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Microencapsulación de Aceite de Sardina Adicionado con Extractos de Cáscara de Nuez en una Matriz de Gliadinas y Metilcelulosa

TESIS

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Por medio de la presente me permito informar a usted que he revisado y hecho las correcciones necesarias al trabajo de tesis presentado por: **Ramón Francisco Dórame Miranda** y el cual se intitula **Microencapsulación de aceite de sardina adicionado con extracto de cáscara de nuez en una matriz de gliadinas y metilcelulosa** por lo que me permito dar mi **VOTO APROBATORIO** al respecto, para que se proceda a la impresión de la tesis.

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CONTENIDO

INTRODUCCIÓN

En México, la industria pesquera captura alrededor de 1.3 millones de toneladas anuales de especies marinas, de las cuales, aproximadamente el 70 % se obtiene de la región noroeste (Estados de Baja California, Baja California Sur, Sinaloa y Sonora) (CONAPESCA, 2015). El estado de Sonora captura y procesa los mayores volúmenes de productos marinos en México. Se ha reportado un promedio anual de 320,000 toneladas en los últimos 5 años. Esta pesquería se encuentra en los primeros lugares a nivel mundial en la captura de pelágicos menores, entre los cuales se encuentra la sardina. Alrededor del 70% de esta sardina es procesada por las plantas productoras de harina de pescado, la cual, tiene importancia comercial como materia prima para la alimentación animal. Por tal motivo, se producen más de 10,000 toneladas al año de aceite de pescado como producto secundario con bajo valor comercial (CONAPESCA, 2015). Sin embargo, el consumo de sardina está en constante aumento, debido a su alto valor nutricional. Los contenidos altos de ácidos grasos de cadena larga omega-3 (ω-3) son reconocidos como sustancias potenciales favorables para la salud; los principales ácidos grasos poliinsaturados (AGPI) encontrados en el aceite de pescado son los ácidos eicosapentaenoico (C20:5 n-3, EPA) y ácido docosahexaenoico (C22:6 n-3, DHA). Dichos AGPI disminuyen el riesgo de enfermedades, tales como: enfermedades cardiovasculares, respuesta antiinflamatoria y ayuda al desarrollo en lactantes (Kolanowski y Berger, 1999; Kolanowski *et al.*, 2004; Walker *et al.*, 2015). Por tal motivo, la FDA recomienda un consumo de 3000 mg de ácidos grasos ω-3, por día para los adultos. Como consecuencia de ello, la industria alimentaria tiene gran interés en desarrollar alimentos funcionales enriquecidos con AGPI ω-3. No obstante, debido a su naturaleza los hace susceptibles a procesos de oxidación lipídica (Vaisali *et al.*, 2016), afectando negativamente el sabor, olor, color y valor nutricional de los aceites durante su almacenamiento (Tan *et al.*, 2002; Comunian y Favaro-Trindade, 2016).

La microencapsulación es una de las alternativas prometedoras para fines de estabilización de AGPI ω-3. Una de las principales técnicas de encapsulación es la técnica de electroaspersión (Kaushik *et al.*, 2015; Gárcia-Moreno *et al.*, 2018; Liu *et al.*, 2018). Este método presenta varias ventajas tales como: no utiliza temperaturas altas, se obtienen materiales con alta eficiencia de encapsulación y es escalable a nivel industrial (Moomand y Lim, 2014; García-Moreno *et al.*, 2018). Además, su tamaño permite su fácil incorporación a los alimentos

sin afectar las cualidades sensoriales. Por tal motivo, esté método ha sido ampliamente utilizado para la encapsulación de ingredientes activos utilizando diferentes materiales de pared. Uno de los principales retos en la fabricación de microparticulas es la selección de los materiales de pared. Los materiales de pared hacen posible la liberación de los componentes encapsulados de forma controlada e incluso dirigida durante el procesamiento de alimentos o en el tracto gastrointestinal. Por lo cual, se han utilizado diversos materiales para la microencapsulación de los ω-3, entre los que destacan los subproductos agroindustriales de origen natural, los cuales podrían ser aprovechados para la obtención de materiales de pared. Entre estos destacan las proteínas, antioxidantes y carbohidratos.

Las gliadinas son un grupo de proteínas que se encuentran en el trigo. Estas pueden ser fácilmente extraídas del gluten en soluciones de etanol al 70%. Las gliadinas son proteínas monoméricas con un peso molecular entre 25,000 y 70,000 Da, que carecen de puentes disulfuro intermoleculares y son fácilmente solubles en soluciones alcohólicas (He *et al.*, 2013). Estas proteínas son polimórficas y se pueden clasificar sobre la base de su movilidad electroforética en cuatro fracciones: α (25-35 kDa), β (30-35 kDa), γ (35 de 40 kDa) y ω (55-70 kDa). La composición de aminoácidos muestra que las gliadinas tienen cantidades iguales de aminoácidos polares y neutros, principalmente glutamina (aproximadamente 40%), además de un contenido alto de prolina (14%) (Balaguer *et al.*, 2013). Además, de presentar otras ventajas como: amplia disponibilidad, fácil extracción y bajo costo.

Otro subproducto importante es la cáscara de nuez pecana (*Carya illinoinensis*), el cual ha sido estudiado debido a su variada composición de compuestos fenólicos y capacidad antioxidante alta (Balasundram *et al.*, 2006; De la Rosa *et al.*, 2010; Hilbig *et al.*, 2018). Los principales compuestos identificados han sido el ácido gálico, el ácido clorogénico, el ácido elágico, el ácido p-hidroxibenzoico, catequinas, epigalocatequinas y galato de epicatequinas (do Prado *et al.*, 2014; Alvarez-Parrilla *et al.*, 2018; Hilbig *et al.*, 2018). Estos compuestos tienen una gran variedad de aplicaciones en alimentos, productos farmacéuticos, medicina y agricultura, entre otros (Alvarez-Parrilla *et al.*, 2018). Los antioxidantes naturales como las catequinas han mostrado ser un material adecuado para la estabilización del aceite de sardina (Vaisali *et al.*, 2016). Por otro lado, las catequinas además de tener una potente capacidad antioxidante, también presentan actividad antimicrobiana.

Otro de los materiales de recubrimiento que están llamando la atención es la celulosa bacteriana (CB), la cual se puede obtener mediante fermentación con diferentes microorganismos, destacando las del género *Gluconoacetobacter* (Shi *et al.*, 2014; Lin *et al.*, 2013). Es un subproducto de la producción de ácido acético a nivel industrial (Mohammadkazemi *et al.*, 2015; Pacheco *et al.*, 2017). Este es un polímero natural atractivo en la fabricación de materiales con interés en el área de alimentos y la industria farmacéutica. Este material es reconocido como un material GRAS por la FDA.

Se ha observado que *G. xylinus* (PTCC, 1734), produce un alto rendimiento (43.5 g L-1), aunque éste rendimiento puede variar dependiendo del medio de cultivo, especie y condiciones de incubación (Lin *et al.*, 2013; Shi *et al.*, 2014). Para la especie *entanii* son escasos los trabajos donde se reporta su producción, purificación y moficación química. Un material de interés a partir de la CB es la metilcelulosa, la cual se encuentra entre los principales compuestos utilizados en el área de alimentos y la industria farmacéutica (Lin *et al.*, 2013; Mohammadkazemi *et al.*, 2015; Pacheco *et al.*, 2017). Con este material se podrían obtener películas claras, duras y flexibles con excelentes propiedades de barrera para la protección de aceites y grasas.

La microencapsulación con este tipo de materiales podría ser utilizada en una gran cantidad de productos alimenticios secos y en polvo, debido a su escasa solubilidad.

Por otro lado, durante la elaboración de harina de pescado, se genera aceite como subproducto. El cual debido a su contenido alto de AGPI omega-3 (EPA y EHA), podría considerarse un ingrediente de alto valor nutricional. Sin embargo, esto mismo, el contenido alto de AGPI hace al aceite de pescado susceptible a los factores ambientales, disminuyendo su estabilidad oxidativa y su valor nutricional, convirtiéndolo en un riesgo potencial para la salud.

Por tal motivo, el aceite de sardina debe ser estabilizado mediante técnicas como la microencapsulación. El uso de subproductos agroindustriales como fuentes de compuestos antioxidantes y material de pared podrían ayudar a estabilizar el aceite de sardina mediante técnicas de microencapsulación. Por lo tanto, en la presente tesis, se tiene como objetivo evaluar la microencapsulación de aceite de sardina adicionado con extractos de cáscara de nuez en una matriz de metilcelulosa o gliadinas.

En este trabajo se evaluó la optimización de un proceso de microencapsulación de aceite de sardina adicionado con extractos de cáscara de nuez en una matriz de metilcelulosa o

gliadinas, a fin de prolongar su vida de anaquel. Para lo cual se establecieron las condiciones de obtención de celulosa bacteriana a partir de *Gluconacetobacter entanii* usando cáscara de nuez pecana (*Carya illinoinensis*) como fuente de carbón. Así, como la conversión de la celulosa de la bacteria *Gluconacetobacter entanii,* a su derivado metilcelulosa.

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DESARROLLO DEL TRABAJO DE INVESTIGACIÓN

Para probar la hipótesis planteada, que fue que la microencapsulación del aceite de sardina en una matriz de gliadinas y metilcelulosa, adicionado con compuestos antioxidantes de la cáscara de nuez pecan (*Carya illinoinensis*), permitirá proteger a los ácidos grasos contra factores ambientales y facilitará su uso como ingrediente nutracéutico, el trabajo experimental, se dividió en tres etapas, las cuales se describen en los siguientes tres capítulos.

Capítulo 1: Obtención de celulosa bacteriana a partir de *Gluconacetobacter entanii* usando cáscara de nuez pecana (*Carya illinoinensis*) como fuente de carbón

Este capítulo consiste en un manuscrito titulado: "Bacterial cellulose production by *Gluconacetobacter entanii* using pecan nutshell (*Carya illinoinensis*) as carbon source and its chemical functionalization", publicado en la revista Carbohydrate Polymers. El manuscrito contiene información acerca de la obtención de celulosa bacteriana empleando como fuente de carbono para el crecimiento de la bacteria *Gluconacetobacter entanii* la cáscara de la nuez pecanera (Fecha de publicación: 23 de noviembre de 2018, Vol. 207, Pág. 91-99, Doi: https://doi.org/10.1016/j.carbpol.2018.11.067).

Capítulo 2: Establecimiento de las condiciones óptimas para la microencapsulación del aceite de sardina adicionado con extractos de cáscara de nuez pecana (*Carya illinoinensis*) como antioxidante en una matriz de metilcelulosa o gliadinas.

Este capítulo consiste en un manuscrito titulado: "Microencapsulation of sardine oil by electrospray deposition with gliadin proteins and pecan nutshell extracts", sometido a la revista LWT- Food Science and Technology. El manuscrito contiene información acerca de la optimización del proceso microencapsulación del aceite de sardina empleando metilcelulosa gliadinas, adicionado con compuestos fenólicos de la cáscara de nuez pecana (*Carya illinoinensis*) y de las propiedades físicas y químicas de las microcapsulas obtenidas.

Capítulo 3: Caracterización de los compuestos fenólicos con actividad antioxidante presentes en la cáscara de nuez pecana [*Carya illinoinensis* (Wagenh) K. Koch]

Este capítulo consiste en un manuscrito titulado: “Influence of solvent on the phenolic compounds from pecan nutshell [*Carya illinoinensis* (Wagenh) K. Koch] and the antioxidant capacity”, que se contempla someter a la revista Food Analytical Methods. Este estudio proporciona una descripción completa del contenido de compuestos fenólico presentes en la cáscara de nuez pecana.

CAPÍTULO I

Bacterial cellulose production by *Gluconacetobacter entanii* using pecan nutshell (*Carya illinoinensis)* as carbon source and its chemical functionalization

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Bacterial cellulose production by *Gluconacetobacter entanii* using pecan nutshell as carbon source and its chemical functionalization

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ABSTRACT

Pecan nutshell is an abundant waste with a high content of carbohydrates. According to its chemical composition, pecan nutshell could be used as carbon source for *Gluconacetobacter entanii*, a bacterium that produces cellulose with high purity and nanometric characteristics. Bacterial cellulose (BC) was obtained from a static culture medium using pecan nutshell as carbon source and saccharose as control. Results showed that the pecan nutshell could be used as carbon source for production of BC. The cellulose yield ranged around 2.816 ± 0.040 g/L for 28 days. The morphological, structural and chemical properties of the cellulose produced were similar to those reported for others BC. The spectroscopic characterization indicated the chemical functionalization of BC and the reduction of its crystallinity. The production of BC with *G. entanii* using pecan nutshell as carbon source, is the first report. The BC could have potential use in chemical functionalization and in the preparation of biocomposites.

