PONTIFICIA UNIVERSIDAD CATOLICA DE CHILE
ESCUELA DE INGENIERIA

MUCILAGE FROM CHIA SEEDS (*Salvia hispanica*): MICROESTRUCTURE, PHYSICO-CHEMICAL CHARACTERIZATION AND APPLICATIONS IN FOOD INDUSTRY

LORETO MUÑOZ HERNÁNDEZ

Thesis submitted to the Office of Research and Graduate Studies in partial fulfillment of the requirements for the Degree of Doctor in Engineering Sciences

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Santiago de Chile, July, 2012

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A mi maravillosa familia, Diego, Kta y Rafa; por el gran apoyo, paciencia y amor, por estar conmigo en todos los momentos de la vida. Gracias por permitirme emprender esta aventura en la que en muchos momentos significó que los dejara solos.

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ABSTRACT

Salvia hispanica L. is an oilseed commonly known as chia. It was one of the main crops of the pre-columbian cultures, being exceeded just by the corn and beans. In the Conquer era, a series of botanical species existed, four of them protruded in the nutritional point of view: amaranth (*Amaranthus hypochondriacus*), beans (*Phaseulus vulgaris*), chia seeds (*Salvia hispanica L*.) and corn (*Zea mays*). The whole and ground chia seed was used as food, but moreover through pressing, oil was obtained, which was subsequently used as base for face and body paintings. The Aztecs received the chia seed as an annual tribute from the people under their domain and was given to the gods as an offer in religious ceremonies.

This seeds have a small, oval and flatted shape and they measure between 2 and 2.5 mm length, 1.2 and 1.5 mm width and 0.8 to 1 mm of thickness. Its colour varies from dark brown to black, sometimes grey or even white. The dry and clean seed can be kept for years because it has antioxidants that prevent the deterioration of essential oils. One of the main properties of this seed is that it is natural source of omega – 3 (corresponding to 75 % of the total seed oil) and omega – 6. It also has significant concentrations of natural antioxidants, primary and synergistic such as chlorogenic acid, caffeic acid, quercetin and kaempferol, soluble and insoluble fiber, vitamins and minerals. The seed also has significant amount of dietary fiber compared with other

fruits and seeds. When the seed is placed in water, it exudes a mucilaginous polysaccharide that surrounds it. This mucilage has interesting properties for food, care and pharmaceutical industries. It has been reported that when mucilage from chia is consumed, it aids digestion while the whole seed is a nutritive food.

To determine the potential use of this mucilage as food ingredient, it is necessary, in first instance to study the seed microstructure to assess where the mucilage is located within the seed and the exact mechanism by which mucilage is released during extraction.

The seed morphology was evaluated using stereomicroscopy (SM), optical microscopy (OM) and scanning electron microscopy (SEM). For stereomicroscopy, the seeds were separated into groups by color and conditioned at three levels of relative humidity (RH), 20-25%, 40-50% (room temperature) and 75% inside desiccators. Silica gel was used to adjust the RH to 20 and 25% with measurement every 2 days for 15 days until equilibrium was attained, before the seeds were put into the desiccator. For the 75% RH, saturated solution of NaCl was used in a similar manner, while for the 40 – 50% RH, no chemical was used. RH in all cases was accurately measured using a hygrometer and after 15 days (equilibrium), the seeds were placed inside the desiccator and kept in these conditions at 20°C for other 15 days. After this time the seeds were removed from the desiccators and 100 units of each sample were placed vertically and horizontally in a slide. The three main dimensions: length, width and thickness, as well diameter, sphericity, surface area, density and weight of 1000 units were measured.

For microscopic observations, a light microscope was used at magnification of 10x and 40x. Whole seeds were dyed with three stains: firstly, seeds were hydrated with safranin distilled water solution (0.01%) for 24 hours; the second group was stained with fast green and safranin, and lastly, stained with lugol and fast green. Images were taken with a digital camera connected to the microscope and data stored in a computer. Afterwards, the images were analyzed with the software Image Pro Plus 6.0.

For scanning electron microscopy, mature dried seeds without any treatment, were sputter coated with gold and subsequently examined. ADDA II was used as interface

between microscope and computer; the images were taken and then analyzed with the software AnalySIS® ver. 3.2

The extraction of the mucilage was optimized using Box-Behnken experimental design with the independent as variables temperature (20-80 °C), pH (4 - 8) and proportions seed:water (1:20; 1:40) and the dependent variable (response) was the extraction yield. After the mucilage was extracted, it hydration was also studied using experimental design of Box Behnken model 3^3. High and low levels were selected for each independent variable: temperature (20 – 80 °C), pH (3 – 9) and ionic strength (0 – 1%), determined by $CaCl_2$, NaCl and KCl respectively. Thereafter, the mucilage was analyzed for chemical, thermal and functional properties as well as proximate analysis, organic elemental analysis, FT-IR, thermogravimetric, DSC, monosaccharide determination, solubility, rheology and emulsifying and foaming properties. Finally, some applications of the mucilage in the food industry were studied. Firstly, the mucilage was used to produce edible films and also a dairy dessert was produced where the commercial stabilizer was totally replaced with the mucilage from chia seeds. Edible films were obtained using a blend of the mucilage and whey protein concentrate in proportions 1:3 and 1:4 at pH 7 and 10 using glycerol as plasticizer. Transmission electron microscopy was used to investigate each dispersion from the previously formed film and each edible film was also observed by scanning electron microscopy while physical properties, color, opacity, moisture content, solubility, water vapour barrier and mechanical properties, were analyzed for. Finally, a dairy dessert was formulated using a commercial formula and subsequently was subjected to sensory evaluation with triangular and preference test, rheological characterization, syneresis, color and viscosity.

Chia seeds were small (1.87± 0.1 mm length, 1.21 ± 0.08 mm width and 0.88 ± 0.04 mm thickness) with an oval flattened shape and ranged in colour from dark coffee to beige with small darker spots. Length, width and thickness in the two groups of seeds increased when the relative humidity increased. Adsorption of moisture by seeds induced changes mainly when they were exposed to 75% RH resulting in increase in

geometric mean diameter and decrease in sphericity and surface area in beige seeds. However, there was no significant difference between the bulk density of beige and dark seeds (25 % RH 0.70 ± 0.006 g/cm3 ; ambient RH 0.71 ± 0.02 g/cm3; 75 % RH 0.62 ± 0.01 g/cm3).

It is presumed that the mucilage is located in the outer cells that form the seed coat, called mucilaginous cells. The seed coat or testa has a thickness of 13 ± 0.41 μm and was composed of three layers: an outer layer, formed by rectangular thin-walled cells with sizes of 4.2 ± 0.26 μm, where presumably the mucilage is localized; a scleroid layer of long and thin cells resembling fibers, and the endocarp, a thin and inner layer. This conformation has not been reported on *Salvia hispanica L*.

When the chia seed was hydrated, the mucilage was exudated and spiral filaments (mucilage fibers) become apparent. These filaments begin to expand until fully stretched to achieve maximum hydration after 2 hours and new structures on seed surface became apparent. These new structures called columella, have a *"volcano-shaped"* conformation and were uniformly distributed on the surface. At the base of this structure was a small cluster of spheres of 11.6 ± 1.4 μm diameter that could be easily seen using a safranin reagent. The mucilage is present inside the testa epidermal cell of mature chia seeds and when they come into contact with water it immediately expands rupturing the primary cell layer that protrudes from these epidermal cells thus surrounding the seed.

When the mucilage is fully hydrated it forms a transparent "capsule" surrounding the seed adhered with great tenacity and when many seeds were hydrated in water, a highly viscous solution was formed. Preliminary studies of hydration of whole seeds demonstrated that after 2 hour of hydration, the total weight of seeds became constant and water absorption was completed. This was considered the maximum time to perform the extraction and hydration experiments.

The optimization of the extraction process was achieved at temperatures close to 80°C and 1:40 seed:water ratio with a 7% of yield. Chia isolated mucilage had a great capacity of hydration because a sample of 100 mg of mucilage was able to absorb 2.7 g

of water, 27 times its own weight, while the whole seed hydration capacity is only 12 times.

An increase in salt concentration induced a decrease in water absorption capacity. The higher hydration was achieved at basic pH close to 9; low salts concentration and temperatures close to 80°C.

Proximate analysis shows that the total sugar content is 48±0.55%, protein is 4±0.05%, ash is 8±0.57% and fat is 1.78±0.02% while elemental analysis showed levels of nitrogen, carbon, hydrogen and oxygen of 1.38±0.04%, 37.99±1.19%, 5.64±0.13% and 47.27±1.02% respectively. . The monosaccharide composition was 16.78±0.59% D-xylose+D-mannose, 2.11±0.18% D-arabinose, 6.77±0.30% D-glucose, 3,9±0.32% galacturonic acid and 12.1±2.30% glucuronic acid, with 41.66% total sugars. The mucilage showed an extrapolated thermal decomposition temperature at 271.2°C. Additionally FT – IR was used to compare functional groups from mucilage and xanthan gum. The main bands observed were assigned to –OH from hydroxyl group, C-H aliphatic, (C=O) to carboxylates and carboxylic groups, -COO corresponds to carboxylate from uronic acids and finally C-O as ester group. The main bands to both polysaccharides were similar, only differences in intensity were observed. DSC spectrum together with fusion point show the mucilage temperature stability and the feasibility of using it in processes that involves high temperature since it does not have an exact fusion point but only degraded at 320 °C. The mucilage was totally soluble in water at studied conditions: temperature (30, 60, 70 and 90°C), concentration (0.15; 0.25; 0.5%) and centrifugation conditions (800 and 2000 g), the mechanism responsible for this behavior can be attributed to its complete dispersion followed by the solubilization; this allows water to penetrate into the swollen particles resulting a complete interaction between the macromolecules.

Edible films formed by dissolving mucilage and whey protein concentrate in two proportions (1:3; 1:4) at pH 7 and 10, using glycerol as plasticizer showed good mechanical properties and low water vapor permeability. The effects of pH and polysaccharide:protein ratio on forming dispersions were studied using TEM. When the

two components were mixed, microstructures with sphere and ovoid conformation were observed. This elements or aggregates take the form of white masses due to the negative staining procedure. The formation of aggregates could be attributed to electrostatic interactions between mucilage and whey proteins concentrate. However, at the investigated pH, which is higher than isoelectric point (pI) of whey proteins (4.5), the components are negatively charged, promoting electrostatic repulsion between them, resulting in the formation of aggregates. In this case, soluble complexes can be formed when the two biopolymers have a negative net charge (pH>pI) due to existence of positively charged patches on the protein interacting with the anionic polysaccharide.

The microstructural differences in edible films can also be attributed to aggregates formation produced for the interaction between polysaccharides and proteins.

The addition of mucilage to edible films had significant influence on tensile strength, elongation at break and less influence but significant on water vapour permeability. Films produced with higher proportion of mucilage showed better water vapor barrier properties. The addition of mucilage in the film formulation also increased the film resistance and flexibility. The pH had positive influence on tensile properties and in terms of water vapor permeability, significant differences were observed in the samples with edible films prepared at pH 10 having superior barrier properties. The use of this new polysaccharide is an option to modify and improve the physical properties of hydrophilic edible films.

In conclusion, Salvia hispanica seeds have a great potential to be used as a source of nutrients and nutraceutical, of great interest to science, technology and food engineering. The seeds have, among other nutrients, mucilage. This mucilage is mainly composed of polysaccharides located in the three layers forming the seed coat and can be easily removed after hydration. The mucilage is a potential source of hydrocolloids with different and attractive functional properties such as, water absorption capacity, emulsifying and foaming properties and highly soluble in cold and hot water. The mucilage can also been incorporated into different food and formulations, has ability to produce edible film in combination with proteins improving mechanical and functional

properties of the films. And also is possible to replace totally the stabilizer used in the commercial formula by mucilage from chia seed.

Finally, the mucilage from chia seed is a new functional ingredient with great potential to be exploited in industries such as food, feed and pharmaceutical.

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PONTIFICIA UNIVERSIDAD CATOLICA DE CHILE
ESCUELA DE INGENIERIA

MUCÍLAGO DE SEMILLAS DE CHÍA (*Salvia hispanica*): MICROESTRUCTURA, CARACTERIZACIÓN FÍSICO-QUÍMICA Y APLICACIONES EN LA INDUSTRIA ALIMENTARIA

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LORETO MUÑOZ HERNÁNDEZ

RESUMEN

Salvia hispanica L. es una semilla oleaginosa conocida comúnmente como chía. Fue uno de los cultivos principales de las sociedades precolombinas, siendo superado sólo por el maíz y los porotos (frijoles). En el tiempo de la Conquista existían una serie de especies botánicas, cuatro de ellas sobresalían desde el punto de vista nutricional: amaranto (*Amaranthus hypochondriacus*), frijoles (*Phaseulus vulgaris*), chía (*Salvia hispanica L.*) y maíz (*Zea mays*). La semilla de chía entera y molida era usada como alimento, pero además a través de prensado se obtenía aceite, el cual se utilizaba posteriormente como base para pinturas para el rostro y el cuerpo. Los aztecas recibían semilla de chía como tributo anual de los pueblos bajo su dominio y era entregada a los dioses como ofrenda en las ceremonias religiosas. Las semillas son pequeñas con forma ovalada y achatada, miden entre 2 y 2,5 mm de largo, entre 1,2 y 1,5 mm de ancho y 0,8 – 1 mm de espesor. Su coloración va de café oscuro a negro, a veces gris o blanco; las semillas blancas son de mayor peso, ancho y espesor que las oscuras.

La semilla limpia y seca puede conservarse durante años ya que posee antioxidantes que evitan el deterioro de los aceites esenciales que contiene.

Una de las principales propiedades que posee esta semilla es ser fuente natural de ácidos grasos omega – 3, que corresponde al 75% del aceite total de la chía y omega – 6,

posee concentraciones importantes de antioxidantes naturales, primarios y sinérgicos, como son el ácido clorogénico, ácido caféico, miricetina, quercetina y kaempferol, fibra soluble e insoluble, vitaminas y minerales. También posee una cantidad importante de fibra dietética, que se encuentra en mayor cantidad en comparación a otras frutas y semillas y que el sistema digestivo no puede digerir. Una gran particularidad de la semilla es que cuando es puesta en un medio acuoso exuda un polisacárido mucilaginoso que la rodea. Este mucílago posee interesantes propiedades para la industria alimentaria, cosmética y farmacéutica.

Se ha reportado que el consumo del mucílago de chía facilita la digestión y que junto con la semilla forma un alimento nutritivo.

Para determinar la potencial utilización de este mucílago como ingrediente es necesario en primer lugar, estudiar la microestructura de la semilla con el objetivo de adquirir mayor información a cerca de la ubicación del mucílago dentro de la semilla y conocer el mecanismo por el cual el se libera durante la extracción.

La morfología de la semilla de chía se estudió por medio de estereomicroscopía (SM), microscopía óptica (OM) y de barrido electrónico (SEM). Para el uso del estereomicoscopio las semillas fueron separadas en grupos por color y acondicionadas a tres niveles de humedad relativa (RH) dentro de desecadores, 20-25%, 40-50% y 75%. La humedad relativa entre 20-25% se ajustó empleando silica gel; previamente se acondicionó el desecador durante 15 días midiendo la RH por medio de un higrómetro cada 2 días hasta alcanzar el equilibrio. La humedad relativa del 75% se alcanzó usando una solución saturada de NaCl y se procedió de igual manera y para humedad relativa entre 40 – 50% el desecador se mantuvo vacío. Para todos los casos la humedad relativa fue controlada y al cabo de 15 días (equilibrio) las semillas fueron situadas dentro de cada desecador y mantenidas en estas condiciones a 20°C por otros15 días. Al cabo de este tiempo las semillas se retiraron de cada desecador y se dispusieron 100 unidades de cada muestra en portaobjetos en forma vertical y horizontal. Posteriormente se determinaron sus tres principales dimensiones para cada humedad relativa: largo, ancho

y espesor, además de diámetro, esfericidad, superficie, densidad y peso de 1000 unidades.

Las observaciones microscópicas se realizaron a través de un microscopio de luz con aumentos de 10x y 40x. Las semillas fueron teñidas con tres diferentes técnicas; inicialmente se tiñeron con una solución safranina en agua (0,01%) por 24 horas, otro grupo se tiñó con "fast green" y safranina y el último grupo con lugol y "fast green". Posteriormente se tomaron fotografías usando una cámara digital conectada al microscopio y las imágenes se almacenaron en computador hasta su posterior análisis por medio del programa Image Pro Plus 6.0.

Para microscopía de barrido electrónico las semillas maduras secas sin tratamiento fueron recubiertas con oro a través de pulverización catódica y posteriormente examinadas. Se usó una interfase ADDA II entre el computador y el microscopio y las imágenes fueron capturadas y analizadas posteriormente por medio del programa AnalySIS® ver. 3.2

La extracción del mucílago se investigó empleando diseño de experimentos con modelo Box-Behnken y posterior optimización, las condiciones incluyeron como variables independientes temperatura (20 – 80 °C), pH (4 – 8) y proporción semilla:agua (1:20; 1:40) y la variable respuesta fue el porcentaje de extracción. Luego que el mucilago fue extraído, se procedió a estudiar su hidratación empleando también un diseño de experimentos modelo Box-Behnken 3^3. Se seleccionaron niveles altos y bajos para cada variable independiente: temperatura (20 – 80 °C), pH (3 – 9) y fuerza iónica (0 – 1%) determinada por $CaCl_2$, NaCl y KCl en forma separada. A continuación el mucílago fue sometido al estudio de sus propiedades químicas, térmicas y funcionales a través del análisis químico proximal, orgánico elemental, FT-IR, termogravimetría, DSC, determinación de monosacáridos, solubilidad, reología, propiedades emulsificantes y espumantes. Finalmente se estudiaron algunas aplicaciones en la industria de alimentos. Como primera aplicación se estudió la elaboración de películas comestibles y posteriormente se elaboró un postre lácteo donde el estabilizante empleado comercialmente se remplazó en su totalidad por el mucílago de chía. Las

películas comestibles fueron obtenidas empleando una mezcla del mucílago y proteínas de suero concentrado en proporción 1:3 y 1:4 a pH 7 y 10 y usando glicerol como plastificante. Cada dispersión formadora de película fue examinada usando microscopía de trasmisión electrónica y cada una de las películas fue analizada posteriormente a través de microscopía de barrido electrónico. Se determinaron a su vez las propiedades físicas, análisis de color, opacidad, contenido de humedad, solubilidad, propiedades de barrera al vapor de agua y propiedades mecánicas de cada una de las películas del estudio. Finalmente se elaboró un postre lácteo en base a una formulación comercial y se procedió a realizar una evaluación sensorial que constó de un test triangular y de preferencia, caracterización reológica, determinación de sinéresis, color y viscosidad.

Las semillas de chía son pequeñas (1,87± 0,1 mm largo, 1,21 ± 0,08 mm ancho y 0,88 ± 0,04 mm espesor) de forma oval aplanada y de colores que van desde café oscuro a crema con pequeños puntos oscuros. Cuando la humedad relativa aumentó, se observó un aumento en el largo, ancho y espesor de los dos grupos de semillas. La adsorción de humedad induce cambios en las semillas principalmente cuando fueron expuestas a 75% de humedad relativa, resultando en un aumento en el diámetro medio geométrico y una disminución de la esfericidad en las semillas color crema. Sin embargo, no se observaron diferencias significativas en la densidad aparente para semillas crema y oscuras (25 % RH 0.70 ± 0.006 g/cm^3; ambiente RH 0.71 ± 0.02 g/cm^3; 75 % RH 0.62 ± 0.01 g/cm^3).

Se presume que el mucílago está localizado en las células externas que forman la testa (cáscara), estas estructuras son denominadas células mucilaginosas. La testa tiene un grosor de 13 ± 0.41 μm y está compuesta de tres capas, una externa formada por células delgadas rectangulares de 4.2 ± 0.26 μm donde aparentemente se encuentra el mucílago, una capa de escleroides, compuesta por células largas y delgadas similares a fibras y el endocarpio, una delgada capa interna. Este tipo de conformación no había sido reportada para la semilla de *Salvia hipanica*.

Inmediatamente cuando la semilla se pone en contacto con agua el mucílago comienza a aparecer y filamentos en forma de espiral (fibras del mucílago) se hacen evidentes en la superficie. Estos filamentos comienzan a expandirse hasta estar completamente estirados al alcanzar la hidratación máxima al cabo de 2 horas y se revelan nuevas estructuras en la superficie de la semilla. Estas nuevas estructuras se denominan *columella*, poseen forma de volcán y están uniformemente distribuidas en toda la superficie de la semilla. En el extremo de cada *columella* se observó además un racimo de pequeñas esferas de 11.6 ± 1.4 μm de diámetro que fueron fácilmente visibles cuando se tiñeron con safranina. El mucílago está presente dentro de las células epidérmicas de la testa de la semilla madura y cuando ésta se pone en contacto con agua se rompe la primera capa de células permitiendo la salida de las fibras de mucílago que posteriormente rodean la semilla.

Cuando el mucílago se encuentra completamente hidratado forma una cápsula transparente que rodea la semilla adherida con gran tenacidad y cuando muchas semillas se hidratan forman una solución altamente viscosa y estable. Estudios preliminares demostraron que al cabo de 2 horas de hidratación el peso total de las semillas permanece constante y la absorción de agua está completada. Este tiempo fue considerado el máximo para la realización de la extracción y posterior hidratación.

La optimización en el proceso de extracción se logró a temperaturas cercanas a 80°C y proporción semilla:agua de 1:40, obteniéndose un 7% de rendimiento. Se observó que el mucílago aislado posee una gran capacidad de retener agua debido a que 100 g fueron capaces de absorber 2,7 g de agua, es decir, tiene la capacidad de retener 27 veces su peso en agua, a diferencia de la semilla que se hidrata solamente hasta 12 veces.

Se determinó que un aumento en las concentraciones de las sales estudiadas induce una disminución en la capacidad de retención de agua. Se observó que la máxima hidratación se logró a bajas concentraciones de sales, pH cercano a 9 y temperaturas alrededor de 80 °C.

El análisis químico proximal mostró un contenido de azúcares totales de 48±0,55 %, proteínas de 4±0,05 %, cenizas 8±0,57 %y lípidos de 1,78±0,02%; mientras que el análisis elemental mostró que el mucílago posee niveles de nitrógeno, carbono, hidrógeno y oxígeno de 1,38±0,04%, 37,99±1,19%, 5,64±0,13% y 47,27±1,02% respectivamente. La composición de monosacáridos fue 16,78±0,59% D-xilosa+D-manosa, 2,11±0,18% D-arabinosa, 6,77±0,30% D-glucosa, 3,9±0,32% ácido galacturónico y 12,1±2,30% ácido glucurónico, con un 41,66% de azúcares totales. El mucílago mostró una temperatura de descomposición térmica extrapolada (TDT) de 271,2 °C. Adicionalmente se usó FT-IR para comparar los grupos funcionales del mucílago con goma xantana. Las principales bandas registradas fueron asignadas al –OH del grupo hidroxilo, -C-H alifático, (C=O) para el grupo de ácidos carboxílicos y aromáticos, –COO correspondiente al grupo carboxilato de los ácidos urónicos y finalmente C–O como grupo ester. Las bandas observadas en ambos polisacáridos fueron muy similares, solo se visualizaron diferencias en intensidad. El espectro DSC en conjunto con el punto de fusión, mostraron una temperatura estable y la posibilidad del empleo del mucílago en procesos que involucren altas temperaturas, ya que no presenta un punto de fusión exacto, solamente se degrada a los 320°C. El mucílago fue completamente soluble en agua bajo las condiciones estudiadas: temperatura (30, 60, 70 y 90 °C), concentración (0,15; 0,25; 0,5 %) y condiciones de centrifugación (800 y 2000 g); este comportamiento puede deberse a la completa dispersión del mucílago seguida de la solubilización, lo que permite que el agua penetre en las partículas hinchadas provocando una completa interacción entre las macromoléculas.

Las películas comestibles formadas a partir del mucílago de chía (MC) y proteínas de suero concentrado (WPC), en proporciones 1:3 y 1:4 MC:WPC a pH 7 y 10 usando glicerol como plastificante, presentaron excelentes propiedades mecánicas y baja permeabilidad al vapor de agua.

