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# Effect of Processing on Structure and Morphology of Amaranth Starch

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**ABSTRACT**

The structure of Raw and Processed Amaranth Grains starch was studied by using Scanning Electron Microscopy (SEM). In this study, starch morphological differences were observed between raw and processed (Boiled, Roasted and popped) grains derived from the same cultivar. Among food carbohydrates, starch occupies a unique position. It is the major carbohydrate storage material in many higher plants and is considered the second largest natural biopolymer next to cellulose. Starch contributes to the physicochemical properties of food products made from cereals, tubers, roots, legumes, and fruits. It is the basic source of energy for the majority of the world's population. In human nutrition, starch plays a major part in supplying the metabolic energy that enables the body to perform its different functions. In this study SEM revealed that starch granules of grains were much smaller in size that many of them were not separated from amyloplasts. Cooked grains have wider, more defined cracks, suggesting that they serve as channels for water migration into the grain during cooking. Water penetrates unequally into the grain during cooking, low water penetration produces dense regions with minimal starch gelatinization, and high water penetration produces large voided areas. The voids occur in the transverse orientation of the grain and are the main of cause grain expansion during cooking than the other processing such as roasting and popping. But the structural changes at micro and nano-scales can be dramatic, as revealed by scanning electron microscopy. Especially when water traces are present, a very rich network of polymer chains can be observed, remaining after the granule structure collapses. This is considered a suitable structural basis for different new products were interactions could be modeled, such as carriers for nutritional interesting substances.
**KEY WORDS:** Amaranth grain, starch, Scanning Electron Microscopy, processing, boiling, roasting.

## INTRODUCTION

Grain amaranth belongs to a group of cereal-like grain crops or pseudocereals. According to the definition proposed by Shewry (2002), pseudocereals are dicotyledonous species which are not closely related to each other or to the monocotyledonous true cereals. Amaranth grain has significant nutritional value. Its protein, mineral meters, fat and cellulose percentage are higher compared to cereals (Saunders and Becker, 1984; Bodroza Solarov, 2001). Since this plant has similar application as cereals, it is classified as pseudocereal (BodrozaSolarov, 2001). In recent years, beans has received attention because of having positive effects on the physiological function of the gastrointestinal tract (Pirman *et al.,* 2001).

*Amaranthuscruentus* grains contain 62 % of starch and about 14—16 % of proteins it is used as an ingredient in some foods such as puddings, soups, salad dressings. The nutrient composition of amaranth grains — total protein, its amino acids, as well as minerals and vitamins, has been comparable or even better than that of common cereal grains (Becker *et al.,* 1981). There are only a few reports on isolation and some of the properties of starch from amaranthus grains, therefore, as a part of our studies on alternative starch sources, investigation on the physicochemical and pasting properties and amylolytic susceptibility monitoring the structure of branching part of starch seemed to be worthwhile.

Starch is an important industrial raw material for food products as well as for technical products. Corn starch is relatively easily isolated by wet-milling procedure. Amaranth starch is interesting because of its small granular size, 1–2 μm, which provides specific functional properties, as good freeze–thaw stability and resistance to mechanical shear. Amaranth starch content is approximately 63%, while protein content is 15%. The starch has „waxy" characteristics and specific molecular composition, while proteins have high quality and major content of S–amino acids and lysine (Asp *et al.,* 1989).

The starch swelling and its property of forming gel depends upon it source and variety from where it is derived because the amylose content and molecular weight of amylose differ as with the starch origin. The difference in granule structure results in different degrees of amylase leaching. For example, tuber starches form

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gels much more slowly than cereal starches at similar conditions (Svegmark K, 1993). The starch granules hydration results in its swelling, and rupture to a great extent, the starch paste loses its viscosity and produces a weak bodied, stringy, and cohesive paste. For some starches, the paste may remain fluid or form a semi-solidor solid gel demonstrating considerable strength (Qiang Liu, 2005).

Starch is widely used as a functional ingredient in a variety of food products such as sauces, puddings, confectionery, comminuted meat, fish products, and low fat dairy products. In order to meet the requirement of some food products, starch is chemically and/or biotechnologically modified as an ingredient (Qiang Liu, 2005).