1. Introduction

Cellulose is one of the most abundant biopolymer on the planet and the main by-product worldwide (Cairul, Mohd, Abadi, & Katas, 2014; Castro et al., 2012). This polymer is mainly synthesized by plants (Somerville, 2006). Additionally, microorganisms from protista, fungi, and bacteria kingdoms can also synthesize cellulose (Keshk, 2014).

In the present, nanomaterials obtained from cellulose have attracted great attention due to the unique thermal, optical and mechanical properties (Chen, Zhu, Baez, Kitin, & Elder, 2016). These nanomaterials of cellulose are commonly obtained by acid hydrolysis from lignocellulosic components (Chen et al., 2015). However, in obtaining by hydrolysis are used large volumes of concentrated acid and high temperatures. For this reason, these methods remain environmentally unsustainable, very expensive, and economically out of reach for development of applications. Besides, low yields are reported (approximately 30% by weight) (Chen et al., 2015, 2016). Nowadays, the use of bacteria to generate cellulose with high purity is of great interest. The cellulose produced by bacteria has nanometric characteristics, good thermal and mechanical properties (Shi, Zhang, Phillips, & Yang, 2014; Lin et al., 2013). Also, not require aggressive treatments for purification and is environmentally sustainable. The bacteria of the genus *Gluconacetobacter* have been identified as good producers of bacterial cellulose (BC) in static culture medium. *G. entanii* has been identified as producer of industrial vinegar (Yucel & Karabiyikli, 2011). However, the reports about its activity to produce BC and their physicochemical characterization are scarce.

By the other side, the main challenge in bacterial cellulose production is to find suitable carbon sources that are cheap, abundant and that do not compete with the food production (Kiziltas, Kiziltas, & Gardner, 2015). Agroindustrial by-products such as grape skin (Carreira et al., 2011), rice wine (Wu & Liu, 2012), wheat straw (Chen, Hong, Yang, & Han, 2013), dry olive (Gomes et al., 2013), coffee cherry husk (Rani & Appaiah, 2013), and cashew tree (Pacheco et al., 2017) have been analyzed and used as carbon source for BC production. Nevertheless, new sources of carbon need to be investigated.

Pecan nutshell (*Carya illinoinensis*) is a by-product that has not been investigated as carbon source in the production of BC. Around 40–50% of the total pecan production represents a waste generated worldwide, which is not used (do Prado et al., 2014; Hilbig et al., 2018). According to the Food and Agriculture Organization (FAOSTAT, 2016), nutshell production is more than 460×10^3 tons. In addition, it has been

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CAPÍTULO II

Microencapsulation of sardine oil by electrospray deposition with gliadin proteins and pecan nutshell extracts

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Highlights

Sardine oil microparticles with gliadin proteins as wall material and pecan nutshell extracts as antioxidants were obtained.

Optimal conditions to obtain sardine oil microparticles were determined.

The microparticles obtained showed a high encapsulation efficiency and oxidative stability.

Gliadin proteins and nutshell extracts are good sardine oil stabilizers.

Electrospray deposition is a good method to obtain sardine oil microparticles.

Abstract

Sardine oil (SO) was microencapsulated by electrospray deposition using gliadin proteins and pecan nutshell extracts as stabilizers. The diameter of the SO microparticles was optimized through the response surface methodology (RSM). The optimal conditions of the process to get microparticles with a spherical and smooth structure, and characteristics micrometric were obtained. The diameter of the microparticles was around 1.47 µm and the encapsulation efficiency was 98%, confirmed the presence of SO in the microparticles. Also, the physicochemical stabilization of the SO microparticles systems was investigated. FT-IR results showed interactions between SO and protein gliadins, these interactions could help oxidative stability of SO. The pecan nutshell extract prevented the lipid oxidation by the Rancimat test. The SO microparticles had greater oxidative stability compared to SO non-encapsulated. Overall results indicated SO microparticles obtained from gliadins and pecan nutshell extracts there are a good materials for stabilization of SO.

Keywords: Microencapsulation, Sardine oil, Electrospray deposition, Gliadins, Pecan nutshell, Optimization.

Introduction

Sardine oil (SO) is one of the major sources of polyunsaturated fatty acids (PUFAs), specially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Both EPA and DHA have been considered essential fatty acids associated with health benefits. Because SO had these compounds, SO can be used as a nutraceutical ingredient or in the development of functional foods in the pharmaceutical and food industries, respectively (Kadam & Prabhasankar, 2010; Calder, 2017). However, due to the high content of PUFAs SO has a low oxidative stability during the processing and storage. Therefore it is of great importance to increase the half-life of the sardine oil to maintain the nutritional properties

The most common methods for stabilization of the edible oil are refining techniques and the use of antioxidants (Carvajal, Mozuraityte, Standal, Storrø, & Aursand, 2014). The natural antioxidants have been used as an alternative of the synthetic antioxidants in the stabilization of

fish oil (Leonardis, Pizzella, & Macciola, 2008; Wang et. al., 2011; Rovira, Venskutonis, & Damas, 2011; Vaisali, Belur, & Regupathi, 2016;). Among them, catechin and quercetin have shown higher antioxidant activity in fish oils than other compounds (Vaisali, Belur, & Regupathi, 2016). Pecan nutshell (*Carya illinoinensis*), is a by-product generated from the nut industry, and is a rich source of catechins (Prado et. al., 2014; Hilbig et. al., 2018). Approximately, 460 x 10^3 tons of pecan nutshell are generated worldwide, indicating that this by-product could be an alternative for the extraction of phenolic compounds and fiber (Dórame-Miranda et. al., 2019). The antioxidants obtained from pecan nutshell have not been used to stabilize the fish oil. Such application could be of interest to the fish oil industry.

Among other methods for stabilization of fish oil is the microencapsulation, which has the ability to increase the shelf-life (Bakry et. al., 2016). The preparation of microparticles requires a careful selection of the wall material to protect the compound of interest, PUFAs in this case. Different wall materials have been used for the microencapsulation of PUFAs, among them are the zein, prolamine, gelatin, pullulan, dextran, glucose syrup, poly (vinyl alcohol) (PVA), gum arabic and gliadins (Torres-Giner, Martinez-Abad, Ocio, & Lagaron, 2010; Moomand & Lim, 2014; Kaushik, Dowling, Barrow, & Adhikari, 2015). Gliadins are a sub-product of the wheat starch extraction for the production of bioethanol. Furthermore, gliadins are promising proteins in the production of microparticles because they are abundant and cheap compared to another proteins. Additionally, the use of gliadin proteins to encapsulate lipophilic compounds can improve the bioavailability of these compounds due to the bioadhesive capacity of these proteins in the gastric mucosa (Arangoa, Campanero, Renedo, Ponchel, & Irache, 2001).

On the other hand, there are a large number of methods used to obtain microparticles of fish oil. Spray dried, freeze dried, fluidized bed drying, extrusion, complex coacervation, electrospinning and electrospray deposition are some of them (Kaushik, Dowling, Barrow, & Adhikari, 2015). Electrospray deposition is a newfangled method, which presents high encapsulation efficiency and that not requires high temperatures during the processing of the materials (Torres-Giner, Martinez-Abad, Ocio, & Lagaron, 2010; Moomand & Lim, 2014; García-Moreno et. al., 2018; Tang et. al., 2019). In this method a thermodynamic instability in polymeric solutions with high-voltage supply is created. The liquid solution sprayed out from the metal nozzle forms a meniscus, which is elongated due to the electric field, and it is

disintegrated into droplets, easing the formation of smaller particles (Taylor cone). Before the formation of the small particles (Rayleigh disintegration or Coulomb fission), a high solvent evaporation and charge concentrations occurs; also, colloidal reactions occur within the solution. Finally, the droplets are solidified and the forms of the material obtained have a micro or nanoparticulate structure (Jaworek & Sobczyk, 2008; Xie, Jiang, Davoodi, Srinivasan, & Wang, 2015). Parameters such as the morphologic characteristics, encapsulation efficiency, and stability oxidative are greatly affected by the composition of the wall material core, viscosity, molecular weight, solvent system and conditions of the process (such as flow rate solution, applied voltage and distance between the needle and the collector plate). Many factors have been determined in the microencapsulation by electrospray; however, the conditions to obtain microparticles with an average diameter that allows the release of the compound encapsulated need to be studied (Jaworek & Sobczyk, 2008; Ghayempour & Mortazavi, 2013). According to Abyadeh, Aghajani, & Gohari (2018) the increment of the applied voltage and decrease of molecular weight of the cell wall material let the formation of small particles. Optimal conditions to obtain microparticles with different wall material depending of its application must be optimized.

Therefore, the aim of this study was to optimize the microencapsulation process of SO by electrospray deposition with gliadin proteins as wall material and pecan nutshell extracts as antioxidant. In addition, the physical and chemical properties of the microencapsulated SO were evaluated. Such information will be useful to give an added value to by-products generated by the fish, pecan nut, and bioethanol industries.

2. Material and methods

2.1. Samples

Sardine oil (*Sardinops sagax caerulea*) by the group Guaymex, S.A. de C.V., Mexico was provided. Wheat Gluten (WG) proteins were obtained from Sigma-Aldrich Chemical Company (St. Louis, MO), and the gliadins fraction was extracted from WG. Pecan nutshell (*Carya illinoinensis*) by the group Alta, S.A. de C.V., Mexico was supplied.

2.2. Chemicals

2,2′-azinobis(3-ethylbenzothiazoline-6 sulfonic acid) (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ) and TEAC (Trolox Equivalent Antioxidant Capacity), were purchased from Sigma-Aldrich. All the other chemicals used were of analytical grade.

2.3. Preparation of phenolic extracts from pecan nutshell

The pecan nutshell (*Carya illinoinensis*) was ground in a Wiley mill (Thomas-Wiley Laboratory Mill Model 4) to a particle size up to 50 microns. The powder was keep in amber bottles at -20 °C in the absence of light. Ultrasonic-assisted treatment described by Liu, Wei, & Liao, (2013) for the extraction of pecan nutshell phenolic compounds was used. The sample (1 g) was mixed with 30 mL of 60% (v/v) ethanol using a vortex. Subsequently, the homogenate was subjected to ultrasonic movements (21 min) (Branson Sonicador, model 1510, USA) and centrifuged (3000 g, 4ºC, 15 min). The supernatant was mixed and filtered with Whatman No. 4 paper. The extract was rotaevaporated and freeze-dried. The extract powder was stored at -20ºC until further analysis.

2.3.1. Antioxidant capacity

Antioxidant capacity of pecan nutshell extracts was estimated using the radical scavenging reaction system with DPPH• and ABTS•+ radicals. The dry extract (0.1 mg mL^{-1}) was dissolved with 60% ethanol.

2.3.1.1. ABTS•+ method

The Trolox equivalents of antioxidant capacity (TEAC) value was determined according to Re et. al. (1999). The methodology is based on the ability of antioxidants present in an extract to inactivate the radical ABTS•+ (2,2'-azino-bis 3-ethylbenzthiazoline-6-sulfonic acid). The reaction was carried out using 280 µL of radical ABTS•+ with 20 µL of extract. The reaction was allowed to stand during 7 min in darkness, and the radical reduction was monitored at a wavelength of 734 nm in a 96-well microplate reader (Veloskan™ LUX multimode microplate reader, Thermo Scientific, USA). The antioxidant capacity was expressed as micromoles of Trolox equivalent antioxidant capacity per gram of extract (µmol of TEAC/g of extract).

2.3.1.2. DPPH• method

The ability of the extracts to inactivate the stable radical DPPH • was calculated according to Palafox-Carlos, Yahia, & González-aguilar (2012) with some modifications. The reaction was carried out with 280 μL of radical DPPH• and 20 μL of sample. The reaction was allowed to stand for 30 min in the dark. The absorbance of the samples was read at 515 nm in a microplate reader using a 96-well microplate (Veloskan ™ LUX multimode microplate reader, Thermo Scientific, USA). The antioxidant capacity was expressed in terms of the concentration as μmol of TEAC/g of extract.

2.3.1.3. FRAP method

The FRAP test was carried out using the method of Benzie & Strain (1996) with some modifications. This method is based in the increase of the absorbance of the reaction sample due to the formation of the complex 2,4,6-tripyridyl-s-triazine (TPTZ) -Fe (II) in the presence of reducing agents. The FRAP reagent was prepared mixing 10 μM TPTZ, 40 mM HCl, 20 μM FeCl3, and 300 μM acetate buffer. An aliquot of sample (20 μL) was placed in each well in a microplate and mixed with 280 μL of FRAP solution. The samples were incubated at room temperature in the dark for 30 min and the absorbance was measured at 630 nm in a microplate reader. The results were reported as μmol of TEAC/g of extract**.**

2.4. Preparation of gliadins and sardine oil solutions

Samples were prepared according to th following procedure: gliadin solutions at 6%, 7%, and 8% (w/w) were dissolved in 70% aqueous ethanol with continuous stirring during 30 min at room temperature and used as a control samples. Same gliadin solutions were prepared, but in this case 30% (w/w) SO was added respect to the concentration of the gliadin solution. The gliadin solution was mixed with the SO during 15 min at room temperature under dark conditions. From the gliadin solutions with SO, the concentration with better morphology and average diameter was chosen. The gliadin concentration with an adequate average diameter and morphology was selected to obtain the microparticles at optimal conditions (kV, cm). Finally, microparticles with SO and pecan nutshell extract (150 ppm in ethanol) at the optimal conditions were obtained. The concentration of pecan nutshell extracts was chosen based on previous results of SO oxidative stability with different concentration of pecan nutshell extracts.