Se analizó el efecto del pH y proporción polisacárico:proteína sobre las dispersiones formadoras de películas a través de TEM. Se observa que cuando ambos componentes son mezclados aparecen microestructuras de conformación esférica y

ovoide. Estos elementos o agregados, adquieren la forma de masas blancas debido al procedimiento de tinción negativa. Esta formación de agregados puede atribuirse a interacciones electrostáticas entre el mucílago y las proteínas del suero concentrado. Sin embargo, al pH investigado, que es más alto que el punto isoeléctrico (pI) de las proteínas (4,5), los componentes están negativamente cargados; esto promueve una repulsión electrostática entre ellos y da como resultado la formación de agregados. En este caso, los complejos solubles pueden formarse cuando los dos biopolímeros están cargados negativamente (pH>pI) debido a la existencia de parches positivamente cargados en la proteína interactuando con el polisacárido aniónico. Las diferencias microestructurales en las películas terminadas observadas a través de SEM también pueden ser atribuidas a la formación de los agregados producidos por la interacción entre polisacáridos y proteínas.

La proporción de mucílago adicionada tiene influencia sobre los valores registrados en resistencia a la tracción, alargamiento a la ruptura y menor influencia, pero no menos significativa sobre la permeabilidad al vapor de agua. Las películas elaboradas con mayor proporción de mucílago mostraron mejores propiedades de barrera al vapor de agua. Con la incorporación del mucílago en las películas además mejoró la resistencia y la flexibilidad. El pH tuvo una influencia positiva sobre las propiedades de tracción y en términos de permeabilidad; los films preparados a pH 10 mostraron mejores propiedades de barrera. El uso de este nuevo polisacárido es una opción para modificar y/o mejorar las propiedades físicas de películas comestibles hidrofílicas.

En conclusión, las semillas de *Salvia hipanica* poseen un enorme potencial como fuente de nutrientes y nutracéuticos, de gran interés para la ciencia, tecnología e ingeniería de los alimentos. Las semillas poseen, dentro de otros nutrientes, un mucílago compuesto principalmente por polisacáridos que se encuentra ubicado en las tres capas que forman la testa (cascarilla) y puede ser extraído fácilmente previa hidratación. El mucílago obtenido de la semilla es una potencial fuente de hidrocoloides con diferentes propiedades funcionales atractivas para la industria, tales como; gran capacidad de

retención de agua, emulsificante, espesante, estabilizante en la formación de espumas, altamente soluble en agua fría y/o caliente. El mucílago de Salvia hispanica además puede ser incorporado en diferentes alimentos y formulaciones; tiene la capacidad de formar películas comestibles en combinación con proteínas mejorando las propiedades mecánicas y funcionales de las mismas. El mucílago puede remplazar en una formulación comercial el 100 % del estabilizante empleado tradicionalmente, proporcionando a un postre lácteo iguales y/o mejores propiedades sensoriales.

Finalmente se concluye que el mucílago de *Salvia hispanica* es un nuevo ingrediente funcional con grandes posibilidades de ser explotado en las industrias de alimentación humana, animal y farmacéutica.

Miembros de la Comisión de Tesis Doctoral
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Santiago, Julio, 2012

LIST OF PAPERS

This thesis is based on the following manuscripts referred in the text by their respective chapters:

Chapter 3 Muñoz, L. A., A. Cobos, Diaz, O., Aguilera, J. M., (2012). **Chia seeds: Microstructure, mucilage extraction and hydration.** Journal of Food Engineering 108(1): 216-224.

Chapter 4 Muñoz, L.A., Aguilera J.M, Cobos, A., Díaz, O., Zúñiga M.C., Marambio O. **Chemical, functional and thermal properties of the mucilage extracted from chia (Salvia hispanica) seeds.** Carbohydrate Polymers (submitted)

Chapter 5 Muñoz L. A., Aguilera J.M., Rodriguez-Turienzo L., Cobos A., Diaz O., **Characterization and microstructure of films made from mucilage of Salvia hispanica and whey protein concentrate**, Journal of Food Engineering, 111(3): 511-518.

PROCEEDINGS

Parts of this work have also been presented at International Congresses according to the following references:

Muñoz L., Marambio O., Díaz O., Aguilera J.M, Cobos A (2009) **Effect of pH and ionic strength on hydration capacity of mucilage of chia (Salvia hispanica L.)** Congreso Iberoamericano de Ingeniería de Alimentos - CIBIA VII – Colombia

Muñoz L., Aguilera J.M., Díaz O., Cobos A. (2010) **Mucilage of Chia, Salvia hispanica: structure, microstructure, extraction and hydration as affected by different conditions.** 15 th World Congress of Food Science and Technology – South Africa

Muñoz L., Aguilera J.M., Díaz O., Cobos A. (2011) **Development and characterization of composite edible films from mucilage of Salvia hispanica and whey protein concentrate.** International Congress of Engineering and Food – ICEF 11 Greece

Muñoz L., Aguilera J.M., Díaz O., Cobos A.(2011) **Microstructure of chia polysaccharide:whey protein concentrate aggregates.** VIII Congreso Iberoamericano de Ingeniería Alimentaria- CIBIA VIII – Perú

Muñoz L., Cobos A., Díaz O., Zúñiga M.C., Marambio O., Aguilera J.M. (2012) **Chemical, functional and thermal properties of polysaccharide gum extracted from chia (*Salvia hispanica*) seeds.** 16th World Congress of Food Science and Technology – XVII Latin American Seminar of Food Science and Technology – IUFOST - ALACCTA

1. INTRODUCTION

The current trend in the developed world as far as food is concerned, indicates a clear increase by consumers for foods that are not only nutritionally balanced and safe, but also easy to prepare or "ready to eat" and provide wellbeing and pleasure. People are increasingly avoiding foods that contain preservatives, artificial additives or that simply induce weight gain. Scientific studies have shown that many chronic diseases such as cancer, cardiovascular disease, hypertension and obesity, among others, are directly related to food (Jones, 2002).

This change in the tastes, preferences and the demands of modern consumers creates a new area of development and challenges in food and nutritional science.

Within this context, the food industry needs to meet such demands by, for example, incorporating additional ingredients in the manufacture and development of new products. The current trend is to incorporate natural and/or functional ingredients that provide food with the organoleptic and sensory characteristics that are equal to or better than artificial flavors, so providing consumers with "healthy" foods.

One alternative that has great potential within the food industry is the use of ancestral seeds and products. These types of seed, such as amaranth, quinoa and chia, were recognized for their nutritional and medicinal properties by the ancient civilizations that inhabited South America (Whistler, 1982; Ayerza and Coates, 2005a).

In 1991 as part of the Northwestern Argentina Regional Project, seeds such as chia were reintroduced as "new industrial crops" (Ayerza and Coates, 1996).

Salvia hispanica L., commonly known as chia, is an oilseed plant that was once used by the Aztecs not only as a foodstuff, but also as an offering to the gods. This seed is a natural source of omega-3 and omega-6 (α-Linolenic acid), fiber (+30%), proteins of high biological value, and natural antioxidants that protect the seed against certain adverse conditions (Craig, 2004), in addition to other important nutritional components such as vitamins and minerals (Bushway et al., 1981; Ayerza, 1995; Ayerza and Coates,

2005b). Chia helps prevent cardiovascular diseases, inflammatory and nervous system disorders, and diabetes, among others (Vuksan et al., 2007). Thanks to its properties, the seed is considered a dietary supplement by the U.S. Food and Drug Administration.

Today the chia seed offers a huge potential in the industries of food, animal feed, cosmetics and pharmaceuticals, among others, due to its nutrient content.

At present only a few studies of the seed have been carried out, and most of these were focused on omega-3 fatty acids (Bushway et al., 1981; Lin et al., 1994; Ayerza, 1995; Ayerza, 1996; Ayerza and Coates, 2001b; Beltrán-Orozco and Romero, 2003; Cahill, 2003; Ayerza and Coates, 2004; Ayerza and Coates, 2005b; Ayerza and Coates, 2007; Nieman et al., 2009). However, very little research has been undertaken about the mucilage produced by this seed. Upon hydration, chia seeds can absorb up to 12 times their weight in water, becoming enveloped in a copious, mucilaginous polysaccharide that comes from the outer shell of the seed (soluble fiber). To date, the mucilage has not been studied in detail and little is known about those industrial properties that could provide useful components (thickeners, stabilizers, emulsifiers, formation of edible films, etc.). The only study on the structure of chia mucilage was conducted by Lin et al. (1994).

The purpose of the present study is focused at the searching of new ingredients for the food industry not just in terms of nutritional properties. These ingredients when are incorporated in foods should also provide the consumer with features that offer improvements related to different attributes such as for example texture and functionality among others; and useful properties that ultimately contribute to the development and exploitation of Latin America's ancient cultures. Thus, the whole seed and the mucilage of *Salvia hispanica* show interesting properties to be studied and applied in science and food technology.

The objective of this research is to generate more information about the location of mucilage within the structure of the seed and then continue with a study of the mucilage, which will form the basis for investigating its future commercial applications. In this context, a study will be undertaken of the microstructure of the seed and mucilage by

light microscopy and scanning electron microscopy (SEM) in order to reveal the release mechanism for mucilage during extraction and to optimize results.

In the following, the proximal chemical, elementary and sugar composition will be determined, along with the physical, thermal and functional properties of chia.

Finally, once the mucilage has been characterized it will be used in the preparation of edible films.

1.1. Hypothesis and objectives of this thesis

The hypothesis of this thesis is that the mucilage from chia seeds is a potential ingredient with functional properties feasible to be used in food industry. According to this, the general objective is extract and characterizes the mucilage of *Salvia hispanica* to be used such as functional ingredient in food industry.

In order to achieve this aim, the thesis was divided into the following specific objectives:

- Study the microstructure, morphology and physical properties of *Salvia hispanica* to access the location of the mucilage within the seed
- Optimization of mucilage extraction
- Study the physico-chemical, thermal and functional properties of the isolated mucilage
- Application of mucilage in food matrix.

1.2. Outline of this thesis

The general theme of this thesis is the study and characterizes the mucilage from chia seed (*Salvia hispanica*) such as potential food ingredient, to subsequently determine its potential application in food industry.

In Chapter 2, a general review of chia seed, botanical description, nutritional and medical properties and applications in food industry is presented.

The Chapter 3 presents an original study about chia seed microstructure and mucilage location using optical and scanning electron microscopy (SEM). Also include the effect of temperature, pH and seed:water ratio on mucilage extraction; and finally the hydration of the extracted mucilage in function of temperature, pH and ionic strength produced by NaCl, CCl_2 y KCl.

In the Chapter 4 is presented for first time a chemical and thermal characterization of the isolated mucilage, as well as some important functional properties for potential use as food ingredient (water solubility, viscosity and emulsifying and foaming properties), among other advanced analytical techniques such as FT – IR and DSC.

Chapter 5 discusses a relevant application of chia mucilage which is the formation of edible films. The dispersions used for the production of films were characterized through TEM and subsequently the films were studied by SEM. Additionally, the water vapour barrier and mechanical properties of the films were also studied.

In Chapter 6, the main findings of the previous chapters and future perspectives are discussed.

Finally, the Chapter 7 includes the general conclusions of this thesis.

A summary of the contents of Chapters 3 to 5 is schematically presented in Figure 1.1 showing the relationship between the different Chapters of this thesis.

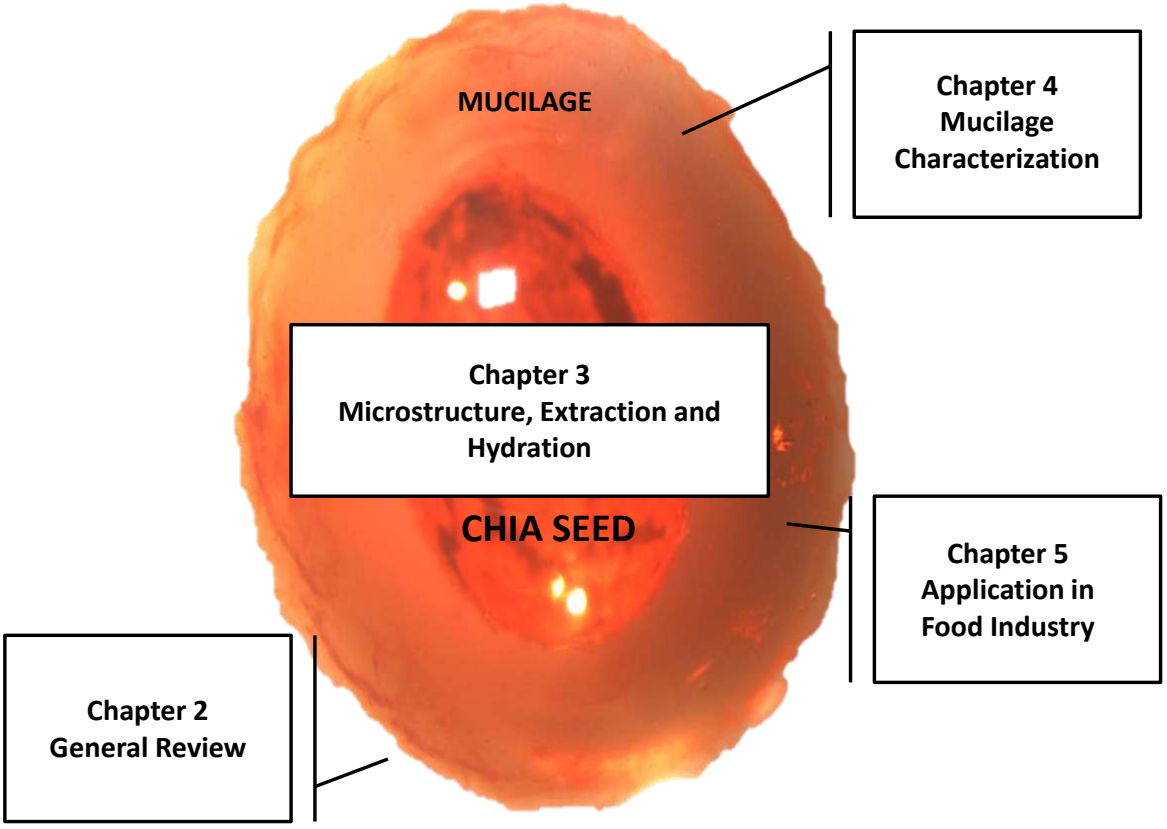

Figure 1.1 Overview of the studies comprising this thesis.

2. REVIEW

2.1. Chia seed history

Chia is native to the central valleys of Mexico and northern Guatemala, where the species of the *Labiatae* family are concentrated, and scientific observations can be found in one of the twelve volumes of the Florentine Codex, written by Fray Bernardino de Sahagun at the time of the conquest of America between 1548 and 1580. Chia seeds began to be used in human food around 3500 BC, and acquired importance as a staple crop in central Mexico between 1500 and 900 BC (Cahill, 2003; Ayerza and Coates, 2005a). Aztecs and Mayans used the seeds for the preparation of various medicines, food and paintings. It was one of the main crops of pre-Columbian societies, surpassed only by corn and beans. At the time of the Conquest there was a number of plant species, four of which stood out from a nutritional point of view: amaranth (*Amaranthus hypochondriacus*); beans (*Phaseulus vulgaris*); chia (*Salvia hispanica L.*) and corn (*Zea mays*) (Craig, 2004).

The seeds of chia, corn, beans and amaranth formed the main components of the diet of the Pre-Columbian peoples of the Americas, diets that, compared with their modern counterparts, met the dietary requirements established today by the Food and Agriculture Organization and the World Health Organization (FAO, 2011).

Whole and ground Chia seeds were consumed as food, but were also pressed to extract their oil, which formed the base for face and body paints. The Aztecs received chia seeds as annual tributes of the people under their rule, and it was used as an offering to the gods in religious ceremonies (Hentry et al., 1990; Beltrán-Orozco and Romero, 2003).

The word "chia" is a Spanish adaptation of *chian or chien* in its plural form, meaning "oily", which comes from *Nahuatl*, the language of the Aztecs. The name "chia" itself was adopted by the Swedish botanist Karl Linnaeus. The former territory of *Nahuatl Chiapan*, meaning "River of Chia", took its name from the plant, and on the banks of the Grijalva River the plant has been grown since ancient times. Today these

lands form the Mexican state of Chiapas. The pre-Columbian peoples also used chia in the preparation of a popular beverage called "chia fresca" (fresh chia), which is still consumed today.

The Spanish conquest suppressed many of the traditions of the pre-Columbian peoples and destroyed most of their agricultural production and marketing system. Many of the crops that constituted the daily diet of pre-Columbian America were destroyed because of their close association with religion, to be replaced by foreign species such as wheat, barley and carrots, which were in great demand among the conquerors (Craig, 2004; Ayerza and Coates, 2005a).

Modern science has since concluded that pre-Columbian diets were, in the main, nutritionally superior to what people eat today (Hentry et al., 1990).

Recent studies have helped explain why the ancient civilizations considered chia a basic component of their diet. The chemical composition of the seed and its nutritional value provide it with a huge potential for commercialization, and technological advances have created an excellent opportunity to establish an agricultural industry fully capable of offering the world a new and old crop at the same time (Scheer, 2001; Ayerza and Coates, 2005a; Ayerza and Coates, 2005b; Fernández et al., 2006).

2.2. Botanical and taxonomic description

The chia plant belongs to the *Lamiaceae* family, which in turn is part of the mint family (Hentry et al., 1990). Its taxonomic description is as follows:

Kingdom	: Plantae
Subkingdom	: Tracheobionta – Planta vascular
Superdivision	: Spermatophyta – Planta de semillas
Division	: Magnoloiphyta – Planta con flores
Class	: Magnoliopsida - Dicotiledónea
Subclass	: Asteridae
Order	: Lamiales

Family : Lamiaceae
Genera : *Salvia*
Specie : *hispanica*

Among the common names used for this plant are: chia; Spanish sage; Mexican chia and black chia.

Chia (*Salvia hispanica L.*) is an annual herb (Figure 2.1) that blooms during the summer months. It is approximately a meter tall, with opposite, petiolate and serrated leaves that are 4 to 8 cm long and 3 to 5 cm wide. The flowers are hermaphrodite (Figure 2.2) and grow in numerous clusters in a spike protected by small bracts with long pointed tips. The seeds are oval, smooth and shiny, and are mottle-colored with brown, grey, dark red and white, and are generally found in groups of four (Ayerza and Coates, 2005a, USDA, 2008). The plant has quadrangular stems that are ribbed and hairy. It grows in light to medium, clay and sandy soils, and even in arid soils that have good drainage but are not too wet. The plant is semi-tolerant to acid soils and drought. Chia is grown mainly in mountainous areas and has little tolerance to abiotic phenomena, such as freezing and sunless locations.

Morphologically, wild and domesticated plants differ very little, and today chia has been classified within the cultivated lands of Mesoamerica (Cahill, 2003).

Figure 2.1 Chia Plant (Courtesy of Benexia)

Figure 2.2 Chia flower (Courtesy of Benexia)

The leaves contain essential oils that act as insect repellents, thus the plant can be grown without pesticides or other chemical compounds (Pascual-Villalobos et al., 1997). In Mexico it grows like weeds and mainly in juniper, oak, pine and pine-oak forests, spreading by seed dispersal, with the wild type an average height of 1.9 meters. In the state of Jalisco, Mexico, chia is grown on farmland from late spring to early summer. In European countries it is grown in greenhouses during March and April, where germination lasts for approximately two weeks, to then be transferred to pots when the plants are high enough (Beltrán-Orozco and Romero, 2003).

As the plant is resistant to very dry areas, this crop is very attractive for developing countries such as Bolivia, Colombia and Argentina, where it is grown in provinces including Salta, Jujuy, Tucumán and Catamarca. The harvested seed (Figures 2.3 and 2.4) continues to be much more profitable than the cultivation of beans (Ayerza and Coates, 1999).

The commercial yield per hectare is normally 500 to 600 kg of seed; however, experimental plots located in Salta, Argentina, provide a yield of around 2500 kg per hectare, with the assistance of irrigation and nitrogen fertilization (Ayerza and Coates, 2005a).

This type of crop is a substitute for tobacco crops in Northwestern Argentina and Southern Bolivia, where they can be rotated in the fields so that soil nutrients are not depleted.

Figure 2.3 Chia Planting (Courtesy of Benexia)

Figure 2.4 Chia crop (Courtesy of Benexia)

2.3. Chia seed description

The seeds are small, oval-shaped and flat, and measure between 2 and 2.5 mm long, 1.2 and 1.5 mm wide, and 0.8 to 1 mm thick. Their color ranges goes from dark brown to black, and sometimes gray or white, as shown in Figures 2.5 and 2.6; the white seeds are greater in weight, width and thickness than the darker ones (Ixtaina et al., 2008).

Figure 2.5 Chia seeds

Clean and dry seeds can be kept for years as they contain antioxidants that prevent the deterioration of the essential oils held within. A principal feature of the seed is that when placed in an aqueous medium, it secretes a mucilaginous polysaccharide that surrounds the seed. It has been reported that consumption of this mucilage aids digestion and that, together with the seed, constitutes a nutritious food source (Salgado-Cruz et al., 2005).

Figure 2.6 Chia seeds

2.3.1. Nutritional Value

Chia was one of the four major crops of the Aztec civilization, and today is classified as a non-conventional seed, as it is not consumed in the normal diets of different countries. It contains high proportions of the essential fatty acid α-linolenic (18:3 n-3), which is associated with certain physiological functions.

The seed contains significant concentrations of primary and synergistic natural antioxidants, such as chlorogenic acid, caffeic acid, myricetin, quercetin and kaempferol (Taga et al., 1984; Ayerza and Coates, 2001a; Ayerza and Coates, 2001b).

One of the main properties of this seed is the fact it is a natural source of omega-3, which represents 75% of the total oil content of chia (Taga et al., 1984). It also has a significant amount of dietary fiber, found in greater proportions compared to other fruits and seeds that the digestive system cannot digest (Vázquez-Ovando et al., 2009).

Furthermore, the seed contains more protein in relation to any other grain and is gluten free. Finally, it is a major source of vitamins and minerals. Table 2.1 details the nutritional composition of the chia seed.

Table 2.1 Nutritional composition of chia seed

Nutrient	**100 g**	**1 portion (25 g)**
Energy (Kcal.)	486	121,5
Proteins (g)	16,54	4,14
Total fat (g)	30,74	7,69
Fatty acids, total saturated	3,33	0,83
Fatty acids, total monounsatured (g)	2,309	0,58
Fatty acids, total polyunsaturated (g)	23,67	5,92
Fatty acids Trans	0,14	0.04
Fatty acids Omega-3 (g)	17,83	4,46
Cholesterol (mg)	0	0
Carbohydrate (g)	42,12	10,53
Fiber, total dietary(g)	34,4	8,6

Source: USDA Nutrient Database for standard Reference, Release 24 (2011)

2.3.2. Fiber

Chia seeds produce between 36 and 40 g of dietary fiber per 100 g, equivalent to 100% of the daily recommendations for the adult population; the defatted flour possesses 40% fiber, 5% of which is soluble and forms part of the mucilage (Bushway et al., 1981; Reyes-Caudillo et al., 2008).

This high fiber content, when compared with other vegetables (Table 2.2), means that the seed can be used in the prevention of many cardiovascular diseases and diabetes,

among others, as demonstrated by a number of epidemiological studies (Ayerza and Coates, 2001a).

Table 2.2 Differences in fiber content in foods

For 100 g of edible product			
Chia	**37,7 g**	Walnuts	5,2 g
Fava beans (dried)	19,0 g	Cokies	5,0 g
Figs and plums (dried)	17,0 g	Olives	4,4 g
Dried peas	16,7 g	Breakfast cereals	4,0 g
Mashed potatoes	16,5 g	Banana	3,4 g
Chickpeas, lentils	12-15 g	Sprouts and cabbage	3,3 g
Almonds	14,0 g	Carrots	2,9 g
Hazalnuts	10,0 g	Figs	2,5 g
Corn	9,2 g	Pear	2,3 g
Dates	8,7 g	White bread	2,2 g
Whole wheat bread	8,5 g	Coliflower	2,1 g
Peanut	8,1 g	Apricot, plums	2,1 g
Quince	6,4 g	Apple, oranges	2,0 g
Spinach	6,3 g	Kiwi	1,6 g
Chard	5,6 g	Leek	1,3 g

Source: Fernández et al., 2006.

2.3.3. Proteins

The protein content of the seed varies between 15% and 23% depending on the geographic location of the crop, exceeding that of traditional cereals such as wheat, corn, rice, oats and barley (Ayerza y Coates, 2001a).

Table 2.3 shows the protein content of chia seeds compared to other commonly consumed cereals.

The seed does not contain gluten, so any food prepared using chia as its basis can be ingested by celiac disease patients. Table 2.4 shows the amino acid content of the seed.

