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Research Article

PROTEOMIC ANALYSIS OF DIFFERENT EXTRACTS FROM AMARANTH (AMARANTHUS TRICOLOR) GRAINS.

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ABSTRACT

Amaranth seed has a high nutritional value due to the balanced composition of its protein fractions (i.e albumins, globulins, prolamins and glutelins). These were isolated by sequential extractions. The amount of albumins, globulins, prolamins and glutelins were 2.90, 2.20, 0.47 and 5.73 g/100g of seed flour. Upon electrophoresis, albumins were found to be most polymorphic, while prolamins were made up of fewer and less abundant components. Gel electrophoresis analysis revealed the occurrence of glutelins as the major proteins in the seed. Amaranth protein isolates were prepared by (a) extraction at different alkaline pHs and precipitation at pH 5 and (b) extraction at pH 9 and precipitation at different pHs. The isolates were compared with protein fractions. Acidification at pH 5 and precipitation at lower pHs lead to denaturation of the proteins which was partially reversed by returning to pH 7.

Keywords: Proteomics, grain protein extraction, pHs, protein pattern.

INTRODUCTION

Amaranthus species have different centers of domestication and origin, being widely distributed in N. America, Central America and S. American Andes. It is estimated that there are 87 species of *Amaranthus* [1]. Some species are cosmopolitan, being both introduced and naturalized plants, with a weed-like behaviour, such as *A. retroflexus*, *A. hybridus*, *A. powellii*, and *A. viridis* [2-4] Amongst the cultivated species, *A. cruentus, A. hypochondriacus* and *A. caudatus* stand out and considered as pseudocereals, with high seed protein content and balanced amino acid composition [5]. The amaranth protein have good digestibility and composed of albumins, globulins and glutelins in similar proportions and prolamin in minor amount [6 and 7]. Although the properties of amaranth protein isolates and concentrates were studied by several researchers [8-9], but very little data are available in the literature on functional properties of different fractions of *A. tricolor*. The aim of research presented in this paper was to study the functional properties of different amaranth protein preparations.

MATERIAL AND METHODS

Protein extraction

The seeds of *Amaranthus tricolor,* were collected and grounded for flour. The defatted flour was separated by centrifugation and then air dried for 2 days at room temperature and finally stored at 4° C until used. Total protein was estimated according to the Lowry's method [10].

Sequential extraction was performed according to the method of Sammour [11] with minor modifications. *Amaranthus* flour (1 g) was extracted consecutively in four different solvents. First, the flour sample was extracted with distilled water (10ml, w/v). The suspension was stirred at room temperature for 20 minutes and then centrifuged at 8,000 rpm for 20 minutes. The supernatant was used as the extract 1(Albumin fraction). The remaining insoluble sample was mixed with aqueous 5% (w/v) NaCl (10ml) solution, with the repetition of extraction procedure and the extract 2 was collected as globulin fraction. Subsequently extractions were followed with aqueous 70% (v/v) ethanol and aqueous 0.2% NaOH solution, the extract 3 as prolamin fraction and the extract 4 as glutelin fraction were obtained.

Extraction at different pH and precipitation at pH 5

The flour was suspended in water (10% w/v) and proteins were extracted at pH 8, 9,10, and 11 from the suspension and each pH were adjusted by adding 0.5 N NaOH. The suspensions were shaken for 30 minutes at room temperature and then centrifuged at 9,000 rpm for 20 minutes. The supernatants were adjusted to pH 5 with 1 N HCl and again centrifuged at 9,000 rpm for 20 minutes at 4°C. The precipitates were resuspended in water, neutralized with 0.1 N NaOH and freeze dried. Although four isolates obtained were termed as P8, P9, P10 and P11 according to the extraction pH.

Extraction at pH 9 and precipitation at different pH

The flour was suspended in water (10% w/v) and proteins were extracted at pH 9 according to the above described method. The supernatants of the extraction were adjusted to pH 4, 5, 6 and 7 with 1 N HCl. The precipitates were again resuspended in water, neutralized with 0.1 N NaOH and freeze dried. The freeze dried precipitates were termed as P4, P5, P6 and P7.

Electrophoresis

All gels were run in minislabs; (Bangalore Genei Vertical mini gel system). SDS-PAGE was carried out according to the method of Laemmli [12]. The runs were carried out in the following discontinuous buffer system: 0.5 M Tris-HCl pH 6.8 (4x stacking gel buffer), 1.5 M Tris-HCl at pH 8.8 (4x separating or resolving gel buffer) and 0.025 M Tris-HCl, 0.192 M glycine/1% (w/v) SDS, pH 8.3 for the running buffer. The protein samples (100 µl) were dissolved in the 100 µl of loading buffer(0.5 M Tris-HCl pH 6.8, 20% (v/v) glycerol, 1% (w/v) SDS/0.05 % (w/v) bromophenol blue and centrifuged at 5,000 rpm for 5 minutes: 25-30 µl/lane supernatants were loaded in the gel. The electrophoretic runs were conducted for about 3-4 hours at a constant voltage of 80 V. The gels were stained with 0.5% R-250 Coomasie Brilliant Blue in water/methanol/acetic acid (4:5:1) for overnight and destained with water:methanol:acetic acid (9:9:1). The molecular weights of polypeptides were calculated by using the protein standard molecular marker.

RESULTS AND DISCUSSION

Total protein in *A. tricolor* was 11.70 g. Grain flour was successfully extracted with distilled water (albumin), NaCl (globulin), ethanol (prolamin) and an alkaline solution (globulin). A protein content of 2.90g/g and 2.20g/g seed flour was found in water and salt extract.

Glutelin content (5.73g) was maximum in amount, in comparison to all the protein fractions.