2.4.1. Physicochemical characterization of solutions for obtained of microparticles

2.4.1.1. Viscosity assay

The viscosity assay was realized according to Nielsen, Horn, & Jacobsen (2013) with modifications. Viscosity measurements were obtained using a rotational viscometer (Brookfield DV-II + Pro Viscometer, mode LVF, USA) with a concentric cylinder geometry. A constant shear rate of 100 s^{-1} at 25 °C was used. The viscosity was recorded during 1 min after starting the measurement. Determinations were made in triplicate.

2.4.1.2. Zeta potential

The gliadins and sardine oil solutions were transferred to a capillary cells for measurement at ambient temperature in a zetasizer nano ZS (Malvern Instruments, UK). For zeta potential measurements, the values were obtained using the Smoluchowsky mathematical model (Nielsen, Horn, & Jacobsen, 2013).

2.5. Sardine oil microparticles by electrospray deposition

The solutions obtained previously were transferred into a plastic syringe with a 5 mL capacity to later take it to the electrospray equipment. The flow rate was controlled using a dual syringe pump (KDS 200, KD Scientific, USA), and the voltage (kV) was applied using a high-voltage power supply (Spellman/Bertan 230-01R, USA). The microparticles formed were collected with an aluminum plate (Figure 1). The variables and operation parameters to optimize the average diameter of the SO microparticles for the RSM are presented in Table 1. The voltage (kV) and distance between the needle and the collector plate (cm) were the independent variables. These variables are of great importance for the formation of SO microparticles. It is worth to mention that the effect of the gliadins viscosity with sardine oil solutions was evaluated in the microparticles formation.

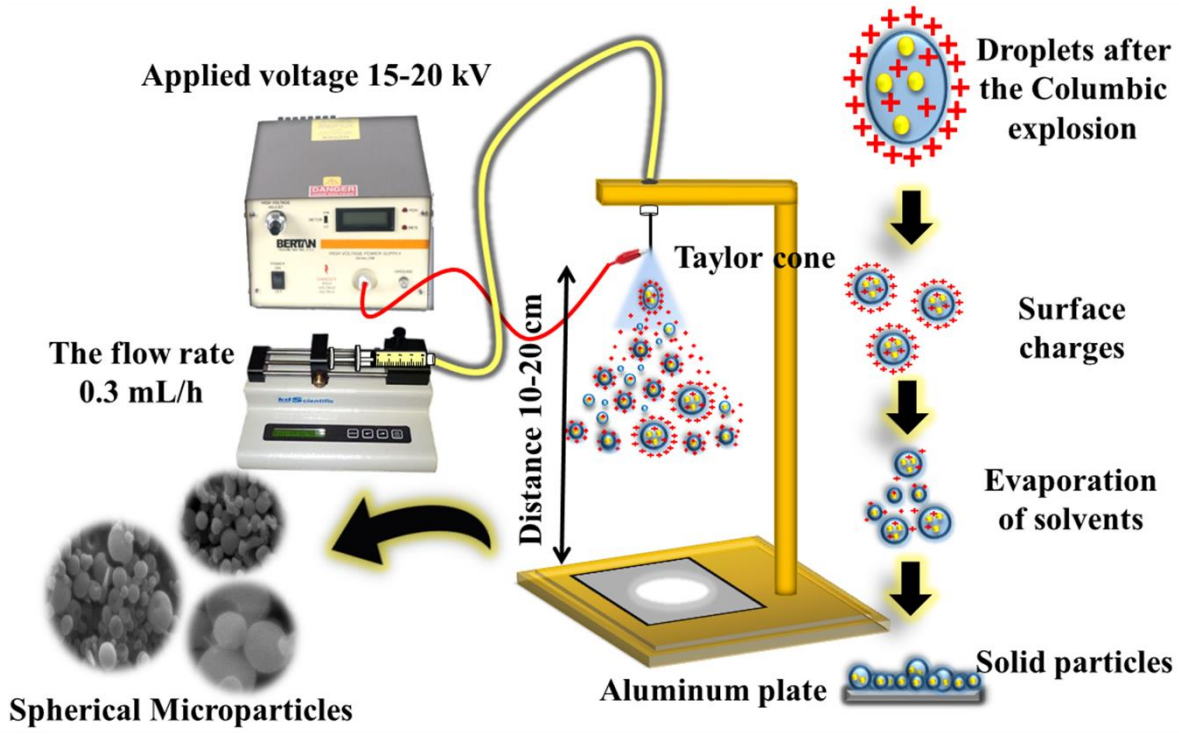

Fig. 1. Schematic diagram of the fabrication of microparticles by electrospray deposition.

2.6. Characterization of microparticles of sardine oil

2.6.1. Morphological characteristics of SO microparticles

Micrographs of SO microparticles were obtained with a scanning electron microscopy (SEM) equipment (XL 30 ESEM Phillips operating at 20 kV at high vacuum), and were used to analyze the microstructure. Samples were prepared in a coal tape and coated with gold under high-vacuum conditions. The average diameter distribution of the microparticles was determined by measuring 100 particles in 5 different fields using the Image J 1.50i (National Institutes of Health, USA) software.

2.6.2. Encapsulation efficiency and loading capacity

The encapsulation efficiency (EE) and loading capacity (LC) were determined as described previously by Moomand & Lim (2014) with some modifications. The microparticles (100 mg)

were submerging in hexane (8 mL) for 30 s to remove the unencapsulated surface oil. The supernatant was filtered on filter paper (Whatman No. 4), and the absorbances of the solutions were measured using a UV-Vis spectrophotometer (Varian Cary 100 Bio, USA) at 320 nm. A calibration curve was used to determine the amount of SO microencapsulated. The EE and LC values were calculated with the following equations:

$$EE = \frac{A-B}{C} * 100 \quad (1)$$

$$LC = \frac{A-B}{A} * 100 \quad (2)$$

Where:

A: the total theoretical amount of SO, B: the free amount of SO in the sample, and C: the weight of the microparticles.

2.6.3. Oxidative stability

The oxidative stability test of the SO, SO with antioxidant (150, 300 and 500 ppm), optimized microparticles and microparticles with antioxidant (150 ppm) was determined using the Rancimat equipment (model 743, Metrohm, Herisau, Switzerland). The method consisted in heat at 65°C 2 mL of liquid samples or 1.5 g microparticles powder, maintaining a flow rate of 20 L h-1. The oxidative stability index (OSI) value of the samples was determined according to the conductivity slope and time observed in the graphic generated by the Rancimat equipment

2.6.4. ATR-FTIR spectroscopy

The SO, gliadins, antioxidants and microparticles were analyzed using the ATR-FTIR spectrometer (FT-IR Spectrum GX, Perkin-Elmxxer, USA) with an average of 64 scans, over a spectral range of 4000–600 cm^{-1}, with a resolution of 4 cm^{-1}.

2.7. Experimental design

Response surface methodology (RSM) was employed to optimize the operating parameters. The applied voltage (X_1) and distance between the needle and the collector plate (X_2), were the independent variables. The Table 1 shows the independent variables and their coded and uncoded levels. The average diameter of SO microparticles (Y) was used as response variable in the design experiment (Table 3). The microencapsulation by electrospray was performed using a two central composite design, with 20 total runs. The model proposed for the response was fitted to a second-order equation (3), using a SAS JMP software, version 8 (SAS, Cary, NC, USA).

$$Y = \alpha 0 + \Sigma \alpha i Xi + \Sigma \alpha ii X2i + \Sigma \alpha ij XiXj + \varepsilon \quad (3)$$

Where: α0, αi, αii, and αij: the intercept and the linear, quadratic, and interaction coefficients respectively. The residual is denoted as ε.

Table 1. Independent variables and their levels used in experimental design

Independent variables	Levels		
	-1	**0**	**1**
Applied voltage (X_1)	15	17.5	20
Distance between the needle and the collector plate (X_2)	10	15	20

2.8. Statistical analysis

The antioxidant capacity assay, phenol content, EE, LC and oxidative stability were expressed as the mean ± standard deviation, n=3 using SAS JMP software, version 8 (SAS, Cary, NC, USA). For the effect of the viscosity, zeta potential and average diameter an analysis of variance was used (ANOVA). The differences between means were evaluated by Tukey's test ($p < 0.05$). For the microparticles of sardine oil process a two factor central composite optimization design was used.

Results and discussion

Antioxidant capacity of pecan nutshell extracts

The results showed that total phenolic compounds and flavonoid content were high in the extract. The total phenols were expressed as mg/g gallic acid equivalents, and for flavonoids cathequin as a standard compound was used. The phenolic content was found in the pecan nutshell extract was of 547.27 ± 2.30 mg/g and for flavonoids was of 179.06 ± 1.64 mg/g (Fig. 2). The higher the total phenolic content in the extracts, were the higher the antioxidant assay by ABTS, DPPH and FRAP values (Noreen, Semmar, Farman, & McCullagh, 2017).

The pecan nutshell extracts have high content of catechins, which are an powerful antioxidant for the stabilization de fish oil (Hilbig et. al., 2018; Vaisali, Belur, & Regupathi, 2016). The sardine oils may be stabilized for these compounds across of donation of a hydrogen atom from a hydroxyl group attached to the aromatic ring to the chemically unstable molecules, electron donating groups and through the metal chelating ability. The metal chelating potential is due the proximity of the hydroxyl groups localized in the B-ring in flavonoids; also, a factor of more important in the antioxidant activity is the effect of structure on the antioxidant activity. Moreover, the phenolic compounds have the ability of inhibiting the action of enzymes such as lipoxygenase that they relate to oxidative changes in fats and oils (Prado et. al., 2014).

The use of phenolic compounds for stabilizing polyunsaturated fatty acids has been reported (Vaisali, Belur, & Regupathi, 2016). However, to our knowledge, recently, there is a lack of information about the application of phenolic compounds extracted from pecan nutshell in the stabilization of marine oils, or used as antioxidant material in microencapsulation systems. Due to the high content of phenolic and flavonoids compounds (Fig. 2), pecan nutshell extracts could be an option for stabilization of PUFAs. The pecan nutshell extracts have high content of catechins, which are a powerful antioxidant for the fish oil stabilization (Vaisali, Belur, & Regupathi, 2016; Hilbig et. al., 2018; Estrada-Flores et. al., 2019). The SO may be is stabilized for this compounds through the donation of an hydrogen atoms from an hydroxyl group attached to the aromatic ring, by the electron donation, or the metal chelating ability of the antioxidant. Moreover, the phenolic compounds have the ability to inhibit the action of enzymes such as

lipoxygenase, relate to oxidative changes in fats and oils (Prado et. al., 2014). Recently, (Rovira Venskutonis, & Damas, 2011) evaluated the effect of garden strawberry leaf extracts on the shelf life and end quality characteristics of fish oil. The extract efficiently retarded fish oil oxidation, particularly when was applied at the concentration of 5%. The results indicate that pecan nutshell as strawberry leaves could be a potential source of natural antioxidants used in the stabilization of SO because of its antioxidant properties (Fig 2).

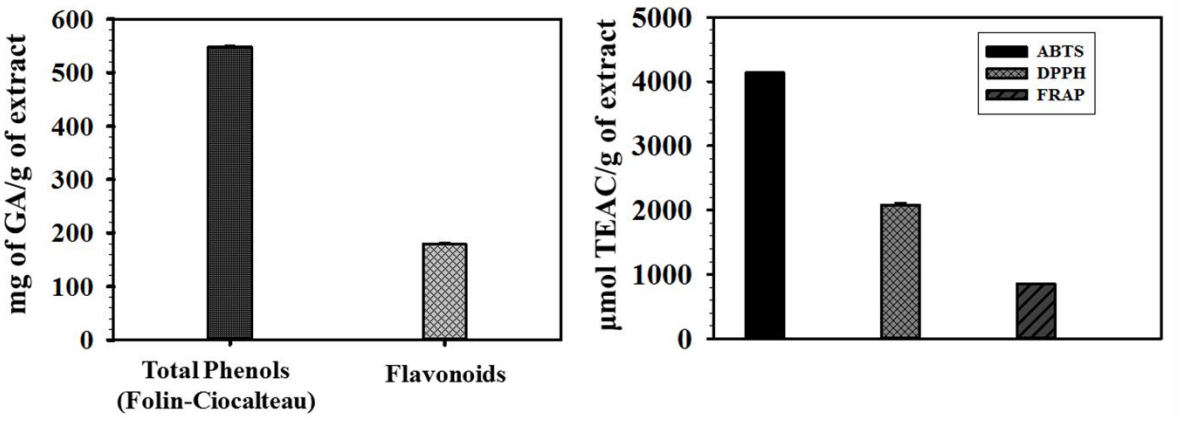

Fig. 2. Natural antioxidants of pecan nutshell extract. a) Total phenols and flavonoids content, a)

antioxidant capacity using different method: ABTS, DPPH and FRAP.