Table 2.3 Comparison between chia seed and cereals

Grain	**% of Proteins**
Rice	6,50
Barley	12,48
Oats	16,89
Wheat	13,68
Corn	9,42
Chía	**20,70**

Source: Ayerza y Coates, 2005b

Table 2.4 Aminoacids content per 100 grams of chia

Aminoacid	**g / 100 gr seed**
Aspartic acid	1,689
Threonine	0,709
Serina	1,049
Glutamic acid	3,500
Glycine	0,943
Alanine	1,044
Valine	0,950
Cysteine	0,407

Table 2.4 continuation

Aminoacid	**g / 100 gr seed**
Methionine	0,588
Isoleucine	0,801
Leucine	1,371
Tryptophane	0,436
Tyrosine	0,563
Phenylalanine	1,016
Lysine	0,970
Histidine	0,531
Arginine	2,143
Proline	0,776

Source: USDA National Nutrient Database for Standard Reference, Release 24 (2011)

2.3.4. Vitamins

Chia is a good source of B vitamins (table 2.5). Compared to other cereals, the seed has higher niacin content than corn, soybeans and rice. As for its thiamine and riboflavin content, this is similar to that found in rice and corn (Bushway et al., 1981; Beltrán-Orozco and Romero, 2003).

Table 2.5 Vitamins content

Vitamins	Units	Quantity
Vitamin C (total ascorbic acid)	mg	1,6
Thiamine	mg	0,62
Riboflavin	mg	0,17
Niacin	mg	8,83
Folate	µg	49
Vitamin A	IU	54
Vitamin E (alpha-tocopherol)	mg	0,5

Source: USDA National Nutrient Database for Standard Reference, Release 24 (2011),

IU: International unit

2.3.5. Minerals

Chia is an excellent source of minerals (Table 2.6), and contains six times more calcium, eleven times more phosphorus and four times more potassium than 100 g of milk, besides possessing magnesium, iron, zinc and copper (Beltrán-Orozco and Romero, 2003)

Table 2.6 Minerals content

Macroelements	
Nutrient	**mg**
Calcium	631
Potassium	407
Magnesium	335
Phosphorus	860

Table 2.6 continuation

Microelements	
Nutrient	**mg**
Selenium (µg)	55,2
Copper	0,924
Iron	7,72
Manganese	2,723
Molybdenum	0,2
Sodium	16
Zinc	4,58

Source: USDA National Nutrient Database for Standard Reference, Release 24 (2011)

Chia contains 13 to 354 times more calcium, 2 to 12 times more phosphorus, and 1.6 to 9 times more potassium than 100 g of wheat, rice, oats and corn.

The iron content of chia is also quite high compared to most other seeds: it has six times more iron than spinach, 1.8 times more than lentils, and 2.4 times more than liver (Bushway et al., 1981; Beltrán-Orozco and Romero, 2003).

2.3.6. Antioxidants

Chia seed has a number of compounds that can act as antioxidants, an asset that makes the seed even more attractive (Table 2.7). Among the most important are the flavonols, chlorogenic acid and caffeic acid, as well as its content of myricetin, quercetin and kaempferol (Taga et al., 1984). These compounds are primary and synergic antioxidants and make a proportionally greater contribution to the antioxidant activity of chia (Fernández et al., 2006).

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Table 2.7 Concentration of antioxidants in chia seed

Compound	mol/kg seed
Not Hidrolyzed	
Caffeic acid	$6,6 \times 10^{-3}$
Chlorogenic acid	$7,1 \times 10^{-3}$
Hidrolized	
Myricetin	$3,1 \times 10^{-3}$
Quercetin	$0,2 \times 10^{-3}$
Kaempferol	$1,1 \times 10^{-3}$
Caffeic acid	$13,5 \times 10^{-3}$

Source: Ayerza y Coates, 2001a

Quercetin is a powerful antioxidant capable of preventing the oxidation of fats, proteins and DNA, and these antioxidant properties are significantly more effective than other flavonoid compounds. Caffeic acid and chlorogenic acid protect against free radicals and inhibit the peroxidation of fats. These compounds present in the seed have much stronger antioxidant properties than those of ferulic acid, vitamin C (ascorbic acid) and vitamin E (α-tocopherol) (Taga et al., 1984; Ayerza and Coates, 2001a; Reyes-Caudillo et al., 2008)

2.4. Uses and Applications

2.4.1. Human consumption

Today, the chia seed is used for different purposes in different countries, such as Mexico, Argentina, Chile, New Zealand, Japan, the United States, Canada and Australia. In 2009, it was approved as a Novel Food by the European Parliament and Council of

Europe According to scientific opinion; chia does not cause any adverse allergenic, anti-nutritional or toxic effects.

Some of the seeds most important applications include its use as a nutritional supplement and as an ingredient in cereal bars, biscuits, pasta, bread, snacks and yogurt, among others.

Another important quality of this seed is its use in the production of oil, light in color, and which has the advantage of containing a large quantity of essential oils. The main application of this product is in the production of capsules that provide a nutritional supplement of omega-3. Oil can also be extracted from chia leaves, and can be consumed as a condiment or used as a fragrance (Ahmed et al., 1994; Beltrán-Orozco and Romero, 2003).

Chia is one of the most significant natural sources of omega-3, the main effect of which is to reduce the level of triglycerides, moderately increasing blood levels of HDL cholesterol and lowering levels of LDL cholesterol. By preventing the formation of clots and plaques in the arteries, it helps prevent cardiovascular disease. The seed is rich in fiber, making it ideal for the proper functioning of the intestine, and contains highly nutritious proteins, more than traditional cereals. It provides a good source of B vitamins plus minerals such as calcium, magnesium, phosphorus, zinc, potassium and others (De Tucci, 2006).

2.4.2. Pest control

Most plants that possess dark-colored or black grains, including chia, are not attacked by insects, as they contain various compounds that offer protection. In the case of chia, an essential oil can be extracted from the leaves that has the following main components: β-caryophyllene; globulol; γ-muroleno; β-pinene; α-humoleno; germacren-B and widdrol, and these have a repellent effect against insects (Ahmed et al., 1994; Pascual-Villalobos et al., 1997).

2.4.3. Medical uses

To date, no evidence has been found of adverse or allergenic effects caused by eating whole or ground chia seeds (EFSA, 2005; EFSA, 2009).

Some of the most important medical studies related to chia have been carried out at the St. Michael Hospital in Toronto, Canada (Vuksan et al., 2007; Vuksan et al., 2010). Dr. Vuksan has confirmed that 100 g of chia contain:

- The same amount of omega-3 as 790 g of salmon
- Almost the same amount of calcium as 3 cups of milk
- Almost as much iron as five cups of spinach

Chia is rich in soluble fiber and gluten free. Only 12 g of seed provide more than 5 g of dietary fiber. The insoluble fiber has the ability to absorb several times its weight in water, which helps provide a feeling of fullness and slows digestion, leading to a stable increase in blood sugar levels and a more stable release of insulin.

Research carried out by Vuksan in 2007 suggested that a high-fiber diet may help control diabetes. The study was conducted on 20 healthy diabetic patients who were given bread made with chia flour and additional whole seeds to be sprinkled on foods at home. The total consumption of chia seed was 37 g per day.

The study subjects then had their blood pressure measured in order to observe any changes. Results showed a small decrease in blood glucose, although the most important findings were as follows:

- Blood became less prone to coagulation, which is a risk factor that may lead to heart attacks and strokes.
- Decreased levels of internal inflammation as measured by C-reactive protein (CRP).

- Chia had an effect on blood pressure, decreasing systolic blood pressure by an average of 6 mmHg.

Additionally, and due to its high content of omega-3 and omega-6, various studies (Bushway et al., 1981; Ayerza and Coates, 2001a; Ayerza and Coates, 2001b; Fernandez et al., 2008) have attributed the following medicinal properties to the seed:

- Helps to reduce cholesterol.
- Inhibits blood clotting and promotes tissue regeneration.
- Helps to reduce the digestion time of carbohydrates, so assisting the control of blood sugar levels.
- Helps prevent brain diseases, depression and epilepsy.
- Improves the immune system.
- In the fetus chia helps the development of the retina and brain, and also has beneficial effects on children under the age of 2.

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3. CHIA SEED: MICROSTRUCTURE, MUCILAGE EXTRACTION AND HYDRATION

Abstract

Microstructural features of the Chia seeds (*Salvia hispanica L.)* were studied by light and scanning electron microscopy. The study reports the effect of temperature (4-80 °C), pH (4-8) and seed:water ratio (1:20 and 1:40) on extraction of the mucilage of Chia seeds and the effect of temperature (20-80°C), pH (3-9) and ionic strength (0-1%) on hydration of the extracted mucilage. The mucilage was localized in cellular structures in the first three layers of the seed coat and upon full hydration filaments (mucilage fibers) became apparent and conformed to a transparent "capsule" attached to the seed. During extraction, temperature and seed:water ratio were found to have a significant effect on yield. Hydration of the extracted mucilage was significantly increased at high pH values, and was higher when salt concentration decreased, being maximal when the temperature reached values close to 80°C.

3.1. Introduction

Chia (*Salvia hispanica L.*) cultivation goes back to pre-Columbian times, but since it was used in religious pagan ceremonies, while the Spaniards attempted to eradicate the seed, they introduced new species which were brought from the old continent. The plant produces brown to black and beige (without pigmentation) seeds. In 1991, a group of producers, scientists and commercial entities associated in the Regional Northwestern Argentina Project started again the cultivation of chia on a small scale (Ayerza, 1995; Coates and Ayerza, 1996).

Nowadays, chia seed is a potential source of nutrients for the food and animal feed industries. Although chia is not a well-known food, its global production is increasing due to its healthy properties and popularity. Chia seeds are also used as nutritional

supplements as well as in the manufacturer of bars, breakfast cereals and cookies in the USA, Latin America and Australia (Dunn, 2010). It possesses a significant quantity of oil (about 40 % total weight of the seed), almost 60% as α linolenic acid (omega 3) and also dietary fiber (over 30 % of the total weight), both important components of the human diet; and proteins of high biological value (around 19 % of the total weight). In addition, it contains natural antioxidants such as phenolic glycoside-Q and K, chlorogenic acid, caffeic acid, quercetin and kaempferol (Reyes-Caudillo et al., 2007) which protects consumers against some adverse conditions, such as protection against some cardiovascular diseases and some types of cancer; as well as vitamins and minerals (Ayerza and Coates, 2001a; Ayerza and Coates, 2004; Craig, 2004). In 2009, it was approved as novel food by the European Parliament and the European Council (Commission, E.U., 2009). There is no evidence of adverse effects or allergenicity caused by whole or ground chia seeds (EFSA, 2005, 2009), thus, chia seeds and derived products are promising sources of food.

Moisture-dependent mechanical properties provide basic data for storage, handling and optimum design of equipment and processing of chia seeds (Mohsenin, 1986; Wilhelm et al. 2005). The fiber content of the chia seed corresponds to a polysaccharide with a high molecular weight (0.8-2 x 10^6 Daltons) (Lin et al., 1994). In 1996, FAO described it as a potential source of polysaccharide gum because of its exceptional mucilaginous properties at low concentration in aqueous solutions (Hulse, 1996). A tentative structure of the basic unit of the polysaccharide was proposed by Lin et al. (1994) as a tetrasaccharide with 4-O-metil-α-D-glucoronopyranosyl residues occurring as branches of β-D-xylopyranosyl on the main chain.

The monosaccharides β-D-xylose, α-D-glucose and 4-O-metil-α-D-glucoronic acids were obtained by acid hydrolysis in the proportion 2:1:1, respectively.

In general, hydrocolloids are widely used in different applications in the food industry, due to their ability to retain water. They are also notable for their thickening and gelling properties, syneresis control, emulsion stabilization, etc. (Phillips and Williams, 2000). Chia seeds contain 5-6% mucilage that can be used as dietary fiber

(Ayerza and Coates, 2001b; Reyes-Caudillo et al., 2008). One patent refers to its extraction using sonication and high pressure filtration (Marin et al., 2008, WO/2008/0044908), but further information is needed on extraction conditions, yields and properties of the extracted hydrocolloid. Some efforts have been previously channeled towards investigating hydration and extraction of mucilage from some agricultural seeds. These reports indicated varied levels of yields usually dependent on extraction and hydration methods. Mucilages from *Prosopis flexuosa* seeds (Ibañez and Ferrero, 2003); whole white mustard seeds (Balke et al., 2000); *Gleditsia triacanthos* seeds (Sciarini et al., 2009); and *Sinapis alba L*. seeds (Cui et al., 1993) were reported to give about 13%, 10-15%, 11.90-34.16 % and 5% yield level, respectively. Since we have not come across any literature detailing the microstructure, extraction and hydration of mucilage of chia seeds, this study was undertaken. Hence, the first objective of this work was to study the microstructure of the chia seed and seed coat as well as how the mucilage was released during hydration. Secondly, we assessed the effects of different factors on the aqueous extraction and hydration of mucilage of *Salvia hispanica.*

3.2. Materials and Methods

3.2.1. Materials

Chia seeds produced commercially in the Salta region of Argentina were obtained from Benexia (Functional Products Trending S.A, Santiago, Chile). The geometrical dimensions of chia seeds were determined for dark and beige seeds separately.

3.2.2. Physical properties and morphology of Chia seed

The seed morphology was evaluated using stereomicroscopy (SM), optical microscopy (OM) and scanning electron microscopy (SEM). For stereomicroscopy, the

seeds were separated into groups and conditioned at three levels of relative humidity (RH), 20-25%, 40-50% (room temperature) and 75% inside desiccators. Silica gel was used to adjust the RH to 20 and 25% with measurement every 2 days for 15 days until equilibrium was attained, before the seeds were put into the desiccator. For the 75% RH, saturated solution of NaCl was used in a similar manner, while for the 40 – 50%, no chemical was used. RH in all cases was accurately measured using a hygrometer (Vaisala HM 34C, Finland). Then the samples were kept at room temperature (20°C) for 15 days. To determine the average size of the seeds, a sample of fifty units of each colour was randomly selected and positioned in two different orientations; vertically and horizontally (Abalone et al., 2004; Ixtaina et al., 2008; Singh and Goswami, 1996; Tunde-Akintunde and Akintunde, 2004; Vilche et al, 2003). Whole seeds were observed using an Olympus Stereomicroscope SZ X7, (Optical Co. Ltd., Tokyo, Japan) equipped with a Digital Camera (Cool Snap Pro Colour, Photometrics Roper Division, Inc., Tucson, AZ, USA). The images were binarized (black & white) and the three principal dimensions, length (L), width (W) and thickness (T) were measured. The images obtained were examined with the Image Pro Plus 6.0 Program (Media Cybernetics, Inc.) and then analyzed statistically. The geometric diameter (*Dg)* and the sphericity (Φ) were calculated using the relationships shown in equations 3.1 and 3.2 (Mohsenin, 1980; Tunde-Akintunde and Akintunde, 2004):

$$D = (LWT)^{1/3} \qquad (3.1)$$

$$\Phi = (LWT)^{1/3} / L \qquad (3.2)$$

Where L is the length, W the width and T is the thickness in mm. Similarly, the surface area in mm2 was determined using (equation 3.3):

$$S = \pi D g^2 \qquad (3.3)$$

To determine W1000, the weight of 1000 seeds, five sample groups of 100 seeds each were selected and weighed in an electronic balance with 0.0001 g accuracy (Sartorius Handy, model H110, Germany) and the weight extrapolated to that of 1000 seeds. The average bulk density of the chia seed was determined by filling a 100 ml test tube with seeds and weighing the contents (Ixtaina et al., 2008; Paksoy and Aydin, 2004; Singh and Goswami, 1996). True density (ρt) was evaluated using the liquid (hexane) displacement method in a picnometer. Due to the short duration of the procedures, the absorption of hexane was considered negligible.

For microscopic observations, a light microscope (Olympus BX50, Japan) was used at magnification of 10x and 40x. Whole seeds were dyed with three stains: firstly, seeds were hydrated with a safranin distilled water solution (0.01%) for 24 hours; secondly, they were stained with fast green and safranin, and lastly, stained with lugol and fast green. Images were taken with a digital camera (Cool Snap Pro Colour, Photometrics Roper Division, Inc., Tucson, AZ, USA) and data stored in a computer. Afterwards, the images were analyzed with the software Image Pro Plus 6.0.

For SEM, mature dried seeds without any treatment, were sputter coated with gold and examined with a JEOL JSM 5300 scanning electron microscope (Jeol Ltd., Tokyo, Japan), operated at an acceleration voltage of 15 kV. ADDA II was used as interface between microscope and computer and the images were analyzed with the software AnalySIS® ver. 3.2 (Soft Imaging System GmbH 1986-2003, Münster, Germany). Moisture content was determined according to AOAC official method 931.04 (AOAC, 1995) and expressed in dry basis (d.b.).

3.2.3. Mucilage Extraction

Mucilage extraction was performed at different seed:distilled water ratios, pH and temperature conditions. A Box Behnken experimental design (BBD) was used for the evaluation of mucilage extraction conditions that included 30 experimental runs with 3 center points at high and low levels of each independent variable: temperature (20 –

80°C), pH (4 – 8) and seed:water ratio (1:20; 1:40). The response (dependent variable) was the extraction percentage under the different conditions.

Samples of 10 g of whole seeds were placed in a 1L beaker and distilled water was added in 1:20, 1:30 and 1:40 proportions. The pH was adjusted and maintained at 4, 6 and 8 through continuous adjustments using 0.2 M NaOH or HCl solutions and the temperature during extraction was maintained at 4, 40 and 80 ± 1.5 °C using temperature controller. The mixtures were stirred with a magnetic stirrer and hydrated for 2 hours. Then the aqueous suspension was spread on a drying tray and exposed to temperature of 50°C for 10 hours. The dried mucilage was separated from the seed by rubbing over a 40 mesh screen, and the weight was recorded.

3.2.4. Mucilage Hydration

The mucilage and whole seed hydration capacities were determined under constant temperature conditions (18°C). Samples of 100 mg of dry mucilage and seeds were placed separately inside a filter paper sachet and immersed simultaneously in distilled water. During the initial 15 min the net weight of each sachet was registered every 3 minutes and then weighed every 15 minutes to a constant value.

A second study was undertaken to predict the mucilage swelling behavior under different conditions. A Box Behnken Design 33, selecting high and low levels for each independent variable: temperature (20 – 80 °C), pH (3 – 9) and ionic strength determined by CaCl2, NaCl and KCl salts in a 0 to 1% concentration. Samples of 25 mg of dried mucilage were hydrated in distilled water and in the aqueous solutions, respectively. The pH was controlled continuously and adjusted using 0.2 M NaOH and HCl. The temperature of each aqueous solution were kept constant by using a temperature controller (Thermocouple, Microprocessor based thermometer W502 K/J Type, Taiwan). The experimental runs were performed in triplicate. After a period of 6 hours of immersion each solution was filtered so that the weight of the swollen gel could

be determined (Medina-Torres et al., 2000). The swelling value (%) (Pourjavadi et al., 2008) was calculated with the following equation (3.4):

$$Sw = \frac{\text{(Weight of the swollen hydrogel – weight of mucilage dried)}}{\text{(Weight of mucilage dried)}} \times 100 \qquad (3.4)$$

3.2.5. Statistical Treatment

Results were analyzed using the Statgraphics Centurion XV Program version 15.1.02 (2006, StatPoint, Inc.). The statistical significances of data for size and the effects of extraction and swelling were analyzed by ANOVA ($p < 0.05$).

3.3. Results and discussion

3.3.1. Morphology and Physical Properties of Chia seeds

Data on physical properties are presented in Table 3.1 Chia seeds were small (1.87± 0.1 mm length, 1.21 ± 0.08 mm width and 0.88 ± 0.04 mm thickness) with an oval flattened shape and ranged in colour from dark coffee to beige with small darker spots. Although, some of them were gray or white in colour as shown in Figure 3.1a. Length, width and thickness in the two groups of seeds (beige and dark) increased when the relative humidity increased. The length was significantly different in both beige and dark seeds at 75 %RH. Adsorption of moisture by seeds induced changes mainly when they were exposed to 75% RH resulting in increase in geometric mean diameter and decrease in sphericity and surface area in beige seeds. However, there was no significant difference between the bulk density of beige and dark seeds (25 % RH 0.70 ± 0.006 g/cm3 ; ambient RH 0.71 ± 0.02 g/cm3; 75 % RH 0.62 ± 0.01 g/cm3). Measurements at each relative humidity were homogeneous and as RH increased to 75%, the bulk density decreases.

Table 3.1 Physical properties of dark and beige seeds

Relative Humidity	Length L (mm)	Width W (mm)	Thickness T (mm)	Arithmetic mean diameter (L+W+T/3) (mm)	Geometric mean diameter $(LxWxT)^{1/3}$ (mm)	Sphericity $\Phi = (LWT)^{1/3}/L$	Surface $S=\pi Dg^2$ (mm^2)	W_{1000} (g)	Moisture (%) d.b.	True Density (g/cc)	Bulk Density (g/cc)
Beige seeds											
25%	1.84 ± 0.09[a]	1.21 ± 0.06ab	0.89 ± 0.06ab	1.32 ± 0.05	0.67 ± 0.08[a]	0.35 ± 0.08[a]	1.51 ± 0.50a	1.068 ± 0.002[a]	5.50 ± 0.10a	1.10 ± 0.002	0.72 ± 0.006a
Ambient (40 - 50%)	1.87 ± 0.10[a]	1.21 ± 0.08a	0.88 ± 0.04a	1.32 ± 0.06	0.66 ± 0.06[a]	0.37 ± 0.06[a]	1.58 ± 0.44a	1.127 ± 0.01b	7.24 ± 0.13b	1.06 ± 0.004	0.70 ± 0.003ab
75%	1.90 ± 0.09b	1.24 ± 0.07b	0.91 ± 0.05b	1.35 ± 0.05	0.72 ± 0.09b	0.34 ± 0.10b	1.48 ± 0.55b	1.121 ± 0.001b	10.02 ± 0.25c	1.06 ± 0.002	0.66 ± 0.004b
Dark seeds											
25%	1.82 ± 0.08[a]	1.19 ± 0.07a	0.90 ± 0.06b	1.31 ± 0.05	0.65 ± 0.09[a]	0.35 ± 0.08ab	1.44 ± 0.47a	1.106 ± 0.001a	5.57 ± 0.01a	1.10 ± 0.01	0.68 ± 0.006a
Ambient (40 - 50%)	1.84 ± 0.11ab	1.20 ± 0.08[a]	0.88 ± 0.06a	1.31 ± 0.07	0.65 ± 0.07[a]	0.36 ± 0.06a	1.52 ± 0.44ab	1.110 ± 0.001a	7.38 ± 0.07b	1.15 ± 0.004	0.72 ± 0.03a
75%	1.88 ± 0.11b	1.22 ± 0.07a	0.91 ± 0.05b	1.33 ± 0.06	0.70 ± 0.09a	0.33 ± 0.10b	1.37 ± 0.49b	1.178 ± 0.01b	9.85 ± 0.35c	0.98 ± 0.002	0.58 ± 0.02b

Values are given as mean ± SD for sixty determinations to geometrical dimensions and nine determinations to the others.
Different letters in the same column in each type of seed mean significant differences ($P<0.05$)

The true density value was between 1.065 and 1.10 g/cm3 and decreased when RH increased in both groups. These small differences, also reported by Ixtaina et al. (2008), may be attributed to the seed variety, geographical location and also agricultural practices. The thousand seed weight (W1000) was similar in dark and beige seeds and as expected, the W1000 increased when increased RH due to moisture adsorption.

As shown in Figures 3.1b and 3.1c, the mucilage in the fully hydrated seed formed a continuous and transparent capsule with an average thickness of around 414±35 μm. This transparent mucilagenous gel reached its maximum thickness after two hours of hydration and when dyed with safranin, two layers were observed; an inner layer, formed by branched structures (Figure 3.1d) and an outer layer that was cloudy and homogeneous. The mucilage appeared to be firmly bonded to the seed and was difficult to separate.

It is presumed that the mucilage is located in the outer cells that form the seed coat, called mucilaginous cells (Windsor et al., 2000). The seed coat or testa has a thickness of 13 ± 0.41 μm (fig 3.1e) and was composed of three layers: an outer layer, formed by rectangular thin-walled cells with sizes of 4.2 ± 0.26 μm, where presumably the mucilage is localized; a scleroid layer of long and thin cells resembling fibers, and the endocarp, a thin and inner layer. This conformation has not been reported on Salvia hispanica L., but a similar structure was found in Arabidopsis thaliana (Beeckman et al., 2000).

