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Research Article

ISOLATION OF PROTEINS FROM SACHA INCHI (*PLUKENETIA VOLUBILIS* L.) IN PRESENCE OF WATER AND SALT

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ABSTRACT

Objective: The aim of this study was to obtain protein isolate from sacha inchi using alkaline pH at different pHs of precipitation with water and salt and to analyze protein isolate with electrophoresis.

Methods: Sacha inchi protein isolates were obtained using isoelectric precipitation method at different pHs. Proteins were analyzed using electrophoresis native-polyacrylamide gel electrophoresis (PAGE), one-dimensional, two-dimensional-sodium dodecyl sulfate-PAGE.

Results: A yield of 20.88% of protein isolate of defatted sacha inchi flour at pH 4.0 with a 75.31% of protein was obtained. The yield of protein isolate using water and salt was similar. Polypeptides profile is between 14 and 70 kDa.

Conclusions: Sacha inchi seed is a good source of proteins. Globulins and albumins were identified in the sacha inchi protein isolate in the presence of water and salt.

Keywords: Albumins, Globulins, Proteins, Protein isolate, Sacha inchi.

INTRODUCTION

Sacha inchi (*Plukenetia volubilis* L), also named as Inca peanut, is a plant that grows in the wild and is native to the rain forests of the Andean region of South America. This plant belongs to the *Euphorbiaceae* family and is composed of nineteen species [1]. Food proteins from plants are important for human and animal nutrition, particularly in developing countries where average protein intake is less than required [2]. 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. Presently, it is possible to find products derived from plant proteins isolate such as soybean, quinoa, amaranth lupin seed, and walnuts [3,4]. It is known that sacha inchi seeds have a high content of oil (54%) and protein (27%) [5]. Sacha inchi seed proteins are soluble in aqueous buffers, and a water-soluble albumin has been reported to constitute 31 % of total proteins in the seed. A sub-product named tort or flour with high content of protein reported between 50% and 70% is obtained, during the production of sacha inchi oil. Sacha inchi proteins are soluble in 2 M NaCl at pH 4.0 [6]. In this study, we reported sacha inchi protein isolates with water and salt at different pHs of precipitation.

METHODS

Sacha inchi flour and proximate analysis

Sacha inchi 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 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 from sacha inchi

Sacha inchi isolate was prepared according to Martinez and Añón (1996) [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 2 M 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 electrophoresis of sacha inchi protein isolate was carried out according to the method proposed by Laemmli (1970) [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).

Two-dimensional (2D) electrophoresis (isoelectric focusing [IEF]-SDS-PAGE)

Samples were analyzed by 2D electrophoresis as described by Quiroga *et al*., (2015) [11]. IEF was run using 7 cm linear immobiline pH gradient (IPG) strips (pH 3-10) in the PROTEAN i12 IEF Cell system (Bio-Rad). Samples were dissolved in the rehydration buffer, and the IPG strips were rehydrated with these samples. Following IEF, the gel strips were incubated with equilibration buffer and were placed onto 12 g acrylamide/100 ml of resolving gels and were run in mini slabs (Bio-Rad). All gels were fixed and stained with Coomassie Brilliant Blue.

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. 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 sacha inchi flour (DSF) (n=3) was analyzed for proximate analysis. Table 1 shows the results of this analysis. The content of protein was high with a 57.6%. This result is comparable with similar results reported in the bibliography between a 53% and 59% [7,12,13] while the carbohydrates content was 15.2%.

Sacha inchi protein isolates were obtained using the isoelectric precipitation method with water and NaCl 1 M as solvents. Using water as solvent, the highest yield was obtained at pH 4.0 with a 20.88% of protein isolate content, whereas using NaCl 1 M as solvent, the highest yield at pH 4 was 77.90%. NaCl was apparently effective for solubilizing protein from sacha inchi flour. However, samples using NaCl as solvent were dialyzed with a membrane with porous of 5000 Da to eliminate the content of salt. Yield after dialysis has not statistical differences with respect to use only water as solvent. Yield results using water as solvent or dialyzed NaCl solution were similar in all pHs assayed. Moreover, the content of protein was determined using Biuret method registering a high content of protein at pH 4.0 with a 75.31% (Table 2).

All protein isolate from DSF were analyzed by electrophoresis native-PAGE, one-dimensional-SDS-PAGE, and 2D-SDS-PAGE. Protein isolate at pH 4 was analyzed with SDS-PAGE at a concentration of 5 mg/ml. Bands between 14 and 200 kDa were found. Bands of 16 and 43 kDa have strong expression (Fig. 1). The profile of protein is complex with bad resolution as protein loaded in each well was 5 mg/ml. It was determined that the optimum concentration of protein was 20 µg/ml for each well of the gel for all assays.

Electrophoresis native-PAGE was assayed to analyze protein isolate obtained with water at different pHs of precipitation. Bands of 14-97 kDa were found. Bands with molecular weight corresponding to 14, 30, 60, and 97 kDa presented high expression (Fig. 2).

