

# Salts and pH Induced Changes in Functional Properties of Amaranth (*Amaranthus tricolor* L.) Seed Meal

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ABSTRACT

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Amaranth meal is a rich source of proteins, carbohydrates, and minerals with a low amount of anti-nutritional factors. It exhibits good functional properties. The effect of NaCl and NaHCO<sub>3</sub> salts and pH level on the functional properties of amaranth meal was studied. The water absorption capacity and protein solubility were improved in the presence of the salts. Protein solubility was high at extreme pH values and minimum at pH 4. Foaming capacity was poor in the presence of the two

salts, while foam stability was better at lower concentrations of NaCl (0.2–0.6M). Changes in pH had a pronounced effect on the foaming properties of amaranth meal. Salts did not change the emulsification properties but significantly increased the relative viscosity of amaranth seed meal at higher concentrations of NaCl and NaHCO<sub>3</sub> (0.6–1.0M). Relative viscosity was highest at pH 10 and lowest at pH 4.

Functionality has been defined as any property of a food ingredient, except its nutritional value, that has a great impact on its utilization. The functional properties directly or indirectly affect the processing applications, food quality, and ultimately their acceptance and utilization in food and food formulations. The use of soybean as a source of nutritious food and substitute for meat is due to the knowledge of the functional properties of its flour and other products (Volkert and Klein 1979).

Amaranth is the world's most nutritious pseudo-cereal grain with multiple uses (Teutonico and Knorr 1985). It is one of those rare plants whose leaves are eaten as a vegetable while the seeds are used as cereals. Amaranth grows vigorously; resists drought, heat, and pests; and adapts readily to new environments, including some that are inhospitable to conventional cereal crops.

Amaranth produces a higher yield of seeds than most common cereals. With a protein content of  $\approx 16\%$ , amaranth seed compares well with the conventional cultivars of wheat (12–14%), rice (7–10%), maize (9–10%), and other widely consumed cereals (Chang and Sosulski 1985). Amaranth proteins have nearly twice the lysine content of wheat protein, three times that of maize, and as much as milk, the standard of nutritional excellence. With so much to its credit, amaranth crops are still playing a secondary role to crops like rice and wheat on our dinner plates. Therefore, the challenge is to incorporate it into existing food formulations to modify the functional and nutritional qualities, and to create new products from it.

A good amount of work has been done on the proximate composition, nutritional value, and genetic potential of this crop (Raina and Datta 1992; Bejosano and Corke 1998), but very little is known about its functional attributes. This investigation deals with the functional properties of amaranth meal in the presence of salts and at various pH levels, to evaluate its utilization in various products. The effect of salts is important because salts are integral constituents of food product formulations; concentrations of 0.2–0.3M are common in most food products. The pH level of the foods changes in the presence of various ingredients added during cooking and thermal processing.

## MATERIALS AND METHODS

Seeds of amaranth (*Amaranthus tricolor* L.) were procured from M/S Durga Seeds, Chandigarh, India. They were crushed and defatted with petroleum ether at 40–60°C four times to remove the fat completely. The chemical composition of the defatted meal was determined.

Moisture and ash contents were calculated by an official method (AOAC 1984), proteins were calculated by Lowry's method (Lowry et al 1951), and carbohydrates were calculated using the procedure of Yemm and Willis (1954). Lipids were extracted with petroleum ether and estimated with sulfophosphovanillin reagent (Frings and Dunn 1970). Phytic acid was calculated by estimating the phosphorus released from the meal (Thompson and Erdman 1982); phenols were measured according to the method of Swain and Hillis (1959).

## Functional Properties

WAC values were determined after defatted meal (1 g) was homogenized with 10 mL of distilled water using a vortex mixer and then stirred for 30 min at 30°C using a metabolic shaker at 1,200 rpm and centrifuged at  $2,350 \times g$  for 10 min at room temperature (30°C). The supernatant was carefully decanted and the residue, along with the centrifuge tube, was weighed. To study the effect of salts, 10 mL of NaCl and NaHCO<sub>3</sub> in concentrations of 0.2–1M was added to the meal instead of water. The WAC values were expressed as g of water absorbed/g of meal (Sosulski 1962).