Physicochemical properties of solutions

In this work, the effect of the gliadins viscosity in aqueous ethanol on the formed microparticles was studied. Table 2 shows the viscosity of gliadin solutions without and with SO. In general, the viscosity increased with the increase of the gliadins concentrations. Same trend was observed in gliadin solutions with SO, but the viscosity was higher and showed significant differences that those ones without SO (Table 2). Both gliadin solutions without and with SO were used to obtain the microparticles by electrospray deposition and observed their morphology and measure their average diameter (Fig. 3). Figure 3a-c shows the effect of the concentration and viscosity on the microparticles formed, where it is observed that gliadin microparticles had a broad size distribution, flat globular shape and wrinkle surface. The average

diameter was around 0.183, 0.652 and 1.00 µm for 6, 7 and 8% (w/w) gliadin concentration, respectively. Respect to the gliadin microparticles at 6, 7 and 8% with SO, the viscosity values resulted in microparticles with an average diameter of 0.564, 0.999, and 0.957 µm, respectively (Fig. 3 d-f). After the addition of SO, the microparticles presented a more spherical morphology, broad size distribution and smooth surface. From all the gliadin solutions, the one at 7 % (w/w) showed higher number of particles with spherical shape and few particles with wrinkle surface (Fig. 3e). Comparing the average diameter with of all the solutions, only the gliadin solution at 6% without SO, presented significant differences. However, the solution at 7 % showed more spherical microparticles. For this reason, this solution was chosen as the concentration for the optimization process. An important factor in the preparation of nano and microparticles by electrospray deposition is the selection of the solvent and the polymer concentration. The polymer concentration is directly related to the viscosity; then when the viscosity of the solution is low, microparticles of gliadins are obtained. On the other hand, the use of polymers with high concentration allows the formation of nanofibers with different morphology (Jaworek & Sobczyk, 2008; Xu et. al., 2017).

Fig. 1 shows the schematic diagram of the microparticles formation by electrospray deposition. First, a strong electric field is applied to the solution to generate a charge on the surface and a thermodynamic instability in the polymeric solution. Due to the high voltage and low fluid flow rate applied, the solution assumes a conical shape (Taylor cone). The viscosity is related with the Taylor cone stability, which in turn is related with morphology of the particles formed (size and form) (Liu, Zhang, Yu, Wu, & Li, 2018; Yang, Zhang, Liu, Wang, & Yu, 2018). Therefore, when the viscosity of the solution increase, particles of major average diameter are obtained, but when the viscosity is low, smaller particles are formed (Ghayempour & Mortazavi, 2013). Apart from the viscosity, the zeta potential value is another relevant parameter to take into account in the electrospray deposition in this experiment. According to Table 2 the gliadin solutions showed a positive charge around 6.08-11.90 mV, and as the concentration increases there is an increase in the zeta potential (Table 3). These zeta potential values could be related to the amino groups linked to the carboxylate groups on the surface of the proteins (Wang, Adhikari, & Barrow, 2014). Thus, the positive charge can be a determining factor for the formation of microparticles.

Gulfam et. al. (2012) synthesized gliadin and gliadin-gelatin composite nanoparticles for the delivery and controlled release of an anticancer drug. The gliadin solution used was at 7% and dissolved in 70% ethanol. The nanoparticles presented an average diameter of 218.66 nm, greater than the value reported in this study. These differences maybe are due to the applied voltage and low flow rate variables used in the electrospray deposition. For this reason, it is very important to find the optimal conditions of the process to get microparticles with an optimized average diameter.

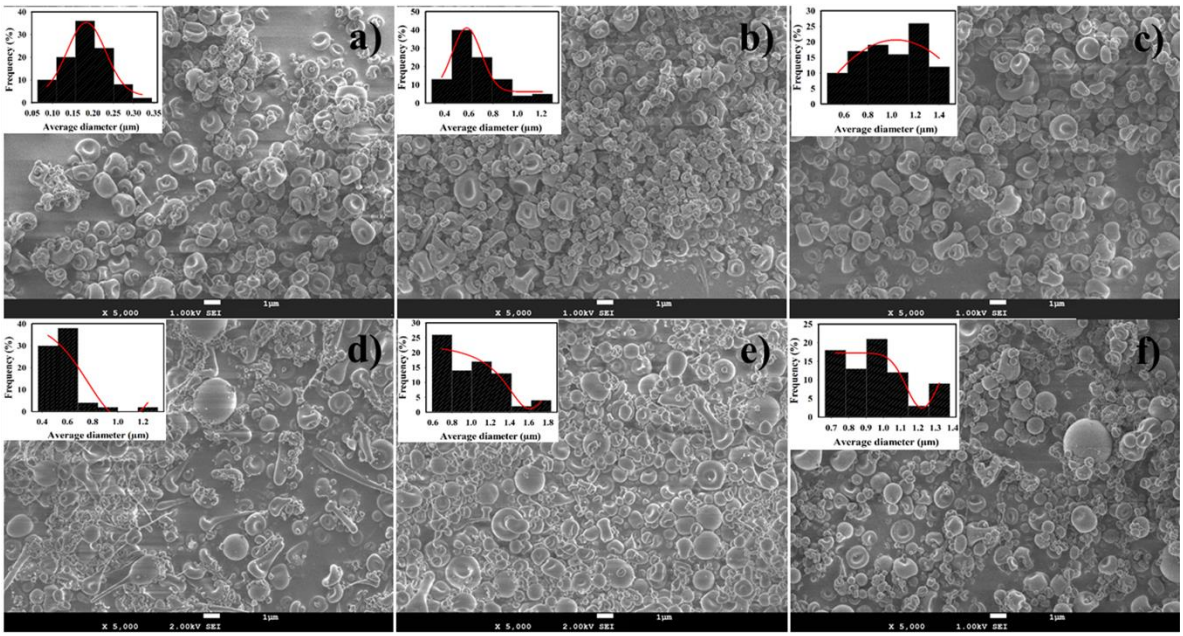

Fig. 3. SEM images of gliadin microparticles without and with SO. Microparticles from a Gliadin concentration of a) 6% (w/w), b) 7% w/w and c) 8% w/w without SO. Microparticles from gliadin concentration at d) 6% w/w, e) 7% w/w and f) 8% w/w with 30% (w/w) SO. Microparticles were obtained using these parameters: 15 kV voltage, 20 cm distance and 0.3 mL/h flow rate.

Table 2. Effect of the viscosity, zeta potential and average diameter on concentrations of gliadin solutions used in electrospray deposition.

Gliadins solutions (% w/w)	**Sardine oil (% w/w)**	**Viscosity (cP)**	**Zeta potential (mV)**	**Average diameter (µm)**
6	0	5.09 ± 0.01^{a}	6.08 ± 0.63^{a}	0.183 ± 0.05^{a}
6	30	5.22 ± 0.03^{b}	6.67 ± 0.26^{a}	$0.564 \pm 0.169^{a,b}$
7	0	6.06 ± 0.03^{c}	7.72 ± 1.79^{a}	$0.652 \pm 0.211^{a,b}$
7	30	6.41 ± 0.06^{d}	$8.56 \pm 0.45^{a,b}$	0.999 ± 0.307^{b}
8	0	6.69 ± 0.03^{f}	$10.49 \pm 1.25^{b,c}$	1.00 ± 0.262^{b}
8	30	7.03 ± 0.04^{g}	11.90 ± 0.60^{c}	$\pm 0.205^{b}$

*Value with different lowercase letters in one row are significantly different ($p < 0.05$).

Microencapsulation of sardine oil

A central composite design was applied to evaluate the effect on the variation of average diameter respect to the operating parameters in electrospray deposition including voltage (kV) and tip-to-collection plate distances (cm) (Table 3). The model applied for microencapsulation of SO showed significant differences in the average diameter ($P < 0.0001$), with a $R2 = 0.9147$ and $R2adjusted = 0.8842$. These results showed that only 8.5284 % of the total variations by the model are not explained (Table 4). The relationship with the adjusted determination coefficient (R2adjusted) and determination coefficient (R2) should be close for have a good statistical model. In Table 4, the difference between R2adjusted respects to R2 is from 0.005, value that is very close to R2. This confirms that the model is highly significant. The analysis ANOVA indicated that the model was adequate to describe the influence of the variables studied (Table 4). The experimental results showed a lack of fit value of 0.2217, which indicate than the model

is adequate to explain the experimental data. When the p-value is smaller than greater F-value indicates that the model is significant and could be used to optimize the microencapsulation process.

Fig. 4 shows the surface response plot (a) and the contour plot (b), representing the relationship between dependent and independent variables. The elliptical contour shapes indicated that interactions between the variables were significant (Liu, Wei, & Liao, 2013). It was observed the average diameter of microparticles increased rapidly with effect of the applied voltage (X1), however, the distance between the needle and the collector plate (X2) did not showed significant differences (15 kV and 10 cm), respectively (Fig. 4a). The characteristics morphological and surface of microparticles with SO under optimized conditions were analyzed with a SEM. The optimal conditions for microencapsulation of SO with average diameter are predicted as 1.4775 µm, using 13.91 kV and 8.3658 cm, respectively. Under these conditions, the experimental average diameter is around1.473 µm, which is well close with the predicted value mentioned above. The difference in the average diameter could be due to the different fields analyzed or by the others variables effect of the gliadin-SO interactions such as molecular weight, viscosity, surface tension, humidity and temperature in gliadins and gliadin-SO. A previous study has found that increasing voltage applied, the size of the nanoparticles decreases (Abyadeh, Aghajani, & Gohari, 2018). The smaller microparticles were obtained from high voltage, due to the formation of a stable and most uniform cone jet mode, also to increased surface charges on polymer jets (Coulomb force).

Table 3. Experimental design matrix with results.

Run	**Independent variables**		
	Applied voltage (X_1)	**Distance (X_2)**	**Experimental Average diameter (µm)**
1	15	10	1.4795
2	15	20	1.1897
3	20	10	1.0682

4	20	20	1.2667
5	15	15	1.3964
6	20	15	1.3088
7	17.5	10	1.3798
8	17.5	20	1.3340
9	17.5	15	1.4539
10	17.5	15	1.4582
11	15	10	1.4691
12	15	20	1.2169
13	20	10	1.0693
14	20	20	1.2806
15	15	15	1.4111
16	20	15	1.2941
17	17.5	10	1.3746
18	17.5	20	1.3636
19	17.5	15	1.3472
20	17.5	15	1.3269

Table 4. Analysis of variance (ANOVA) of the predicted quadratic model to the responses total and regression coefficients calculated.

Source	Sum of squares	Degree of freedom	Mean square	F-value	p-Value (Prob. > F)
Model	0.2432	5	0.0487	29.5468	<0.0001*
(A) Applied voltaje (kV)	0.0637	1		38.6537	<0.0001*
(B) Distance (cm)	0.0029	1		1.8007	0.2010
AB	0.1132	1		68.6135	<0.0001*
A^2	0.0302	1		18.3567	0.0008*
B^2	0.0229	1		13.9090	0.0022*
Lack of fit	0.0075	3	0.0025	1.7764	Prob. > F 0.2097
Pure error	0.0155	11	0.0014		
Total error	0.0231	14			
R^2	0.9134				
$R^2_{adjusted}$	0.8825				

*Significant parameters of the model ($p < 0.05$).

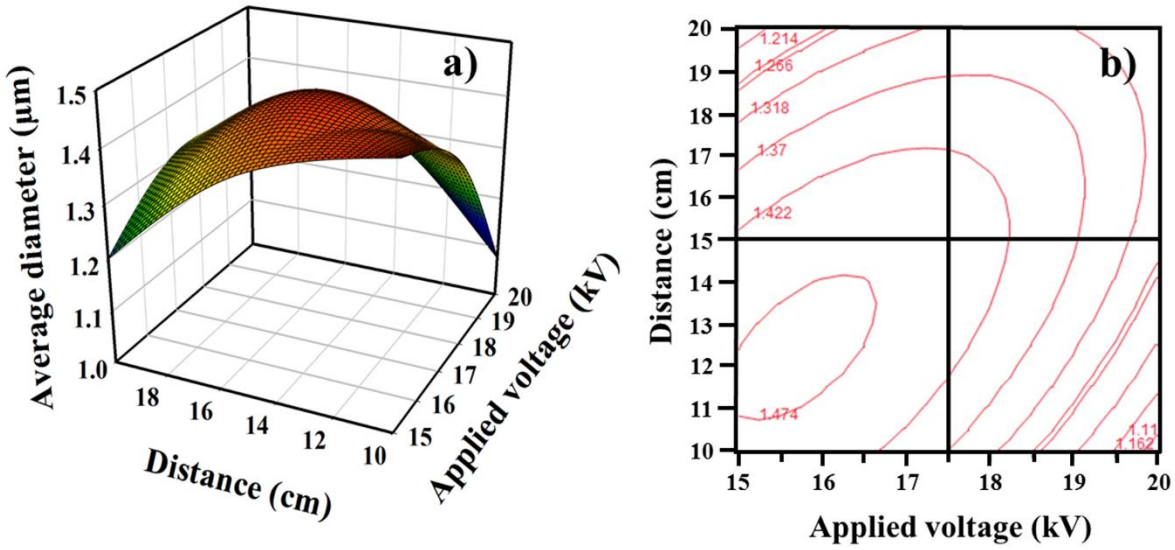

Fig. 4. Surface response plot (a) and the contour plot (b) showing effect of applied voltage (X_1) and distance between the needle and the collector plate (X_2) in the microencapsulation of sardine oil.