On the other hand, the samples were analyzed with electrophoresis SDS-PAGE with 2-β-mercaptoethanol. Soluble proteins were characterized by polypeptides 16-70 kDa range. These results are in accordance to other authors. Bands of 45 and 73 kDa were found in all pHs assayed. The 45 kDa band corresponds to 11S globulin and the band with 73 kDa corresponds to 7S globulin. 11S and 7S fractions are more abundant. Finally, a band with 16 kDa can be 2S albumin (Fig. 3).

Electrophoresis SDS-PAGE without 2-β-mercaptoethanol was used to analyze protein isolate obtained with water. Fig. 4 shows one polypeptide of 45 kDa with high expression and present in all pHs assayed.

Protein isolates obtained with NaCl 1 M at different pHs were analyzed by electrophoresis SDS-PAGE. Fig. 4 shows a band of 66 kDa and one dimer of 45 KDa, other dimer of 28 kDa and other dimer of 16 kDa. All bands have high expression because those bands are more soluble in this solvent (Fig. 5).

Electrophoresis 2D-SDS-PAGE indicates that protein isolate contains a high content of globulins. Moreover, these proteins have the same molecular weight but the different isoelectric point, for these reasons these proteins maybe isoforms (Fig. 6).

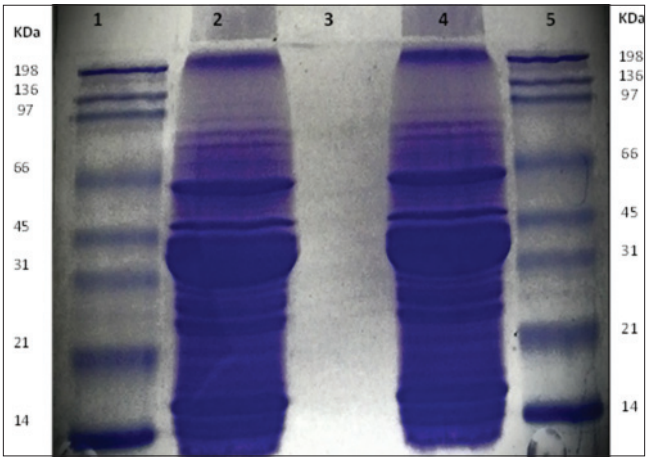

Fig. 1: Electrophoresis sodium dodecyl sulfate-polyacrylamide gel electrophoresis with 2-β-mercaptoethanol of sacha inchi protein isolate at pH 4.0. (5 mg/ml) (20 µl of sample for well), Lane 1: Molecular weight; Lane 2: Sacha inhi isolate at pH 4.0; Lane 3: Water; Lane 4: Sacha inhi isolate at pH 4.0 replicate; Lane 5: Molecular weight

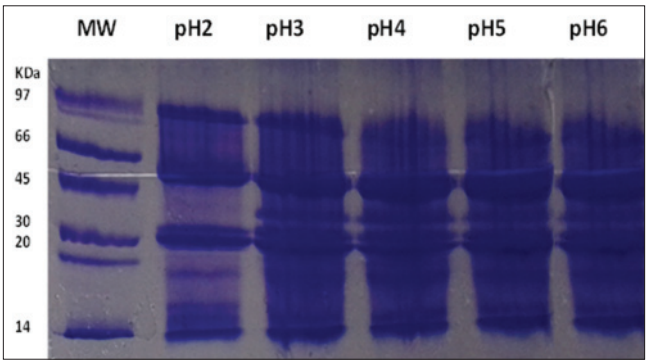

Fig. 2: Electrophoresis native-polyacrylamide gel electrophoresis of sacha inchi protein isolate precipitated at different pHs

Table 1: Proximate analysis of DSF

%	Protein	Fat	Moisture	Total fiber	Soluble Solids	Carbohydrates
DSF	57.60±0.1	11.2±0.01	4.08±0.03	5.72±0.1	5.78±0.2	15.62±0.2

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

Table 2: Content of sacha inchi protein isolate obtained at different pHs

Sample (%)	pH 2.0	pH 3.0	pH 4.0	pH 5.0	pH 6.0
Isolate with water	15.65±0.8^a	16.73±1.2^a	20.88±1.3^a	18.53±0.2^a	19.10±0.2^a
Isolate with NaCl	49.47±2.5^a	48.73±2.5^a	77.90±3.4^b	46.41±1.9^a	10.03±2.0^c
Isolate after dialysis	15.98±2.5^a	16.93±2.5^a	20.50±2.5^a	18.42±2.5^a	10.0±2.5^a
Protein biuret	49.52±1.5^a	52.08±1.5^a	75.31±1.9^b	61.49±0.1^a	69.29±1.2^b