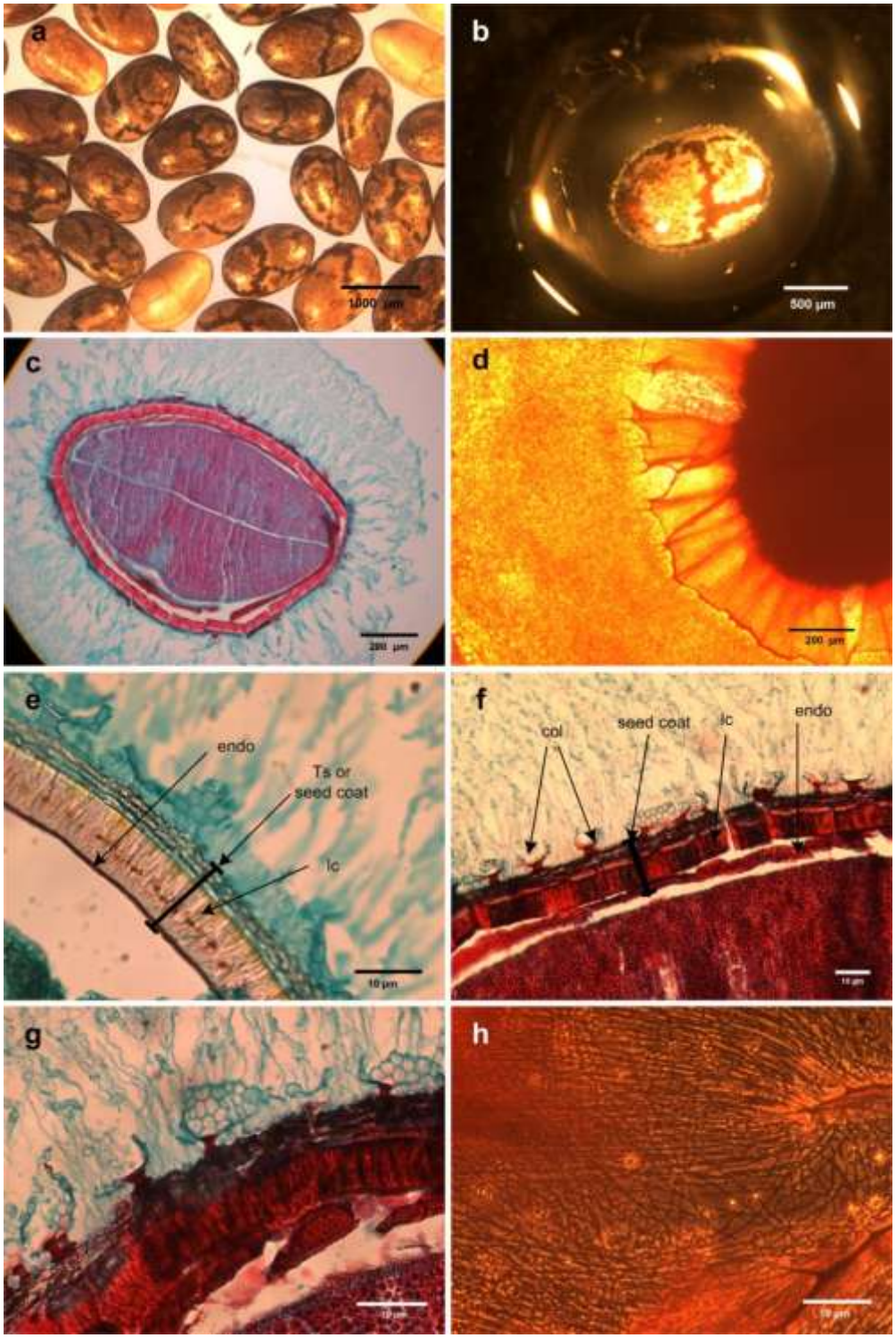
Immediately the seeds came in contact with water, small filaments appeared on the surface that began to stretch slowly until they became fully extended (Figure 3.1d). When the seeds were totally hydrated, these filaments (mucilage fibers) were completely developed and a "volcano-shaped" columella structures became apparent (Figure 3.1f and 3.1g) which was uniformly distributed on the surface around the seed. At the base of this structure was a small cluster of spheres of 11.6 ± 1.4 μm diameter (Figure 3.1g) that could be easily seen using a safranin reagent. This type of structure was described by Beeckman et al. (2000) and Penfield et al., (2001) in Arabidopsis seeds (an inedible seed). The mucilage is present inside the testa epidermal cell of mature chia seeds and

when they come into contact with water it immediately expands rupturing the primary cell layer that protrudes from these epidermal cells thus surrounding the seed. This phenomenon was described in detail by Beeckman et al. (2000) and Penfield et al. (2001) in Arabidopsis seeds but has not been described in edible seeds. After immersion in water and drying by warm air, the seeds appear to be surrounded by a thin film, the remnants of the mucilage. An almost identical phenomenon was observed with Arabidopsis seeds (Windsor et al., 2000). Regarding composition and location (in the external layer of the seed), the mucilage of chia appears to be similar to the mucilage produced by Arabidopsis thaliana. In an hydration study of Arabidopsis seeds under similar conditions (Dean et al., 2007), a capsule surrounding the seed was described as having similar characteristics with those presently observed in seeds of Salvia hispanica (Penfield et al., 2001; Windsor et al., 2000).

Figure 3.2 shows the SEM images of whole chia seed. Figure 3.2a corroborates the main differences in length and width between groups of seeds with different colour and between batches of both colours. Figure 3.2b shows a longitudinal section of chia seed where M represents the dry mucilage surrounding the seed; Ts corresponds to the seed coat or testa and the two cotyledons are visible inside the seed. When the seeds were hydrated and dried, hexagonal epidermal cells with thickened radial cell walls were observed (figure 3.3). Presumably, in the center of each cell the volcano-shaped columellae is located. These observations reaffirmed the results of the optical essays where this type of structures was easily visible.

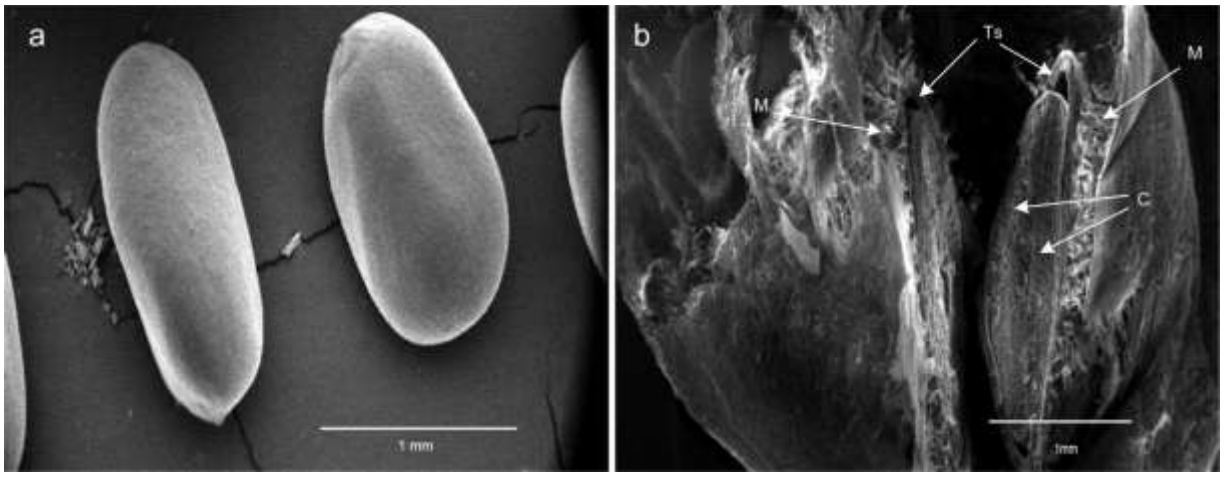
When many seeds were hydrated in water, a highly viscous solution was formed, and this same phenomenon occurred when the extracted mucilage was hydrated. Some researchers believe this same gel-forming phenomenon may occur inside the stomach when foods containing these gummy fibers or mucilage are consumed (Scheer, 2001). The gel creates a physical barrier between carbohydrates and the digestive enzymes that break them down, thereby decreasing the conversion of carbohydrates into sugars while increasing the satiety sensation (Rubio, 2002).

Figure 3.1 Chia seed – Optical images



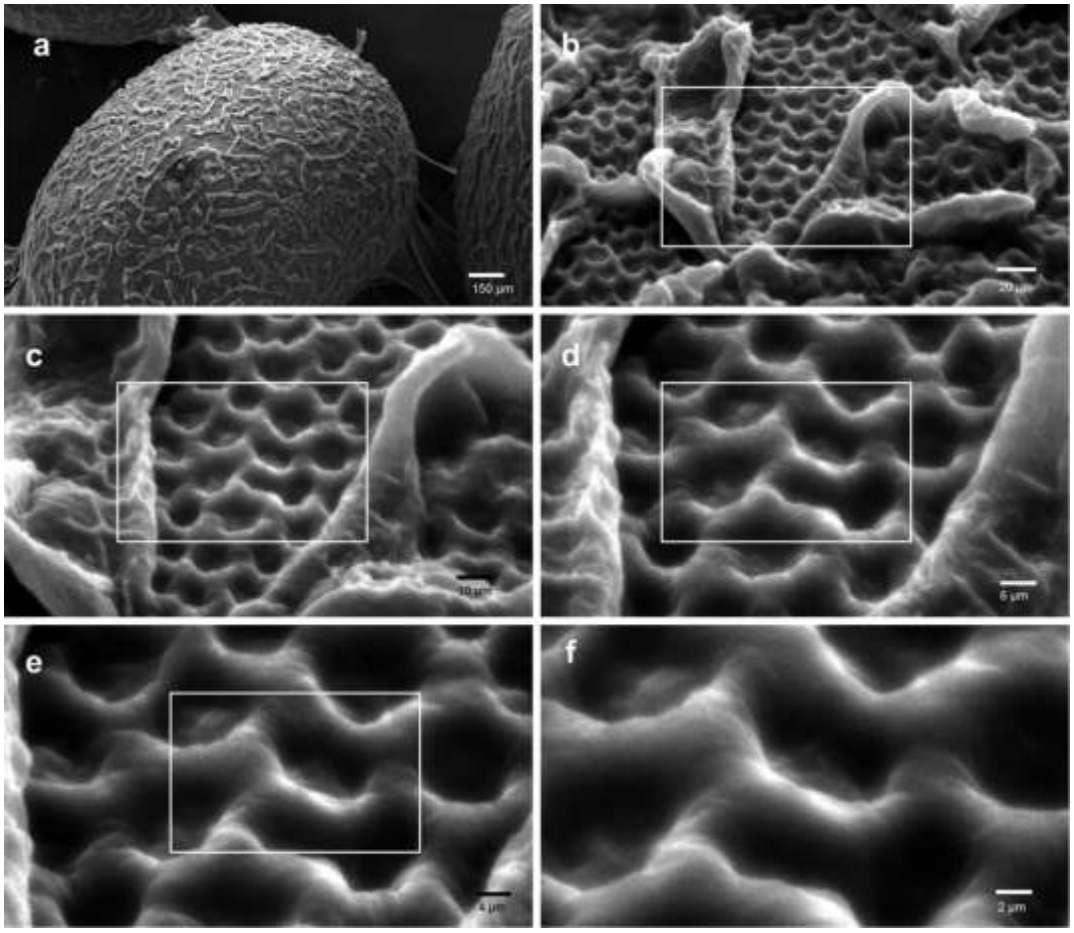
(a) Whole dry seed: beige and dark seeds.
(b) Whole seed hydrated forming mucilaginous capsule surrounding the seed.
(c, d, e, f, g and h) Histological section of whole seed fixed under water conditions.
(c and d) The outer cell wall of the epidermal cells has burst and mucilage has been released to surround the seed.
(e) seed coat: the three layers of rectangular cells forming seed coat are observed; endo: correspond to endocarp layer; lc: sclereid layer.
(f) col: Collumella uniformly distributed on the surface.; seed coat: testa; lc: sclereid layer; endo: endocarp layer.
(g) Cell wall material attached to the columella and small clusters of sphere cells.
(h) The presence of elongated branched aggregated, long from the seed, is observed.

Figure 3.2 SEM photomicrographs of whole Chia seed



(a) Lateral view of whole seeds.
(b) Longitudinal section, internal structure of sead coat; Ts:testa; C:cotiledons; M:mucilage.

Figure 3.3 Hydrated Chia seed - SEM Images



(a) Whole chia seed hydrated and lately dried, note a thin film surrounding the seed.
(b) (c) and (d) Reveals an hexagonal structure. Membranes observed correspond to dry mucilage on the surface of the seed
(e) and (f) in the center of each hexagonal structure shows the base of the columella.

3.3.2. Mucilage Extraction

Preliminary studies of hydration of whole seeds demonstrated that after 2 hour of hydration, the total weight of seeds became constant and water absorption was completed (Figure 3.4). This was considered the maximum time to perform the extraction and hydration experiments.

Figure 3.5a shows the estimated surface response of % yield at pH value of 6 as a function of seed:water ratio and temperature. As the temperature increase from 20 to 80°C, the % yield increase regardless of the ratio. The same pattern occurred when pH increase from 4 to 8, but the highest % yield was obtained at a ratio 1:40 (figure 3.5b).

Figure 3.4 Hydration Whole chia seeds and mucilage v/s time

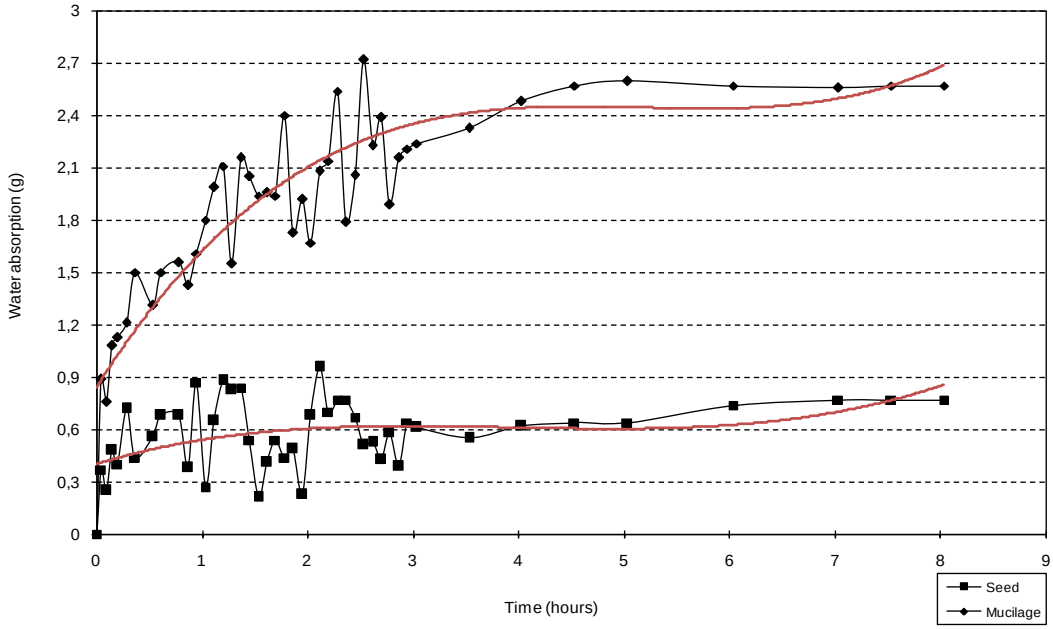
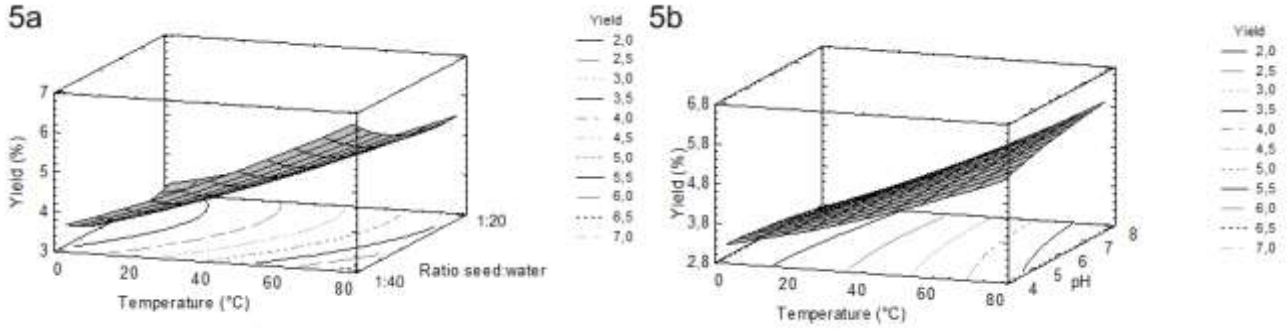


Figure 3.5 Response surface for extraction



(a) Response surface for extraction at pH 6
(b) Response surface for extraction at ratio 1:30

The main effects for extraction are shown in Table 3.2 with temperature and seed:water ratio having the highest influence on % yield. An optimum yield value of 6.97% was achieved at 80ºC with pH of 8 and seed:water ratio of 1:40. This optimum value is different from those reported by Marin et al. (2008), 15.1%; Reyes-Caudillo et al. (2008), 6%; and Ayerza and Coates (2001b), 5%.

Table 3.2 Anova and effects for Extraction

Source	Estimated effect	Stnd. Error	p-Value
A:Temperature	2.5125	0.289129	**0.0000**
B:pH	0.125	0.289129	0.6704
C:Ratio	-0.7625	0.289129	**0.0162**
AB	0.475	0.40889	0.2598
AC	-0.4	0.40889	0.3402
BC	-0.175	0.40889	0.6735
Average	4.3	0.236073	

Standard errors are based on total error with19 d.f.
p-value<0.05 means statistical significant differences

3.3.3. Mucilage Hydration

The preliminary studies of hydration of mucilage are shown in Figure 3.4 The water absorption was very fast in the first few minutes and later it became slower but equilibrium conditions was achieved at about 2 hours of hydration. Similar behavior has been reported in studies carried out on psyllium plants (genus *Plantago),* whose seeds are used commercially for production of mucilage (Singh et al. 2006; Singh et al., 2007). Chia mucilage had a great capacity of hydration because a sample of 100 mg of mucilage was able to absorb 2.7 g of water, 27 times its own weight. This value, almost double that reported by Vazquez- Ovando et al. (2009), in which only the fibrous fraction was hydrated.

It is interesting to observe the behavior of mucilage during hydration as induced by the incorporation of salts, because it relates to many chemical and physical processes in biological systems. Some hydrogels, such as that of chia mucilage, can absorb little or no water in the presence of ions. The selection of temperature, pH and ionic strength as independent variables and the low and high limit values were chosen after several preliminary experiments of hydration. The combination of factors and levels reflects conditions that may occur in food processing, and they cannot be studied separately because they are always inter-related (Montero and Pérez-Mateos, 2002). Significant effects between interactions of temperature-salt content and pH-salt content were observed using the three types of salts (NaCl, $CaCl_2$ and KCl) and negative effects on hydration can be also observed in the three cases (Table 3.3).

Figure 3.6 shows the response surface for hydration with 0.5 % NaCl (Fig. 3.6a), 1% NaCl (Fig. 3.6b), 0.5% CaCl2 (Fig. 3.6c), 1% CaCl2 (Fig. 3.6d), 0.5% KCl (Fig. 3.6e) and 1% KCl (Fig. 3.6f) where a different behavior of hydration is apparent for each salt. An increase in hydration up to maximum at pH 6.5 and temperature over 60° C with 0.5 % NaCl (Figure 3.6a) was observed whereas with 0.5% CaCl2 and KCl (Figures 3.6c and 3.6e), hydration improved with an increase in pH at the same temperatures, but the hydration with $CaCl_2$ was lower compared with the other two salts.

When 1% NaCl was used, a high level of hydration was achieved at pH 4 and temperature over 60°C, but decreased at higher pH values (Figure 3.6b). With 1% CaCl2 and KCl the highest hydration was observed at acidic pH and low temperatures (Figures 3.6d and 3.6f).

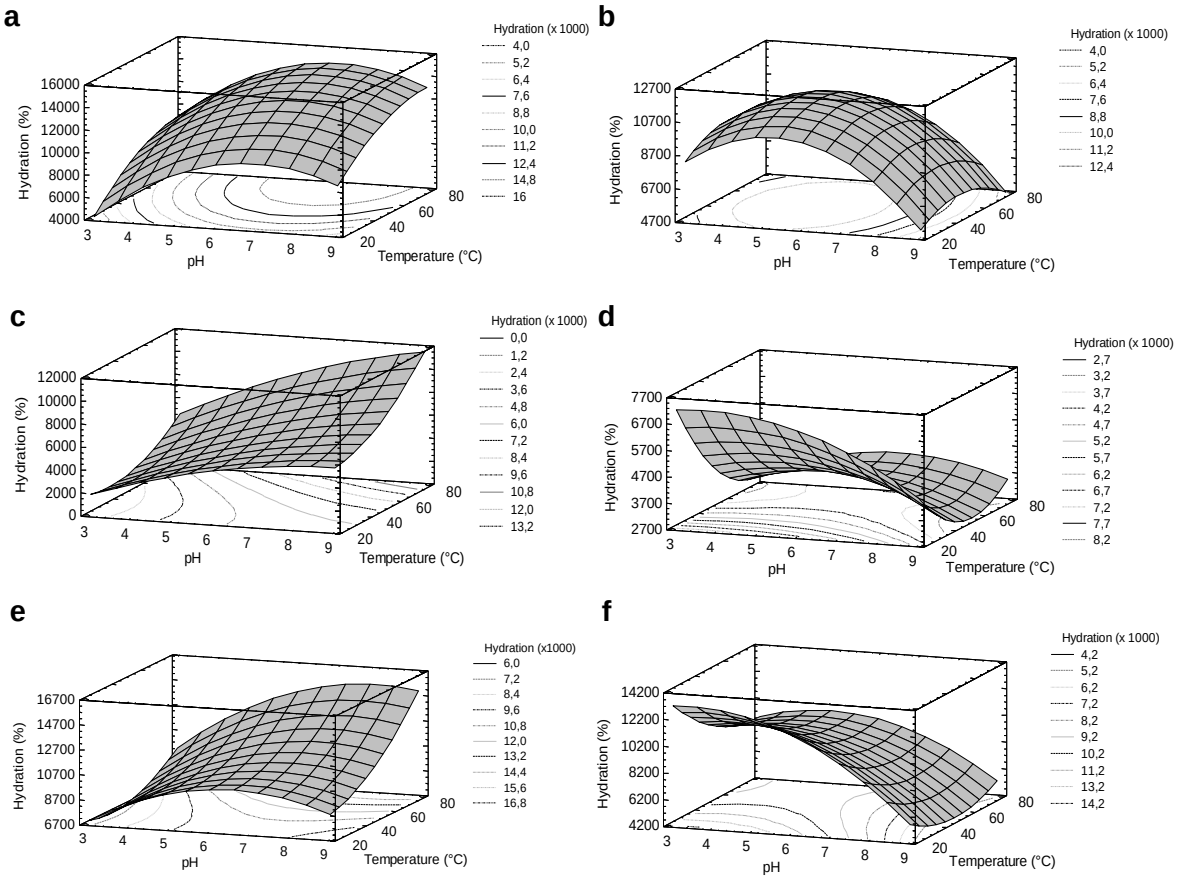
In these figures, it can also be observed that the water absorption was higher at 0.5% than at 1% salt content for the three salts. So, the water absorption of the mucilage was strongly dependent on the concentration and the type of salt. A similar behavior has been reported by other authors for several polysaccharides (Montero and Pérez-Mateos, 2002; Pérez-Mateos and Montero, 2002; Pourjavadi et al., 2008) who described that the addition of monovalent salts over 0.5% produced a decrease in water holding capacity.

Table 3.3 ANOVA and effects for Hydration

	Hydration with NaCl			Hydration with $CaCl_2$			Hydration with KCl		
Source	**Estimated effect**	**Standard Error**	**p-value**	**Estimated effect**	**Standard Error**	**p-value**	**Estimated effect**	**Standard Error**	**p-value**
A:pH	4015.75	1439.58	**0.0117**	5366.38	1280.77	**0.0005**	3764	974.248	**0.001**
B:Temperature	4903	1439.58	**0.003**	4455	1280.77	**0.0025**	4450.63	974.248	**0.0002**
C:salt content	-16372.3	1439.58	**<0.0001**	-20507.4	1280.77	**<0.0001**	-16123.9	974.248	**<0.0001**
AB	-244.5	2035.88	0.9057	1543	1811.28	0.4049	2034.75	1377.79	0.1561
AC	-7351	2035.88	**0.0019**	-6556.75	1811.28	**0.0018**	-9396.25	1377.79	**<0.0001**
BC	-5122	2035.88	**0.021**	-6969	1811.28	**0.0011**	-6634.5	1377.79	**0.0001**
Average	13121	1175.41		5609.17	1045.75		11163.3	795.47	

Standard errors are based on total error with19 d.f.
p-value<0.05 means statistical significant differences.