In a food system, the roles of starch are to stabilize the structure and interact with other components to deliver or maintain nutrient and flavor. For example, starch can serve as thickening agents for sauces, cream soups, and pie fillings; colloidal stabilizers for salad dressing; moisture retention for cake toppings; gel-forming agents for gum confections; binders for wafers and ice cream cones; and coating and glazing agents for nut meats and candies. The properties of foods are not only governed by the starch and its molecular structure. Processing conditions and the amount of water available are crucial determinants of the final characteristics of the product.

The scanning electron microscope (SEM) is one of the most versatile instruments for determining the microstructure morphology. A scanning electron microscope was used for steam flaked, popped and micronized sorghum grain starch granules to observe the structural changes and hydrolytic patterns (Harbers, 2013).

In a scanning electron microscope a beam of electrons interacts with the sample in a number of ways. The generation of secondary electrons is highly dependent on topography and as such these can be used to image the morphology of the sample. Backscattered electrons can give additional information regarding atomic mass variations of the sample and the energy of the X-rays that are generated when an electron beam hits a solid specimen can be used to determine what elements are present (this is known as Energy Dispersive X-ray Spectroscopy) (Bryony James, 2005).

EDS is not a surface analysis technique. It doesn't take much magnification in the SEM to reach the point where the pixel size on the specimen approaches this dimension (Bob Hafner, 2008).EDS is a technique that may be described as semi-quantitative. The intensity of each peak in the spectrum is related to the quantity of material present. When compared to a standard sample then the technique is fully quantitative (Bryony James, 2005).

## MATERIALS AND METHODS

**Sourcing and processing of amaranth seed:**

Mature seeds of *Amaranthuscruentus*, were used. Sample was obtained from Sree Krishna agro foods shop, salem, tamilnadu. The preliminary cleaning works on grain was done in a systematic manner and dried grains were divided into four batches and processed under the following procedure.

**Preparation of processed samples**

**Raw Amaranth Grains**

Raw grains were cleaned and washed with distilled water and dried under sunlight. After drying the grains were stored were stored for further processing.

**Boiled Amaranth Grains**

The selected amaranth grains were boiled as per the procedure given by Hefnawy, 2011. The procedure is as follows, poured three cups water, one cup of amaranth grains and ½ tsp. of salt in the saucepan and mixed them together and the mixture heated up to the boiling point (100 °C). Once the boiling point is reached, the stove turned into 'low'. The mixture further heated for 25 minutes with lid placed on the saucepan. Turned off the stove and allow saucepan sitting for 10 more minutes. Dry the cooked grains in the sun light using a white muslin cloth.

**Roasted Amaranth Grains**

The selected amaranth grains were roasted as per the procedure given by Nithya *et al* ; 2007. The grains were placed on a hot plate at 120°C for about 5 min to prepare roasted grains, stirring and checking was done often to prevent from charring. Grains were transferred from hot plate when they are lightly browned and crispy.

**Popped Amaranth Grains**

Amaranth grains were heated on a hot plate at 180°C for about 10 seconds until they popped as per the procedure outline by (Hefnawy, 2011). Keep stirring the grains with a spoon to prevent burning. As soon as popping stopped, empty the pan, cool and store it.

The raw and processed grains were packaged in zip-lock polyethylene sample bags and stored at 4°C until needed for analysis.

**Morphology and Particle Size Distribution of Starch Granules**

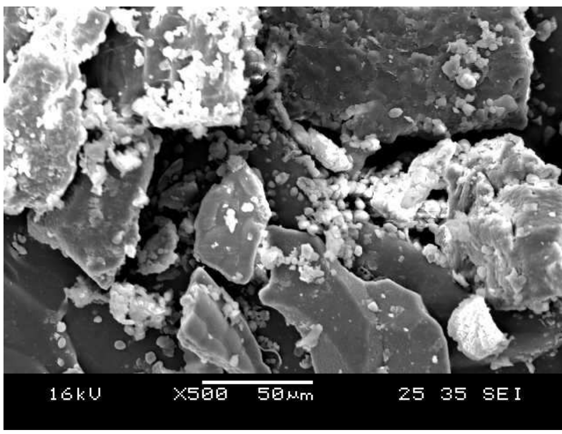
The representative sample was prepared using epoxy resins, polished and made conductive by carbon coating in a Dentom vacuum, DV-502A. The morphology of the processed and raw grain flour was analyzed in a JEOL JSM-6400 scanning electron microscope at accelerating voltage of 20KVA, real time of 21-36 and live time of 60 seconds in various magnifications from 500 to 2000 x. It was configured with an ultra-thin window Energy Dispersive X-ray Spectrometry, three WDS spectrometers and a Geller dSpec automation system controlling the spectrometers and motorized stage. Images were made using the back scattering electron detectors. The chemical elements of the sample were determined by the EDS.

