

## ANALYSIS OF SESAME PROTEINS ISOLATE (*SESAMUM INDICUM* L) WITH WATER AND SALT TREATMENT

POVEDA T, VILCACUNDO R, CARPIO C, CARRILLO W\*

Laboratory of Functional Foods, Faculty of Foods Science and Engineering, Technical University of Ambato, Av. Los Chasquis y Rio Payamino, Campus Huachi, CP 1801334, Ambato-Ecuador. Email: wi.carrillo@uta.edu.ec

Received: 14 March 2016, Revised and Accepted: 22 March 2016

### ABSTRACT

**Objective:** The aim of this study was to obtain protein isolate from sesame using alkaline pH at different pHs of precipitation with water and salt and to analyze protein isolate with sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

**Methods:** Sesame protein isolates were obtained using isoelectric precipitation method at different pHs using water and salt as solvents. Proteins were analyzed using native-PAGE and SDS-PAGE.

**Results:** A yield of  $14,727\% \pm 0.3$  of protein isolate of defatted sesame flour at pH 7.0 with a  $47.4\% \pm 0.6$  of protein was obtained. The yield of protein isolate using water and salt was similar. Polypeptides profile is between 6.5 and 50 kDa.

**Conclusions:** Sesame seed is a good source of proteins. Globulins and albumins were identified in the sesame protein isolate in the presence of water and salt.

**Keywords:** Sesame, Protein isolate, Proteins, Globulins and albumins.

### INTRODUCTION

Food proteins from plants are important for human and animal nutrition, particularly in developing countries where average protein intake is less than required [1]. The production of plant protein isolates is of growing interest to the food industry because of the increasing applications of plant proteins in food markets, nutraceutical products, and functional foods. Sesame (*Sesamum indicum* L.) is one of the most important oilseed crops cultivated in Central America, Tropical Africa, and Eastern Asia. Global production, in 2008, was 3.6 million tons [2]. Sesame seeds and oil are used in the food industry as well as in the pharmaceutical and cosmetic industries due to their high content of oil and protein with 19% oil content and 25% protein content. Most of the proteins present in sesame seeds are storage proteins composed of globulins (67.3%), albumins (8.6%), prolamins (1.4%), and glutelins (6.9%) [3,4]. Some sesame protein subunits (belonging to 2S albumin, 7S and 11S globulins) have been characterized by electrophoresis and described as major allergens.

Presently, it is possible to find products derived from plant proteins isolate such as soybean, quinoa, amaranth lupin seed, and walnuts [5,6]. In this study, we obtained sesame protein isolates with water and salt at different pHs of precipitation.

### METHODS

#### Sesame flour and proximate analysis

Sesame flour was defatted through extraction with hexane (1:10 w/v) at room temperature during 24 hrs, under continuous stirring during the first 5 hrs. After drying at room temperature, the flour was stored at 4°C until used. Analytical methods such as moisture, fat, total fiber, and soluble solids content were determined, according to the methods of Association of Official Analytical Chemists (AOAC) [7], numbers 9250.10, 930.09, 985.29, and 923.03, respectively. The protein content of the samples was determined by the micro-Kjeldahl method AOAC number 920.152, % (N × 6.25). Carbohydrates percentage was calculated with the formulas: % Carbohydrates = 100 - (% moisture + % proteins + % fat + % soluble solids + % total fiber). Contents were expressed on a dry weight basis.

#### Protein isolate obtained from sesame flour

Sesame isolate was prepared according to Martínez and Añón [8] with modifications. The defatted flour was suspended in water in a 1:10 w/v, and the suspension was adjusted to pH 8.0 by adding 2M NaOH. The suspension was stirred during 1 hr and then centrifuged at 4500 g for 30 minutes at 25°C. The supernatant was adjusted to pHs 2.0, 3.0, 4.0, 5.0, and 6.0 with 2 N HCl and centrifuged for 20 minutes at 4500 g. The pellet was suspended in a small volume of water, neutralized with 0.1 M NaOH, and lyophilized and then frozen at -20°C. The content of protein isolate was determined using the method biuret [9].

#### Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

Native-PAGE and SDS-PAGE of sesame protein isolate were carried out according to the method proposed by Laemmli [10] using 4-8% and 4-12% polyacrylamide gel in a mini-protean electrophoresis system (Bio-Rad, Hercules, CA, USA). Polypeptide bands were stained in Coomassie Brilliant Blue G-250 for 12 hrs. Relative molecular masses of protein were determined by a comparison to molecular weight markers (Bio-Rad, Hercules, CA, USA) and software Quantity One of Chemidoc (Bio-Rad).