Figure 3.6 Response Surface for Hydration



(a) 0.5 %NaCl; (b) 1.0 %NaCl; (c) 0.5 % $CaCl_2$; (d) 1.0 % $CaCl_2$; (e) 0.5 % KaCl: (f) 1.0 % KaCl

The effect of the salt concentration could be due to the increase in the differences of osmotic pressure between the forming hydrogel and the watery phase. Consequently, the water absorption of hydrogel diminishes at high salt concentration, as earlier described by Pourjavadi et al. (2008) in a superabsorbent hydrogel from chitosan. This also can be attributed to the loss of the hydrophilic-hydrophobic balance of the polymer networks in the presence of salts (Mishra et al., 2008; Singh et al., 2007). The water holding capacity improves when pH increased from 3 to 9. This result was different to that observed by Pourjavadi et al. (2008) in a chitosan-sucrose polysaccharide, in which

the hydrogel diminished its water absorption when the pH increased. These authors related the phenomenon to the presence or absence of amino groups.

The results of optimization are summarized in Table 3.4. The maximum hydration was produced using low concentrations of salt, pH close to 9 and temperature around 80°C. By the manipulation of these relationships, either a dry or an hydrated mucilage can be produced, a valuable process with real food applications.

Table 3.4 Optimization of the hydration of mucilage of chia

Factor	Low	High	Optimum NaCl	Optimun $CaCl_2$	Optimun KaCl
pH	3.0	9.0	8.9991	9.0	9.0
Temperature	20.0	80.0	80	80	79.3197
Salt (%)	0.0	1.0	0.0174	0.0070	4.74475E-10

3.4. Conclusions

Mucilage of chia seeds was located in the outer three layers of the seed coat. When the seed came in contact with water, the mucilage appeared immediately and in a short time it formed a transparent “capsule” surrounding the seed.

The optimum extraction process was performed at a temperature of 80°C with a seed:water ratio of 1:40 (7 % yield). The maximum hydration occurred at low concentrations of salt, pH from 6.5 upwards, reaching a maximum at pH 9, and a temperature from 65 °C with best results at 80°C. The results of this study showed that the mucilage can be easily extracted and hydrated to achieve a water retention of 27 times its weight in water. Chia seeds and mucilage have a great potential as a functional ingredient to be used as thickener in foods.

3.5. Acknowledgements

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4. CHEMICAL, FUNCTIONAL AND THERMAL PROPERTIES OF POLYSACCHARIDE GUM EXTRACTED FROM CHIA (*Salvia hispanica*) SEEDS.

Abstract

Chemical, functional and thermal characterization of mucilage from chia seed was investigated. The mucilage contained 48±0.55% of total sugar, 4±0.05% of protein, 8±0.57% of ash and 1.78±0.02% of fat. The monosaccharide composition was 16.78±0.59% D-xylose+D-mannose, 2.11±0.18% D-arabinose, 6.77±0.30% D-glucose, 3.9±0.32% galacturonic acid and 12.1±2.30% glucuronic acid, with 41.66% total sugars. The mucilage contains functional groups - hydroxyl and carbonyl groups of carboxylates and carboxylic acid similar to those found in xanthan gum. DSC spectra together with fusion point show the mucilage temperature stability and the feasibility of using it in processes that involves high temperature. Chia mucilage exhibited shear thinning flow behavior in all concentration studied (0.1, 0.5, 0.8 and 1%). Finally, the mucilage shows high solubility and capacity to form highly viscous solutions at low concentrations, therefore can be a potential ingredient for food industry.

Keywords: *Salvia hispanica,* chia seed, mucilage, functional properties, thermal behavior

4.1. Introduction

Less than 20 crops account for the majority of nutrients available in human diets, thus, there is a need to expand the base of our food supply. Ancient crops that have been neglected in the past by modern agriculture are now major targets in the search for new food sources with unique characteristics. *Salvia hispanica L.,* commonly known as Chia, an oilseed used centuries ago by Mayas and Aztecs in Central and North America is a

natural source of omega-3 and omega-6 fatty acids, soluble fibers, antioxidants, vitamins and minerals. It has been reported that Chia seeds can prevent heart diseases, inflammatory problems, protect the central nervous system and has also been reported to have nutritional food supplements by FDA (Ayerza & Coates, 2005; Craig, 2004; Fernández, Ayerza, Coates, Vidueiros, Slobodianik, & Pallaro, 2006; Fernandez, Vidueiros, Ayerza, Coates, & Pallaro, 2008; Vuksan, Jenkins, Dias, Lee, Jovanovski, Rogovik, et al., 2010; Vuksan, Whitman, Sievenpiper, Jenkins, Rogovik, Bazinet, et al., 2007). One major characteristic of this oilseed is that it exudes mucilage with interesting properties for the food, care and pharmaceutical Industries.

Mucilages are heterogeneous polysaccharides of vegetable origin, formed by different carbohydrates (Ixtaina, Nolasco, & Tomás, 2008; Lin, Daniel, & Whistler, 1994). They form hydrogels that possess three-dimensional polymeric networks and absorb large amounts of water (Singh, Chauhan, Kumar, & Chauhan, 2007). They are characterized by producing colloidal viscous dissolutions and gels in water. These form part of the soluble fibers and their properties can be summarized in the following way: they swell and form gels when in contact with the water, dissolve giving viscous dissolutions, loses some monosaccharide during hydrolysis , but has a more resistant nucleus that can only be hydrolyzed enzymatically (Stephen, Phillips, & Williams, 2006). Besides, they possess different benefit such as diminishing the time of gastric voidance, among others.

The presence of mucilage in the seeds of *Salvia hispanica* has been reported by various authors (Ayerza & Coates, 2001; Lin et al., 1994; Muñoz, Cobos, Diaz, & Aguilera, 2012; Whistler, 1982). Chia seed when is hydrated can absorb up to 12 times its weight in water and the seeds are surrounded by a transparent mucilaginous polysaccharide. Also had been shown that the extracted mucilage has the capacity to hold 27 times its weight in water (Muñoz et al., 2012), an excellent property to be considered as functional ingredient.

Thorough studies about the mucilage of chia are very scarce in the literature. Lin et al. (1994) proposed a tentative structure for the polysaccharide, consisting of a

tetrasaccharide with 4-*O*-metyl-α-D-glucoronopyranosyl residues occurring as branches at *O*-2 of some β-D-xylopyranosyl residues in the main chain of (1→4)-β-D-xylopyranosil-(1→4)-α-D-glucopyranosyl-(1→4)-β-D-xylopyranosil units. Hence, it is very important to study the chemical composition, functional properties (solubility, viscosity as well as emulsifying and foaming properties) and thermal properties of the mucilage from Chia seeds. Therefore, the aim of this research is to study some chemical, functional and thermal properties of the mucilage of *Salvia hispanica* in order to ascertain its potentials as nutraceutical and/or functional food ingredient.

4.2. Materials and methods

4.2.1. Materials

Chia seeds were obtained from Benexia (Functional Products Trending S.A. Santiago, Chile) which are produced commercially in the Salta region, Argentina and xanthan gum was purchased from Sigma-Aldrich.

4.2.2. Removal of mucilage

Whole seeds were first hydrated (1:40 w/v seed:water ratio) for 2 hr in distilled water at 20°C while stirring with a magnetic stirrer bar. Then the aqueous system was widespread in a drying tray and exposed to temperature of 50°C for 10 h in an air convection heat oven (Indelab, model IDL.FI 80, Navarra, Spain).. The dried mucilage was separated from the seed by rubbing over a 40 mesh screen (Muñoz et al. 2012).

4.2.3. Chemical Analysis

4.2.3.1. Proximate analysis

All the analyses were performed in triplicate. The total protein concentration in the mucilage was estimated using AOAC official method 950.09. The total ash was determined by calcinations at 550 °C using AOAC official method 950.49. Moisture was determined by thermogravimetric method at 104 °C for 4 h, using AOAC official method 985.05. For determination of total carbohydrate content, the Anthrone method was used (Ludwig & Goldberg, 1956). The fat determination was realized by AOAC Official Method 920.39C with hexane as solvent (AOAC, 1995).

4.2.3.2. Organic elemental analysis

Organic Elemental Analysis (OEA) is based on the combustion of samples which allows determination of accurate level of carbon ©, hydrogen (H), nitrogen (N), sulfur (S) and oxygen (O) in different natural and synthetic materials. The analysis for C, H, N and S was performed with a Fisons model EA 1108 CHNS-O Micro analyzer, Milan Italy with 0.3 % precision absolute while for oxygen determination, a Carlo Erba model EA 1108 Microanalyzer was used, both previously calibrated by BBTO [2,5-Bis-(5-ter-butyl-benzoxazol-2-yl)-thiophen] standard (Thermoquest, Italia). Proteins from the extracted mucilage were removed using the Sevag method (Wang, Chang, & Chen, 2009) and subsequently analyzed. The samples were studied in triplicate. The method is based on the complete and instant combustion of the sample for subsequent determination of gases from the combustion through a thermal conductivity detector (Marcó, Companyó, Rubio, Pueyo, & Vilalta, 2007).

4.2.3.3. Fourier transform infrared spectroscopy (FT-IR) and Thermal analysis

The FT-IR spectrum of mucilage of chia seeds and xanthan gum were recorded on a Bruker Vector 22 (Bruker Optics GmbH, Inc., Ettlingen, Germany) with a frequency range of 4000 – 250 cm-1 using KBr pellets. Thermogravimetric analysis (TG) and Differential Scanning Calorimetry (DSC) to obtain the thermograms were performed under nitrogen atmosphere (flow rate 150 and 50 mL/min, respectively). A sample of 3-4 ± 0.1 mg was used in each experiment. Thermal stability studies were performed using a TG (STA-625 Termobalance) at a heating rate of 10°C min-1. DSC measurements were carried out with a Mettler Toledo Star System 822e to determine the Lower Solution Critical Temperature (LSCT) at a heating rate of 10°C min-1.

4.2.3.4. Monosaccharides determination

The composition of monosaccharides and uronic acids in the mucilage of Salvia hispanica were determined by HPLC as modified by Sciarini, Maldonado, Ribotta, Pérez, & León (2009).

The standards D-arabinose, D-xylose, D-mannose, L-rhamnose, D-glucose, D-galactose, glucuronic and galacturonic acid were purchased from Sigma-Aldrich. The composition of neutral sugars and uronic acids was carried out after hydrolysis of the mucilage. 100 mg of the polysaccharide was dissolved in 10 ml of 0.5M sulphuric acid, the tube was sealed and heated for 12 and 24 h at 95 °C each, cooled and later neutralized with 0.2 M NaOH. The volume was graduated at 50 mL with milli-Q water and centrifuged at room temperature at 1300 g for 10 min. The supernatant was filtered through a nylon membrane of 0.45 mm diameter.

For quantification of sugars present in the mucilage, the hydrolysis was performed on the sample of mucilage alone and the same amount of mucilage sample was doped with known amounts of the different standards of neutral sugars and uronic acids present

in the mucilage. Then, 20 µL of the hydrolyzates were injected into the HPLC using Shimadzu HPLC System with autosample and refraction index detector (Labchrom RI) for determination of sugars and spectrophometric detector (210nm) for determination of uronic acids. Monosaccharide separation was performed using a BioRad HPX 87-H column 250x4.6 mm at 65 °C. The mobile phase was sulphuric acid 5 mM and the flow of 0.5 mL/min. Each triplicate sample was injected three times.

4.2.4. Functional Properties

4.2.4.1. Solubility

Solubility was determined by modified method of Sciarini *et al*. (2009) and Betancur-Ancona, López-Luna, & Chel-Guerrero, (2003) using equation (4.1). Samples of 30 mL at 0.15; 0.25 and 0.5% were placed in water bath at 30, 60, 70 and 90 °C respectively, for 30 min and constantly stirred. Each suspension was centrifuged at room temperature at 800 g and 2000 g separately for 15 min and the supernatant was dried at 125°C overnight until constant weight.

$$\%\, Solubility = \frac{(wf - wi)\, x\, 30}{10}\, x\, 100 \qquad (4.1)$$

where *wi* correspond to initial and wf is final weight.

4.2.4.2. Rheological behavior

The basic rheological characterization of MC in comparison with XG in aqueous system was performed using a Rheolab MC20 Rheometer (Physica Inc. Spring, TX. USA) with Z2 spindle and equipped with temperature controlled water bath. Data was analyzed according to the Power law model $\sigma = K\dot{t}^n$ and *K* (consistency index) and *n*

(flow behavior index) were calculated from the data and statistically evaluated using nonlinear regression. Dispersions of 0.3; 0.5; 0.8 and 1 % w/v mucilage of chia and xanthan gum were used as control. The values were determinate in triplicate.

4.2.4.3. Emulsifying Properties

Solutions of 50 ml of 0.8 % w/v of mucilage in water was prepared (pH 7) and used as continuous phase. These solutions were used to prepare the emulsions at 40 and 60 % by mixing commercial sunflower oil as dispersed phase. Emulsions were prepared by homogenizing two times at 11,000 rpm for 2 min in Ultraturrax IKA Labortechnik model T 25 basic. An aliquot was diluted 1/3000 using a 0.1% Sodium dodecyl sulfate (SDS) solution in phosphate buffer at pH 7 and the absorbance of the diluted emulsions were determined at wavelength of 500 nm using a spectrophotomenter (Zuzi model UV-4210). The emulsifying capacity was determined by turbidimetric procedure (Pearce & Kinsella, 1978) and expressed as Emulsify index activity (EAI) using Pearce and Kinsella´s equation (4.2) as modified by Cameron (1991):

$$EAI\ (\frac{m^2}{g}) = \frac{2 \text{ x } 2.303 \text{ x } A500 \text{ x } L \text{ x } C}{\emptyset} \qquad (4.2)$$

where: A500 corresponds to absorbance of dilute emulsion at 500 nm; $\emptyset$ the volume fraction of dispersed phase; L, path length of cell (in m), and C, weight of product per unit of product dispersion (g/m3).

4.2.4.4. Emulsion Stability

The Emulsifying Stability Index was determined in triplicate (Tornberg & Hermansson, 1977; Tornberg & Lundh, 1978). Aliquots of the emulsions prepared as previously described were transferred to centrifuge tubes and stored at 4°C for 24 hr. After this time the samples were centrifuged at 1000 g for 15 min at 20 °C. Fat content

of the bottom fraction and initial emulsion were determined by Hanson and Olley Method (Hanson & Olley, 1963). Index of emulsion stability (IEE) is given by the following formulae (4.3):

$$\text{IEE}\ (\%) = \frac{\text{Fat content in lower phase}\ (\%)}{\text{Fat content inicial emulsion}\ (\%)} \text{x}\ 100 \qquad (4.3)$$

4.2.4.5. Foaming Properties

Polysaccharide hydration and dispersion was carried out using the standard solubilization procedure by Morr and the method proposal by Phillips (Morr, German, Kinsella, Regenstein, Van Buren, Kilara, Lewis & Mangano, 1985; L. G. Phillips, Schulman, & Kinsella, 1990).

Solutions of 1% (w/v) of mucilage were prepared, and ovalbumin was used as reference (Sigma Aldrich 67% purity) at 5% w/v. The suspension was whipped at 10,000 rpm (Braun mixer model MR 550) for 10 min at room temperature (20°C). The foam volume was recorded and the overrum was calculated as shown in equation 4.4:

$$\%\ \text{Overrum} = \frac{(\text{weigth of 100 ml dispersion} - \text{weigth of 100 ml foam})}{\text{weigth of 100 ml foam}} \qquad (4.4)$$

4.2.4.6. Foam Stability

According to Patel, Stripp & Fry (1988) the foam prepared as noted above was quickly transferred into a 2 L measuring cylinder and the total volume was recorded. The foam was allowed to stand for 30 min at room temperature and afterwards the volume of drained liquid was measured. Foam Stability (FS) was calculated as follows (equation 4.5):

$$FS(\%) = 100 - [\ \frac{liquid\ drainage}{Initial\ Volume}\ x\ 100\ ] \qquad (4.5)$$

4.3. Results and Discussions

4.3.1. Chemical Analysis

4.3.1.1. Proximate and Organic Elemental Analysis.

Results of the proximate analysis of the crude mucilage are shown in table 4.1.

Table 4.1 Proximate Composition of mucilage from chia seeds

Nutrient	Composition (%)
Moisture	15.15 ± 0.33
Carbohydrates	48.09 ± 0.55
Proteins	4.43 ± 0.05
Lipids	1.78 ± 0.02
Ash	8.07 ± 0.57
Uronic Acid	23.22 ± 1.32

Values are means of triplicate ± standard deviation.

The mucilage of Chia seeds contains about 71.22 % of polysaccharides slightly lower than the results obtained for the polysaccharides of mucilage of flaxseed (75 %) (Warrand, Michaud, Picton, Muller, Courtois, Ralainirina & Courtois, 2005) and yellow mustard seed (80 %) (Cui, Eskin & Biliaderis, 1993). Comparing this composition with that of other industrial hydrocolloids, different amounts of carbohydrates in their composition were found, for example guar gum possess about 23.7 % and xanthan gums contain 98%, approximately (Ahmed, Hamed, Ali, Hassan & Babiker, 2006). Uronic acid content obtained was 23.22% which agrees with the work of Lin et al. (1994), where the percentage value corresponds to about 23%. In the work reported by Wu, Cui, Eskin and Goff (2009), where the mucilage of mustard was studied, they reported similar composition which is very common in this type of mucilages. The acidic nature of this mucilage can be attributed to the presence of uronic acid.

The elemental analysis showed the chia mucilage contains 1.38±0.04% nitrogen; 37.99±1.19% carbon; 5.64±0.13% hydrogen and 47.27±1.02% oxygen, the remaining

7.72 % could correspond to sulfur. In comparing with the results obtained by Lin et al. (1994) who reported 0.62%, 34.4% and 5.27% for nitrogen, carbon and hydrogen respectively, similarities were observed in carbon and nitrogen composition. These differences may be attributed to differences in seeds varieties, geographical location, agricultural practices and the method of proteins removal.

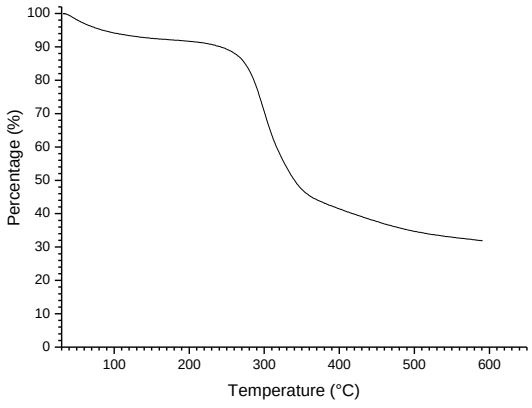
4.3.2. Thermal Behavior

The TGA results for the mucilage are summarized in table 4.2. The mucilage showed a one-step degradation with an extrapolated thermal decomposition temperature (TDT) of 271.2°C (figure 4.1).

Table 4.2 Thermal decomposition behavior of the mucilage by thermogravimetric analysis (TG) at different temperatures (°C).

	Weight loss (%) at different temperatures (°C)						
	100°	200°	300°	400°	500°	600°	TDT
Mucilage	5.9	8.3	29.5	58.6	65.3	68.1	271.2

Figure 4.1 TG Thermogram for mucilage from chia seeds

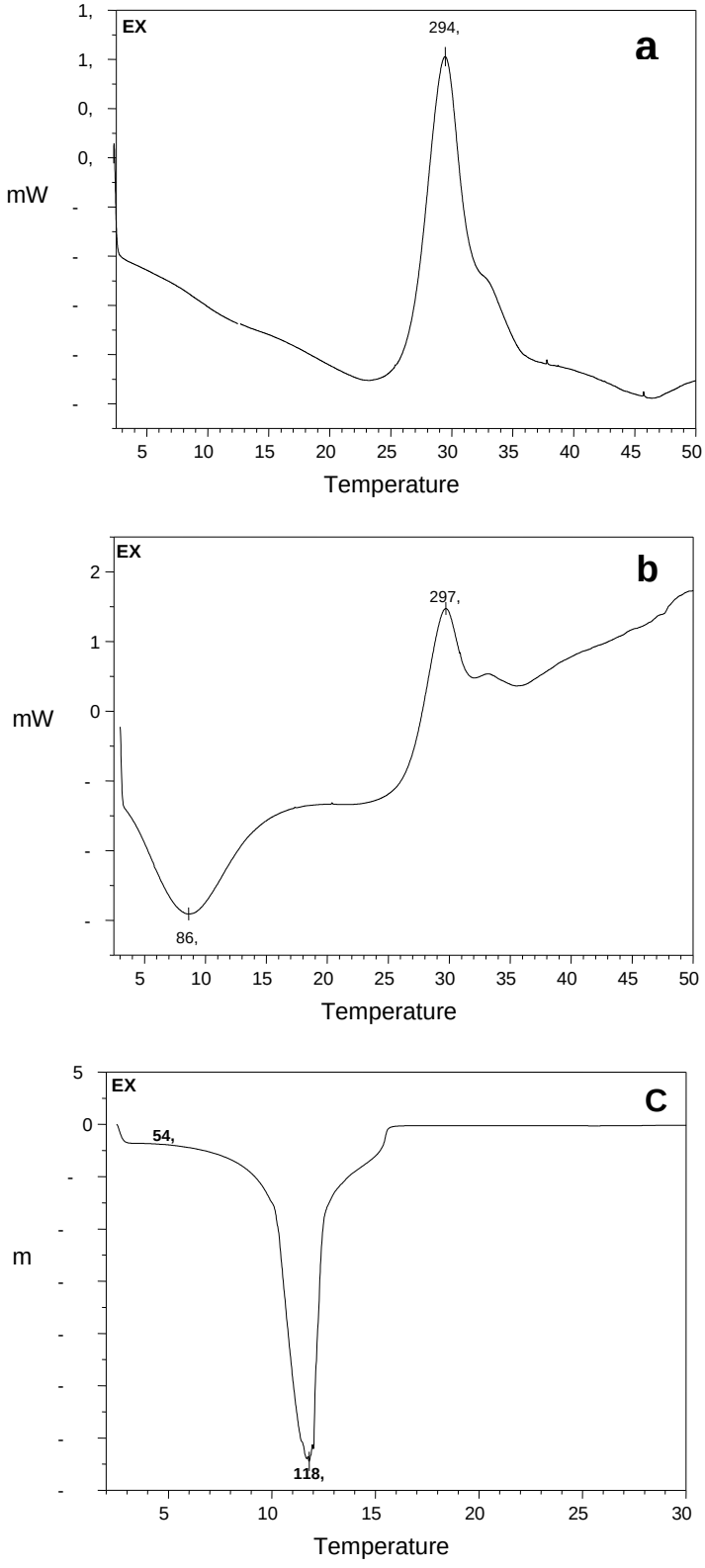


The DSC thermograms for mucilage from chia seeds are shown in figure 4.2. Figure 4.2a corresponds to dry mucilage (Xerogel).

The oxidative degradation temperature with a heating rate of 10°C min^{-1} was estimated from the first DSC run. Figure 4.2b shows the thermogram of the hygroscopic mucilage with an endo transition peak at 86.2°C for the lost hygroscopic water and an exo transition at 297.2°C for the oxidative degradation of the sample. Figure 5.2c shows the thermogram of the hydrogel with an endo transition at 54.2°C for the lower critical solution temperature (LCST). The reason for this sharp phase transition is a good balance between hydrophilic and hydrophobic interactions in the mucilage (Kuckling, Adler, Arndt, Ling & Habicher, 2000).

The mucilage undergoes a temperature induced collapse from an extended coil into a globule structure and this was revealed on the macroscopic scale by a sudden decrease of the degree of swelling of the mucilage (Kuckling et al., 2000; Tanaka, 1993). Increasing the temperature of an aqueous mucilage solution above the LCST causes a coil-to-globule transition, followed by a phase separation. This phase transition is accompanied by a release of water bound to the mucilage chain, which is an endothermic process. Similar results on LCST determination to detect transition from coil to globules using different methods have been reported in the literature for different mucilages (Guohua & Hoffman, 1995; Shibayama, Suetoh & Shunji, 1996; Wu et al., 2009).