## RESULT AND DISCUSSION

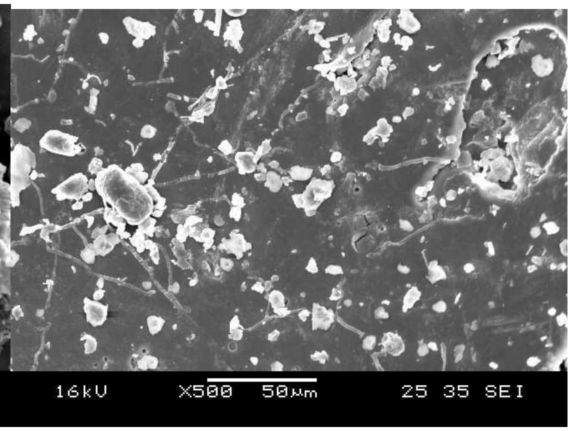
**Morphology, Size Distribution, and EDS of Amaranth Starch Granules**

**Microstructure of Processed seeds by SEM**

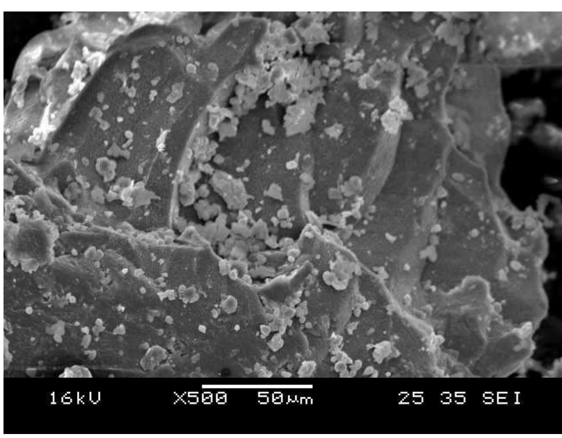
Distribution of particles of amaranth starch accordingto their size and shape of granule is illustrated from Fig. 1 to 4. Microscopic granules of Starch grains in plants store food and energy. They act as a storage organ, such as roots, tubers and seeds, but can also be found in smaller quantities in leaves and other plant parts (Haslam, 2004).



