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Research Article

IDENTIFICATION OF PROTEINS ISOLATE FROM AMARANTH (*AMARANTHUS CAUDATUS*) BY SODIUM DODECYL SULFATE-POLYACRYLAMIDE GEL ELECTROPHORESIS WITH WATER AND NACL 0.1 M SOLVENTS

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ABSTRACT

Objective: The aim of this study was to obtain protein isolate from amaranth using alkaline method at extraction pH 8 and extraction pH 12 with different precipitation pHs and to analyze protein isolate with electrophoresis.

Methods: Amaranth protein isolates were obtained using isoelectric precipitation method at different pHs. Proteins were analyzed using sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

Results: A yield of 20.52% of protein isolate of defatted amaranth at pH 4.0 was obtained. The content of protein isolate was higher than 53% in all pH assays. Globulins and albumins in protein isolate at different pHs were observed. A band with 50 kDa corresponding to 7S globulin was found. The bands 36-38 kDa and 18-20 kDa correspond to 11S globulin. Bands less to 14.4 kDa correspond to albumins.

Conclusions: Amaranth protein isolate is possible to obtain in extreme conditions of pH. The treatment with water was optimum to obtain amaranth protein isolate.

Keywords: Amaranth, Globulins, Albumins, Proteinisolate, Sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

INTRODUCTION

Amaranth (*Amaranthus caudatus*) is a pseudocereal cultivated around the world in the Andean regions of South America, Asian, Africa and Europe and belongs to the family Amaranthaceae. Amaranth has more of 250 species but only three species are comestible as grain: *A. caudatus, Amaranthus hypocondriacus,* and *Amaranthus cruentus.* The protein content of amaranth is higher (13-19%) than most cereals [1-3]. This is an option as a source of proteins, producing bioactive peptides that prevent chronic diseases. The use of protein isolate has increased in the food industry because of different factors such as higher protein levels, good functionality, bioactive components, and lower content of anti-nutritional factors [4]. The most used method to obtain protein isolate is alkaline pH (8-11) through solubilization of proteins at acid pH (4-6) for their isoelectric precipitation [5]. The aim of this study was to obtain protein isolate from amaranth using alkaline pH at different pHs of precipitation using water and NaCl as solvents and to analyze these proteins with sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

METHODS

Amaranth flour and proximate analysis

Amaranth 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 contents were determined according to the methods of AOAC [6], numbers 9250.10, 930.09, 985.29, and 923.03, respectively. The protein content of the sample 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 amaranth

Amaranth isolate was prepared according to Martıínez and Añón [7] with modifications. The defatted flour was suspended in water and 0.1 M NaCl in a 1:10 w/v, and the suspension was adjusted to pH 8.0 and 12.0 by adding 1 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 [8]. The amaranth protein isolate obtained with salt were dialyzed in Milli-Q water with a membrane of 5000 Da of pore size (spectra/por) during 24 hrs at room temperature. The samples were subsequently lyophilized and then frozen at −20°C.

SDS-PAGE

SDS-PAGE of amaranth protein isolate was carried out according to the method proposed by Laemmli [9] using 4-12% polyacrylamide gel in a Mini-Protean electrophoresis system (Bio-Rad, Hercules, CA, USA). Polypeptide bands were stained in Coomassie Brilliant Blue R-250 for 12 hrs. Relative molecular masses of protein were determined by a comparison to molecular weight (MW) markers (Bio-Rad, Hercules, CA, USA) and software Quantity One of Chemidoc (Bio-Rad).

Statistical analysis

Results are presented as means±standard deviation (SD) 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's test. All tests were considered significant at $p<0.05$. A statistical analysis was performed using the software package Prism 4 for Windows, version 4.3 (GraphPad Software Inc., www.graphpad.com).

RESULTS

Composition analysis

Table 1 shows the approximate composition of defatted amaranth flour obtained with water. The protein content was 13.9%; this result is in accordance with other authors [10,11]. Moreover, we can see that the approximate composition of amaranth protein isolate at pH 4.0 shows the protein content increase to 50.8% using Kjeldahl method for determination of protein content (%N×6.25). Carbohydrates content in this protein isolate resulted to be low at 30.47%. There is then a statistical difference ($p<0.05$) between defatted flour and protein isolate as shown in Table 1. The content of protein also was determined with biuret method; the content of protein increases from 13.9% in the amaranth flour to 82.33% in the proteins isolate at pH 4.0 with water solvent. All pHs analyzed resulted in a content of proteins higher than 50% (Table 2).

Results represent the average of three determinations±SD. Different letters show a statistical difference between the groups (columns) (<0.05) ANOVA and Turkey's test.