Encapsulation efficiency and loading capacity

The encapsulation efficiency (EE) and loading capacity (LC) was determined to assess the quality of microencapsulated SO. The SO microparticles under optimized conditions showed an EE of 98.68% and LC of 26.11%. It has been reported the content of residual oils on the surface of the microcapsules have a negative effect on the oxidative stability. Then these conditions should be important during oil encapsulation. In addition to the SO microparticles formed, pecan nutshell extracts (150 ppm) were evaluated too under optimized conditions, and their EE and LC was determined and depicted in Fig. 5 These extracts were incorporate to the SO and used as natural antioxidants to decrease or avoid the oxidation of lipids. The SO microparticles with antioxidants showed high EE and LC without any significant differences ($p > 0.05$) under optimized conditions. The high EE it is the result of migration of SO towards the core of the

ethanol-based microparticles as a result of phase separation occurred during the electrospray deposition. The lipids migrate towards the core due to the low boiling point of ethanol in contrast with water. So, the solvent in the surface decreases and causes the lipid migration towards the core. Also, the lipids migration could produce interactions between the protein and lipid molecules, and increase the SO oxidative stability (Moomand & Lim, 2014). Additionally, the SO interactions with non-polar regions on the protein surface across hydrophobic attraction undergo conformational changes in the protein, which increase their concentration at the core. The analysis of SEM confirms this theory because not SO was found on the surface of the microparticles.

There is a lot of research about the low stability of PUFAs, and their susceptibility to oxidation by environmental factors. However, the microencapsulation of PUFAs with natural antioxidants would help to protect PUFAs stability (Liu et. al., 2016; Pourashouri & Shabanpour, 2014). These results indicated that SO was well encapsulated and can be protected against adverse factors.

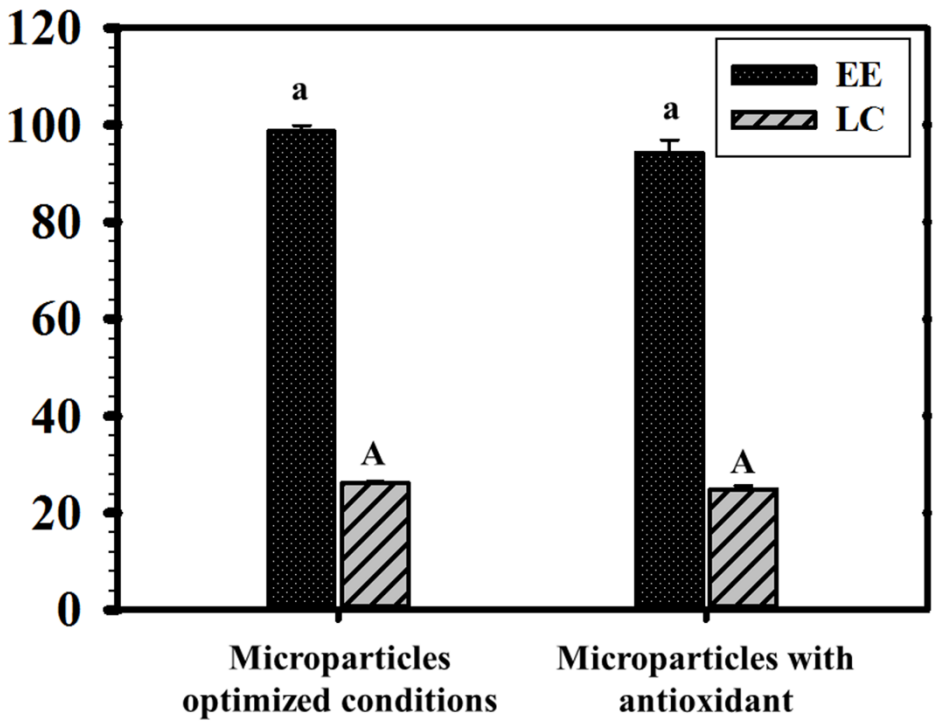

Fig. 5. Encapsulation efficiency (EE) and loading capacity (LC) of microparticles of SO under

optimized conditions. Values with different letters in the bars are significantly different ($p<0.05$).

Characterization of optimized SO microparticles

The size and outer morphology of the optimized particles powder by SEM was verified (Fig. 6). The microparticles obtained under optimal conditions were spherical, with smooth surface and some with dented surfaces, and polydispersed. The average diameter of spherical structures was 1.473 µm. The morphology o the microparticles it is important to prevent the SO oxidation and controlled release of it in the gastrointestinal tract. The dented surface occurs during the drying process or high temperature. As well this effect could be attribute to the conductivity, surface tension, concentration of polymers, viscosity of the polymer solution and applied voltage. These parameters have a high influence on the electrospray process and the particles generated (e.g. morphology and stability of Taylor cone). Gomez-Estaca, Balaguer, Gavara, & Hernandez-Munoz (2012) investigated the effects of key parameters on the process of electrospray (polymer concentration, flow rate and applied voltage) using zein protein polymer. The most important data generated by the authors indicate that low concentration and viscosity of the zein solution resulted in an increase of the droplet formation, particles of greater size and non-spherical morphologies. This information can be related to the structures observed in this study. When low viscosities were tested, particles of greater size and non-spherical morphologies were obtained.

The molecular weight profile of a polymer is also an important factor that must be known before undertaking an electrospray process. Polymers with low molecular weight lead to the formation of particles and debris, whereas high molecular weight polymers allows the formation of a great number of entanglements and the formation of fibers (Balaguer & Gavara, 2012). Gliadins are proteins with a molecular weight (MW) between 30-80 kDa. They can be easily extracted from wheat gluten using 70% (w/w) aqueous ethanol. These proteins are composed of three structurally different subclasses: ω-, α+β-, and γ-gliadins (Joye, Davidov-pardo, Ludescher, & Mcclements, 2015). In addition, as showed in the gel electrophoresis (Lane A, B and C, Fig.

S1), wheat gluten proteins mainly consisted of low molecular weight glutenins (LMW-GS) and high molecular weight glutenins (HMW-GS), also of all different subclasses of gliadins. In contrast with gliadins extract, these presented three subclasses of α+β-, and γ-gliadins and LMW-GS (less than 50 kDa) proteins. In the case of SO microparticles, a similarly molecular weight in comparison to gliadins was observed (Lane B, Fig. S1). The gliadins used in this work presented a molecular weight of 30-66 kDa (Fig. S1). During the gliadins extraction others type of proteins as high molecular weight glutenins (HMW-GS) and ω-gliadins were removed. This phenomenon suggested that the network of wheat gluten was disrupted during the extraction process and less entangled structure was formed (Liao, Luo, Zhao, & Wang, 2012).

Oxidative stability.

The oxidative stability index (OSI) value was determined by locating rapid change in the slope of the conductivity versus time curve. The OSI values for SO are presented in Table 5.The SO without antioxidant presented a OSI value around 9.48 h. When the antioxidants of pecan nutshell extract (150, 300 and 500 ppm) were added to the SO, the OSI value increased significantly ($P > 0.05$). Antioxidants at concentrations of 150 and 300 ppm did not showed significant differences, however, at 500 ppm of the antioxidants, significant differences were observed compared to the SO without antioxidants. The natural antioxidants of pecan nutshell extracts had a positive effect on the stabilization of the PUFAs presented in the SO. In this study, the most abundant fatty acids found in SO were palmitic acid, oleic acid, eicosapentaenoico acid (EPA) and docosahexaenoic acid (DHA). The DHA and EPA accounted for 82.74 % of the total PUFAs (Table S1). It is good to mention due that catechin in the major antioxidant presented in pecan nutshell extract, the catechin effect on SO oxidative stability was tested to a concentration of 150 ppm. The OSI value detected with such compound was around 16.83 h, greater than that obtained for SO with antioxidants from the pecan nutshell extract.

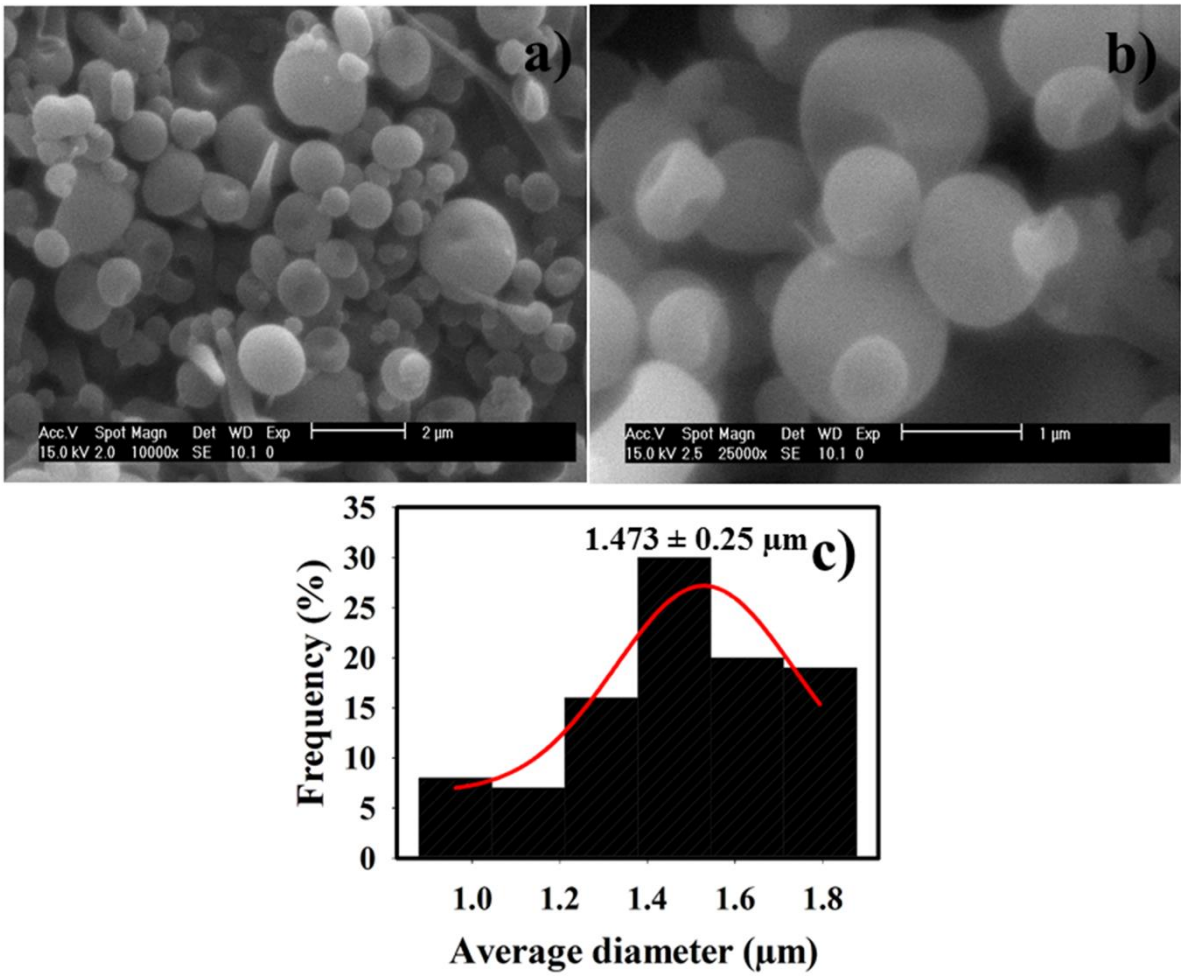

Fig. 6. Microparticles of SO under optimized conditions. SEM images of microparticles at 10,000 (a),

25,000X (b), and frequency histogram of particle size (c).

