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Research Article

ANALYSIS OF PROTEIN ISOLATE FROM QUINOA (*CHENOPODIUM QUINOA* WILLD)

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ABSTRACT

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

Methods: Quinoa protein isolates were obtained using isoelectric precipitation method at different pHs. Proteins were analyzed using electrophoresis native-polyacrylamide gel electrophoresis (PAGE) and sodium dodecyl sulfate - PAGE.

Results: A yield of 6.29% of protein isolate of defatted quinoa at pH 4.0 was obtained. The content of protein isolate was higher than 64% in all pH assays. Globulins and albumins in protein isolate at different pHs were observed. One band near 130 kDa was found. A band with MW 60 kDa corresponding to 7S globulin was found. The bands, MW 33-36 kDa and MW 20-22 kDa, correspond to 11S globulin. Bands less to 15.4 kDa correspond to albumins.

Conclusions: Quinoa is a good source of proteins. Globulins and albumins were identified in the quinoa protein isolate.

Keywords: Quinoa, Globulins, Albumins, Polypeptides, Protein isolate.

INTRODUCTION

Quinoa (*Chenopodium quinoa* Willd) is a pseudo-cereal native of the Andean regions of South America and belongs to the family Chenopodiaceae [1,2]. It was the main crop of the Incas, a cereal-like crop with high yield seed. Quinoa has been selected by FAO, 2014 as one of the crops destined to offer food security in the 21st century, quinoa plants are tolerant to salinity and drought stress, and able to grow in marginal regions [3]. The seed protein content is high (about 12-15%), and its essential amino acid balance is excellent due to a wider amino acid spectrum than cereals and legumes, with higher lysine (5.1-6.4%) and methionine (0.4-1.0%) contents. The use of protein isolate has increased in the food industry because of different factors such as higher protein level, 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 quinoa using alkaline pH at different pHs of precipitation and to analyze these proteins with electrophoresis sodium dodecyl sulfate - polyacrylamide gel electrophoresis (SDS-PAGE).

METHODS

Quinoa flour and proximate analysis

Quinoa 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 (2012) [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 quinoa

Quinoa isolate was prepared according to Martinez and Añón (1996) [7] 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 one hour and then centrifuged at 4,500 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 4,500 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].

SDS-PAGE

Native-PAGE and SDS-PAGE electrophoresis of quinoa protein isolate were carried out according to the method proposed by Laemmli (1970) [9] 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 hours. 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).

RESULTS

Composition analysis

Table 1 shows the approximate composition of defatted quinoa flour obtained with water. The protein content was 13%; this result is in accordance with other authors [4,10,11]. Table 2 shows the protein yields from quinoa protein isolate. At pH 4.0, the protein yield obtained was the highest with 6.29% of protein isolate. The content of protein increased from 13% in the quinoa flour to 84.32% in the proteins isolate at pH 2.0. In all pHs, the content of proteins was higher than 60% (Table 2).

Effect of pH on the extraction of quinoa proteins

Quinoa seed shows proteins fractions of globulins and albumins as storage protein. Albumins and globulins are the highest protein fractions (44-77% of total, respectively) while the percentage of prolamins is low (0.5-7.0%) [12]. Globulins have two groups depending on its sedimentation coefficient: 11-12 S and 7-8 S. Quinoa storage protein predominant are globulins 11S and 7S. Recently, globulin 11S from quinoa has been named Chenopodin. This protein has two

Table 1: Proximate analysis of DQF

%	Protein	Fat	Moisture	Total fiber	Soluble solids	Carbohydrates
DQF	13.0±0.1	4.99±0.01	9.05±0.03	1.01±0.1	2.09±0.03	69.9±0.4

DQF: Defatted quinoa flour, SD: Standard deviation, results represent the average of three determinations±SD

Table 2: Content of quinoa protein isolate obtained at different pHs

Sample	pH 2.0	pH 3.0	pH 4.0	pH 5.0	pH 6.0
% isolate	3.37±0.10	3.82±0.04	6.29±0.01	5.66±0.01	3.93±0.07
% protein	84.32±2.5	82.76±0.07	65.01±0.04	73.65±1.09	64.78±0.55

Values are expressed in grams per 100 g of protein. Values are means±SD of three determinations, SD: Standard deviation

subunits consisting of an acid polypeptide (AS) (32-39 kDa) and a basic polypeptide (AB) (22-23 kDa). The 2S albumin has been described as a band of low MW near 6-8 kDa [5,13,14].

Electrophoresis pattern

Quinoa isolate proteins were compared using electrophoresis Native-PAGE, and six similar protein profile (Fig. 1) were found by native-PAGE in all pHs assays with high expression at pH 4, 5, and 6.