Table 1: Proximate analysis of DAF and AIP

%	Protein	Fat	Moisture	Total fibre	Soluble solids	Carbohyidrates
DAF	13.9[a]	6.64[a]	9.58[a]	0.70[a]	2.74[a]	66.4[a]
AIP	50.82[b]	3.57[a]	6.75[a]	0.51[a]	8.10[b]	30.47[b]

DAF: Defatted amaranth flour, AIP: Amaranth isolate protein, SD: Standard deviation, results represent the average of three determinations ±SD. Different letters show statistical difference from the control group (p <0.05) ANOVA and Turkey's test

Table 2: Protein content in amaranth isolate obtained at extraction pH 8 and extraction pH 12 with different precipitation pHs

Treatment	Isolate at pH 8% of protein content (%)		Isolate at pH 12% of protein content (%)	
	Agua	NaCl	Agua	NaCl
pH 2.0	73.83±3.91[a]	1.01±0.60[a]	53.39±2.38[a]	16.41±0.92[a]
pH 3.0	59.30±2.18[b]	2.22±0.79[a]	83.42±1.39[b]	16.88±0.77[a]
pH 4.0	82.33±2.37[c]	23.91±1.01[b]	73.10±3.37[c]	19.30±3.51[a]
pH 5.0	56.70±2.60[b]	37.66±4.61[c]	53.01±2.40[a]	17.75±1.35[a]
pH 6.0	58.98±2.18[b]	0.02±0.00[a]	73.61±2.11[c]	30.32±3.09[b]

SD: Standard deviation, results represent the average of three determinations ±SD. Different letters show statistical difference from the control group (p <0.05) ANOVA and Turkey's test

Results represent the average of three determinations±SD. Different letters show a statistical difference between the groups (columns) (<0.05) ANOVA and Turkey's test.

Effect of pH on the extraction of amaranth proteins

Extraction at pH 8 at different pHs

The protein yields of amaranth protein isolate solubilized at pH 8 precipitate at different pHs (2.0, 3.0, 4.0, 5.0 and 6.0) were obtained using water and NaCl 0.1 M as solvents.

Using water as solvent, the highest yield was obtained at pH 4.0 with a 20.52% of protein isolate content, whereas using NaCl 0.1 M as solvent, the highest yield at pH 4.0 was 53.77%. NaCl was apparently effective for solubilizing protein from amaranth flour. However, samples using NaCl as solvent were dialyzed with a membrane with porous of 5,000 Da to eliminate the content of salt. Yield after dialysis has statistical differences with respect to using only water as solvent. Yield results of dialyzed NaCl solution were low in all pHs assay with values of 8.46% to pH 4.0 (Table 3).

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 (file) (<0.05) ANOVA and Turkey's test.

Extraction at pH 12 at different pHs

The protein yields from amaranth protein isolate solubilized at pH 12 precipitate at different pHs (2.0, 3.0, 4.0, 5.0, and 6.0) were obtained using the isoelectric precipitation method with water and NaCl 0.1 M. At extraction pH 12 using water as solvent at pH 4.0, the protein yield obtained was 19.31% while the protein yield obtained with NaCl 0.1 M was 44.57%. After dialysis, the protein yield was 9.33%. At precipitation pH 6.0, the protein isolate presents the highest yield with a value of 17.46% compared to the extraction pH 8.0 with precipitation pH 6.0 which presents a value of 8.31% (Table 4). The extraction of amaranth protein isolate at pH 12 using NaCl as solvent presents a higher protein content than extraction pH 8.0 using NaCl (Table 2).

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 (file) (<0.05) ANOVA and Turkey's test.

SDS-PAGE

Amaranth seed shows proteins fractions of globulins and albumins as storage protein. Globulins, albumins, and glutelins are the major proteins fractions in amaranth seeds. Its globulin is composed primarily of the 11S globulin. 7S globulin is also present but only in minor quantity [12]. Globulins have two groups depending on its sedimentation coefficient:

Table 3: Content of amaranth protein isolate obtained at extraction pH 8.0 with different precipitation pHs

% Yield	pH 2.0	pH 3.0	pH 4.0	pH 5.0	pH 6.0
Isolate/water	18.66±0.39[a]	18.66±0.26[a]	20.52±0.38[a]	15.02±0.20[b]	8.31±0.39[c]
Isolate/NaCl (0.1 M)	40.53±0.39[a]	53.62±1.45[b]	53.77±0.15[b]	50.61±0.63[b]	48.32±0.29[c]
Isolate after dialysis	6.64±0.15[a]	8.41±0.11[a]	8.46±0.40[a]	7.21±0.73[a]	6.67±0.59[a]