On the other hand, the oxidative stability of microparticles was higher than that of untreated SO. The microparticles under optimized conditions were more stable to oxidation (OSI = >48 h), but there were no observed significant differences ($p > 0.05$) respect to the SO microparticles with antioxidants (Table 5). The use of electrospray deposition helps to obtain microparticles with smooth surface structure according to SEM images. This smooth structures are very important for the prevention of the inward oxygen diffusion and better protection of the encapsulated SO. In other microencapsulation methods such as spray drying highly porous structure have been

reported due to the shell rupture of the microcapsules and porous structure. The oxidative stability index reported for spray drying solid microcapsules was between 22.10-26.75 h. These values are low compared with those for electrospray deposition (Carvalho, Silva, & Hubinger, 2014; Eratte, Wang, Dowling, Barrow, & Adhikari, 2014). According to the results, the use of electrospray deposition to microencapsulate SO with antioxidants resulted in good OSI values due to the high stability of the SO to the temperature tested.

Table 5. Oxidative stability index of SO and microparticles without and with antioxidants by the

Rancimat method at 65 °C.

Samples	Oxidative stability index (h)
SO	9.48 ± 0.028^{a}
SO + 150 ppm of antioxidant*	12.34 ± 0.138^{b}
SO + 300 ppm of antioxidant*	12.20 ± 0.100^{b}
SO + 500 ppm of antioxidant*	13.133 ± 0.152^{c}
Microparticles under optimized conditions	$> 48^{d}$
Microparticles with antioxidant*	$> 48^{d}$

* Pecan nutshell extract as natural antioxidant. Parts per million (ppm).

**Values are the mean ± standard deviation, n=3. Values in the same column with the same letter are not significantly different (P > 0.05).

ATR-FTIR spectroscopy.

The ATR-FTIR spectra of SO showed two main peaks (Fig. 7). The first one was at ~3011 cm^{-1}, which is related to the vibration of C–H stretching of *cis*-alkene –HC=CH– of the PUFAs. The second peak was at 1743 cm^{-1} and correspond to the vibration of group C=O, primarily from lipids and fatty acids. Besides, peaks at 2960, 2923 and 2853 cm^{-1} were observed, and they are related to the asymmetrical stretch of (C–H) from methyl (–CH3), asymmetrical stretch of group

(C–H) from methylene (–CH2) and symmetrical stretch of group (C–H) from methylene (–CH2) of lipids (Vongsvivut et. al., 2012). The peaks at ~3011 and 1743 cm^{-1}, have been related to high content of DHA and EPA in marine oils. The band at 1145 cm^{-1} in spectra of the SO have been linked to the presence of EPA and DHA in free form (Liao, Luo, Zhao, & Wang, 2012). The high content of EPA and DHA is consistent with the values obtained from quantification in gas chromatography (Table 2).

In Fig. 7 the three main peaks at 3293, 1650 and 1545 cm^{-1} were observed for gliadins spectra. These peaks correspond to the O–H vibration of water absorbed, stretching vibration of (C=O) associated with proteins (amide I band), whereas the latter one was attributed to bending vibration of (N–H) associated with proteins (amide II band) (Balaguer et. al., 2013; Liao, Luo, Zhao, & Wang, 2012). The antioxidants obtained from the pecan nutshell extracts also were analyzed by ATR-FTIR. The antioxidants showed similar spectra with other reported by other authors (Prado et. al., 2014). The peaks at ~3293, 1650 and 1145 cm^{-1} have been related to stretch of vibration of O–H group, assigned with the vibration of group C=O and which are related to C=C (aromatic ring) and C=O bonds (pyranose heterocyclic chain). After the electrospray deposition method, the peaks at 3011 and 1743 cm^{-1} are present in both treatments, suggesting that SO is present in microparticles. Besides, the displacement of amide I band indicate the formation of new chemical interactions as hydrogen bonds, electrostatic and hydrophobic interactions (Moomand & Lim, 2015; Yang et. al., 2017). The factors as the heating due at higth voltaje or an molecular rearrangement, could modify the structure of proteins and change the position of functional groups such as C=O, N–H, hydrophobic groups and chain polar. These groups that are engaged in intramolecular hydrogen bonding and electrostatic interactions, accordingly, these functional groups become available for further intermolecular interaction. Also, the unfolded proteins chains they have higher possibility to form intermolecular interactions, leading to the formation of a network that acts as the matrix for entrapping bioactive components. The β-sheet structure has been seen that intervened in the intermolecular hydrogen bondingand could be the cause of these structures (Guerrero, Kerry, & de la Caba, 2014). These chemical interactions could have a positive effect on the OSI values, and improve the shelf life of the SO.

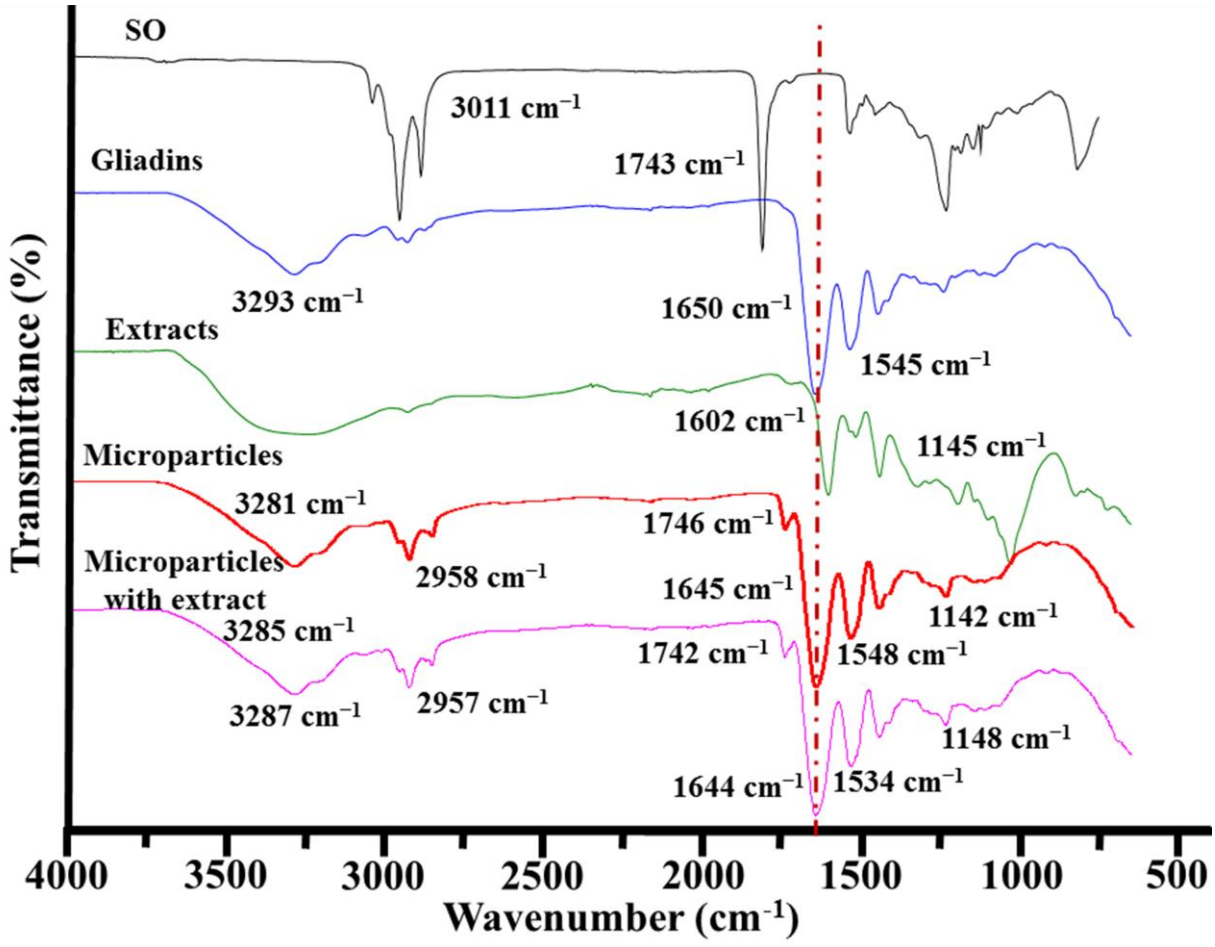

Fig. 7. ATR-FTIR spectra of SO, gliadins, antioxidant, microparticles of SO in optimized conditions and microparticles of SO with antioxidants.

Conclusions

The microparticles of SO obtained by electrospray deposition with gliadin proteins and pecan nutshell extracts were polydispersed and presented a spherical structure and smooth surface. The gliadin proteins can be used as a wall material to microencapsulate SO and improve its chemical stability. The antioxidant properties of pecan nutshell extracts can be an option for the stabilization of SO. The physicochemical characteristics of microencapsulate SO are important to improve the OSI index of SO. The FTIR spectra evidenced that SO was encapsulated into microparticles through interactions the SO, antioxidants, and gliadins. Finally, the optimal

operating conditions to obtain microencapsulated SO with average diameter and physicochemical properties adequate were obtained.

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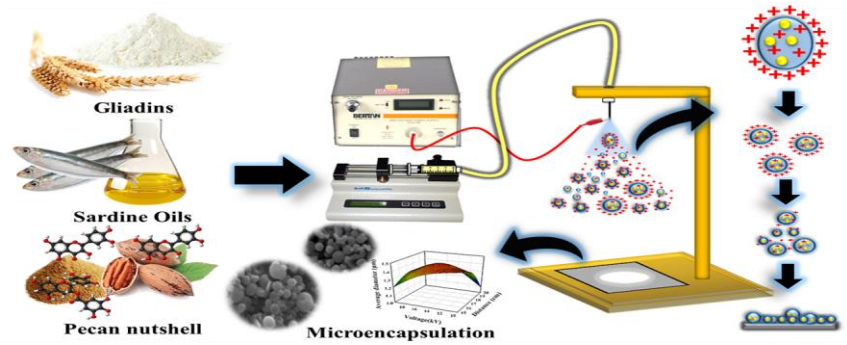
Graphical abstracts



CAPÍTULO III

Influence of solvent on the phenolic compounds from pecan nutshell [*Carya illinoinensis* (Wagenh) K. Koch] and the antioxidant capacity

Article type

Original article, Research paper

ABSTRACT

The pecan nutshell is one of the by-products abundant worldwide. Pecan nutshell by-products can be used to obtain phenolic compounds. The phenolic compounds could be using different methods of extraction like or different solvent system. Ultrasound-assisted extraction was used for the extraction of phenolic compounds, using water, ethanol and an acidified solvent. The profile of phenolic compounds was affected, due to the type of solvent used. Gallic acid, protocatechuic acid, vanillic acid, syringic acid, ellagic acid, also various types of catechins as: epigallocatechin, catechin and epicatechin were identified and quantified. This study provides the most complete description of the phenolic content of, due to identification previously not found compounds. Besides, the antioxidant activity was reduced was reduced when the acid extraction system was used. The extract with greater activity was water and ethanol. Due to the type of extraction and powerful antioxidant activity these extracts could have potential application in the food industry as natural antioxidant, for the prevention of lipid oxidation.

Keywords

Pecan nutshell. Phenolic compounds. Antioxidant activity. Solvent systems. Byproducts.

Highlights

- The main phenolic compounds extracted from pecan nutshell by different solvents were identified and quantified.
- The different solvent systems used had a great impact on the content of phenolic compounds and antioxidant activity.
- The water and ethanol showed a powerful antioxidant activity in contrast with acid-extractions.

Introduction

The deterioration of food is one of the main problems facing the food industry. The Food and Agriculture Organization of the United Nations (FAO), estimate that around of 1300 million tons of food are lost annually (FAO, 2012). The main factors contributing to the loss of food worldwide, is the chemically, physical and microbial growth. The microorganisms can produce secondary metabolites causing bad odor, texture change and pH in food. Resulting in a decrease in shelf life and an increase in the risk of foodborne diseases (Amit, Uddin, Rahman, Islam, & Khan, 2017).

For this reason, the food industry used various methodologies are used to prevent the growth of microorganisms, such as the use of antimicrobial agents. The main compounds used by the industry are of synthetic origin, for example, organic acids, organic salts, phosphates, nitrates and nitrites (Bedale, Sindelar, & Milkowski, 2016; Honikel, 2008; Sallam, 2007). Besides, they are use of synthetic antioxidants such as, propyl gallate (PG), butylated hydroxytoluene (BHT), t-butylhydroxyquinone (TBHQ) and butylated hydroxyanisole (BHA) (Liu, Wei, & Liao, 2013). These compounds are preservation tool commonly used by the food industry, and are used for to retard the growth of pathogenic, protect the safety, oxidation process and quality of foods.