Protein solubility was determined in the presence of NaCl and NaHCO<sub>3</sub> in one set of experiments and at different pH levels in another set. Meal (100 mg) was mixed with 5 mL of NaCl and NaHCO<sub>3</sub> (at concentrations of 0.2–1M). To study the effect of pH, 100 mg of meal was mixed with 5 mL of distilled water in a series of test tubes. The pH level was adjusted from 2 to 12 by adding 0.1N HCl or 0.1N NaOH. After shaking for 1 hr in a metabolic shaker at 30°C and 1,200 rpm, dispersions were centrifuged at  $2,940 \times g$  for 15 min at room temperature (30°C). Protein content of all supernatants was estimated applying the method of Lowry et al (1951) using bovine serum albumin as standard.

## Other Properties

Foaming properties, emulsification properties, and viscosity were also measured. Supernatants were produced from 20 g of meal in 100 mL of salt solution homogenized using a metabolic shaker for 1 hr, followed by centrifugation at  $4,100 \times g$  for 15 min at 30°C. To study the effect of pH, 100 mL of distilled water was adjusted to specific pH values and substituted for salt solution.

Foaming properties were estimated by the method of Lawhon et al (1972). Meal extract (50 mL) was whipped at  $1,000 \times g$  for 3 min using a metabolic stirrer (Rider Solid State) and the mixture was poured into a 100-mL cylinder. Total volume was recorded immediately and foam capacity (FC) was expressed as percent increase in volume. To determine foam stability (FS), foam volume was recorded at 60 min after whipping and calculated as  $FS = \text{foam volume after time } (t) / \text{initial foam volume} \times 100$ .

Emulsification properties were studied using the procedure of Yasumatsu et al (1972). Groundnut oil (10 mL) was added to 20 mL of meal extract; this mixture was homogenized for 3 min at  $1,760 \times g$  using a metabolic stirrer. The resulting emulsion was centrifuged at

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2,940 × g at 30°C. The height of the emulsion layer was noted in a graduated centrifuge tube and emulsifying activity (EA) was expressed as EA = height of emulsified layer/height of total content in the tube × 100.

Emulsion stability (ES) was determined in a similar manner, but the emulsion was heated to 80°C for 30 min in a water bath and cooled under running tap water for 15 min before centrifugation. The emulsion stability was expressed as a percentage of the emulsifying activity remaining after heating.

Viscosity of the meal extract was determined using an Ostwald viscometer at room temperature (30°C). Meal extract (15 mL) was put into the viscometer and flow time of each solution was noted. Relative viscosity was calculated as  $\eta_{rel} = \eta/\eta_0 = t/t_0 \times d/d_0$  where  $t$  is the time of flow of the meal extract,  $t_0$  is the time of flow of water,  $d$  is the density of the protein solution, and  $d_0$  is the density of the water.

Density of the protein solution was determined with a bicapillary pycnometer as Density = weight of the protein solution/weight of the distilled water × 1.

**TABLE I**  
Effect of Salts on Water Absorption Capacity (WAC) and Protein Solubility of Amaranth (*Amaranthus tricolor* L.) Seed Meal<sup>a,b</sup>

Salt Conc.	WAC (g/g of meal)		Protein Solubility (%)	
	NaCl	NaHCO <sub>3</sub>	NaCl	NaHCO <sub>3</sub>
DW	1.800 ± 0350	1.800 ± 035	24.60 ± 0.80	24.60 ± 0.80
0.2M	1.872 ± 0280	1.980 ± 0320	31.42 ± 1.25*	39.00 ± 1.10*
0.4M	2.580 ± 0250*	2.028 ± 0380*	45.92 ± 1.62*	20.71 ± 0.80
0.6M	2.330 ± 0360*	2.076 ± 0400*	31.85 ± 1.35*	20.42 ± 1.00
0.8M	1.220 ± 0400*	2.000 ± 0320	32.00 ± 1.35*	41.50 ± 1.65*
1.0M	1.240 ± 0340*	1.800 ± 0340	30.21 ± 1.20	29.50 ± 1.30*

<sup>a</sup> DW = distilled water (control).