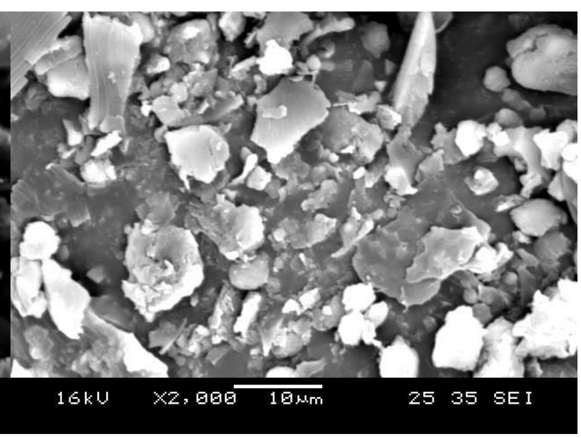

*Fig, 1- Microstructure of Raw seed*




*Fig, 2- Microstructure of Boiled seed*




*Fig, 3- Microstructure of Roasted seed*




*Fig, 4- Microstructure of Popped seed*

The SEM images indicate structural changes between Raw, Boiled, Roasted and Popped grains. This method analyzed the quantify fracture pattern and microstructural heat damage through image analysis was proposed. Furthermore, the result of boiled seed shows that the particles were clustered together with smaller air cell diameters in the way of shapeless or mud-like structure. In this sample starch grains are distributed evenly over the entire area and it had irregular-shape with relatively smooth surfaces. According to Ljubica P. *et al.,* (2009) amaranth has reduced expansion properties compared to corn. The Reduced expansion resulted in a denser product with smaller air cell diameters. It is difficult to produce expanded products by extrusion cooking of amaranth grain alone, due to its high fat content (6-8% in whole grain). Fat provides a powerful lubricant effect in extrusion cooking and reduced product expansion. Highest expansion ratio provides crispy structure during eating (Ilo S, 1999). The increase in size of the starch granules of cooked rice is due to water absorption. The swelling and gelatinization, results in a "melted" appearance of grain periphery. Two types of structures are evident in the cooked grains: one where the starchy endosperm is intact and the other containing voids in the central endosperm (Yukiharu Ogawa. *et al.,* 2005). The voids are responsible for the deformation and swelling of the grain during cooking and are probably the result of rapid pressure buildup (steaming) and subsequent expansion or localized explosion within the grain. Voids or "internal hollows" in cooked rice grains were also reported by Horigane *et al.,* (1999 &2000). The starch gelatinization increased, resulting in swelling and melting which led to strong deformation of the granules and ultimately the molten granules connected with one another given an internal hollow structure to the grain.

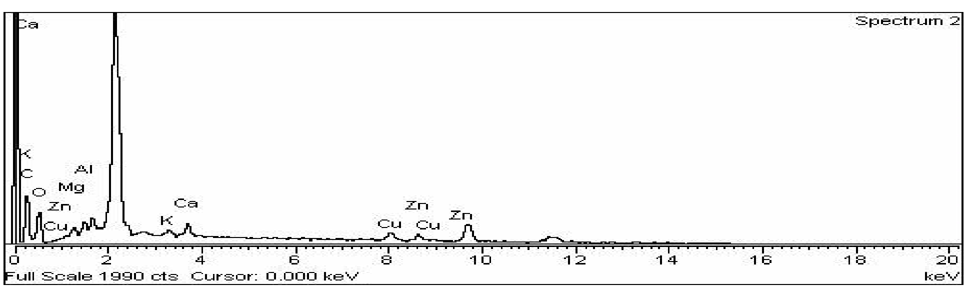
Presence of the spherical grains in the microstructure image of the samples is shown in raw and roasted grains. However, the starch grains are spread evenly in the raw grain and it is clearly visible. The image of popped grain sample, which is present in anomalous/agglomerate form. This is indicating that the degree of crosslinking of the polymer binder was low (yongliu *et al.,* 2010). The starch grain possesses different shapes (such as small, large and tunnel, etc.) of particles and had much more compacted appearance in this sample. Popped starch granules expanded from the hilus to the edge and formed large, thin sheets that fused with adjacent granules (Harbers LH, 2000). The SEM images of samples popped at in the temperature of 180°. The SEM provides a clear view of changes of intact starch granules in popped samples. When the sample processed for a short time e.g. 20 min, the lentil-shaped and circular starch granules of various sizes, protein matrix, and adhesive protein areas attached to starch granules were clearly observed. However, when processing for a longer period, starch gelatinization increased, resulting in swelling and melting which led to strong deformation of the granules and ultimately the molten granules connected with one another. Other components of the grains such as protein, pentosan and gluten also absorbed water and could be denatured when exposed to heat for a longer period. The combination of these factors resulted in the changes of starch microstructure which were progressively more shapeless or mud-like structure at more than 130-140°C.