Figure 4.2 DSC Thermograms

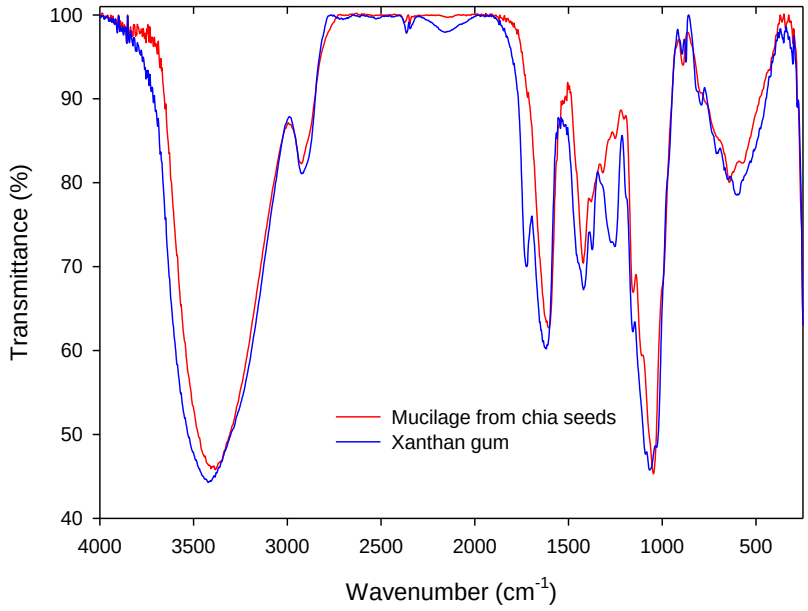


(a) DSC for dry mucilage "Xerogel"; (b) DSC for hygroscopic mucilage; (c) Lower critical solution temperature (LCST) of hydrogel mucilage. Heating rate of 10°C min^{-1}

4.3.3. FT-IR spectroscopy

FT-IR spectroscopy was additionally used to compare functional groups between mucilage of chia seeds and xanthan gum as reference. Figure 4.3 shows the FT-IR spectra of XG and MC in KBr pellets and table 4.3 shows the main functional groups found in MC in comparison with commercial XG.

Figure 4.3 FT-IR (KBr pellets) spectra of mucilage from *Salvia hispanica* and xanthan gum



The most important bands recorded were assigned to the –OH stretching of hydroxyl group, aliphatic –C-H stretching vibration, group (C=O) stretching vibration of carboxylic acid and aromatic group, $–COO^-$ stretching and finally C-O as ester group . The absorption bands 1604.5 and 1618.6 cm^{-1} to MC and XG respectively can be

attributed to ring stretching of mannose (Freitas, Alves, Pais, Costa, Oliveira, Mafra, Hilliou, Oliveira & Reis, 2009). Bands at 1421.4 to MC and 1419.9 cm^{-1} to XG respectively can be assigned to the carboxylate group of uronic acid. The presence of uronic acid was confirmed based on the results obtained by FT-IR (stretching >C=O, carboxylic acid group: –COOH) which establish the presence of two functional groups present in the structure. The carbohydrate peaks of the mucilage of chia seeds were similar to xanthan gum because they have similar monosaccharide composition, the identified absorption bands only differ in intensity. Comparing FT-IR MC spectrum with other commercial gums (Freitas et al., 2009; Prado, Kim, Ozen & Mauer, 2005) as well with xanthan gum (in this study), they show bands close to 3400, 2939 and 990 – 1200 cm^{-1} common to all polysaccharides representing O-H , C-H bonds of CH_2 groups and saccharides respectively. Finally, the presence of uronic acid could be also corroborated by the decrease of pH of the aqueous solutions, a phenomenon associated with the presence of carboxylic acids (Matsuhiro, Lillo, Sáenz, Urzúa & Zárate, 2006).

Table 4.3 FT-IR absorption bands to MC in comparison with XG

Funcional Group class	**Band position (cm^{-1})**	**Intensity of absorption**	**Wavenumber MC (cm^{-1})**	**Wavenumber XG (cm^{-1})**
–OH stretching of hydroxyl group	3200-3400	Strong/ broad	3384.8	3421.8
–C-H stretching vibration	2926 (±10)	strong	2924.8	2923.5
(C=O) stretching vibration of carboxylic acid	1700-1725	strong	1718.8	1721.5
(C=O) bonded to aromatic group	1600-1700	strong	1604.5	1618.6
$–COO^-$ stretching (ion)	1400-1550	medium	1421.4	1419.9
C-O as ester group in a cycle	1230-1270 1020-1075	medium weak	1251.7 1047.2	1253.6 1068.6

4.3.4. Polysaccharide determination

The hydrolysis of the mucilage carried out for 24 h was more reproducible and resulted in a high amount of neutral sugars and uronic acids. The retention times obtained for monosaccharides and uronic acid standards are shown table 4.4. Retention time for D-mannose and D-xilose were very similar (11.46 and 11.55 min respectively), therefore the results were quantified with D-xylose as standard.

The results for the hydrolyzed mucilage samples show the presence of D-xylose, D-mannose, D-arabinose, D-glucose, glucuronic and galacturonic acid but L-rhamnose and D-galactose were absent; while two unknown peaks with retention times of 8.67 and 9.84 min were observed.

Table 4.4 Sugar composition of mucilage from chia seeds

Compound	Retention Time (min)	Percentage of contribution (%)
Glucose	10.8	6.77 ± 0.30
Xylose+Mannose	11.55	16.78 ± 0.59
Arabinose	12.45	2.11 ± 0.18
Ramnose	12.15	ND
Galactose	11.09	ND
Galacturonic acid	7.35	3.9 ± 0.32
Glucuronic acid	8.30	12.1 ± 2.30
unknown	8.67	–
unknown	9.84	–

ND: not detected. Values are means of triplicate ± standard deviation.

These peaks were only observed in the chromatogram obtained by refraction index detector, so it can be assumed to be two unidentified sugars, the peak of 8.66 min was 10 times bigger than the area given to 9.84 min retention time. The percentages of sugars obtained from the sample of mucilage were: 16.78±0.59% D-xylose+D-mannose, 2.11±0.18% D-arabinose, 6.77±0.30% D-glucose, 3.9±0.32% galacturonic acid and

12.1±2.30% glucuronic acid, giving a total of 41.66% of sugars. The difference in results obtained with total sugar determined with Anthrone method may be due to the presence of two unknown peaks and could be attributed to some losses during hydrolysis which could contribute to this results. The quantification of monosaccharides of mucilage of chia seed has not been published but Lin et al. (1994) reported only the presence of D-glucose, D-xylose and uronic acids in chia seed polysaccharide exudate. In flaxseed mucilage, with some similar characteristics to chia seed mucilage (Warrand, Michaud, Picton, Muller, Courtois, Ralainirina & Courtois, 2003) was reported the presence of D-xylose, D-galactose, D-arabinose, L-fucose, L-rhamonose and galacturonic acid.

4.4. Functional Properties

4.4.1. Solubility

Solubility represents the quantity of molecules that have been solubilized in a period of time at controlled temperature (Wang & Wang, 2003). The mucilage was completely soluble (100%) in water at the investigated temperatures (30, 60, 70 and 90°C), concentrations (0.15, 0.25 and 0.5%) and centrifugation conditions (800 and 2000g). These results are different from those observed with other gums in literature. For example, Larch gum had up to 60% solubility at room temperature, Arabic gum was highly soluble in hot water, but less soluble in cold water while Karaya gum had <0.02 % solubility in cold water and 0.06 % soluble in hot water (Williams, Phillips, Stephen & Churms, 2006). The possible mechanism responsible for this kind of behavior in mucilage from Chia seeds could be due to complete dispersion followed by solubilization that aids water penetration into the swollen particles thus complete interactions between the macromolecules (Doublier & Cuvelier, 2006).

4.4.2. Rheological behavior

The rheograms of MC and XG at 20°C are shown in figure 4.4 generated from SigmaPlot 11.0 software using measurements of strain rate to shear stress at 0.3, 0.5, 0.8 and 1% w/v of MC and XG as control (figures 5.4a and 5.4b, respectively). Xanthan gum such as mucilage of chia seed does not have the ability to form gel, but both exhibited a great capacity to form highly viscous solutions at low concentrations.

Chia mucilage had similar behavior compared with xanthan gum and the solutions showed a non-Newtonian flow. The flow behavior index (*n*) and consistency index (*K*) values were recorded to shape the rheogram (rotational speed v/s apparent viscosity) in order to select the appropriate model to characterization of the mucilage. Finally, the model of Oswald de Waele or Power Law model was selected to describe the rheological properties of mucilage dispersions, because using Herschel-Bulkley model ($\sigma = \sigma_0 + t^n$) obtained negative values of yield stress (σ_0), with no physical meaning, therefore the model was not suitable in this case (Steffe, 1996). Thus, the Power of law model was appropriate to describe the rheological behavior of the mucilage. *K* and *n* values are showed in table 4.5.

Table 4.5 K and n values for MC and XG

MC and XG Concentration (%)	XG							MC						
	K			***n***			$\mathbf{R^2}$	***K***			***n***			$\mathbf{R^2}$
0.3	162.53	±	7.83	0.30	±	0.004	0.99	3.05	±	0.22	0.65	±	0.006	0.99
0.5	577.89	±	34.69	0.23	±	0.005	0.97	5.86	±	0.23	0.63	±	0.003	0.99
0.8	990.92	±	58.83	0.21	±	0.005	0.96	74.72	±	2.89	0.51	±	0.003	0.99
1	1243.20	±	54.58	0.22	±	0.004	0.97	111.88	±	4.12	0.49	±	0.003	0.99

In both gums the *K* values decrease when decrease the solution concentration, but significant differences between MC and XG were observed. However, for both gums, an

increase in concentration was complemented with an increased in pseudoplasticity, shown by a decreased values of flow behavior index (*n*) when increased the concentration. Comparatively, the magnitude of consistency index was lower for MC than XG within the range of concentration studied. These differences could be attributable, in the case of pseudoplastic fluids, to shear stress what is a nonlinear function of shear rate and depends on the particular polysaccharide, their physicochemical characteristics, molecular weight, solubility, concentration, temperature, pH, ionic strength, size and shape, morphology, between others (Wang & Cui, 2005). Viscosity to MC and XG decreased with increasing shear rate, thus dispersions exhibited shear thinning behavior (Marcotte, Taherian Hoshahili & Ramaswamy, 2001; Phillips & Williams, 2000). It must be noted that steady-shear viscous flow properties of some important hydrocolloids such as xhantan gum are similar to that shown for mucilage of chia.

Figure 4.4 Viscosity and Shear stress as a function of strain rate of chia mucilage and xanthan gum

Figure 4.4a

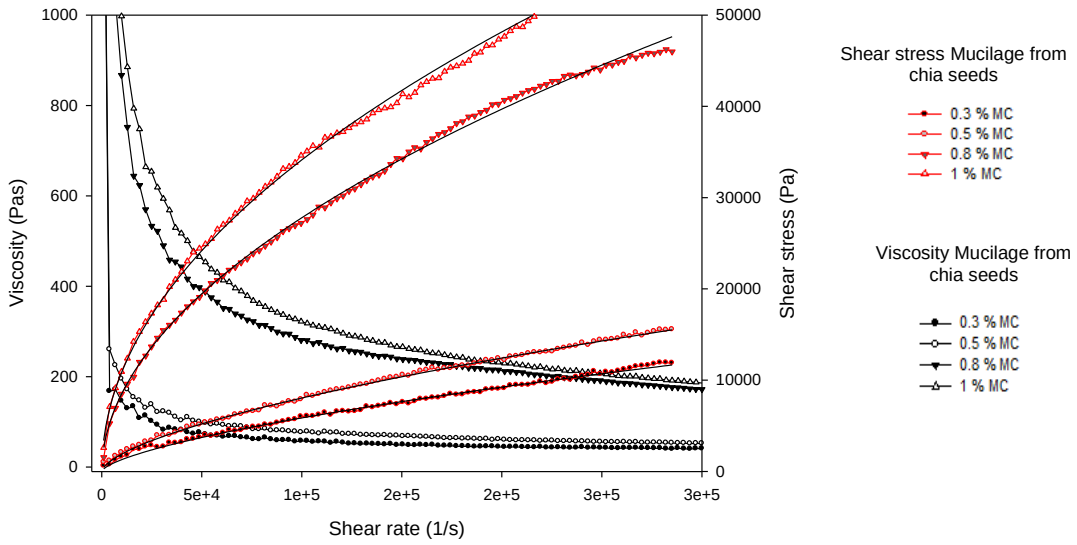
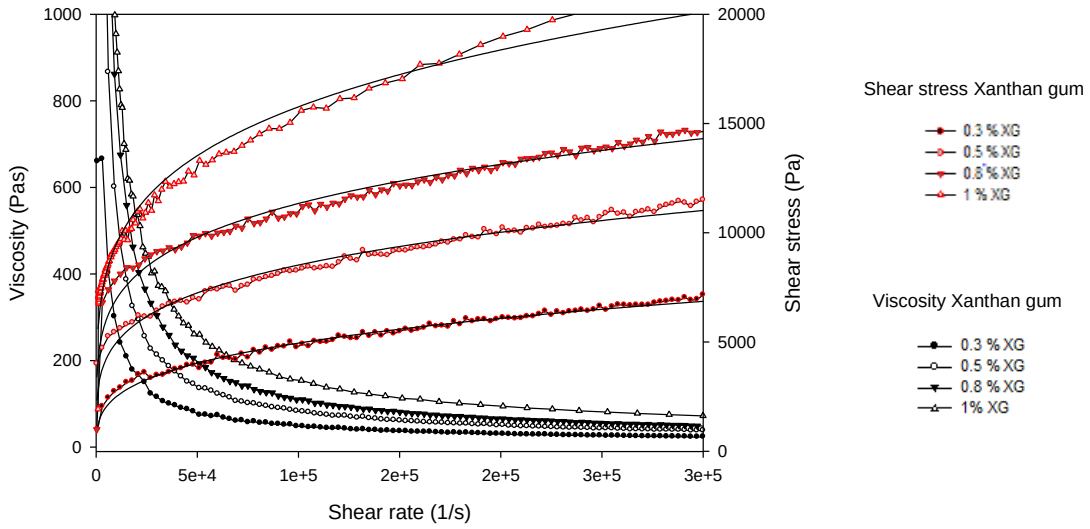


Figure 4.4b



The implication of this is that, the mucilage exhibited pronounced shear thinning behavior at low concentrations but when this system is more concentrated it can be considered as a solid-like material (Doublier & Cuvelier, 2006). Viscosity values of the different mucilage solutions are comparable with xanthan gum at the same concentration with both having high viscosity at low shear rates. This behavior can be attributed also to the rheological effect of molecules that increases with the concentration, which in turn increases the intermolecular hydrodynamic interaction (Chen & Chen, 2001). The most important functional properties of food polysaccharides are water binding capacity and viscosity (Wang & Cui, 2005) and the mucilage from chia seed has them.

4.4.3. Emulsifying activity index and index of emulsion stability

Emulsifying capacity is an important functional property of proteins and polysaccharides in various emulsion-based food systems. Most hydrocolloids can act as stabilizers of oil-water emulsions, but only a few can act as emulsifiers (Dickinson, 2009). Emulsifying activity index of mucilage of chia is low compared with other

polysaccharides such as gum Arabic, modified starch and celluloses (Dickinson, 2003; Garti & Leser, 2001). Mucilage showed an EAI of 41.41± 0.104 m^2/g with 40% oil and 4.46 ± 0.015 m^2/g with 60 % oil. Moreover, the mucilage presents greater capacity to stabilize the emulsion at 78.42 ± 1.96 % using 40% oil and at 42.31 ± 0.79 % using 60 % oil. The mucilage of chia possess a 4% w/w of proteins, in the continuous phase corresponds to 0.032 %; this small quantity of protein can be almost negligible because the amounts reported with emulsifying activity are around 0.2-10 % w/w proteins (van Dam, Watts, Campbell & Lips, 1995). The fact that mucilage of chia has the capacity to stabilize an emulsion can be explained based on its capacity to adsorb onto solid or liquid interfaces and can stabilize oil in water emulsions without any chemical or enzymatic modification. This can due to the addition of soluble polymer which increase the viscosity of the dispersed phase (Garti & Leser, 2001) thereby reducing the velocity of coalescence, especially when the polymer forms gel, i.e. the polymer chain form a network that needs minimum force to disrupt and thus the droplets are physically captured by the network and the emulsion will be stable (Bloom, 2008).

4.4.4. Foam expansion and foam stability

Stability is the most important property of a bubble containing product which seeks to maintain the structure until product consumption (Niranjan & Silva, 2008). Mucilage of chia does not have the capacity to form foams by itself, but have a great capacity to stabilize foams formed by ovalbumin. This is different from the results reported for yellow mustard mucilage (Cui et al., 1993). Table 4.6 shows the results of foaming properties of mucilage studied and the values obtained for foaming of ovalbumin were used as comparison.

Table 4.6 Foam expansion and Foam stability of mucilage from chia seeds

Dilution	% Overrum	Foam Stability (%)
5% OV	590.99 ± 13.70a	90.95 ± 0.21a
5% OV + 0.1 % MC	533.06 ± 14.67b	94.05 ± 0.27b
5% OV + 0.3 % MC	505.94 ± 25.28c	98.26 ± 1.52c

Values are means of triplicate ± standard deviation.

The highest overrum was found with ovalbumin 5% (control) but the stability is less than foams stabilized with 0.3 % MC. The results are inversely proportional, that is, when the level of overrum increase, the stability decreases. Highest value of 98.26 ± 1.52 % for foam stability was obtained with 0.3 % MC where the drainage liquid was minimal. This behavior can be explained due to the formation of small bubbles within the protein-polysaccharide matrix which confers an aerated structure that remains with time as well as interaction of polysaccharide with proteins presenting usually synergistic properties (Narchi, Vial & Djelveh, 2009).

4.5. Conclusions

Salvia hispanica seeds are source of hydrocolloids and the composition of mucilage obtained is mainly polysaccahrides and uronic acids. It possesses excellent properties as emulsifier and foam stabilizer. The mucilage showed a one-step degradation of TDT at 271°C, assigned at oxidative degradation of the sample. An endo transition at 54.2°C was assigned to the lower critical solution temperature (LCST). In view of the results obtained in this work, mucilage of chia can be a potential ingredient for food industry, especially as thickening agent, foam stabilizer, emulsifier and can be used as a surfactant to stabilize emulsions against coalescence.

4.6. Acknowledgment

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5. CHARACTERIZATION AND MICROSTRUCTURE OF FILMS MADE FROM MUCILAGE OF SALVIA HISPANICA AND WHEY PROTEIN CONCENTRATE

Abstract

Microstructural, physical and functional properties of thin films (90-110 μm) made from blends of the mucilage of *Salvia hispanica* (MC) and whey protein concentrate (WPC) were studied. Two proportions of MC:WPC were used (1:3; 1:4) at pH 7 and 10 in distilled water using glycerol as plasticizer. The effects of MC:WPC ratio and pH on colour, solubility, water vapor permeability, mechanical properties and microstructure were investigated. Transmission and scanning electron microscopy were used to investigate the microstructure of aggregates and films. Films produced at pH 10 and MC:WPC ratio of 1:3 has superior mechanical properties than the other films, with higher resistance and flexibility. Also, films produced at pH 10 demonstrated better water vapour barrier (0.620±0.08 g mm/Kpa h m^2) than films at pH 7. The pH and higher proportion of polysaccharide had a positive influence on mechanical and barrier properties achieving the highest value at pH 10 and higher proportion of polysaccharide.

5.1. Introduction

Several biopolymers such as proteins, polysaccharides and lipids as well as their combined forms have been used to develop coatings and edible films (Arvanitoyannis, Nakayama, & Aiba, 1998; Osés, Fabregat-Vázquez, Pedroza-Islas, Tomás, Cruz-Orea, & Maté, 2009; The, Debeaufort, Voilley, & Luu, 2009). In particular, these materials have attracted greater attention and are been used as protective barrier at low or intermediate relative humidities (Perez-Gago & Krochta, 2002). Polysaccharides and proteins possess the ability of establishing polymer interaction, thus creating a continuous network responsible for the functional properties of edible films (Giancone et

al., 2009; Perez, Carrara, Sánchez, Rodríguez Patino, & Santiago, 2009). The desired barrier properties of edible films are similar to those of other packaging materials which include protection of food from relative humidity, light, atmosphere, enzymatic and chemical reactions, lipid oxidation, colour, flavour and nutritional changes (Guilbert, Gontard, & Cuq, 1995; Han & Gennadios, 2005; Haugaard, Udsen, Mortensen, Høegh, Petersen, & Monahan, 2001; Robertson, 2010).

Edible films from whey protein concentrate (WPC) and isolates (WPI) (Banerjee & Chen, 1995; Kester & Fennema, 1987; McHugh, Aujard, & Krochta, 1994; McHugh & Krochta, 1994; Perez-Gago & Krochta, 1999) as well as polysaccharides (Carneiro-da-Cunha, Cerqueira, Souza, Souza, Teixeira, & Vicente, 2009; Donhowe & Fennema, 1994; Guilbert et al., 1995; Nieto, 2009) have been widely studied. The studies of the properties of composite edible films from proteins and polysaccharides are scarce, despite the fact that the combination seems to improve the physical characteristics of edible films (Coughlan, Shaw, Kerry, & Kerry, 2004; Osés et al., 2009; Perez et al., 2009). Mucilage of Salvia hispanica, a natural exudates from chia seeds is a novel ingredient. It is mainly composed of xylose, glucose and glucuronic acid forming a branched polysaccharide (Lin, Daniel, & Whistler, 1994; Muñoz, Cobos, Diaz & Aguilera, 2012).

Of recent, chia seeds is seen as a potential source of nutrients as it is been used as nutritional supplements as well as in the manufacture of bars, breakfast cereals and cookies in USA, Latin America and Australia (Muñoz et al. 2012). In 1996, it was described by FAO as a potential source of polysaccharide gum because of its exceptional mucilaginous properties at low concentration in aqueous solution. Reyes-Caudillo, Tecante, & Valdivia-López, (2008) reported earlier that chia seeds contain about 5 – 6 % mucilage that can be used as dietary fiber. In fact, in our previous report (Muñoz et al., 2012) we highlighted about 7 % yield of extraction of mucilage of chia seeds.

According to Whistler (1982), this mucilage has numerous potential food applications, but it has been poorly studied. Therefore, the mucilage obtained from chia seeds could be a new source of polysaccharides with the potentials of generating

different polymer blends to produce films and coatings with improved properties. Hence, the aim of this study was to develop thin films from mixtures of mucilage of Salvia hispanica and whey protein concentrates and to evaluate their physico-chemical properties.

5.2. Materials and methods

5.2.1. Materials

Protarmor 800 a whey protein concentrate (WPC), was purchased from Armor Proteines (Saint-Brice en Coglès, France). According to the manufacturer, the composition of the product was: 80% protein, 4% moisture, 3.5% ash, 3.5% fat and 9% lactose. Chia seeds were provided by Benexia (Functional Products Trending S.A., Santiago, Chile).

5.2.2. Mucilage extraction

Mucilage extraction was performed as described by Muñoz et al. (2012). Samples of about 20 g of the whole seeds were placed in a 1L beaker and distilled water was added in 1:40 seeds:water proportion. The dispersion was stirred with a magnetic stirrer (ARE, VELP Scientifica, Italy) and hydrated for 2 hours at room temperature. Thereafter, the aqueous suspension was spread on a drying tray and exposed to temperature of 50°C for 10 hours in an air convection heat oven (Indelab, model IDL.FI 80, Navarra, Spain). The dried mucilage was separated from the seed by rubbing over a 40 mesh screen. Proximate analysis of the dried mucilage was determined using AOAC methods (AOAC, 1995). For determination of total carbohydrate content, Anthrone method was used (Ludwig & Goldberg, 1956).

5.2.3. Preparation of film forming dispersions

Film forming solutions with 1.6 % total solids (total solids was kept constant in order to form films of uniform thickness) were prepared by mechanically stirring mixtures of seven proportions of mucilage of Salvia hispanica (MC) and WPC (1:0; 1:1, 1:2, 1:3, 1:4, 2:1 and 0:1; w/w) in de-ionized water for 20 min at 18°C. The pH was adjusted to 7 or 10 with 0.1 M NaOH. Then, glycerol or sorbitol (Panreac, Barcelona, Spain), in proportion of 2:1 of mixture:plasticizer was added. The solutions were stirred with a magnetic stirrer (ARE, VELP Scientifica, Italy) for additional 10 min and then heated in a circulating water bath (Selecta, model Tectron 200, Barcelona, Spain) at 80°C for 30 min. The dispersions were then cooled immediately in an ice-water after which 30 g of each film forming solution were poured into Plexiglas Petri dishes of 9 cm diameter. The films were dried in an air convection heat oven (Indelab, model IDL.FI 80, Navarra, Spain) at 50° C for 8 hours and then kept in a desiccator containing a saturated potassium carbonate solution at 50 ± 5 % relative humidity (RH) for 72 hours (Hygrometer Testo, model 645, Lenzkirch, Germany). The films were peeled from the dishes and stored for 48 hr at 50 ± 5 % RH. All experiments were done in triplicates. Film thickness was measured using a 0-25 mm electronic digital micrometer (Selecta, Barcelona, Spain) with 0.001 mm resolution. Measurements were performed at 5 points of three films selected randomly.