#### Statistical analysis

Results are presented as means ± standard deviation from three replicates of each experiment. Differences between mean values were determined by the analysis of variance (ANOVA). The *post-hoc* analysis was performed by the Tukey test. All tests were considered significant at  $p < 0.05$ . Statistical analysis was performed using the software package Prism 4 for Windows, version 4.3 (GraphPad Software Inc., www.graphpad.com).

### RESULTS AND DISCUSSION

The defatted sesame flour (n=3) was analyzed for proximate analysis. Table 1 shows the results of this analysis. Content of protein was high with 40.10%. Achouri and Boye, 2013 have been reported a protein content of 59% [4], while the carbohydrates content was 22.22%.

Sesame protein isolates were obtained using the isoelectric precipitation method with water and NaCl 0.1 M as solvents. Using water as solvent, the highest yield was obtained at pH 7.0 with a 14.72% of protein isolate content. Using NaCl 0.1 M as solvent, the highest yield at pH 4 was 44.6%. NaCl was apparently effective for solubilizing sesame flour protein. Samples, using NaCl as solvent, were dialyzed with a membrane with porous of 5000 Da (Spectra/Por) to eliminate the content of salt. Yield after dialysis (17.2%) has not statistical differences with respect to using only water as solvent. Protein isolate yield after dialysis was low at pHs 3.0-6.0. Moreover, content of protein was determined using biuret method registering the highest content of protein at pH 7.0 with a 47.4% (Table 2).

All defatted sesame flour protein isolate were analyzed by electrophoresis native-PAGE and SDS-PAGE.

Electrophoresis native-PAGE was used to analyze protein isolate obtained with water at different pHs of precipitation. Bands of 35-198 kDa were found. Bands with molecular weight corresponding to 198 kDa presented high expression at pH 5.0, 6.0, and 7.0. They can be 11S aggregates with high molecular weight. Achouri *et al.*, 2012 [3] described 11S aggregates with a molecular weight of 140 and 669 kDa of sesame protein isolate (Fig. 1).

On the other hand, the samples were analyzed with electrophoresis SDS-PAGE with 2-β-mercaptoethanol. Soluble proteins were characterized by polypeptides 6.5-50 kDa range. These results are in accordance to other authors. Bands of 6.5, 18-20, 28-30, and 50 kDa were found in all pHs assayed. The 50 kDa band corresponds to 7S globulin and the bands with 18-20 corresponding to 11S basic subunits and 28-30 corresponding to 11S acid subunits. 11S and 7S fractions are more abundant at pH 5.0, 6.0, and 7.0 finally, a band with 6.5 kDa is 2S albumin (Fig. 2).

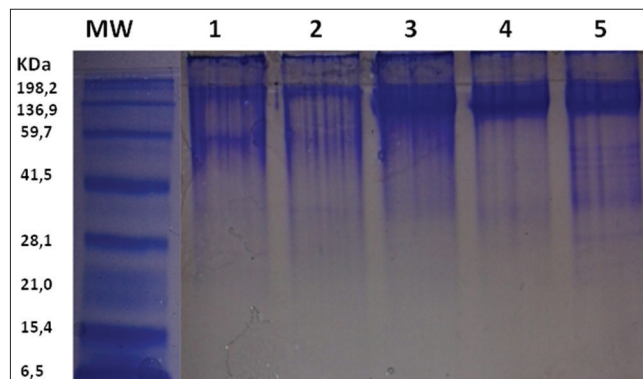
Electrophoresis SDS-PAGE without 2-β-mercaptoethanol was used to analyze protein isolate obtained with water. Fig. 3 shows polypeptides of 6.5 kDa with high expression and present in all pHs assayed. At pH 5.0, 6.0, and 7.0, we observed bands near 50 kDa.

Protein isolates obtained with NaCl 1M at different pHs were analyzed by electrophoresis SDS-PAGE under reducing conditions. Fig. 4 shows differences in the electrophoretic between the sesame proteins isolate with water and those extracted in the presence of NaCl 0.1 M. The extracts obtained at pH 3.0, 4.0, 5.0, 6.0, and 7.0 with NaCl 0.1 M showed similar profiles of proteins. Proteins isolate were composed of identical polypeptides bands with molecular weight (28-30 kDa) corresponding to basic subunit of 11S sesame and bands with molecular weight (18-20 kDa) corresponding to basic subunit of 11S sesame proteins globulins. 11S is the most abundant fraction, with intense bands at pH 5.0, 6.0, and 7.0. 7S globulin corresponds to bands between 45 and 50 kDa. Those bands are present in all pHs assays. Molecular weight bands of 6.5 kDa correspond to 2S sesame albumins. Those bands