<sup>b</sup> \*, Significant at  $P \leq 0.05$ .

**TABLE II**  
Effect of Salts on Foaming Properties (%) of Amaranth (*Amaranthus tricolor* L.) Seed Meal<sup>a,b</sup>

Salt Conc.	NaCl		NaHCO <sub>3</sub>	
	FC	FS	FC	FS
DW	90.69 ± 1.30	61.53 ± 1.30	90.69 ± 1.30	61.53 ± 1.30
0.2M	76.19 ± 1.10*	100.00 ± 2.70*	74.12 ± 1.90*	65.00 ± 1.40*
0.4M	85.17 ± 2.30*	94.44 ± 2.50*	63.00 ± 2.50*	68.23 ± 2.25*
0.6M	85.17 ± 2.50*	88.88 ± 2.32*	38.21 ± 1.32*	40.65 ± 1.32*
0.8M	66.66 ± 1.30*	83.33 ± 1.70	30.00 ± 1.20*	52.67 ± 2.50*
1.0M	61.16 ± 1.25*	81.81 ± 1.70*	32.15 ± 1.25*	72.32 ± 2.60*

<sup>a</sup> DW = distilled water (control); FC = foam capacity; FS = foam stability.

<sup>b</sup> \*, Significant at  $P \leq 0.05$ .

**TABLE III**  
Effect of Salts on Emulsification Properties (%) and Relative Viscosity of Amaranth (*Amaranthus tricolor* L.) Seed Meal<sup>a,b</sup>

Salt Conc.	NaCl			NaHCO <sub>3</sub>		
	EA	ES	RV	EA	ES	RV
DW	52.12	43.02	1.249	52.12	43.02	1.249
0.2M	± 0.70	± 0.60	± 0.012	± 0.40	± 0.60	± 0.012
	50.00	45.19	1.256	45.78	52.00	1.269
0.4M	± 0.70*	± 0.30*	± 0.023	± 0.70*	± 0.80*	± 0.026
	53.33	45.00	1.285	51.68	51.00	1.298
0.6M	± 0.60*	± 0.30*	± 0.016*	± 0.60	± 0.70*	± 0.032*
	53.26	47.82	1.337	53.33	49.48	1.348
0.8M	± 0.60*	± 0.50*	± 0.035*	± 0.70*	± 0.60*	± 0.025*
	51.57	47.80	1.359	48.93	50.58	1.384
1.0M	± 0.40	± 0.60*	± 0.032*	± 0.50*	± 0.50*	± 0.032*
	52.12	52.27	1.449	53.93	49.09	1.480
	± 0.60	± 0.60*	± 0.032*	± 0.60*	± 0.60*	± 0.038*

<sup>a</sup> DW = distilled water (control); EA = emulsifying activity; ES = emulsion stability; RV = relative viscosity.

<sup>b</sup> \*, Significant at  $P \leq 0.05$ .

## Statistical Analysis

All the experiments were performed at least three times. Data was analyzed by one-way analysis of variance (ANOVA) to test the significance among values between the salts. The  $t$ -test was conducted to analyze the significance of the results at the 5% level.