5.2.4. Transmission Electron Microscopy

The microstructure of polysaccharides:protein aggregates was observed by transmission electron microscopy (TEM) using a negative staining method. One drop of each polysaccharide:protein dispersions (1:3; 1:4) at pH 7 and 10, prepared two hours before analysis, was diluted 20 times and deposited onto a carbon support film on a copper grid. Excess sample was removed after 30 s using filter paper. Contrast was achieved by negative staining via the addition of a droplet of 1% aqueous uranyl acetate

solution (Sigma-Aldrich, USA) for 60 s. Any excess was removed again with a filter paper. After drying the grid at room temperature (18°C) for 5 min, images were taken using TEM (Philips Tecnai 12 Bio Twin, Eindhoven, The Netherlands) operating at 80 kV. The images were recorded on Kodak film SO163 and the negative film scanned at 300 dpi resolution in a digital scanner CanoScan 9950F (Canon Inc.,Tokio, Japan).

5.2.5. Scanning Electron Microscopy

The microstructure of films was observed using a JEOL-JSM 6360LV scanning electron microscope (Jeol, Ltd., Tokio, Japan) operated at 15 kV. Before the analysis each film was fixed on a support using double side adhesive tape, placed horizontally and with an angle of 90°. Samples were dried at critical point with CO_2 in a critical point drier (Baltec, model CDP 030, Balzers, Liechtenstein) and were covered with Au-Pd alloy with a Sputter Coater (Baltec, model SCD 005, Balzers, Liechtenstein).

5.2.6. Film Colour and Opacity

Colour parameters of films were determined using a spectrophotometer (X-Rite, model SP60, Michigan, USA). All measurements were made in the CIE L*a*b* colour space (CIE, 1976) using a D65 illuminant with an opening of 14 mm and the 10º standard observer. The instrument was standardized before measurements with the white and black tiles provided by the manufacturer before sample measurements. The colour values were expressed as L* (lightness), a* (redness/greenness) and b* (yellowness/blueness). For each film preparation, the colour of three different films in three random positions was determined. The difference in colour (ΔE*) was expressed as (equation 5.1):

$$\Delta E* = \sqrt{(\Delta L*)^2 + ((\Delta a*)^2 + (\Delta b*)^2} \qquad (5.1)$$

where ΔL*, Δa* and Δb* represent the differentials between the colour parameter of the sample and the white standard.

The opacity (Y) was calculated as the ratio between the opacity of each sample on the black standard (Yb) and opacity of each sample on the white standard (Yw). The parameters Yb and Yw were determined in three films in three random positions and expressed in percentage (equation 5.2):

$$Y\ (\%) = \frac{Yb}{Yw}100 \qquad (5.2)$$

5.2.7. Moisture content and solubility

The moisture content was determined by film weight loss using oven drying at 105°C for 24 hr (Indelab, model IDL.AI 80, Navarra, Spain). The dry matter was calculated by difference between the moisture content and weight of the original sample. The determinations were done in triplicate. The solubility in water was defined as the percentage of film dry matter solubilized after 24 hr immersion in water (Gontard et al., 1992). Pieces of 0.1± 0.006 g of each film were weighed and immersed in 30 ml distilled water and stored at 24°C for 24 h and were then filtered using dessicated pre-weighed filter paper. The filter paper, containing undisolved pieces of film, was dried for 24 h at 105°C (Indelab, model IDL.AI 80, Navarra, Spain), to determine the weight of insoluble dry matter. The soluble dry matter was calculated by subtracting the weight of insoluble dry matter from the weight of initial dry matter. The determinations were done in triplicate.

5.2.8. Water vapor barrier properties

The water vapor barrier properties of each film was determined according to the method described by Gontard, Guilbert & Cuq (1993) based on the ASTM E96-93 method (ASTM, 1993). Films were cut, adjusted and sealed on glass cup containing

dried silica gel with an exposed area of 3.6 ± 0.17 cm diameter. Sealed glass cup were weighed and placed in a desiccator with distilled water in a chamber conditioned at 24 ± 0.5°C. The water vapor transmission rate (WVT) was calculated using the following formula (equation 5.3):

$$\text{WVT (g/h m2)} = w/A \qquad (5.3)$$

Where w is the weight gain of the cell (g) after 1 h and A is the area of the exposed film.

The water vapor permeability (WVP) transferred through the film and absorbed by the silica gel was calculated as the weight gain of the cups (equation 5.4), measured every two hours until constant weight was recorded:

$$\text{WVP} = \frac{\text{WVT} * \text{L}}{\Delta \text{P}} \qquad (5.4)$$

Where WVT is the water vapor transmission (g/h m2); L is the film thickness (mm) and ΔP is the partial water vapor pressure difference (kPa) across the two sides of the film (2.895 kPa, at 24ºC).

5.2.9. Mechanical properties

Mechanical properties were measured using a texturometer EZ Test, and the Trapezium2 Data Processing System software (Shimadzu Corporation, Tokyo, Japan) version 2.22E (2004), according to the ASTM D882 method (ASTM, 2000; Carneiro-da-Cunha et al., 2009; Osés et al., 2009). For puncture tests, the films were cut in strips of 33 x 45 mm while for tensile tests they were cut in strips of 20 x 70 mm, and then stored in desiccator at 50 % RH for 48 hours. The strips for tensile strength (TS) determination were set in metal grips with an initial separation of 60 mm and stretched at an overhead crosshead speed of 20 mm/min. Force and distance were measured at 8

replicates during extension. TS were calculated by dividing maximum loads by specimen cross-sectional areas. The percentage of elongation at break (EB) was determined by dividing the extension at break of the strip by its original length and multiplying by 100. Strips for the puncture test were placed over an acrylic plate perforated at the center with a hole 13 mm of opening diameter. Puncture was done with a 3 mm diameter probe moving at 60 mm/min. The puncture strength (PS) was measured for 3 films at 6 points each and was calculated by dividing maximum force by the cross-sectional area of the exposed film (Cao & Chang, 2002). Puncture deformation (PD) was calculated using equation 5.5 described by Gontard et al. (1993) and Sobral, Menegalli, Hubinger & Roques (2001):

$$\frac{\Delta l}{l_0} = \frac{\sqrt{D^2 + {l_0}^2} - l_0}{l_0}. \qquad (5.5)$$

Where D is the displacement of the specimen and l0 is the initial length of the film corresponding to the radius of the measurement cell (6.5 mm).

5.2.10. Statistical Analysis

Significant differences among the means was evaluated by analysis of variance (one-way ANOVA), and the means were compared using the least significant difference test with significance at $p < 0.05$. Data were evaluated using Statgraphics Centurion Software XV version 15.1.02 (StatPoint Inc., VA, USA).

5.3. Results and Discussion

5.3.1. Mucilage extraction and Film formation

The method of extraction developed on a laboratory scale produced dried white flakes of mucilage (15% moisture) with a 7 % extraction rate. The composition of chia

mucilage obtained from this work was 85 % total solids, 48% carbohydrates, 23.22% uronic acids, 8% ash, 4 % protein and 1.78% lipids. With respect to formation of films, an unstable film behavior was observed when proportions of polysaccharide:protein was 1:0; 1:1, 1:2, 2:1 and 0:1, while concentrations above 1% of MC (1:0; 2:1) were difficult to dissolve, resulting in the formation of insoluble lumps. In addition, films formed with proportions of polysaccharide:protein (1:1 and 1:2) were too thin, brittle and difficult to peel off, while, no film was formed when pure WPC (0:1) was used which can be attributed to its low total solid and protein content.

Preliminary evaluation was carried out to study the usefulness of either sorbitol or glycerol as plasticizer. It was discovered that glycerol provided better physical and functional properties than sorbitol which produced wet films that are difficult to peel. Dispersions based on 1:3 and 1:4 proportions of polysaccharide:protein using glycerol as plasticizer were able to produce films that were transparent, light yellow, easily peeled and of thickness ranking between 90-110 μm. Proportions of polysaccharide and protein as well as pH have no significant influence on thickness of the films because the amount of total solids remained constant.

5.3.2. Microstructure of MC:WPC aggregates

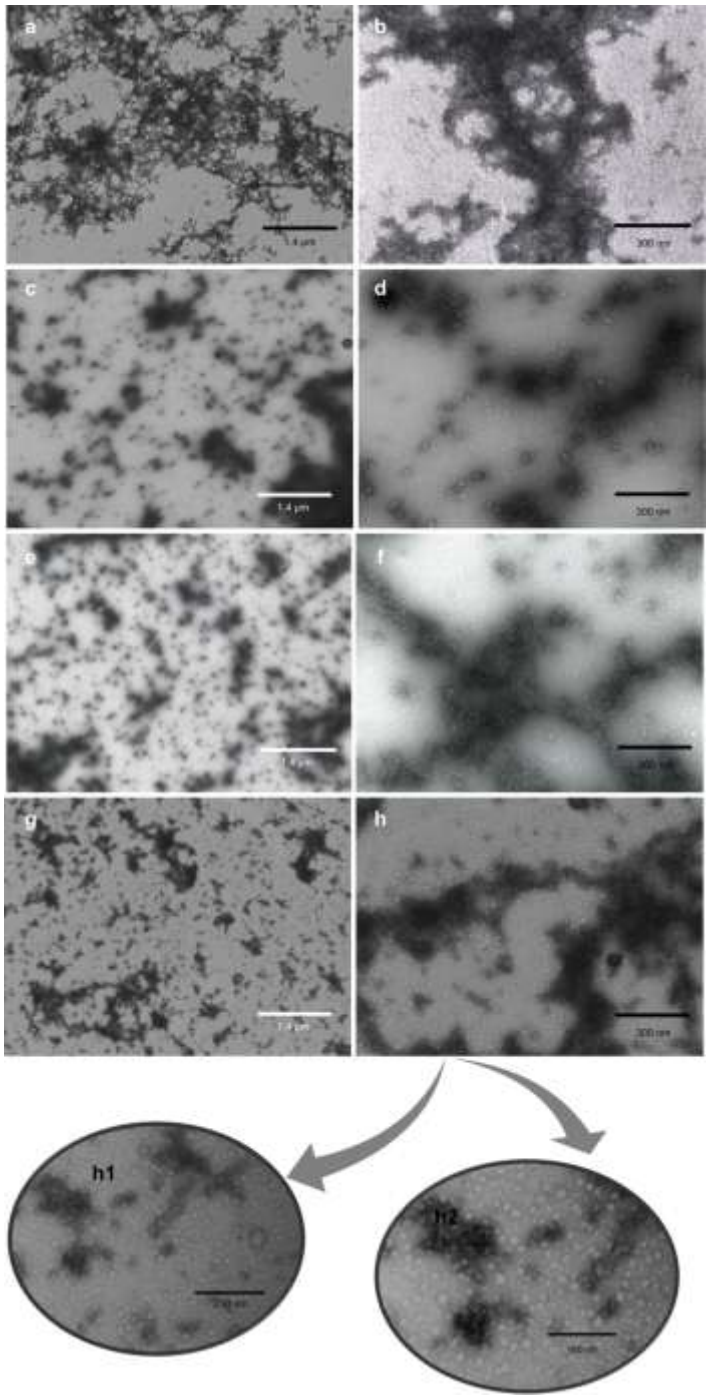
The effect of pH and polysaccharide:protein ratio on microstructure were analyzed at four different length scales (11000X, 49000X, 68000X and 98000X magnification) as shown in Figure 5.1.

When the polysaccharide and protein were mixed, microstructures with sphere and ovoid conformation were observed. These new elements or aggregates take the form of white masses due to the negative staining procedure. Figure 5.1a-h shows the microstructure of MC:WPC at different pH and proportions observed under two magnifications (11000X and 49000X). The structures observed at pH 7 and ratio 1:3 (figures 5.1a and 5.1b) had vesicular or globular conformation and they are homogeneously distributed having an average, minimum and maximum diameters of

20.96 ± 4.02, 12.45 and 36.41 nm, respectively, as compared with irregular sizes from solutions prepared with pH 7 and ratio 1:4 (figures 5.1c and 5.1d). At pH 10 and ratio 1:3 (figures 5.1e and 5.1f), the aggregates observed had irregular shape, while some of them were elongated and linked together. With ratio 1:4 and pH 10 (figures 5.1g and 5.1h), the aggregates observed had 21.04 ± 3.9, 12.66 and 28.66 nm as average, minimum and maximum diameters, respectively, with uniform size and distribution. They were more uniform in diameter, distribution and size as compared with samples shown in Figure 5.1b. Also, in figure 5.1h fewer aggregates were observed per unit area compared with those formed at pH 7 and ratio 1:3 (figure 5.1b) where the aggregates are much more per the same unit area. However, a higher magnification (figures h1 and h2) showed clearly a spherical conformation.

On the other hand, samples with ratio 1:3 and pH 7 had aggregates with more irregular sizes with average, minimum and maximum diameters of 23.51 ± 6.47, 12.45 and 36.41 nm, respectively. According to Dickinson (1998), when two biopolymers are mixed in solution, they can exhibit two interaction possibilities: attractive or repulsive, depending on the solvent, distribution of the charged group and the presence of hydrophilic or hydrophobic, groups. In this study, the formation of aggregates could be attributed to electrostatic interactions between MC and WPC. However, at the investigated pH (7 and 10), which is higher than isoelectric point (pI) of whey protein (4.5) at any temperature (Pelegrine & Gasparetto, 2005), the components (WPC and MC) are negatively charged, promoting electrostatic repulsion between them resulting in the formation of aggregates. This is in agreement with the report of Osés et al. (2009). In this case soluble complexes (polysaccharide-protein) can be formed when the two biopolymers have a net negative charge (pH >pI).

Figure 5.1 Microstructure of MC:WPC aggregates



This phenomenon has been earlier described by Dickinson, (1998) as the existence of positively charged patches on the protein interacting with the anionic polysaccharide. Also, this formation of the aggregates (MC:WPC) at pH 7 and 10 could be based on the attraction between the two molecules by positively charged patches because, WPC and MC posses a large number of ionisable and functional side groups with different pK value that can produce junction zones of inter-biopolymer complexes as described by Tolstoguzov (2007). This behavior could be desirable especially when film to be produced is expected to have higher gel strength and lower gel formation period. It has been earlier shown that the addition of non-gelling polysaccharides to whey protein can improve the gelling properties of the proteins and produce the higher gel strength and diminish gel formation times (Bertrand & Turgeon, 2007).

5.3.3. Microstructure of Edible Films

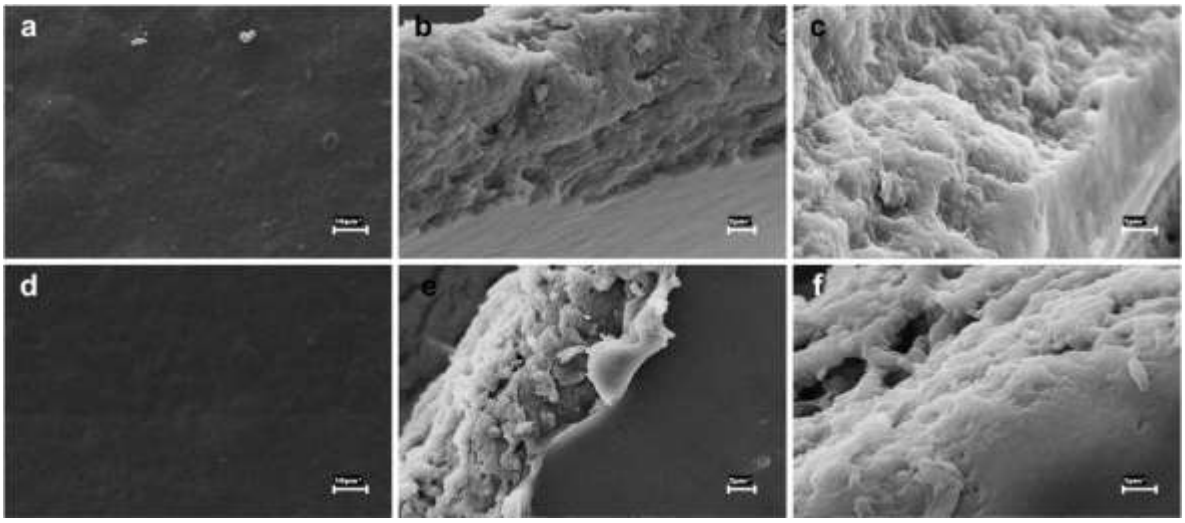
The study of films microstructure and interactions between films components provides important information in material science and practical technologies for possible applications (García, Pinotti, Martino, & Zaritzky, 2009). Figure 5.2 shows microstructure of the surface and cross-section of the edible films formed.

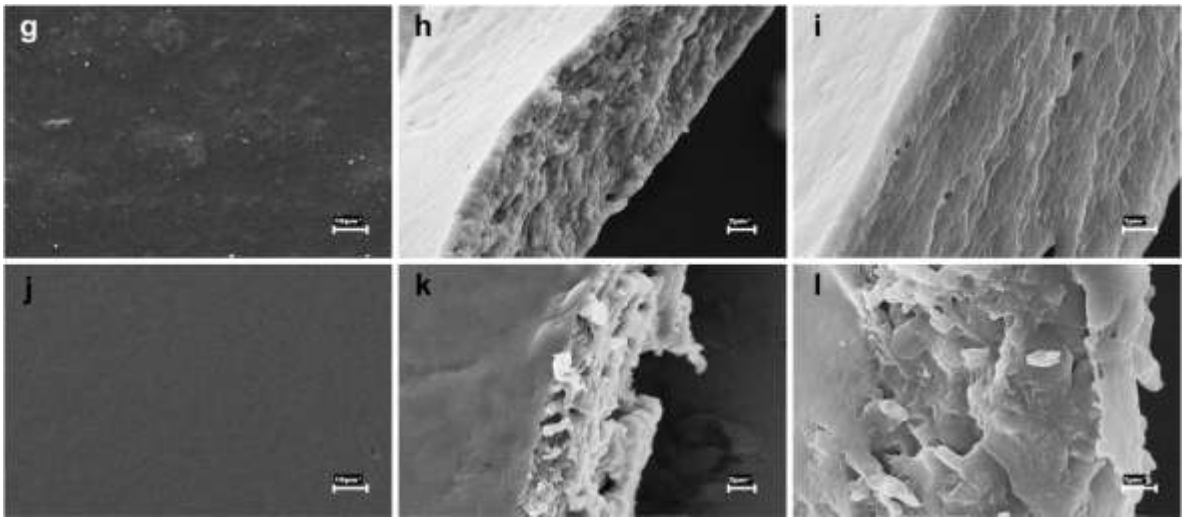
Figures 5.2a-c show the microphotographs of edible films formed with ratio 1:3 at pH 7 under different magnifications while figures 5.2d-f show the edible films produced with ratio 1:3 at pH 10. The microphotographs (figures 5.2g, h and i; and figures 5.2j, k and l) show polysaccharide-protein edible films formed with ratio 1:4 at pH 7 and 10, respectively. In the first column, figures 5.2a, d, g and j corresponds to the surface exposed to water evaporation.

SEM images revealed that the structure of the films surface at pH 7 with both ratios (figures 5.2a and g) shows some cracks presumably caused by air bubbles incorporated during films formation. In the same images, some particles with granular forms were observed, which can be attributed to water loss by evaporation from the surface resulting in water concentration gradient. This resulted in the insolubility of the

particles which are deposited on the surface of the film. This phenomenon has earlier been described by (Murillo-Martínez, Pedroza-Islas, Lobato-Calleros, Martínez-Ferez, & Vernon-Carter, 2011). But at pH 10 using the two ratios (figures 5.2d and j), the surfaces observed were smooth, compact and homogeneous without pores or micro-holes. The other two columns correspond to cross-section micrographs at different magnifications. In edible films at pH 7 with two ratios MC:WPC (figures 5.2b,c,h and i), the microstructure observed was more compact, homogeneous and more orderly in terms of arrangement. For edible films at pH 10, as shown in figures 5.2e, f, k and l, disorganized microstructures were observed, consisting mainly of fibrous-like structures.

Figure 5.2 Microstructure of films





a) Surface micrograph of films at ratio 1:3 at pH 7 exposed to water evaporation.
b) and c) Cross-section micrograph at ratio 1:3 at pH 7 at 4000X and 10000X magnification, respectively.
d) Surface micrograph of films at ratio 1:3 at pH 10 exposed to water evaporation.
e) and f) Cross-section micrograph at ratio 1:3 at pH 10 at 4000X and 10000X magnification, respectively.
g) Surface micrograph of films at ratio 1:4 at pH 7 exposed to water evaporation
h) and i) Cross-section micrograph at ratio 1:4 at pH 7 at 4000X and 10000X magnification, respectively.
j) Surface micrograph of films at ratio 1:4 at pH 10 exposed to water evaporation
k) and l) Cross-section of films at ratio 1:4 at pH 10 at 4000X and 10000X magnification, respectively.

These differences in microstructure may be due to interactions between MC and WPC. In this study, both MC and WPC have net negative charge with the pHs used, therefore, the interaction could be based on the net charge of each components promoting the electrostatic incompatibility between them. However, the presence of positively charged patches on the protein could induce the formation of these aggregates before film formation (Dickinson, 1998; Osés et al, 2009). According to García *et al*. (2009), a compact and homogeneous matrix films is an indication of structural integrity responsible for good mechanical properties such as high resistance and elongation at break.

5.3.4. Color and Opacity Measurements

Colour parameters of the edible films are presented in Table 5.1. For films produced at pH 7 solutions, L* and a* values were slightly higher, while b* and ΔE were lower than those from solutions at pH 10. Lightness also increased when WPC concentration was highest at neutral pH in agreement with the report of Osés et al. (2009) with films from mixtures of whey protein isolate and mesquite gum. These observations could be due to the proportion of the polysaccharide incorporated into the solution as well as the changes produced when the pH was modified.

As reported by Benichou, Aserin, & Garti, (2002) these changes can be explained as a consequence of modifications in optical configuration produced in the molecules due to modifications in polyelectrolites interactions between polysaccharide-protein. Mucilage ratio and pH had significant effect ($p<0.05$) on the opacity of the films. The highest MC ratio and the lowest pH value produced a significant increase in the opacity of all the films, comparable with the results described by Cerqueira, Souza, Martins, Teixeira, & Vicente, (2010). The films obtained in this work have the advantage of providing desirable light barrier properties depending on its application in food products.

Table 5.1 Comparison of Colour parameters of some films

Films Components	Proportion Polysachharide:protein	pH	L*	a*	b*	Opacity	ΔE	Reference
MC:WPC + Gly	1:3	7	90.75 ± 1.22ab	0.01 ± 0.25b	11.74 ± 0.94a	12.87 ± 2.02b	11.50 ± 0.75a	Present study
MC:WPC + Gly	1:4	7	91.30 ± 1.54b	-0.02 ± 0.47b	11.10 ± 4.00a	12.40 ± 1.24b	11.13 ± 3.30a	Present study
MC:WPC + Gly	1.3	10	89.53 ± 1.56a	-0.41 ± 0.09a	14.82 ± 0.60b	10.32 ± 1.34a	14.78 ± 0.84b	Present study
MC:WPC + Gly	1:4	10	90.51 ± 1.45ab	-0.57 ± 0.11a	12.53 ± 1.17a	10.33 ± 1.84a	12.29 ± 1.39a	Present study
OKP: WPI + Gly	1:3	n.r.	84.9 ± 0.7	-1.5 ± 0.1	21.5 ± 0.6	n.r.	87.59**	Prommakool et al., 2010.
MG:WPI + Sor	1:3	6.6	94.5*	0.2*	8*	n.r.	94.85**	Oses et al., 2009.
WPC + Gly	0:1	~6.6	n.r.	n.r	n.r	n.r	n.r.	Baneerje & Chen, 1995.
WPI + Gly	0:1	n.r.	90.8 ± 0.4	-1.3 ± 0.1	5.0 ± 0.3	n.r	90.94**	Prommakool et al., 2010.
GT + Gly	1:0	n.r.	86.77 ± 2.11	5.85 ± 0.68	14.94 ± 2.20	4.94 ± 0.09	88.24**	Cerqueira et al., 2010.
OFI + Gly	1:0	7	92.93 ± 0.52	2.15*	15.76*	n.r.	94.26**	Espino Diaz et al. 2010.