SD: Standard deviation, results represent the average of three determinations ±SD. Different letters show statistical difference from the control group (p <0.05) ANOVA and Turkey's test

Table 4: Content of amaranth protein isolate obtained at extraction pH 12.0 with differentprecipitation pHs

% Yield	pH 2.0	pH 3.0	pH 4.0	pH 5.0	pH 6.0
Isolate/water	14.78±0.35[a]	17.11±0.46[a]	19.31±0.77[b]	19.11±0.46[b]	17.46±0.62[a]
Isolate/NaCl (0.1 M)	48.89±0.50[a]	39.28±0.46[a]	44.57±0.32[a]	41.06±0.61[b]	53.01±0.58[c]
Isolate after dialysis	8.33±0.14[a]	5.25±0.25[a]	9.33±0.28[a]	5.50±0.25[a]	8.41±0.38[a]

SD: Standard deviation, results represent the average of three determinations ±SD. Different letters show statistical difference from the control group (p <0.05) ANOVA and Turkey's test

11-12S and 7-8S. Amaranth storage protein predominant is globulins 7S and 11S. 7S present MW of 41-45 kDa. Recently, globulin 11S from amaranth has been named amaranthine [13]. This protein has two subunits consisting of an acid polypeptide (AS) (33-36 kDa) and a basic polypeptide (AB) (16-19 kDa). The 2S albumins have been described like a band of low MW near 6-10 kDa.

SDS-PAGE at reduced and nonreduced conditions of amaranth-extracted protein in water and salt at extraction pH 8 and 12 with different pHs were assayed. Fig. 1 shows SDS-PAGE of amaranth protein isolate obtained at pH 8 with different precipitation pHs. Proteins mass was determined with software Quantity one of Chemidoc™ PM (Bio-Rad). In the presence of 2-β-mercaptoethanol, proteins with MW ranging between 6.5 and 50 kDa were found in all pHs. The protein bands with 50 kDa corresponding to 7S globulin were found in all pHs assayed. Proteins with MW 36-38 kDa corresponding to 11S AS were found in all pHs with high expressions. On the other hand, proteins with MW 18-20 kDa corresponding to 11S AB were found in all pH values. Proteins with 20-36 kDa correspond to Amaranthine. All proteins bands <15.4 kDa corresponding to albumin components were found in high expressions in all pHs.

SDS-PAGE without 2-β-mercaptoethanol present a similar profile of proteins at all pHs assay, the band with MW 6.5, 15.4, 36, 38 and 50 kDa was found in all pHs with high expression (Fig. 2). Only the bands corresponding at 11S basic (18-20 kDa) not found in the gel at all pHs.

SDS-PAGE with 2-β-mercaptoethanol present complex profile of proteins isolate at pH 12 in all pHs assay. Bands ranging between 6.5 and 50 kDa were observed. This profile has many bands when compared to isolate obtained at pH 8 of solubilization. 7S, 11S globulins and 2S albumins were observed in all pHs assay (Fig. 3).

SDS-PAGE of amaranth isolate solubilized at pH 8 obtained with NaCl 0.1 M was analyzed. Fig. 4 shows profiles at different pHs of precipitation. Bands with MW of 30 kDa were found in all pHs with high expression. At pH 4 and pH 5 bands range between 6.5 and 50 kDa were observed with high expression. When compared with the results of biuret method, we observed a correlation because pH 4 and 5 has high protein content.

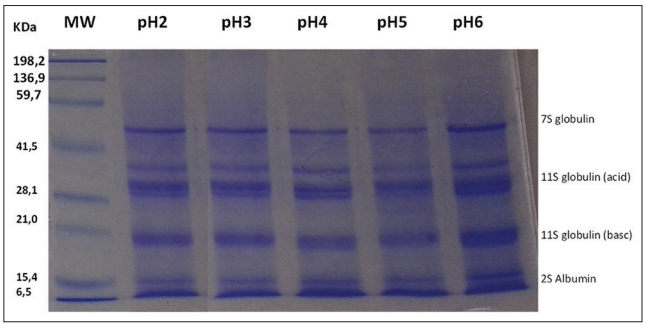

Fig. 1: Electrophoresis sodium dodecyl sulfate-polyacrylamide gel electrophoresis profiles of amaranth proteins obtained at pH 8 with different precipitation pHs extracted under reducing conditions (sodium dodecyl sulfate+2-β-mercaptoethanol). MW: Molecular weight marker

