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Short Communication

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Isolation, biochemical characterization, oxido-reductive and proteolytic activities of globulin protein isolates from seeds of Chia

Abstract

Selective isolation, purification, secondary structure and functional properties of some globulins of Chia (*Salvia hispanica* L.) were studied. These globulins were isolated using water and two different NaCl concentrations (5 and 10%) and testing four enzymatic assays: polyphenol oxidase, catalase, peroxidase and chymoprotease for each extraction. Maximum protein extraction (18.4%) and highest enzymatic activity occurred in the presence of 5% NaCl. The main globulin protein was obtain using gel filtration chromatography and shows an increase of polyphenol oxidase, chymoprotease and proteolytic activities; and a decrease of catalase activity as compared with the crude extract. Further purification using anion-exchange chromatography produces two globulins SB1Q2 and SB1Q3 with molecular weights of 10 and 13 kDa and isoelectric points of 4.5 and 6.8, respectively. Their secondary structure using circular dichroism showed 42, 7%; 42.1, 7.4% of β-sheet and helical structures, respectively. This observation agrees well with the secondary structure of globulins in plant dicotyledonous seeds.

Keywords: isolation, biochemical characterization, enzymatic assays activities, globulin proteins, Chia, *salvia hispanica*

Volume 5 Issue 2 - 2017

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Received: May 11, 2017 | **Published:** June 15, 2017

Introduction

The food system appropriates over 30% of all ice-free land, 70% of available freshwater and 20% of energy. Sustainable food production for more than 7.0billion more people in the next four decades requires societal transition and industrial transformation. Protein supply is crucial, nutritionally and environmentally. The food industry can contribute to a sustainable future by development of novel plant protein products (NPFs) and continual innovations in food preservation and waste reduction.[1] In this context, vegetable proteins are emerging as a promising alternative to replace partially animal for human protein.[2]

Among the nonconventional seeds as sources of proteins, Chia is one of the main capable, with a high protein content 19-23%, %, which is higher than wheat (14%), corn (14%), rice (8.5%), oats (15.3%), and barley (9.2%).[4] On the other hand, Chia has a high content of unsaturated fatty acids; almost 60% is α-linolenic acid (omega-3);[3,4] with high amounts of natural antioxidants such as phenolic compounds including chlorogenic and caffeic acids, quercetin, and kaempferol, as well as high dietary fiber content (>30% of the total weight).[5,6]

Originally from Mesoamerica, Chia (*S. hispanica*) is the most widely distributed species (among approximately 900 species) belonging to the *Salvia* genus, a member of the Labiatae family, and which also includes *S. officinalis*, *S. columbaria* and *S. polystachia*. This plant species has been used mainly for medicinal purposes and has been regaining the popularity it enjoyed before European contact[7] due to its chemical composition and nutritional qualities, such as oil content (between 250 and 320g/kg seed) and fatty acid composition (800g/kg of total are unsaturated fatty acid),[8] health benefits in animal diets[9] and human diets[10] as well as the functional properties of its components.[11]

The recent study was to fractionate and characterize these proteins, as well as to identify, isolate, and characterize proteins from Chia seeds (*Salvia hispanica* L.). The main protein fraction corresponded to globulins (52%). Sedimentation coefficient studies showed that the globulin fraction contains mostly 11S and 7S proteins. The molecular sizes of all the reduced fractions were about 15−50kDa .[12,13] Based on the above exposed, the aim of this work was to characterize the functional behavior of globulin protein isolates from seeds of Chia (*Salvia hispanica*) under different conditions of pH and/or ionic strength.

Materials and method

Materials

Chia seed (*Salvia hispanica* L) harvested in the state of Jalisco was purchased at a local market, The Central de Abasto in Mexico City. In order to certify the correct seeds in our studies, a seed sample was identified as *Salvia hispanica* at the Herbario Nacional de Mexico, Universidad Nacional Autónoma de México in Mexico City. Dust, vain seeds and straw from threshed seeds were separated manually. The seeds were stored in tightly plastic bottles at room temperature (22-25ºC) until used. All chemicals used were analytical grade.