However, today there is a growing demand for minimally processed foods has led the food industry to look for natural alternatives, due to potential health risks related to excessive use synthetic additives. They exist in nature a great number of natural antimicrobials derived from animals, microbial and plant sources. Consequently, the use of natural antioxidants obtained from plants is as alternatives to synthetics additives have attracted global interest in the food industry. Natural antioxidants obtained from plants are primarily phenolic compounds. In general, phenolic antioxidants can be divided into several different groups depending on their basic structure, such as phenolic acids, hydroxybenzoic acids, hydroxycinnamic acids, anthocyanins, tannins, lignans, stilbenes, coumarins, essential oils and flavonoids (Balasundram, 2006). Flavonoids and phenolic acids some of the most persistent group of phenolic compounds in plants (Vaisali, Belur, & Regupathi, 2016). These compounds have been related a powerful biological activity as antioxidants, antimutagenic, anticarcinogen properties, antihypertensive, antimicrobial capacity (Heim, Tagliaferro, & Bobilya, 2002; Palafox-carlos, Yahia, & González-aguilar, 2012).

A rich source of bioactive compounds flavonoids and phenolic acids, are the extract of plants as nutshell of pecan [*Carya illinoinensis* (Wagenh) K. Koch]. In recent years the global of by-product as nutshell of pecan production is more than 460 one thousand tons (FAO, 2016). These by-products is not used nowadays, accordingly represents a waste generated worldwide (Dórame-Miranda et al., 2019; Hilbig et al., 2018). The largest producer of pecan nut is the United States, followed by Mexico (Hilbig et al., 2018b). At the national level, Mexico contribute with 63 one thousand tons and constituting a serious environmental problem (FAO, 2016). Around 40–50% of the total pecan production represents a waste generated worldwide, which is not used. The composition of the pecan nutshell has been previously reported. The main compounds identified are gallic acid, chlorogenic acid, ellagic acid, p-hydroxybenzoic acid, (+) -Catechin, epigallocatechin and epicatechin-gallate have been reported in pecan nutshell extract (Alvarez-parrilla, Urrea-lópez, & Rosa, 2018; do Prado, et al., 2014; Hilbig, et al., 2018). The catechins one of the main antioxidants reported in the literature. These compounds have a myriad of applications as in food, pharmaceutical, medicine, and agriculture and among others. Also, catechins have been linked to potent antioxidant and antimicrobial capacity.

The type of phenolic compounds extracted form pecan nutshell, will depend on several factors such as, the extraction methods, solvent used, solvent concentration, extraction time, pH of solution, temperature, pressure and ratio of solvent to raw material (do Prado, et al., 2014; Liu et al., 2013; Nour, Trandafir, & Cosmulescu, 2016). There is a large number of the extraction methods, among those who stand out maceration, hydro distillation, soxhlet extraction, microwave-assisted extraction, supercritical fluid extraction, and ultrasound-assisted extraction (UAE) (Hilbig, et al., 2018; Nour et al., 2016). Among these, the UAE is a newfangled method of extraction and offers a series of advantages, among those the high efficiency, low solvent consumption, use of low time and temperature, reproducibility and is an easy method. The aim of this study was to analyze the effect of extraction on profile of phenolic compounds of pecan nutshell [*Carya illinoinensis* (Wagenh) K. Koch] and the relationship with antioxidant capacity.

2. Material and methods

2.1. Samples

Pecan nutshell [*Carya illinoinensis* (Wagenh) K. Koch] was supply from the group Alta, S.A. de C.V., Mexico. The Wichita variety was used, samples they were frozen and stored in the dark until its use.

2.2. Chemicals

2,2′-azinobis(3-ethylbenzothiazoline-6 sulfonic acid) (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ) and TEAC (Trolox Equivalent Antioxidant Capacity), were purchased from Sigma-Aldrich Chemical Company (St. Louis, MO). All the other chemicals used were in an analytical grade.

2.3. Ultrasound-assisted extraction of phenolic compounds

Pecan nutshell was ground in a Wiley mill (Thomas-Wiley Laboratory Mill Model 4, Thomas Scientific, USA) with a particle size up to 50 microns. The extractions were carried out in a branson ultrasonic bath (Branson Sonicador, model 1510, USA), according to Liu, Wei, & Liao, (2013) with some modifications. Samples in powder was well mixed with 30 mL of solvent (water and ethanol 60 % (w/w), also acidified solvent was used for obtain extract acid-hydrolyzed according to (Hilbig, et al., 2018) with the help of a vortex. Subsequently, the

ultrasound assisted was applied by 21 min. For increase the extraction performance. Later, the solution was centrifuged (3000 g, 4ºC, 15 min). The supernatant was mixed and filtered with a filter paper Whatman No. 4. The extract was rotaevaporated and freeze-dried. The extract powder was stored at -20ºC until further analysis.

2.3. Identification and quantification of phenolic compounds by UPLC–ESI-MS/MS analyses

The identification and quantification of phenolic compounds was performed using UPLC–ESI-MS/MS analysis was performed Acquity UPLC system (Waters Corporation, Milford, MA) coupled with a tandem Xevo TQ-S triple quadrupole mass spectrometer (Waters). According to (García-villalba, Carlos, Tomás-barberán, & Rocha-guzmán, 2017). Using an Acquity UPLC BEH C8 column (100 mm × 2.1 mm x 1.7 μm; Waters) operated at 30 °C. Concisely, the mobile phases were, acidified water with 7.5 mM formic acid (A) and acetonitrile (B): initial 95% A; 0–0.8 min, 95%; 0.8–1.2 min, 90% A; 1.2–1.9 min, 90% A; 1.9–2.4 min, 85% A; 2.4–3.7 min 85% A, 3.7–4.0 min 79% A; 4.0–5.2 min, 79% A; 5.2–5.7 min, 73% A; 5.7–8.0 min, 50% A; 8.0–9.0 min, 0% A; 9.0–11.5 min, 95% A; then the column was stabilized for 2 min. Data were collected in multiple reaction monitoring mode (MRM). Negative ionization was used for MS assays. ESI conditions were as follows: capillary voltage 1.56 kV; desolvation temperature 350 °C; source temperature, 150 °C; desolvation and cone gas, 650 L/h and 150 L/h, respectively, and collision gas, 0.14 mL/min. MRM transitions were determined from the MS/MS spectra of the existing phenolic acid standards and the results were in accordance with those found in the literature (Strnad, 2008). Data acquisition and processing were performed using MassLynx (Waters) software. Peak identification was based on a comparison of their retention times and MRM transitions with those of pure standards. Quantitative determinations of phenolic acids were carried out using standard calibration curves of the available standards.

2.4. Antioxidant capacity

Antioxidant capacity of pecan nutshell extracts was estimated using the radical scavenging reaction system with DPPH• and ABTS•+ radicals. The dried extract (0.1 mg mL^{-1}) was dissolved with 60% ethanol in triplicate.

2.4.1. ABTS•+ method

The Trolox Equivalents of Antioxidant Capacity (TEAC) value was determined according to Re et al., (1999). The methodology is based on the ability of antioxidants present in an extract to inactivate the radical ABTS•+ (2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid). The reaction was carried out using 280 μL of radical ABTS•+ with 20 μL of sample. The reaction was allowed to stand during 7 min in darkness, and the radical reduction was monitored at a wavelength of 734 nm in a 96-well microplate reader (Veloskan™ LUX multimode microplate reader, Thermo Scientific, USA). The antioxidant capacity was expressed as micromoles of Trolox equivalent antioxidant capacity per gram of extract (μmol of TEAC/g of extract).

2.4.2. DPPH• method

The ability of the extracts to inactivate the stable radical DPPH • was calculated according to Palafox-Carlos, Yahia, & González-aguilar, (2012) with some modifications. The reaction was carried out with 280 μL of radical DPPH • and 20 μL of sample. The reaction was allowed to stand for 30 min in the dark. The absorbance of the samples was read at 515 nm in a microplate reader using a 96-well microplate (Veloskan ™ LUX multimode microplate reader, Thermo Scientific, USA). The antioxidant capacity was expressed in terms of the concentration as μmol of TEAC/g of extract.

2.4.3. FRAP method

The FRAP test was carried out using the method of Benzie & Strain, (1996) with some modifications. This method is based in the increase of the absorbance of the reaction sample due to the formation of the complex 2,4,6-tripyridyl-s-triazine (TPTZ) -Fe (II) in the presence of reducing agents. The FRAP reagent was prepared mixing 10 μM TPTZ, 40 mM HCl, 20 μM FeCl3, and 300 μM acetate buffer. An aliquot of sample (20 μL) was placed in each well in a microplate and mixed with 280 μL of FRAP solution. The samples were incubated at room temperature in the dark for 30 min and the absorbance was measured at 630 nm in a microplate reader. The results were reported as μmol of TEAC/g of extract**.**

2.5. Statistical analysis

All samples were analyzed in triplicate. All data are expressed as mean value ± SD or information about RSD is provided. Graphs of the experimental data were produced using Sigma Plot 12.5 (Systat Software, San Jose, CA).

Results and discussion

The identification and quantification of phenolic compounds from UPLC–ESI-MS/MS analysis

Pecan nutshell [Carya illinoinensis (Wagenh) K. Koch] is a rich source of phenolic compounds. Polyphenols are among the most abundant antioxidant compounds in our diet and may play a key role in the prevention and treatment of non-communicable degenerative diseases. Also, due at its chemical nature could be used in food areas as potential molecules to prevent oxidation. The identification and quantification of phenolic compounds (flavonoids and non-flavonoids) in the pecan nutshell using different solvent systems are shown in Table 1.

Phenolic acids, monomers and dimers of flavan-3-ols, flavonols, flavonones, flavones and dihydrochalcones were determined. The acid extracts showed a greater content of phenols compounds, with a total phenolic content 515.627±39.45 μg/g of extract lyophilized for water-acid extraction, also of 491.816 ± 37.87 μg/g for ethanol-acid extraction. The two acid extractions showed similar content and profile of phenolic compounds. For non-acid extraction the content of phenols was less compared with acid extraction. With a content of 331.130 ± 16.96 μg/g for water and 300.653 ± 18.99 μg/g of extract lyophilized for ethanol. In addition the content of flavonoids were quantified. Water extraction showed the higher flavonoid content with 343.341± 42.46 μg/g, followed by ethanolic extraction 176.858 ± 7.07 μg/g. The ethanoic extraction he only represented the 51 % of phenolic compounds by contrast with water. For acid-extraction the flavonoid content is much less. These results agree well with the reports where it has been seen that the use of acid extractions can promote the release or hydrolysis of phenolic compounds. For example, anthocyanins are usually extracted from plant material with an acidified organic solvent, most commonly methanol. This solvent system destroys the cell membranes, simultaneously dissolves the anthocyanins, and stabilizes them. However, the acid may bring about changes in the native form of phenolic compounds by breaking down their complexes with metals and co-pigments (Garcia-salas, Morales-soto, Segura-carretero, &

Fernández-gutiérrez, 2010; Ross, Beta, & Arntfield, 2009). Besides, the hydrolysis conditions, acid or alkaline only, or in different sequence, can significantly affect the total yield and profile of phenolic acids. Quinic acid, shikimic acid, floridzin, quercetin-3-glycoside, (epi)-gallocatechin, Catechin and (epi) -catechin were found to hydrolyzed under hot acidic conditions. This may partially explain why acid hydrolysis gave ahigth yield of hydroxycinnamic and hydroxybenzoic acids. In previous investigations other groups have reported, the extracts obtained from the red wheat bran showed great amount of phenolic acids existed in a bound form in wheat bran. After of hydrolyzing the bran under alkaline or acidic conditions the phenolic acids can be released (Kim, Tsao, Yang, & Cui, 2006).

Table 1. Phenolic compounds from pecan nutshell using different extraction solvent systems.

				Solvent system			
	Phenolic compounds	**[Quantitative ion (m/z)**	**Rt**	**Water**	**Ethanol**	**Ethanol-acid**	**Water-acid**
1	**Shikimic acid**	173.18>111.07	2.35	55.48±9.28	60.58±9.19	ND	ND
2	**Gallic acid**	169.15>125.05	5.8	36.77±2.71	48.25±3.23	79.53±3.57	70.41±6.95
3	**Protocatechuic acid**	153.15>109.05	6.91	16.10±2.25	21.03±0.52	40.20±1.01	48.29±2.60
4	**4-hydroxybenzoic**	137.04>93.05	8.85	12.46±0.39	16.55±0.62	40.48±2.10	56.50±5.93
5	**Vanillic acid**	167.18>123.09	9.79	15.35±0.99	18.65±2.35	35.13±1.91	44.77±4.98
6	**Syringic acid**	197.21>153.11	10.07	5.48±3.24	9.39±1.09	38.94±3.97	47.77±4.21
7	**Tri-hydroxybenzal-dehyde**	153.15 > 83.04	12.22	15.35±0.99	ND	33.61±1.91	14.42±4.98
8	**Ellagic acid**	301.00>229.00	12.42	60.07±10.72	ND	216.72±24.93	230.12±30.16
9	**Salicylic acid**	137.04 > 93.05	14.36	0.526±0.12	ND	0.68±0.00	1.16±0.02

10	**Quinic acid**	191.20 > 85.06	2.3	117.84±12.63	124.21±6.99	2.56±0.34	1.47±0.14
11	**p-coumaric acid**	163.24 > 119.08	11.85	1.35±0.07	1.96±0.17	0.696±0.04	0.662±0.11
12	**Epigallocatechin**	305.3>125.02	7.32	19.37±2.61	12.66±3.46	ND	ND
13	**Catechin**	289.02 > 245.04	9.01	100.83±14.87	59.04±6.18	3.86±1.20	5.60±1.90
14	**Epicatechin**	289.02 > 245.04	10.32	24.460±4.94	21.26±0.67	3.104±1.18	5.36±0.57
15	**Quercetin-3-O-glucoside**	463.26>300.42	12.24	93.863±4.66	35.16±11.46	ND	ND
16	**Quercetin**	301.2>151.02	16.45	ND	ND	ND	5.98±1.30
17	**Myricetin**	317.21 > 151.03	14.55	1.85±0.30	ND	3.87±1.62	10.52±1.70
18	**Eriodictyol**	287.28 > 135.05	16.03	5.39±1.73	ND	1.51±0.05	9.50±1.77
19	**Naringenin**	271.28 > 119.06	17.73	1.19±0.55	0.451 ± 0.18	4.69±0.92	12.40±2.25
20	**Luteolin**	285.21 > 133.04	16.15	0.92±0.31	ND	ND	ND
21	**Apigenin**	269.27 > 117.04	17.77	0.96±0.62	ND	ND	ND
22	**Phloridzin**	471.34 > 273.15	14.03	94.21±21.23	47.17± 2.58	ND	ND
23	**Phloretin**	273.23 >119.06	17.65	0.26±0.12	0.100 ± 0.04	1.044±0.20	2.756±0.50

*ND= not detect. Rt= Retention time.