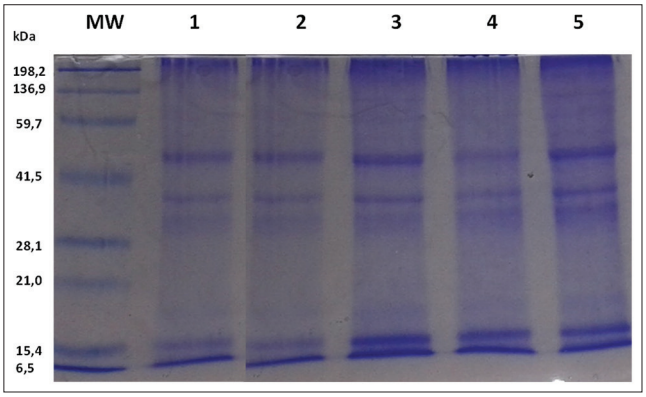

Fig. 2: Electrophoresis sodium dodecyl sulfate-polyacrylamide gel electrophoresis profiles of amaranth proteins obtained at pH 8 with different precipitation pHs extracted without reducing conditions. MW: Molecular weight marker

DISCUSSION

It is known that two of the major types of storage proteins in legume and some no legume seeds are 7S and 11S based on their sedimentation coefficients. Amaranth seeds, due to their high protein content, are actually the subject of many investigations as potential food source and functional food [14,15]. Different studies have reported high isolate protein yield obtained with NaCl. Achouri *et al*. [16,17] have reported that sesame protein isolates obtained with water (12.5%) and salt (54.6%). We can observe that this yield is apparently higher in salt than water. However, after dialysis this difference was clarified as the increase of weight is due to the weight of the salt.

CONCLUSIONS

The content of proteins was higher than 53% in all pHs assays. Albumins and globulins were identified in amaranth protein isolates

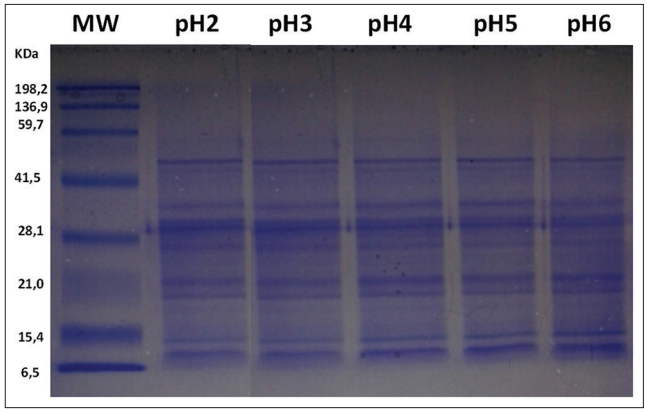

Fig. 3: Electrophoresis sodium dodecyl sulfate-polyacrylamide gel electrophoresis profiles of amaranth proteins obtained at pH 12 with different precipitation pHs extracted under reducing condition (sodium dodecyl sulfate+2-β-mercaptoethanol). MW: Molecular weight marker

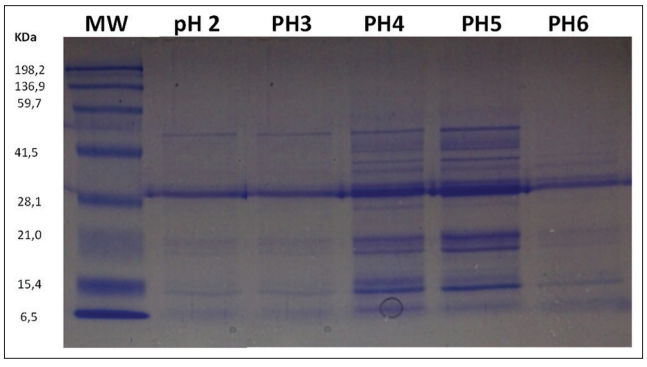

Fig. 4: Electrophoresis sodium dodecyl sulfate-polyacrylamide gel electrophoresis profiles of amaranth proteins obtained at pH 8 with different precipitation pHs using NaCl 0.1 M as solvent, extracted under reducing condition (sodium dodecyl sulfate+2-β-mercaptoethanol). MW: Molecular weight marker

using isoelectric precipitation at different pHs. It is possible to obtain amaranth protein isolate in extreme conditions with extraction pH 12. The isolate yield is higher using water as solvent rather than using salt as solvent because after dialysis the isolate protein yield is low. Amaranth is a good candidate for supplementation of food protein or substitution of common cereal grains and can be a source of bioactive components.

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