L*: Lightness; a*: redness/greeness; b*: yellowness/blueness and ΔE: differences in colour. Values are means of triplicate ± standard deviation. Different letters in the same column mean significant differences (p<0.05) MC: mucilage from chia seeds; WPC: Whey protein concentrate; WPI: whey protein isolate; OKP: okra polysaccharide fraction; Gly: glycerol; MG: Mesquite gum; sor: sorbitol; *: data extracted from graph; **: calculated from data; GT: Gleditsia triacanthos; OFI: Opuntia ficus-indica; n.r.: not reported

5.3.5. Moisture content and solubility

The moisture content of the films was equilibrated at 50% relative humidity. The result of the solubility of MC:WPC edible films are shown in Table 5.2.

All the films showed high moisture content of about 36.44 % in average, which was significantly higher than edible films from whey proteins (21.8 %) in the study of Banerjee and Chen (1995) and composite edible films from WPC and mesquite gum (Osés et al., 2009), as shown also in table 5.2. Considering the hydrophilic nature of hydrocolloids, the MC content in the films was expected to dissolve in water. Generally, high solubility would indicate lower water resistance; however for some applications such as packaging wrap, the high solubility is an indicator of biodegradability which could be an advantage (Stuchell & Krochta, 1994). The solubility of composite edible films from MC:WPC was affected by pH. At pH 7, the total soluble matter increase to 64 % while at pH 10, it remains at 48%. Results showed significant effect ($p<0.05$) of pH on solubility. Total soluble matter was observed to increase when pH was 7. The pH has been reported to induce variation in the solubility of the dispersing medium due to the incorporation of acidic sugars (Williams, Phillips, Stephen, & Churms, 2006). This high solubility can also be explained by the presence of anionic groups from acidic sugars that increase the polarity and water solubility (Nieto, 2009). The increase in moisture and solubility of MC:WPC films can also be due to the hydrophilic nature of this polysaccharide as well as higher solubility exhibited by the MC.

5.3.6. Water Vapor Permeability (WVP) and Water Vapor Transmission (WVT) of films.

Table 5.2 also shows the changes in WVP and WVT produced with the different concentrations of MC:WPC. The average thickness of film samples used in these determinations was 92.77 ± 0.005 μm.

The study of the WVP of edible films formed with blends of polysaccharides and proteins is of special importance due to the posterior tendency to cluster within the polymeric matrix (Arvanitoyannis et al., 1998). The WVP values showed significant differences ($p<0.05$) between the films formed with MC:WPC at pH 7 and 10. Films produced at pH 10, however, showed better water vapour barrier (0.620±0.08 g mm/Kpa h m^2) than films at pH 7 although WVP was higher in the films with the highest MC proportion.

Table 5.2 Moisture, solubility, water vapor permeability and transmission of some edible films

Films Components	Proportion Polysaccharide:protein	pH	WVP (g mm/Kpa h m^2)	WVT (g/h m^2)	Moisture (%)	Solubility (%)	Reference
MC:WPC + Gly	1:3	7	0.678 ± 0.09a	21.25 ± 3.47a	36.90 ± 1.22ab	52.99 ± 14.50ab	Present study
MC:WPC + Gly	1:4	7	0.662 ± 0.14a	20.96 ± 4.86ab	36.24 ± 2.18ab	63.96 ± 15.77b	Present study
MC:WPC + Gly	1:3	10	0.620 ± 0.08b	20.20 ± 2.66b	37.44 ± 1.85b	48.98 ± 14.41a	Present study
MC:WPC + Gly	1:4	10	0.620 ± 0.07b	19.91 ± 2.42b	35.16 ± 2.72a	48.30 ± 10.83a	Present study
OKP: WPI + Gly	1:3	n.r.	2.9 ± 0.1	n.r.	n.r	n.r.	Prommakool et al., 2010.
MG:WPI + Sor	1:3	6.6	2.0 ± 0.1	n.r.	10 ± 0.2	n.r.	Oses et al., 2009.
WPC + Gly	0:1	~6.6	10.64	n.r.	20.18	n.r	Baneerje & Chen, 1995.
WPI + Gly	0:1	n.r.	2.6	n.r.	n.r.	n.r	Prommakool et al., 2010.
GT + Gly	1:0	n.r.	0.235**	n.r.	n.r.	n.r	Cerqueira et al., 2010.
OFI + Gly	1:0	7	4.96	n.r	n.r.	n.r	Espino Diaz et al. 2010.

WVP: Water vapour permeability; WVT: Water vapour transmission. Values are means of triplicate ± standard deviation. Different letters in the same column mean significant differences (p<0.05) MC: mucilage from chia seeds; WPC: Whey protein concentrate; WPI: whey protein isolate; OKP: okra polysaccharide fraction; Gly: glycerol; MG: Mesquite gum; sor: sorbitol; *: data extracted from graph; **: calculated from data; GT: Gleditsia triacanthos; OFI: Opuntia ficus-indica; n.r.: not reported

The films formed with chia mucilage was observed to show better water vapor barrier properties than some composite films produced with WPC and alginate, pectin carrageenan and konjac flour (Coughlan et al., 2004); films of galactomannan (Cerqueira et al., 2010), films formed with whey protein and okra polysaccharide (Prommakool, Sajjaanantakul, Janjarasskul, & Krochta, 2010) and films based on mucilage of *Opuntia ficus indica* (Espino-Díaz, Ornelas-Paz, Martínez-Téllez, Santillán, Barbosa-Cánovas, Zamudio-Flores, & Olivas, 2010). However, films of MC:WPC showed lower WVP values compared with those of whey protein concentrate films of 10.64 g mm/Kpa h m^2 (simple films) and 3.95 g mm/Kpa h m^2 to composite films (Banerjee & Chen, 1995). Good water vapor barrier properties could be attributed to the formation of a continuous phase by protein network that accommodates the polysaccharide chains, acting as a filler of protein network (Tavares and Lopes da Silva, 2003).

Water vapor transmission through a hydrophilic film depends on the diffusivity and solubility of the water molecules in the film matrix (Gontard & Guilbert, 1994). In this study, films at pH 7 showed greater WVT than films at pH 10 and can be also attributed to the interactions between MC and WPC.

5.3.7. Mechanical Properties

The effects of MC:WPC ratio and pH on tensile strength (TS), elongation at break (EB), puncture strength (PS) and puncture deformation (PD) values of edible films from chia mucilage are summarized in Table 5.3. It has been reported that the mechanical properties of the biopolymers mixes depend on the degree of interaction of the components (McHugh et al., 1994). They also propose that cross-links between chains of intermolecular hydrogen and disulfide bonds as well as hydrophobic interaction could be responsible for the integrity and strength in whey protein films, but the negative charged mucilage incorporation could induce new links between both components.

The values obtained are similar to films produced with blends of WPC and alginate, pectin, carrageenan and konjac flour (Coughlan et al., 2004) but slightly lower

than those formed with polysaccharide alone, as earlier reported by previous authors (Parris, Coffin, Joubran, & Pessen, 1995; Yang & Paulson, 2000). These differences could be attributed to interactions between WPC and MC and differences in gel formation. Since protein–polysaccharide interactions are mainly determined by electrostatic interactions, pH plays a fundamental role in their organization. Proteins from whey are positively charged at pH below pI, in this case the pH>pI, hence, the net negative charge tends to undergo phase separation. In our work, the presence of anionic polysaccharide such as chia mucilage could suggest that the formation of aggregates may be caused by attractive forces between positively charged patches on the protein (WPC) responsible for the structure and network formation. Bertrand & Turgeon (2007) had earlier used xanthan gum and whey protein isolate to describe this phenomenon.

The incorporation of MC into edible films increased TS, EB, PS and PD values, providing greater strength and flexibility. pH had significant influence ($p<0.05$) on mechanical properties of edible films. Films produced at pH 10 exhibited superior mechanical properties than the other films. However, EB, which is indirectly related to the elasticity of the film, increased only with 1:3 ratio. The films made with the highest ratio of polysaccharides were more resistant and extend more than those with the lowest amount of polysaccharide. It has earlier been reported that proteins and polysaccharides complexes show more effective functional properties than proteins and polysaccharides individually (Coughlan et al., 2004).

Table 5.3 Mechanical Properties of some edible films

Films Components	Proportion Polysaccharide:protein	pH	Thickness (µm)	TS (Mpa)	EB (%)	PS (N)	PD (%)	References
MC:WPC + Gly	1:3	7	90 -110	3.79 ± 0.72b	16.33 ± 2.81ab	85.35 ± 31.87b	5.45 ± 3.01ab	Present study
MC:WPC + Gly	1:4	7	90 -110	2.67 ± 0.91a	15.20 ± 4.75a	73.32 ± 17.89a	5.49 ± 2.13b	Present study
MC:WPC + Gly	1.3	10	90 -110	4.68 ± 1.05c	17.32 ± 3.82b	117.05 ± 21.76c	5.30 ± 1.01ab	Present study
MC:WPC + Gly	1:4	10	90 -110	3.93 ± 0.98b	15.17 ± 2.80a	93.42 ± 21.33b	4.69 ± 1.00a	Present study
OKP: WPI + Gly	1:3	n.r.	70-100	5*	28*	n.r	n.r.	Prommakool et al., 2010.
MG:WPI + Sor	1:3	6.6	120	11.5 ± 0.9	6.7 ± 1.9	n.r	n.r	Oses et al., 2009.
WPC + Gly	0:1	~6.6	110	3.36	20.24	1.69	n.r.	Baneerje & Chen, 1995.
WPI + Gly	0:1	n.r.	70-100	8.5*	20*	n.r.	n.r.	Prommakool et al., 2010.
GT + Gly	1:0	n.r.	52	n.r.	n.r.	n.r.	n.r.	Cerqueira et al., 2010.
OFI + Gly	1:0	7	100-110	0.4-0.95	14.99	n.r.	n.r	Espino Diaz et al. 2010.

TS: Tensile strength; EB: Elongation at break; PS: Puncture strength; PD: Puncture deformation. Values are means ± standard deviation. Different letters in the same column mean significant differences (p<0.05) MC: mucilage from chia seeds; WPC: Whey protein concentrate; WPI: whey protein isolate; OKP: okra polysaccharide fraction; Gly: glycerol; MG: Mesquite gum; sor: sorbitol; *: data extracted from graph; **: calculated from data; GT: Gleditsia triacanthos; OFI: Opuntia ficus-indica; n.r.: not reported.

5.3.8. Conclusions

Mucilage from chia in combination with whey protein concentrate were successfully used to produce edible films. Edible films formed with mixtures of MC and WPC in proportions 1:3 and 1:4 showed good mechanical properties and low water vapor permeability. The films made with solutions at pH 10 had lower water vapor permeability, solubility, a* and opacity values than those from solutions at pH 7. The highest tensile strength, elongation at break and puncture strength values were higher in films formed with solutions at a ratio polysaccharide-protein of 1:3 and pH 10. All the changes produced in physical properties of edible films by MC:WPC could be attributed to the formation of aggregates polysaccharide/protein; which were observed in the microstructure of the solutions and films. The use of this new polysaccharide is an option to modify and improve the physical properties of hydrophilic edible films combining MC and WPC components.

5.3.9. Acknowledgements

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5.3.10. References

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6. GENERAL DISCUSSION

As stated in the introduction, the purpose of this doctoral thesis is related to the search for new ingredients for the food industry. Within this context, the study focused on the Chia mucilage obtained from the seeds of Salvia hispanica. The prime objective was to study the microstructure of the seed and mucilage, and then continue with the study of physicochemical, thermal and functional properties. Lastly, after the mucilage was characterized it was used as an ingredient for preparing edible films.

In the article "Chia seeds: microstructure, mucilage extraction and hydration" the location of mucilage in the seed was obtained using optical and electron microscope scanning techniques. The mucilage is located on the outer surface of the seed coat or testa. This wall is composed of three layers of rectangular mucilaginous cells from which the mucilage is released when the seed comes into contact with water. This cell formation has not been detailed for *Salvia hispanica L*, but was previously reported by Beeckman et al. (2000) for the seed produced by *Arabidopsis thaliana*. When the seed is placed in contact with water different filamentous structures form in a spiral on the surface, spreading out until they have completely extended once fully hydrated. The mucilage is present within the epidermal cells of the mature seed testa, and these filaments on contact with water begin to expand, breaking the primary cell layer and forming a transparent capsule that surrounds the seed. This mechanism was described in detail by Beeckman et al. (2000) and Penfield et al. (2001) for *Arabidopsis*, but has not been described for edible seeds.

When several seeds have been soaked in water a highly viscous solution forms, and this same characteristic was explored further in isolated mucilage, so determining its capacity to absorb water.

Preliminary studies showed that the entire seed reached its maximum level of hydration within 2 hours. This time period was selected in order to carry out extraction and then hydration. The maximum value for the extraction was 6.97% in conditions of pH 8 and 80°C. The value obtained was higher than that reported by Reyes Caudillo et

al. (2008) of 6% and Ayerza and Coates (2001) of 5%, but less than the value of 15% reported by Marin et al. (2008); these differences are probably due to extraction conditions.

Promising results were obtained from the study of mucilage hydration. The mucilage was able to absorb up to 27 times its weight in water, more than double the quantity reported by Vasquez-Ovando (2009), where the fiber fraction was hydrated. The maximum hydration value was reached at low concentrations of salt, basic pH and temperatures near 8°C.

In order to provide as much information as possible about mucilage as a potential functional food ingredient, its physicochemical, thermal and functional qualities were characterized.

Chia mucilage contains around 71.22% of polysaccharides, a value slightly lower than the 75% reported for flaxseed mucilage (Warrand et al., 2005) and the 80% reported for the mucilage of hydrocolloid mustard seeds. Chia mucilage also contains uronic acids, which are characteristic of some polysaccharides found in seeds (Wu et al., 2009). Some minor differences were observed between the results of the elemental analysis of the mucilage in the present study and the result obtained by Li et al. (1994), which can possibly be attributed to differences in seed variety, location and agricultural practices.

After observing the behavior in the DSC spectrum and melting point, one interesting aspect that should be noted is that the mucilage has considerable stability at high temperatures, making it a potentially attractive material to be used in processes involving such temperatures.

Furthermore, FT-IR spectroscopy revealed the presence of functional groups such as hydroxyl, aliphatic, carboxylic and aromatic ester. The FT-IR spectrum for chia mucilage was performed in comparison with xanthan gum. The results show a great similarity between the two, i.e., the main bands recorded in the chia mucilage and xanthan gum correspond to the same functional groups and differ only in intensity.

Comparing the spectrum with other commercial hydrocolloids, the main absorption bands were observed at 3400, 2939 and 990 to 1200 cm^{-1}, which is common in all polysaccharides, representing O-H and C-H bonds of the CH_2 groups and saccharides , respectively (Freitas, Alves et al. 2009).

The presence of xylose, mannose, arabinose, glucose and uronic acids such as glucuronic and galacturonic acid can also be detected in the mucilage. The percentages of saccharides include: 16.78±0.59% D-xylose+D-mannose, 2.11±0.18% D-arabinose, 6.77±0.30% D-glucose, 3.9±0.32% galacturonic acid and 12.1±2.30% glucuronic acid, reaching a total of 41.66% of sugars. The difference obtained regarding the value of 71.22% in the proximate analysis may be due to the existence in the chromatogram of two unknown peaks, which were not quantified in the results. Quantification of chia mucilage monosaccharides have not yet been published, although previous studies reported that they only possess glucose, xylose and uronic acids (Lin et al., 1994). Comparing chia mucilage with mucilage obtained from flaxseed, it was possible to observe some similarities in their composition, for example the presence of xylose, arabinose and uronic acids (Warrand et al., 2003).

In terms of solubility, it was observed that the mucilage is completely soluble under the conditions investigated. These results differ from others reported in the literature for other polysaccharides. For example, larch gum is 60% soluble at room temperature, gum arabic is highly soluble in hot water, but less soluble in cold water and karaya gum has <0.02% solubility in cold water and 0.06% solubility in hot water (Williams, Phillips et al. 2006). The mechanism responsible for this behavior can be attributed to its complete dispersion in water followed by solubilization, which allows full penetration of water between the particles and complete macromolecular interactions (Doublier and Cuvelier, 2006).

The rheology of the mucilage compared to xanthan gum was also studied as part of its functional properties. None of the two polysaccharides has the ability to form gels, but both have a considerable ability to produce highly viscous solutions at low concentrations. According to the rheograms obtained for the two polysaccharides, it was

observed that both exhibit non-Newtonian behavior. The model that was adjusted to the rheogram was the Oswald de Waele or Power law, given that negative values with no physical meaning were obtained when using the Herschel-Bulkey model, meaning that the latter was unsuitable for the purpose of characterization (Steffe, 1996). The K values decreased in both gums when the concentration was reduced, although significant differences were observed between the two. The increased concentration in both gums induced greater pseudoplasticity, highlighted by the decrease of the values obtained in the flow behavior index (n). Comparatively, the magnitude of the consistency index (K) of chia mucilage was lower compared to the xanthan gum in the range of concentrations studied. These differences may be attributed, in the case of pseudoplastic fluid, to the stress of the cut, which is a nonlinear function of the cutting speed and depends on each polysaccharide in particular and of their physicochemical characteristics, molecular weight, solubility, concentration, temperature, pH, ionic strength, size, shape and morphology, among others (Wang and Cui 2005). The viscosity of the chia mucilage and xanthan gum decreased when the cutting speed rose, thus both dispersions exhibited pseudoplastic behavior according to Marcotte et al. (2001) and Phillips and Williams (2000), although their readings differed. The chia mucilage produces higher viscosities at the same concentration as xanthan gum, which is one of the most important functional properties according to Wang and Cui (2005).

Other functional properties that were studied included the ability to form emulsions and foams. Compared with other hydrocolloids such as gum arabic, modified starch and cellulose, chia mucilage has a low EAI - Emulsifying activity index (Garti and Leser, 2001; Dickinso,n 2003). The mucilage was 41.41±EAI 0,104 m2/g with 40% oil and 4.46±0.015 m2/g with 60% oil. However, mucilage showed a significant ability to stabilize emulsions; 78.42±1.96% using 40% oil and 42.31±0.79% with 60% oil. The fact that the mucilage possesses such a capacity to stabilize emulsions may be due to its ability to adsorb in the solid or liquid interface and stabilize emulsions without chemical or enzymatic modifications. This may be because the addition of soluble polymers

increases the viscosity of the dispersed phase, thereby reducing the rate of coalescence (Garti and Leser, 2001).

Similarly, the mucilage does not have the ability to form foam by itself, but has a great ability to stabilize foams produced with ovalbumin. The differences observed in comparison with mustard seed mucilage may be principally due to the composition (Cui et al., 1993). The high value of 98.26±1.52% obtained for the stability of the foam of 0.3% for chia mucilage, can be attributed to the formation of small bubbles between the protein-polysaccharide matrix that provide a prolonged aerated structure, and also due to the interaction of polysaccharides and proteins that normally exhibit synergistic properties (Narchi et al,. 2009).

One of the applications studied for chia mucilage was the development of edible films.

Mucilage has the ability to form films on its own, but they are extremely delicate and brittle, thus it was decided to use whey concentrate to supplement this application.

In the first instance the formation of aggregates in the dispersions prior to film formation were studied. Through the use of TEM protein:polysaccharide structures were observed that formed under different conditions. According to Dickinson (1998), when two biopolymers are mixed in solution they may exhibit two possible interactions: attractive or repulsive, depending on the solvent, the distribution of the charged functional groups, and the presence of hydrophilic and hydrophobic groups.

In the present study, aggregate formation could be attributed to the electrostatic interactions between proteins of whey concentrate and the polysaccharides of chia mucilage. However, along with the pH employed (7 and 10) which are higher than the isoelectric point (pI) of whey protein (4.5) at any temperature (Pelegrine and Gasparetto, 2005), the components (in this case whey and mucilage), were negatively charged, promoting electrostatic repulsion between these and resulting in the formation of aggregates; this mechanism was previously reported by Oses et al. (2009).

In this case, the soluble complexes (protein:polysaccharide) formed when the two biopolymers possessed a net negative charge (pH> pI). This phenomenon was described

by Dickinson (1998) as the possible existence of positively charged patches on the protein interacting with the anionic polysaccharide. Furthermore, the formation of these aggregates at pH 7 and 10 could be explained on the basis of attraction between the two molecules through these patches, given that the whey protein concentrate and the polysaccharides of chia mucilage possess a large number of ionizable functional groups with different pK, which could produce inter-biopolymer zones of contact. This phenomenon was previously described by Tolstoguzov (2007). This type of behavior may be desired, especially when seeking a film that has a strong gel with a low period of formation.

Complementing the use of TEM, when the films were formed a study of the microstructure using SEM was undertaken. The main differences observed may be attributed to those interactions described above, i.e. the presence of positively charged patches on the molecules which can induce the formation of aggregates.

According to Garcia et al. (2009) a compact and homogeneous matrix in the films is an indicator of structural integrity, and is responsible for beneficial mechanical properties such as high resistance and elongation at brake.

Another parameter studied in the edible films was the color. The main differences detected in the L*, a* and b* values were due to the different proportions of polysaccharide incorporated, as well as the changes produced when the pH was modified. In the study, the polysaccharide ratio and the pH also had a significant effect on opacity. These changes to the CIELAB parameters, according to Benichou et al. (2002), can be explained as a consequence of the modification in the optical configuration of the molecules due to changes between the polyelectrolytic interactions between protein-polysaccharide.

A highly important parameter to evaluate because of the subsequent tendency to form clusters in the polymer matrix, is permeability to water vapor. The films produced in the present study had better barrier properties to water vapor compared to films formed with whey protein concentrate and alginate, pectin, carrageenan and konjac flour

(Coughlan et al., 2004) and films formed by whey proteins and okra (Prommakool et al., 2010).

A good water vapor permeability may be attributed to the formation of a continuous phase of the protein network that accommodate the polysaccharide chains and act as a filler of the protein mesh (Tavares and Lopes da Silva, 2003).

Another property that is characteristic of the edible films are the mechanical properties, in which tension and puncture were studied. It has been reported that the mechanical properties of biopolymer mixtures depend on the degree of interaction of their components (McHugh et al., 1994). In the same report, it was proposed that cross-links created from the intermolecular hydrogen bonds and disulfide bonds, as well as hydrophobic interactions, could be responsible for their integrity and resistance. However, in the case of the present study, the negatively charged mucilage could induce the formation of new links between components.

The values obtained are similar to films produced with mixtures of whey protein with alginate, pectin and carrageenan (Coughlan et al., 2004) and slightly lower than those reported by Parris et al. (1995) and Yang & Paulson (2000).

In the present study, the presence of chia mucilage as anionic polysaccharide could suggest that aggregate formation can be caused by forces of attraction between the positively charged patches (described above), which are responsible for the structure and formation of the film matrix (Bertrand and Turgeon, 2007).

The films made with a higher proportion of chia mucilage were more resilient and extensible; coinciding with the findings reported by Coughlan et al. (2004) which state that protein/polysaccharide complexes show improved and effective functional properties compared to individual proteins and polysaccharides.

As demonstrated in this thesis, chia mucilage has a huge potential for the food, pharmaceutical and nutraceutical industries, as it contains excellent properties, although there is much still to discover.

As regards future research, many different applications are emerging from the whole seed and the mucilage within which can be mentioned:

- Improve the mucilage extraction to offer to the food industry an attractive functional ingredient
- Explore different application in diverse food matrices
- Study its use in medicine, action to of the mucilage on postprandial response in human and animal models
- Study the effect "in vivo" and/or "in vitro" on satiety and dyslipidemia
- Explore potential antioxidant effect of the plant extracts

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7. GENERAL CONCLUSIONS

- The seeds of *Salvia hipanica* have enormous potential as a source of nutrients and nutraceuticals, of great interest to science, technology and food engineering.

- Among other nutrients, the seed possesses mucilage composed mainly of polysaccharides, located in the three layers forming the testa (seed coat) which can be easily removed after hydration and have the capacity to retain water 27 times its weight in water.

- The mucilage obtained from the seed is a soluble fiber and potential source of hydrocolloids with different functional properties that are sought by the food industry, such as: a high water retention capacity; emulsifier; thickener; stabilizer in the formation of foam, and highly soluble in both hot and cold water.

- This polysaccharide has the ability to form edible films in combination with proteins, so improving the film's mechanical and functional properties.

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- Finally, it was concluded that the mucilage of Salvia hispanica represents a new functional ingredient with huge potential for the food, animal feed and pharmaceutical industries.