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

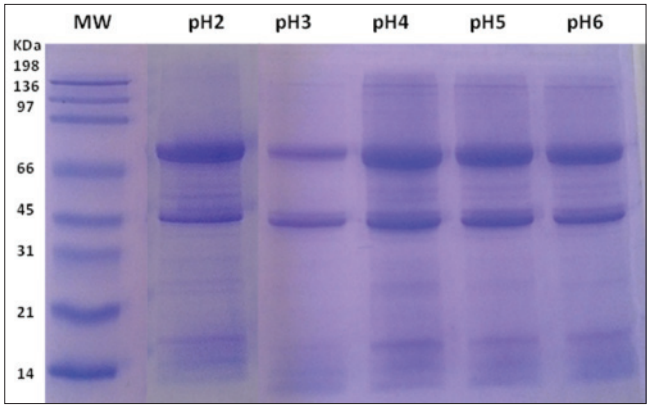

Fig. 3: Electrophoresis sodium dodecyl sulfate-polyacrylamide gel electrophoresis with 2-β-mercaptoethanol analysis of sacha inchi protein isolate obtained at different pHs

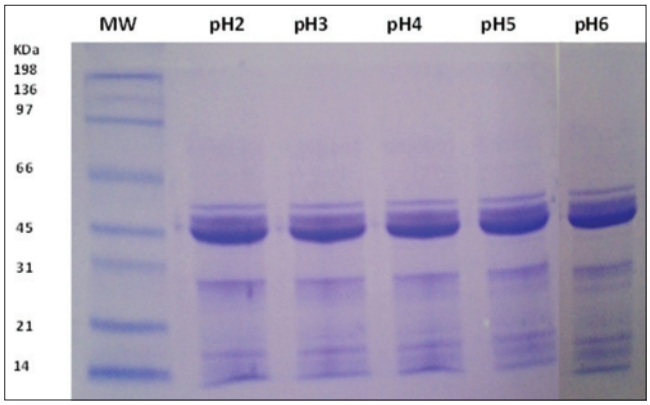

Fig. 4: Electrophoresis sodium dodecyl sulfate-polyacrylamide gel electrophoresis without 2-β-mercaptoethanol analysis of sacha inchi protein isolate obtained at different pHs

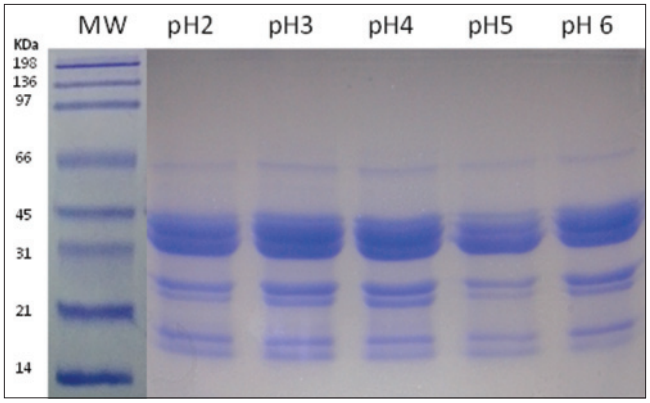

Fig. 5: Electrophoresis sodium dodecyl sulfate-polyacrylamide gel electrophoresis with 2-β-mercaptoethanol analysis of sacha inchi protein isolate obtained with NaCl 1 M at different pHs

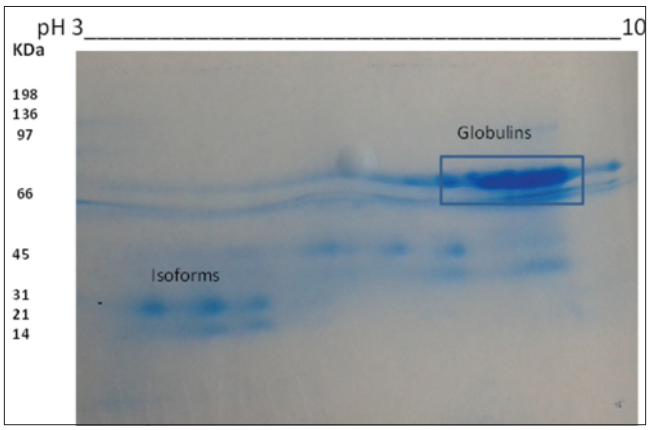

Fig. 6: Electrophoresis two-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis of sacha inchi isolate at pH 4.0

DISCUSSION

It is known that plant protein 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 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 [14]. The occurrence of 11S and 7S type storage globulins in angiosperm seeds has been recognized and accepted [15-18]. 2S albumins have been characterized in different plant proteins for example amaranth, quinoa, lupin seed, soybean, and sacha inchi, as they are considered as allergens [19,20]. In this study, globulins and albumins proteins were obtained from sacha inchi protein isolates at different pHs with water and salt as solvent for extraction. Shridhar *et al*., 2012 reported that they obtained polypeptides with molecular weight between 6 and 70 kDa, although with high expression of polypeptides with mass of 20-40 kDa. In this study, similar results have been obtained with polypeptides between 14 and 70 kDa, polypeptides with the highest expression were 20-70 kDa.

CONCLUSIONS

It was possible to obtain protein isolates from sacha inchi flour with solvents as water and NaCl 1 M with high yields. No major differences were found in yields when using water or NaCl as solvent. Electrophoresis 2D indicate that protein isolates have proteins with isoforms. Sacha inchi flour is a good source of proteins to be used for animal and human nutrition.

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