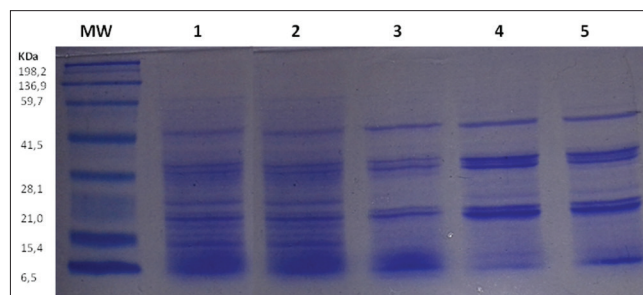
were intense at pH 5.0, 6.0, and 7.0. 11S globulins and 2S albumins bands have a high expression as those bands are more soluble in this solvent (Fig. 4).

**DISCUSSION**

It is known that plant proteins from legume and non-legume plants have two of the main classes of storage proteins. These proteins are named 7S and 11S depending on their sedimentation coefficients. 11S globulins are hexamers with molecular weights between 300 and 400 kDa, consisting of two opposed hexagonal rings, each



**Fig. 1: Electrophoresis native-polyacrylamide gel electrophoresis of sesame protein isolate precipitated at different pHs. Lane 1: Molecular weight standard, Lane 2: Sesame protein isolate at pH 3.0, Lane 3: Sesame protein isolate at pH 4.0, Lane 4: Sesame protein isolate at 5.0, Lane 5: Sesame protein isolate at pH 6.0, and Lane 6: Sesame protein isolate at pH 7.0**



**Fig. 2: Electrophoresis sodium dodecyl sulfate-polyacrylamide gel electrophoresis with 2-β-mercaptoethanol of sesame protein isolate at different pHs. Lane 1: Molecular weight standard, Lane 2: Sesame protein isolate at pH 3.0, Lane 3: Sesame protein isolate at pH 4.0, Lane 4: Sesame protein isolate at 5.0, Lane 5: Sesame protein isolate at pH 6.0, and Lane 6: Sesame protein isolate at pH 7.0**

**Table 1: Proximate analysis of defatted sesame flour**

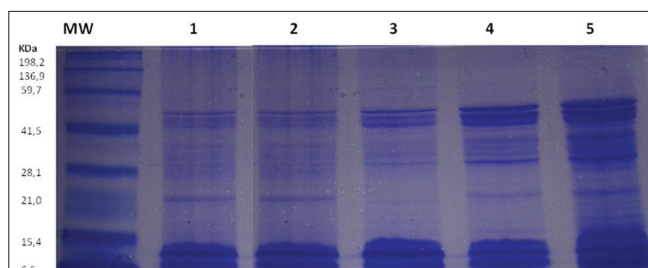
%	Protein	Fat	Moisture	Total fibre	Soluble solids	Carbohydrates
DSF	40.10±0.2	10.8±0.03	10.10±0.03	5.74±0.1	11.1±0.2	22.22±0.2

Results represent the average of three determinations±SD, DSF: Defatted sesame flour, SD: Standard deviation

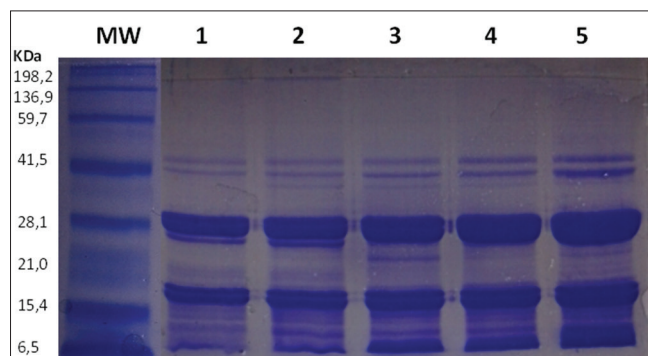
**Table 2: Content of sesame protein isolate obtained at different pHs**

Sample	pH 3.0	pH 4.0	pH 5.0	pH 6.0	pH 7.0
% Isolate with water	8.464±1.8 <sup>a</sup>	8.339±1.3 <sup>a</sup>	9.394±1.3 <sup>a</sup>	9.394±0.2 <sup>a</sup>	14.727±0.3 <sup>a</sup>
% Isolate with NaCl	8.7±0.7 <sup>a</sup>	11.0±0.7 <sup>a</sup>	14.7±0.4 <sup>b</sup>	5.0±1.3 <sup>a</sup>	44.6±0.5 <sup>c</sup>
% Isolate after dialysis	4.2±2.5 <sup>a</sup>	4.7±2.5 <sup>a</sup>	7.0±2.5 <sup>a</sup>	1.8±2.5 <sup>a</sup>	17.2±2.5 <sup>a</sup>
% protein biuret	70.5±0.9 <sup>a</sup>	33.6±5.6 <sup>a</sup>	37.5±1.2 <sup>b</sup>	32.2±2.4 <sup>a</sup>	47.4±0.6 <sup>b</sup>