Chemical composition

Moisture, ash, protein, fat were determined for chia flour, for Chia globulin using standards methods 925.10 (moisture), 923.03 (ash), 920.87 (crude protein), and 920.85 (lipid).[14] Carbohydrate content was based on percent differential from 100%.

Preparation of flour

Seed meal was obtained by grinding whole clean seeds at room temperature in a coffee mill attaining an average particle size of about 0.05mm mesh. The sample was defatted repeatedly by extraction with dichloromethane (1:10, w/v) until essentially fat free. The defatted meal was air-dried at room temperature and stored in a closer container at room temperature until used.

Preparation of chia protein concentrates

Globulins salt-soluble proteins were extracted from defatted flour. One hundred grams of defatted flour was mixed with 1,500mL of 5% NaCl solution for 36 hours at 4°C using a magnetic stirrer, after which it was centrifuged at 8,100g for 30min at 4°C. The supernatant was used for activity determination of oxido-reductive (polyphenol oxidase, catalase and peroxidadse) and chymo-proteolytic activity. The pellet was then twice resuspended with 1,000 and 500mL of 5% NaCl and centrifuged. The protein concentration was measured for each extraction using the Bradford method. The supernatant obtained from the three extractions were pooled and precipitated with saturated (90%) ammonium sulfate ($(NH_4)_2SO_4$) and then centrifuged and the pellet was resuspended with 5% NaCl solution. The concentrated globulin fraction was dialyzed at 4°C against distilled water for 48h to remove the excess of ammonium sulfate. After that, the solutions were centrifuged at 8,100g for 30min at 4°C, the supernatants were discarded and precipitates were analyzed by electrophoresis process. The extract obtained was lyophilized and kept in a refrigerator at about 4°C prior use.

Gel electrophoresis

Nondenaturing gels were used to examine the native protein. This was carried out in a Mini-Protean II electrophoresis cell (Bio-Rad), using 12% polyacrylamide gel. Native gel was loaded with 25µg of protein by lane. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out using the Phast System apparatus (Pharmacia Biotech) according to the Laemmli method .[15]. SDS-PAGE was run on 25 % gels according to separation method 2 using the separation technique file 111 of the Phast System user's manual. Molecular weight standards (low molecular weight calibration kit, Pharmacia Biotech) were used to estimate the protein molecular weight. The gels were stained with Coomassie brilliant blue R-250. Isoelectric Focusing. Isoelectric focusing was performed by the technique described in Phast System technical file 100 using IEF 3-9 gels (Pharmacia Biotech). The isoelectric focusing method contains three steps: a prefocusing step, in which the pH gradient is generated; a sample application step, in which 2µg was loaded per lane; and a focusing step, in which the proteins are separated according to their charge. Isoelectric focusing calibration broad pI kit (pH 3-9, Pharmacia Biotech) was used to calculate the protein isoelectric point. The gel was stained with 0.1% Coomasie brilliant blue R-250. Gels were analyzed using the Quantity ONE 4.6.3 software of BioRad to obtain the molecular weights of these globulins.

Results and discussion

Protein purification

The concentrated globulin fraction was purified by gel filtration and anion exchange chromatography. Sample of 150 mg were dissolved in 5mL of 6M urea and applied on a filtration column. Gel filtration was done using a Sephadex G-75 medium gel (GE Healthcare Biosciences) column (2.0 x 80.0cm) equilibrated with 0.05M Tris-HCl, 0.15M NaCl buffer at pH 8.5. A standard curve was obtained using the low molecular weight gel filtration kit (SIGMA marker SDS6H2): myosin, rabbit muscle (200kDa), β-galactosidase, E. coli (116kDa), phosphorylase B, rabbit (97kDa), albumin, bovine (66kDa), albumin, egg (45kDa) and carbonic anhydrase, bovine (29kDa). Buffer was eluted from the column at room temperature with a flow rate of 24mL/h; 5 mL fractions were collected, and the absorbance at 280nm was measured.