The presence of large air spaces between starch granules (Singh *et al.,* 2006) result in significantly different physico-chemical, morphological, thermal, cooking and textural properties

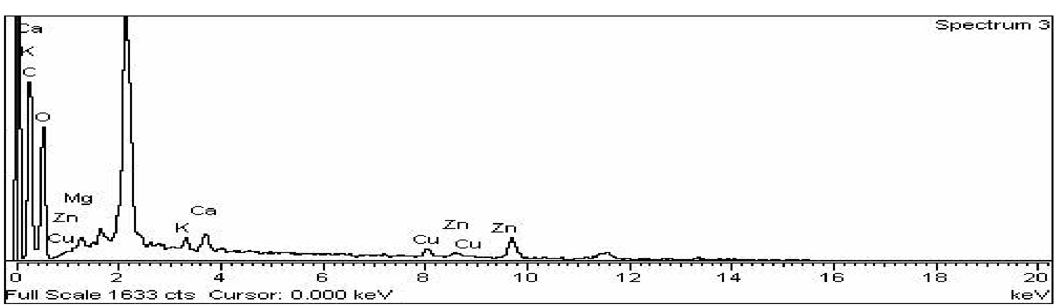
Compared to those of translucent grains (Singh *et al.,* 2003; Cheng *et al.,* 2005).

Under elevated temperatures the initiation of starch granules and the synthesis of endosperm starch did not appear to keep pace with cell division and cell enlargement and the peripheral endosperm cells remained relatively empty of starch granules. This was consistent with the reduction in starch synthesis due to high temperature exposure which has been reported elsewhere (MacLeod and Duffus, 1988; Wallwork et al., 1998a).

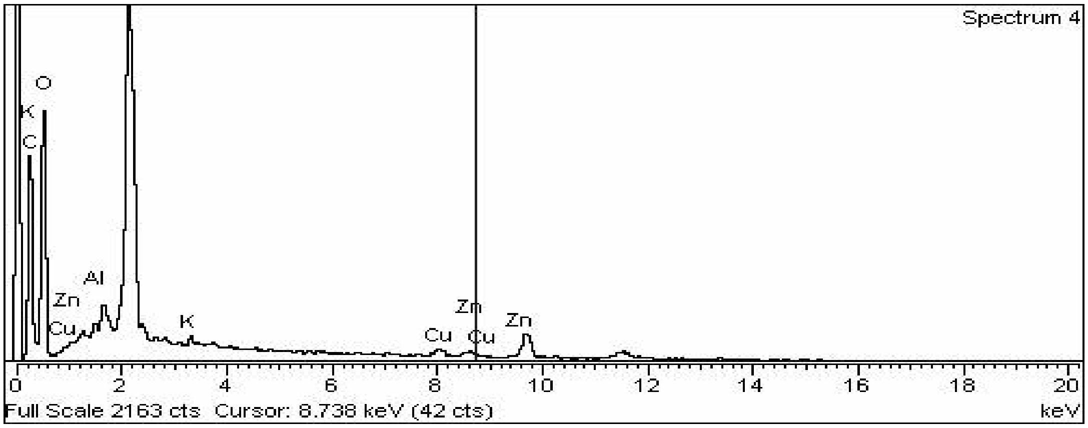
**EDS Sketch of Raw and Processed amaranth grains**



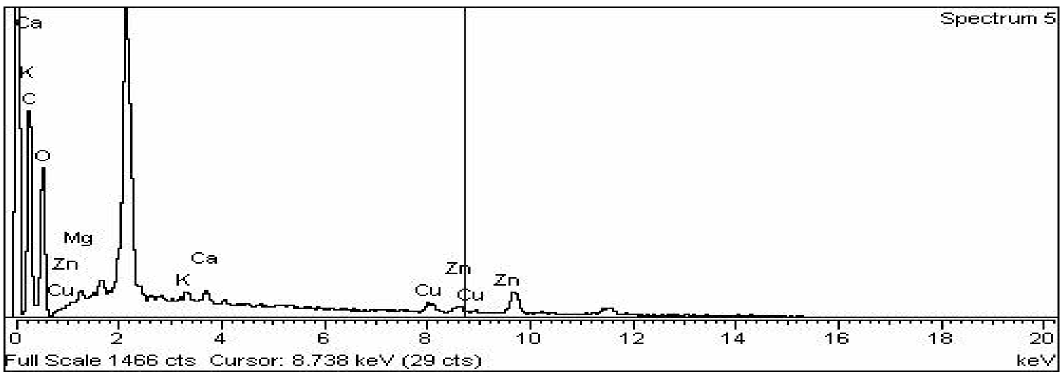

***Fig, 5 - The EDS illustration for raw grains***




***Fig, 6 - The EDS illustration for boiled grains***




***Fig, 7 - The EDS illustration for roasted grains***




***Fig, 8 - The EDS illustration for popped grains***

The elemental composition of the material in the grains was determined using EDS element analysis, enabling the material attributes of the grains and binder to be established. The presence and amount of macro and micro minerals is shown in below table. 1