3.2. Antioxidant capacity

3.2.1. ABTS radicals scavenging assay

The evaluation is based on ABTS radical solution discoloration (green-colored compound) to 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid. The discoloration degree will depend on the electron-donating ability of the particles. Results are shown in Figure 1, which indicate that pecan nut shell extract showed a greater than 90% inhibition at the concentration of 250 μg/mL for water, ethanol and ethanol acid-extraction. However, activity was depending on type of solvent used and the concentration (Figure 1).

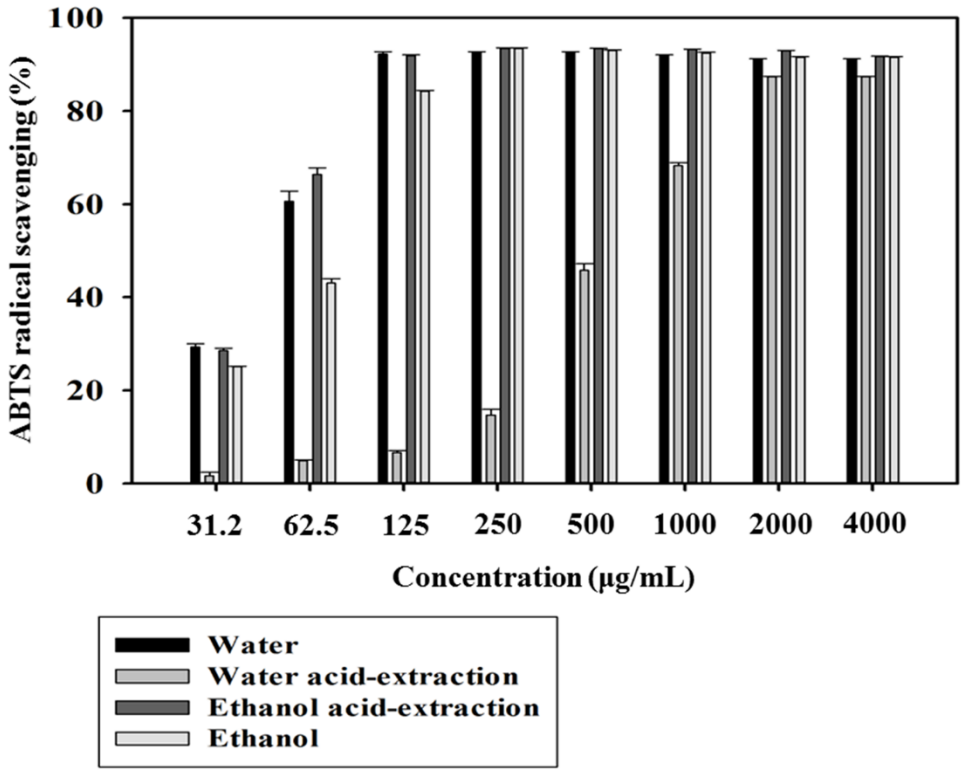


Figure 1. Antioxidant activity by ABTS radical of phenolic compounds from pecan nutshell by different solvent systems. The value is expressed as the mean ± standard deviation (n = 3).

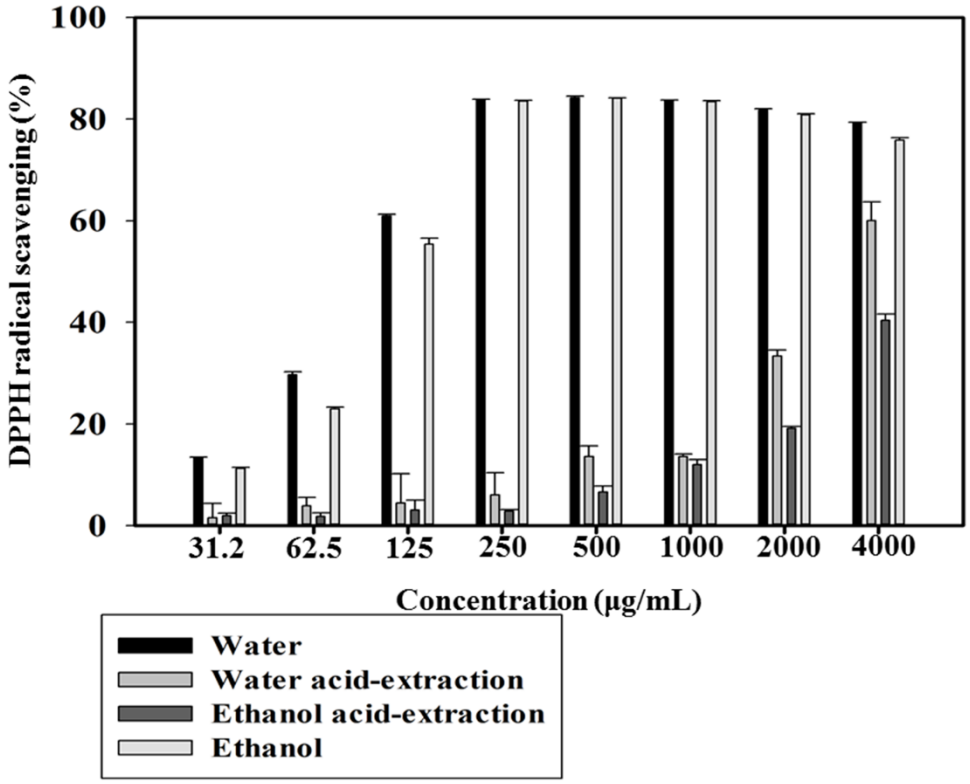


Figure 2. Antioxidant activity by DPPH radical of phenolic compounds from pecan nutshell by different solvent systems. The value is expressed as the mean ± standard deviation (n = 3).

Additionally Table 2 shows the antioxidant activity of value of antioxidant activity of phenolic compounds from pecan nutshell using different solvent systems in Trolox equivalents. The antioxidant activity of phenolic compounds occurs through the donation of a hydrogen atom from a hydroxyl group attached to the aromatic ring to the chemically unstable molecules, free radicals, thus slowing the oxidation process in cell membranes and foods rich in lipids. Moreover, they are capable of inhibiting the action of enzymes such as lipoxygenase, responsible for oxidative changes in fats and oils, improving the stability of such food products. However, several factors may affect the antioxidant capacity of these compounds, including the processing conditions involving technology, solvent use and extraction temperature used in the isolation of phenolic substances (Moure et al., 2001). The concentration, the various chemical groups for the phenolic compounds, the species and variety of pecan being cultivated, in which

antioxidants are synthesized naturally, can also significantly influence the characteristics such as shell color and astringent taste of tea obtained (do Prado et al., 2010).

Table 2. Antioxidant activity of antioxidant activity of phenolic compounds from pecan nutshell using different solvent systems

	Micromoles of Trolox equivalents per g of extract lyophilized (µmol TE/g)		
Samples	**ABTS**	**DPPH**	**FRAP**
Water	4541.40 ± 9.28^{a}	2056.93 ± 2.05^{a}	3545.03 ± 16.91^{a}
Water acid-extraction	208.36 ± 18.57^{b}	97.60 ± 3.71^{b}	183.51 ± 6.72^{b}
Ethanol	4134.79 ± 11.78^{d}	2049.60 ± 4.28^{a}	849.63 ± 9.76^{d}
Ethanol acid-extraction	129.67 ± 3.85^{c}	59.80 ± 1.97^{c}	99.28 ± 3.07^{c}

Note: the data are represented as mean values ± standard deviation (n = 3). Different letters within the same row indicate statistically significant differences ($p<0.05$).

DPPH radicals scavenging assay

The analyses are based on discoloration of a DPPH radical solution (yellow-colored compound) to form diphenylpicrylhydrazine. The degree of discoloration will depend on the hydrogen-donating ability of the antioxidant molecule. The inhibition capacity of the DPPH radical by of phenolic compounds from pecan nutshell using different solvent systems is showed in Figure 3 and Table 2. The results indicate that the acid-extraction showed lower inhibition activity against ABTS and DPPH radical. A similar behavior was reported by do Prado et al., (2014), who evaluated the effect of type of extraction on the antioxidant activity of extract of pecan nutshell. The different type of extraction showed different antioxidant activities.

Ferric-reducing antioxidant power (FRAP) assay.

The FRAP assay is based on the active compounds capacity to directly reduce Fe(III) to Fe(II). The values obtained for different solvent systems are shown in Table 2. The water and ethanol showed greater capacity reduce Fe(III), these capacity could be at the presence of catechins in these extracts, previous studies has been reported the EGCG as an effective iron chelator (Reznichenko et al., 2006). Besides, previous studies that more than one method is essential to calculate the antioxidant capacity of active compounds in vitro, because of the differences in their ability to produce free radicals (Pérez-Jiménez et al. 2008). According to antioxidant activity results in the present study, the extractions of water and ethanol possess the ability to free radical scavenge by multiple mechanisms.

Conclusions

The extracts of pecan nutshell for each of the solvent systems showed the presence of a wide variety of phenolic compounds, these compounds are plant secondary metabolites that possess various biological activities. Gallic acid, protocatechuic acid, vanillic acid, syringic acid, and ellagic acid, also various types of catechins as: epigallocatechin, catechin and epicatechin were identified and quantified. This study provides the most complete description of the phenolic content, due to identification previously not found compounds. Besides, the antioxidant activity was dependent on the type of extraction used. This may be due to the variety of antioxidants identified in each of the extracts. The water and ethanol extracts presented the highest activity by ABTS, DPPH y FRAP, in contrast with acid-extractions. This high activity could be due to the presence of catechins. Finally, this type of extracts could be used in food areas as natural antioxidants for prevent lipid oxidation.

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Conflict of Interest: The authors declare they have no conflict of interest.

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CONCLUSIONES

La cáscara de nuez pecana (*Carya illinoinensis*) representa una excelente fuente de carbono para la producción de celulosa bacteriana con *Gluconacetobacter entanii.*

La metilcelulosa obtenida a partir de la celulosa de la bacteria *Gluconacetobacter entanii* no presentó las propiedades fisicoquímicas adecuadas para microencapsular el aceite de sardina por el método de electroaspersión.

Microcapsulas de gliadinas como material de pared y compuestos fenólicos de la cáscara de nuez como agentes antioxidantes, fueron obtenidas a través de un proceso de optimización por elctrospray, dichas microcapsulas tuvieron la capacidad de encapsular aceite de sardina.

Las catequinas fueron los principales compuestos extraídos de la cáscara de nuez, y a quien se le atribuye la estabilización del aceite de sardina durante su almacenamiento.

La microencapsulación del aceite de sardina en una matriz de gliadinas, adicionado con compuestos fenólicos de la cáscara de nuez pecana (*Carya illinoinensis*), permitió proteger al aceite de sardina contra la oxidación.

RECOMENDACIONES

Estudiar el efecto del grado de sustitución a partir de celulosas funcionalizadas químicamente.

Evaluar el efecto del voltaje, peso molecular y el tipo de solvente en celulosa y metilcelulosa para formación de micropartículas por electroaspersión.

Realizar pruebas de toxicidad y probar los microencapsulados obtenidos utilizando sistemas biológicos *in vivo* o *in vitro*. Tales como: pruebas de digestibilidad, bioabsorción y modelos murinos.

Incorporar los microencapsulados obtenidos en un alimento para ver el efecto potencial como alimento funcional.

Obtener un registro de patente para los materiales obtenidos en este trabajo de tesis.