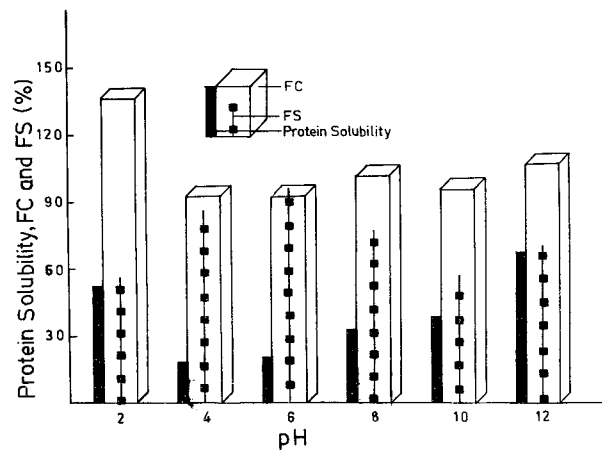
## RESULTS AND DISCUSSION

### Chemical Composition

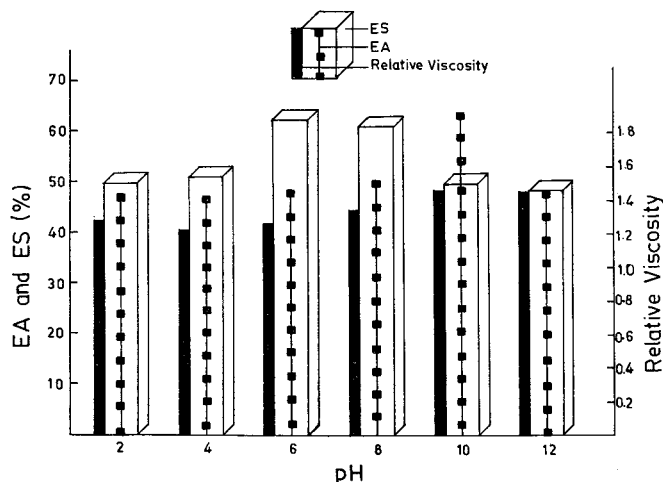
Protein, carbohydrate, and lipid contents of amaranth seed meal were 14.10, 41.23, and 4.50%, respectively. Reported protein content in different species of amaranth has a range of 12–17.6% (Teutonico and Knorr 1985). The ash content of amaranth meal was rather high (8.10%) but the amount of phenol (0.016%) and phytic acid (0.047%) was low. Phenol content in different rapeseed preparations has been reported at 0.329–0.515% (Mahajan and Dua 1998). Phytates have been found in cereal grains and legumes up to a level of ≈5% by weight (De Boland et al 1975). Phytate forms complexes with phosphate, calcium, iron, zinc, and proteins, thus making them unavailable to monogastric animals and humans.

### Water Absorption Capacity

Water absorption capacity (WAC) of amaranth meal was 1.800g/g of meal (Table I). It is comparatively higher than the values reported for soybeans (130–223%), sunflower flour and protein concentrates (107 and 137%) (Venkatesh and Prakash 1993), and pigeon pea flour (138%) (Oshodi and Ekpergin 1989) but lower than that of winged



**Fig. 1.** Effect of pH on protein solubility, foam capacity (FC) and foam stability (FS) of Amaranth (*Amaranthus tricolor* L.) seed meal.



**Fig. 2.** Effect of pH on emulsifying activity (EA), emulsion stability (ES) and relative viscosity of Amaranth (*Amaranthus tricolor* L.) seed meal.

bean (240%) (Sathe et al 1982). Both NaCl and NaHCO<sub>3</sub> enhanced the WAC of the meal; it was maximized at 0.4M NaCl (2.58 g/g of meal) and 0.6M NaHCO<sub>3</sub> (2.076 g/g of meal) (Table I). With increased salt concentrations, there was a progressive decrease in water absorption. Salts markedly affect water absorption and binding by proteins through their effects on electrostatic interactions. Salt effects vary with the cation and anion species involved (Kinsella et al 1985). Generally, low salt concentrations increase water binding.