The globulin sub-fraction eluted into five protein peaks (SB1 to SB5). Each peak was concentrated, and dialyzed against 0.5M sodium acetate buffer at pH 5 and at 5°C for further purification. A SDS-PAGE electrophoresis was carried out to compare the initial globulin sub-fraction with SB1 and SB2 major peaks. The crude globulin subfraction pattern on lane ECG shows eight well-separated major bands; peak on lane SB2 shows only one wide band, which corresponds to the major molecular component (10kDa) of the crude globulin sub-fraction. The electrophoretic pattern on lane SB1 shows five clear bands.

Further purification of SB1 fraction was by a strong anion exchange column using a Q-Sepharose Ion exchange 5mL cartridge (Bio-Rad, Richmond, CA). Samples containing 15 mg were applied on the anionic column, and buffer (0.05M Tris-HCl, 0.15M NaCl buffer at pH 8.5, and a gradient of NaCl ranging from 0 to 1 M was used. This purification process produced 8 peaks mainly SB1Q1 to SB1Q8 that were released at ~0.33, ~0.44, ~0.49, ~0.52, ~0.61, ~0.65, ~0.76 and ~0.78M of the NaCl gradient, respectively. Two major peaks: SB1Q2 and SB1Q3 show clearly one component for each peak with a molecular mass and isoelectric point of 13.2, 10.24kDa; 4.5, 6.8, respectively.

Spectroscopic measurements

Far-UV (from 250 to 187nm) CD spectrum for SB1Q2 and SB1Q3 revealed a predominantly 42, 7%; 42.1, 7.4% of β-sheet and helical structures, respectively. This observation agrees well with the secondary structure of globulins from plants seeds (*11S* from *Brasica Napus L.*; 11S from *Helianthus annuus L.*; *11S* from *Vicia faba*; *7S* from *Phaseolus vulgaris L.*).[16]

Enzymatic assays

Knowledge about seed storage protein mobilization in chia seeds is very scanty. A partial characterization of four enzymatic assays mainly polyphenol oxidase, catalase, peroxidase, chymoprotease and protease were performed to the crude extract and globulins extracted at pH 7 and 8.5. Only protease assay was performed to SB1Q2 and SB1Q3 pure globulins. The results showed an increase on polyphenol oxidase enzyme activity in both globulins extracted at pH 7 and 8.5 as compared to the crude extract. However, the catalase and peroxidase activities decrease or are no detect on both globulins extracted at pH 7 and 8.5. Pure SB1Q3 pure globulins showed high protease activity as compared to SB1Q2 and globulins extracted at pH 7 and 8.5.

Conclusion

Globulin Protein Isolates from seeds of Chia (*Salvia hispanica*) using water and two different sodium chloride concentrations (5 and 10%) and testing four enzymatic assays: polyphenol oxidase, catalase, peroxidase and chymoprotease to each extraction. Maximum protein extraction (18.4%) and highest enzymatic activity occurred in the presence of 5% NaCl. The globulin purification started by gel filtration chromatography using Sephadex G-75, eluting with 0.05M Tris-HCl buffer at pH 8.5. Anion-exchange chromatography was used to obtain two globulins fractions. These two globulins had molecular weights of 10 and 13kDa and their isoelectric points were 4.5 and 6.8, respectively. The spectroscopy studies by circular dichroism showed that these two SB1Q2 and SB1Q3 pure globulins had a predominantly 42.0, 7.0%; 42.1, 7.4% of β-sheet and helical structures, respectively. This observation agrees well with the secondary structure of globulins from plants seeds.

Acknowledgements

This work has been carried during my sabbatical leave (M. Soriano-García) at the área de Química, Departamento de Ciencias

Básicas, División de Ciencias Básicase Ingeniería de la Universidad Autónoma Metropolitana-Unidad Azcapotzalco, México. This work was supported by the Cátedra "Leopoldo Río de la Loza Guillén" del Departamento de Ciencias Básicas. All the authors contributed to the biochemical studies and final editing of the manuscript.

Conflicts of interest

The authors declare no conflicts of interest related to this article.

Funding

None.

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