Values are expressed in grams per 100 g of protein. Values are means±SD of three determinations. Different letters show statistical difference between the groups (p<0.05) ANOVA and Turkey's test, SD: Standard deviation



**Fig. 3: Electrophoresis sodium dodecyl sulfate-polyacrylamide gel electrophoresis without 2- $\beta$ -mercaptoethanol analysis of sesame protein isolate obtained at different pHs. Lane 1: Molecular weight standard, Lane 2: Sesame protein isolate at pH 3.0, Lane 3: Sesame protein isolate at pH 4.0, Lane 4: Sesame protein isolate at 5.0, Lane 5: Sesame protein isolate at pH 6.0, and Lane 6: Sesame protein isolate at pH 7.0**



**Fig. 4: Electrophoresis sodium dodecyl sulfate-polyacrylamide gel electrophoresis with 2- $\beta$ -mercaptoethanol analysis of sesame protein isolate obtained with NaCl 1 M at different pHs. Lane 1: Molecular weight standard, Lane 2: Sesame protein isolate at pH 3.0, Lane 3: Sesame protein isolate at pH 4.0, Lane 4: Sesame protein isolate at 5.0, Lane 5: Sesame protein isolate at pH 6.0, and Lane 6: Sesame protein isolate at pH 7.0**

containing three hydrophobically associated pairs of disulfide-linked acidic (29-35 kDa) and basic (18-28 kDa) subunits. 7S globulins are glycoproteins with molecular weights between 150 and 200 kDa [11]. The occurrence of 11S and 7S type storage globulins in angiosperm seeds has been recognized and accepted [12,13]. 2S albumins have been characterized in different plant proteins for example amaranth, quinoa, lupin seed, soybean, sacha inchi, and sesame as they are considered as allergens [14]. The water-insoluble 11S globulin and soluble 2S albumin are the two major storage proteins of sesame. The 11S sesame globulin has the typical oligomeric structure of 11S seed proteins, being composed of six subunits [15,16]. In this study, globulins and albumins proteins were obtained from sesame protein isolates at different pHs with water and salt as solvent for extraction. Achouri *et al.* 2012 [3] reported that they obtained polypeptides with molecular weight between 10 and 100 kDa. 2S albumin was reported with molecular weight of 13 kDa, and 7S was reported with molecular weight between 45 and 50 kDa, being in accordance to our results. 11S acid subunit (30-35 kDa) and 11S basic subunit (20-25 kDa) were reported. Our results are in accordance to this study. India is the largest producer of sesame with approximately 27% of total production. China, Myanmar, Mexico, Nigeria, Sudan, Bangladesh, Somalia, and Uganda are other major sesame producing countries. Sesame seed contains near 20% protein. Sesame defatted flour protein content is about 50%. The defatted flour also contains some undesired components along with sesame seed protein such as fiber, soluble sugar, phytates, and oxalates that can be eliminated to a large extent by producing protein isolates [17]. Defatted sesame flour (40-50% protein content) is very important as a protein source for

human consumption due to the presence of sulfur-containing amino acids, mainly methionine [18,19]. In this study, we obtain defatted sesame flour with 40.10% of protein content and 10.8% of fat content.

## CONCLUSIONS

It was possible to obtain protein isolates from sesame flour with solvents as water and NaCl 0.1 M with high yields. No major differences were found in yields when using water or NaCl as solvent. Profile of proteins obtained with NaCl 0.1 M as solvent has intense bands corresponding to 11S sesame globulins and 2S sesame albumins at pH 5.0, 6.0, and 7.0. Defatted sesame flour is a good source of proteins to be used for animal and human nutrition.

## ACKNOWLEDGMENTS

This study was supported by Universidad Técnica de Ambato, Ecuador (Project CPU-1373-2014-UTA). This work has been reviewed in the English edition by Emilio Labrador Carrillo W, thanks to Sandra Chavez of AMTECLAB by the advice with instruments of Bio-Rad, Dr. Carlos Rodríguez director (DIDE), and Dra. Jacqueline Ortiz Doyenne (FCIAL) for support and encouragement.

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