (Jia et al., 2010) and antioxidant activities (Salazar-Vega et al., 2012), and the degree of hydrolysis is the main factor affecting the biological activity of the protein hydrolysates of chia. It has been noted that pepsin, trypsin, chymotrypsin, thermolysin and alcalase are efficient enzymes in producing various active fragments.

The degradation action of these enzymes helped the generation of various biopeptides having antioxidative, antihypertensive or immunomodulatory biological functions (Saadi et al., 2015). Segura-Campos et al. (2013a) produced protein hydrolysates from chia.

CONCLUSION

Chia presents a high content of proteins (19 to 23%) that exceeds that of most cereals traditionally used in feed and provides a good source of amino acids. Upon fractionation of chia proteinsare obtained different percentages of albumins, globulins, prolamins and glutelins, which seem to depend on the methods of separation and characterization of the protein groups. In addition, some research has reported protein concentrates and hydrolysates of proteins containing different peptides and molecular masses in chia which feature several biological properties with notorious health effects.

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