**Table 1Macro and Micro minerals content in Raw and Processed Grains**

| S.No | Elements | Raw Grain | Roasted Grains | Boiled Grain | Popped Grain |
|---|---|---|---|---|---|
| **1** | C | 11.29 | 11.61 | 4.98 | 9.64 |
| **2** | Mg | 0.20 | ---- | 0.26 | 0.16 |
| **3** | K | 0.24 | 0.14 | 0.13 | 0.14 |
| **4** | Ca | 0.38 | ---- | 0.32 | 0.19 |
| **5** | Cu | 0.93 | 0.91 | 1.13 | 0.81 |
| **6** | Zn | 0.42 | 0.52 | 0.92 | 0.66 |
| **7** | Al | ---- | 0.25 | 0.26 | ---- |
| | **Total** | **13.46** | **13.43** | **8** | **11.6** |

where carbon content is very high (11.61)in roasted grain than the other processing. But when the grain treated with water carbon content (4.98) was reduced. Mg content is increased (0.26) on boiled amaranth grain than the original value whereas popped grains holds only 0.16g of Mg and it is absent in the roasted grain. Potassium content was reduced highly when grains are subjected heat such as roasted and popped grains had got 0.14 and boiled grain had got 0.13 of Mg which is 0.10% different from the native grain (0.24). Calcium content of raw and processed (boiled and popped) grains were 0.38, 0.32 and 0.19 respectively the same mineral was absent in roasted grain. The copper content was varied between the processing raw amaranth grain was hold 0.93, roasted amaranth grain had 0.91, boiled grain had 1.13 of Cu content however popped grains yielded only 0.81% of Cu. The highest Zinc content (0.92%) was obtained by the boiling process and the least was obtained by raw amaranth grain. The last mineral in the above table is Aluminum which was absent in raw and popped amaranth grain whereas in roasted and boiled grain had more or less same valued Aluminum content (0.25 and 0.26 % respectively). Finally raw and roasted amaranth grain flour had 13.46 and 13.43% of total minerals however the loss of minerals were high in boiling process(8%).

**Conclusion**

This study showed that SEM can be used to observe the morphology of starch granules in the whole grain system without the need for starch isolation. For qualitative purposes or product quality control in food manufacturing, the results should be acceptable. The microstructure morphology of Raw and Processed Amaranth grains observed using SEM with EDS analysis. The size distribution range of the grains was wide, from 10µm to about 50µm. The shape of the grains varied widely and their size was predominately less than 10 µm, with a broad distribution. Due to the different type of thermal treatment the starch granules are getting different structures. Starch granules were completely encased in an amorphous protein matrix. The enhancement of the amorphous region in the matrix has also been confirmed from the SEM images. When the grains are treated with heat starch granules embedded in a protein matrix. Heat treatment appeared to reduce starch granule development and resulted in alterations to starch granule. Higher the temperatures tended to increase the starch, resistant starch, amylose and amylase: amylopectin ratio of samples. Although, resistant starch is formed during seed formation, its proportion may increase during processing, especially heating (Brown, 1996) and storage. Different types of starch granules were observed in the different processing. The starch granules of amaranth have big voids in the fresh grains (Taylor & Belton, 2002). In the present study most of the starch granules were squeezed, some of them overlapped each other and the granules were tightly packed in response to artificial drying. The size of the starch granule is an important factor in determining the energy value of grain, starch with smaller granules having a relatively larger surface area and so a greater potential for hydrolysis by endogenous amylase (Carre, 2004). The EDS report explains that when the grains are subjected to thermal treatment the amount of minerals increased also carbon. From the present study it can be concluded that Raw and Processed Amaranth grains is affected by artificial drying and such treatment may change the chemical composition of the grains considerably. The changes in starch composition may significantly affect the nutritive value of the grains.

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