The fractional composition of amaranth proteins indicates their presence as high digestible proteins. Albumins and globulins comprise approximately 50% of total seed proteins where as prolamins present in 4-5% of total seed protein. The major part was alkali soluble glutelin about 50%, meaning thereby the percentage of nutritive value is very close to sum of albumins and globulins. The amarantha proteins extracted were initially analyzed by SDS-PAGE and compared to protein fractions obtained as described earlier. In SDS-PAGE, the amaranth seed flour protein extracted with distilled water (albumin fraction) showed the dominant bands of low molecular weights of ≤32kDa (Figure 1, Lane 1) and some components of intermediate molecular mass between 43-70 kDa. In globulins (Figure 1, Lane 2), the lane includes some intermediate components of low molecular mass of below 29±1 kDa and a band of 54±1 kDa along with a high molecular mass 70 and 80 kDa and indicated the possibility of the presence of different units of globulin. In prolamins (Figure 1, Lane 3) the most dominant band was about 54±1 kDa and the precipitation of a secondary band of higher molecular mass of about 85 kDa. The distribution of polypeptides of glutelin (Figure 1, Lane 4) showed similar to that of combination of globulin and prolamin. One prominent band of 55±1 kDa and of intermediate polypeptide mass of 70 and 80 kDa along with an abundant protein band of about 85 kDa was observed. In this circumstance it can be assumed that the fractions were extracted at non extreme conditions; therefore possibly, it could have been considerably contaminated with globulins and albumins.

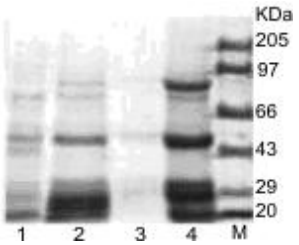

Figure 1:SDS-PAGE of different fractions of amaranth protein: Lane 1 Albumin, Lane 2 Globulin, Lane 3 Prolamin, Lane 4 Glutelin, Lane M standard molecular weight proteins.

Proteins extracted at pH 8,9,10 & 11 and precipitated at pH 5

Protein isolates obtained at different pHs and precipitated at single pH 5. These isolates referred as T8 (Lane 1), T9 (Lane 2), T10 (Lane 3) and T11 (Lane 4) (Figure 2). Total protein concentration ranged between 0.99-2.34 g/100g of seed flour. The variation in protein amount depends on the extraction pH (8-11). The isolates obtained by different pHs were characterized by SDS-PAGE and the results showed that the protein extracted at pH 8, contains high proportion of albumins (low molecular mass peptides 32, 29 and 20 KDa). The electrophoretic pattern of other isolates T9, T10 and T11 indicated that all isolates shared the polypeptide of the intermediate bands of 20±1 KDa, 29±1 KDa, 32±1 KDa, 34 KDa and 85 KDa respectively (Figure 2, Lane 2,3,4). In our study, the observation of high molecular mass peptide of about 80±1 KDa and 85 KDa at pH 9, 10 and 11 indicated the pattern of globulin, glutelin and prolamin. These results evidenced that all are extracted at pH greater than 8 and supported the findings of Martinez and Anon [8].

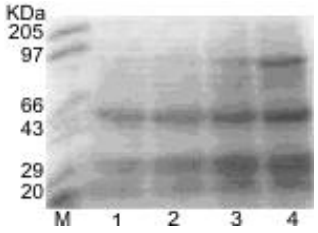

Figure 2:SDS-PAGE of different protein isolates obtained by extraction at different pH and precipitation at pH 5.

Proteins extracted at pH 9 and precipitated at pHs 4.5.6 and 7

Protein isolates obtained at pHs 4,5,6 and 7 were referred as T4 (Lane 1), T5 (Lane 2), T6 (Lane 3) and T7 (Lane 4) (Figure 3). Amount of proteins extracted at above pH showed the range of 1.20-1.96g. The protein yield was significantly higher at pH 4-6 than pH 7 (Figure 3). The electrophoretic profile of protein isolates showed the presence of polypeptide mass 29-32 KDa, 54±1 KDa and 83±1 KDa (Figure 3 Lane 1,2,3). Lane 4 exhibited dominance of high molecular mass peptides of 55±1 KDa.

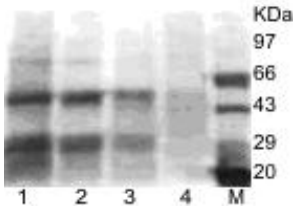

Figure 3: SDS-PAGE of different protein isolates obtained by extraction at pH 9 and precipitation at different pH.

On the basis of above result it seems that the increase of the extraction pH induced the conformational changes in the proteins and precipitation pH affects the composition of protein isolates [8] and the composition of protein isolates could be modified by varying the precipitation pH. As the result showed that precipitation of globulin peptides and some lower molecular mass polypeptides precipitated at pH 6 and 7 and glutelin polypeptides are hardly detected. This has been evidenced that acidification at pH 5 and lower, also causes conformational changes in the protein composition but partially reversed when the pH was higher upto pH 7. The result concludes that composition and structural characteristics of amarantha proteins depend on extraction pH as it is evidenced that albumin and globulin are mostly extracted at pH 8, whereas at pH above 8, pattern of albumin, globulin, glutelin and prolamins were appeared in electrophoretic analysis.

CONCLUSION

According to the results, it could be interpreted that the composition and degree of unfolding of protein isolates are regulated by specific or selecting different combinations of extraction and precipitation pH. The results showed that albumin and globulin are also dominant subunits of main protein. The presence of albumin in all the fractions suggests that the conformation of albumin is stabilized by higher bonding energy similar to that of other plant proteins. All the electrophoretic patterns of proteins were almost similar in all species. It means the pattern of protein isolates are almost similar in expression among the species but they showed variation in content of protein fraction and proteins extracted and precipitated at different pH.

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