SDS-PAGE

Electrophoresis SDS-PAGE at reduced and non-reduced conditions of quinoa-extracted protein in water at different pHs are shown in Fig. 2. Proteins mass was determined with software Quantity one of Chemidoc™ PM (Bio-Rad). In the presence of 2-β-Mercaptoethanol, proteins with high MW MW130 kDa were not found in all pHs, whereas proteins with 60 kDa corresponding to 7S globulin according to Abugoch *et al.* (2008) [5] were found in all pHs. Proteins with MW 33-36 kDa correspond to 11S AS were found in all pHs with high expression. On the other hand, proteins with MW 20-22 kDa corresponding to 11S AB were found in all pH values but with higher expression in pHs 4, 5, and 6. Proteins with 20-36 kDa correspond to Chenopodin according to Abugoch *et al.* (2008) [5]. All proteins bands <14.4 kDa corresponding to albumin components according to Brinegar *et al*. (1996) [14] were found in high expressions in pHs 5 and 6.

SDS-PAGE without 2-β-Mercaptoethanol present similar profile of proteins at all pHs assays, the band with MW 50 kDa was found in all pHs with high expression. Proteins between 28 kDa and 36 kDa have high expression in all pHs (Fig. 3).

DISCUSSION

Srivastava *et al*., 2013 [15] indicated the composition and degree of unfolding of protein isolates are regulated by specific or selecting different combinations of extraction and precipitation pH. We observed this comportment in the quinoa proteins. It knows that two of the major type of storage proteins in legume and some no legume seeds are 7S and 11S based on its sedimentation coefficients. Quinoa seeds, due to its high protein content, are actually the subject of many investigations as a potential food source and functional food [16].

CONCLUSION

The content of proteins was higher than 64% in all pHs assays. Albumins and globulins were identified in quinoa protein isolates using isoelectric precipitation at different pHs. Quinoa 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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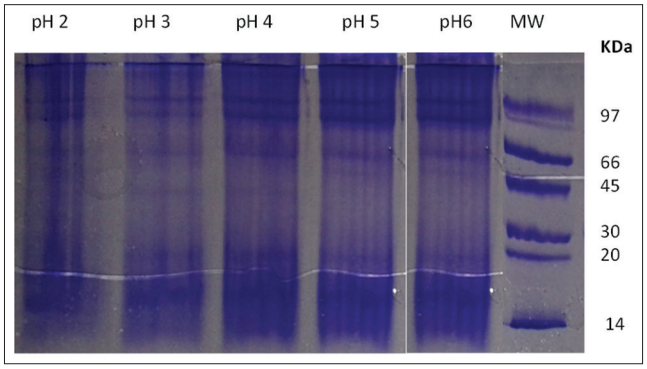

Fig. 1: Electrophoresis native-polyacrylamide gel electrophoresis profiles of quinoa proteins obtained at different pHs molecular weight marker

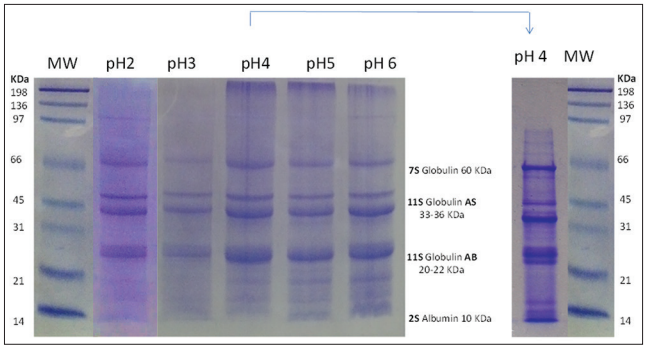

Fig. 2: Electrophoresis sodium dodecyl sulfate - polyacrylamide gel electrophoresis SDS-PAGE profiles of quinoa proteins obtained at different pHs of precipitation extracted under reducing condition (SDS+ 2-β-Mercaptoethanol) molecular weight marker

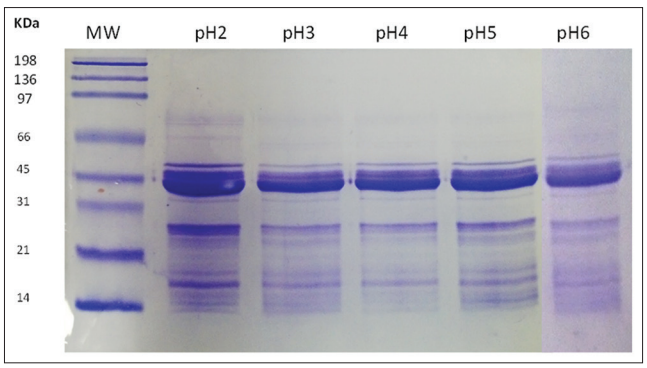

Fig. 3: Electrophoresis sodium dodecyl sulfate - polyacrylamide gel electrophoresis profiles of quinoa proteins obtained at different pHs of precipitation extracted without reducing conditions, molecular weight marker

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