

# Characterization of Seed Storage Protein Pattern of *Amaranthus viridis*: SDS-PAGE Study



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## Abstract

Use of amaranth seed flour in production of functional food, rich in health beneficial components, the study of grain proteins content, their structure and quality are important. Thus, proteins are not only important in the human body but are also widely used in the industry. Proteins from *A. viridis* (AV) were extracted according to the Sammour method. Various extracting agents were tested for protein solubilization. It was found that the best agent for extraction of albumins and globulins was water and NaCl respectively and for prolamins and glutelin was 70% ethanol and 0.2% NaOH solution respectively. The amount of albumin, globulin, prolamin and glutelin were 3.61, 3.56, 0.64 and 6.62 g 100g-1 seed flour.

**Keywords:** Amaranthus Viridis; Protein Extraction; Different Seed Proteins; Protein Pattern

**Abbreviations:** SDS-PAGE: Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis; AV: Amaranthus viridis

## Introduction

*Amaranthus viridis* (AV) belongs to Amaranthaceae family. AV is commonly known as green amaranth or karund, is a common gene, found in many parts of the world. Greek people called it as "never fading flower". In many parts of the world, *Amaranthus* has been rediscovered as leafy vegetables, cereals etc. [1,2]. It is characterized by a high degree of diversity and a wide spectrum of adaptability to different agroecological conditions [3]. Being very cheap and widely distributed Amaranth vegetable is very popular in rural areas because it is very nutritious and provides essential nutrition to them and also prevention of diseases associated with nutritional deficiencies, such as blindness due to vitamin A deficiency [4]. *Amaranthus* has received considerable attention in many countries because of the high nutritional value of some species [5]. The protein of AV includes albumins, globulins, prolamins and glutelins; are of good digestibility nature [6,7]. *Amaranthus* species have different centers of domestication and origin, being widely distributed in North America, Central America and South American Andes, where the greatest genetic diversity is found [8,9]. It is estimated that there are 87 species of *Amaranthus*, 17 in Europe, 14 in Australia and 56 in America [10].

Proteins of amaranth have better balanced amino acid composition and thus higher biological values than those of most cere

als. They can safely be consumed by people suffering from celiac disease and more sustainable than those from animal sources [11-14]. There is currently much interest in the use of pseudo cereals for developing nutritious food products. They contain high levels of starch, protein, dietary fibers, minerals, vitamins and other bio-active. Their proteins have well balanced amino acid composition. Pseudo cereal protein mainly consists of albumins and globulins, the predominant cereal proteins are prolamins and glutelin's [15]. *Amaranthus* is classified as pseudo cereals which in turn good food crop too because it has exceptionally good nutritive values owing to its protein, lipids and essential amino acids like lysine contents [16]. Understanding of the function of biological systems requires knowledge of their chemical composition. The state of life of a cell at any given time is defined by its protein composition, i.e. its proteome.

That is why it is necessary to have a rapid and an efficient tool for characterization to the proteome [17]. There are several ways of protein identification, however majority of them are very time-consuming procedures. Separation techniques became important tools for proteins and proteome analysis [18]. These methods have been successfully applied for the identification of proteins in numerous biological system [19,20]. The principal energy sources within the

grains are protected from infestation by outer coverings. The largest morphological component of all grains is the endosperm and approximately 80% of this is starch. Other important compound classes are the storage proteins, which makes the next largest contribution to endosperm dry weight. Proteins are important both as nutrients and as active chemicals [17].

There are four categories of proteins occurring in seeds:

- a. Albumins- soluble in water and comprise mostly enzymatic proteins.
- b. Globulins- soluble in dilute salt solutions and occur in protein bodies.
- c. Prolamins- soluble in aqueous ethanol solution and are also found in protein bodies as true storage proteins.
- d. Glutelin's- soluble in alkaline or acid solutions or in detergents and are probably mainly structural proteins. Thus, the purpose of this study was to determine the amounts of albumins, globulins, prolamins and glutelin present in the proteins of AV grains.

## Materials & Methods

### Protein Extraction

The seeds of AV were collected and grounded for flour. The de-fatted 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 [21]. Sequential extraction was performed according to the method of Sammour [22] with minor modifications. Amaranthus flour (1g) 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.

### Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

All gels were run in minilabs; (Bangalore Genie Vertical mini gel system). SDS-PAGE was carried out according to the method of Laemmle [23]. 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 Coomassie Brilliant Blue in water/methanol/acetic acid (4:5:1) for overnight and detained with water: methanol: acetic acid (9:9:1). The molecular weights of polypeptides were calculated by using the protein standard molecular marker.

## Results & Discussion

Amaranth seeds flour was successively extracted with distilled water (albumin), NaCl (glutelin), ethanol (prolamin) and an alkaline solution (glutelin). The concentration of proteins extract was determined by the method of Lowry et al. [21]. According to the results, the amount of total protein was 14.9% of seed flour and the content of albumin, globulin, prolamin and glutelin were 3.61, 3.56, 0.64 and 6.62 g 100g-1 seed flour respectively. The content of glutelin was highest in comparison to all the protein fractions and it was approximately 10 folds higher than prolamin, which was lowest in total protein. In SDS-PAGE, the amaranth seed flour protein extracted with distilled water (albumin), showed the dominant bands of low molecular weight of <32KDa (Figure 1 Lane 1) and some components of intermediate molecular mass between 43-70KDa in agreement with our previous studies [5,7]. 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 like that of combination of globulin and prolamin.

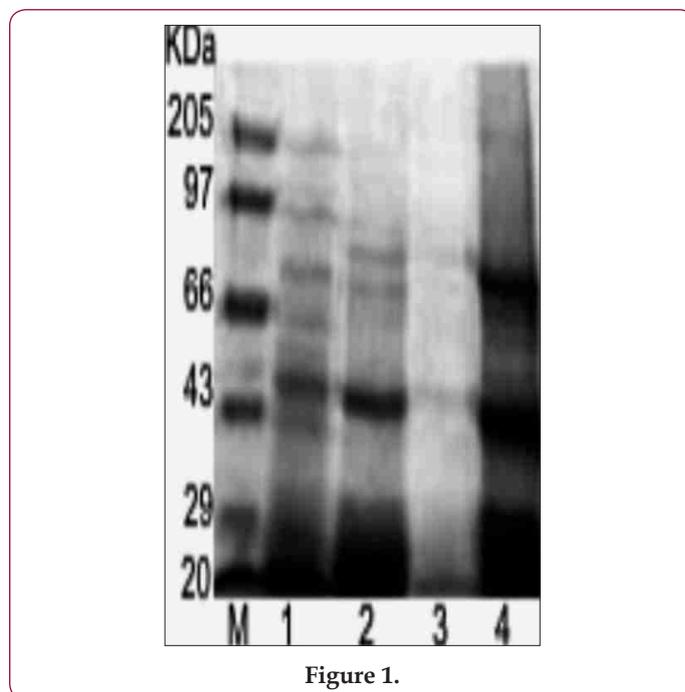


Figure 1.

## Conclusion

Storage proteins accounts for about 50% of the total protein in mature cereal grains and have important impact on their nutritional quality for humans and livestock and on their functional properties in food processing [24]. The storage proteins of grains

are of immense importance in determining the quality and end use properties of the grain. Understanding the structures of these proteins is important to improve the quality of grains. Though plant has been extensively studied for its nutritional properties, but more experimental and clinical trials are warranted.

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