### Protein Solubility

The solubility of Amaranth meal protein was higher than that of the control at all NaCl concentrations (0.2–1M). This may be due to the salting-in effect of NaCl on the hydrophobic interactions, which progressively decrease their aggregating tendency and increase the solubility. However, with NaHCO<sub>3</sub>, the solubility of amaranth protein was lower than that of the control at 0.4M and 0.6M salt concentrations (Table I). This decrease can be explained on the basis of electrostatic shielding of charged groups by salts, which decrease electrostatic repulsion and enhance hydrophobic interactions. Natural salts exert striking effects on the solubility, the association-dissociation equilibrium, the enzyme activity, the stability of fibrillar structure, and the rate of conformational change of proteins, polypeptides, and nucleic acids (Oshodi and Ojoken 1997). Similar results were obtained with wheat gluten in various salt solutions (Preston 1981).

The solubility of amaranth protein was minimum at pH 4 and it increased linearly above and below this level but was maximum at pH 12 (Fig. 1). The low solubility at pH 4 indicates that this is near the isoelectric point of amaranth protein. At the isoelectric point, there is no net charge on the protein, as a result there are no repulsive interactions, and the protein-protein interactions disfavor solubility. But at very low and high pH values, repulsive forces favor protein-solvent interactions, thus solubility increases. Tasneem and Subramaniam (1986) reported 80–90% solubility of protein at extreme acidic and alkaline pH values in guar meal. These types of findings were also noted in rapeseed protein meal (Mahajan and Dua 1995).

### Foaming Properties

There was a significant ( $P \leq 0.05$ ) change in the foaming properties in the presence of salts. At all concentrations, both NaCl and NaHCO<sub>3</sub> decreased the foam capacity (FC) of amaranth meal proteins when compared with that of the control (Table II). However, foam stability (FS) was improved significantly at all the concentrations of NaCl. In general, foaming properties were better in NaCl than in NaHCO<sub>3</sub>. Literature reports on the presence of salts on protein foams are conflicting. A direct relationship between protein solubility and FC has been reported but increase in the protein solubility did not improve the FC of amaranth meal proteins. These salts, therefore, limit the use of amaranth meal in the production of cakes and whipped toppings where foaming is an important property. However, the foams formed were stable in the presence of these salts. Sunflower meals also showed higher levels of foam stabilization in the presence of salts than in water (Venkatesh and Prakash 1993).

Foam capacity (FC) as a function of pH showed a maximum value (136%) at pH 2 and it became lower at higher pH values. It remained marginally same at pH 4–12. However, values of FS were highest at pH 6. (Fig. 1). Similar types of findings have been reported in linseed (Madhusudan and Singh 1985) and poppy seed meal (Srinivas and Narasinga Rao 1986). The high FC at extreme acidic pH 2 may be due to high solubility of proteins at this level. The pH level, by influencing the net charge affects the rate of adsorption and extent of protein-protein interaction in the interfacial film. The higher solubility of protein at pH 12 but lower FC as compared with that at pH 2 shows protein is not adsorbed easily at the interface at pH 12. The reason for highest FS values at low solubility may be that interpeptide interactions are maximum at that level (pH 6). Due to maximum attractions, protein assumes a compact state, and more proteins absorb at interface, resulting in maximum reduction in surface tension.

### Emulsification Properties

Amaranth protein has same emulsifying activity (EA) in the presence and absence of salts (distilled water), while emulsion stability (ES) was better in the presence of salts and the increase was significant ( $P \leq 0.05$ ) (Table III). Both EA and ES depend on the salt concentration and the type of salt under consideration. In alfalfa leaf proteins, NaCl had no effect on EA or ES but increased the emulsifying capacity (Wang and Kinsella 1976). The nature of hydrophobic groups that are exposed to the surface may largely determine emulsification properties of proteins and may affect a balance between net charge on the protein and hydrophobicity.

Emulsifying activity (EA) of amaranth meal was highest (60%) at pH 10 and lowest at pH 4 (47.11%), while emulsion stability (ES) was maximum at pH 6 (62.07%) and minimum at pH 12 (48.95%) (Fig. 2). The protein solubility of amaranth meal underwent the same type of changes as the EA. Similar pH dependence of emulsifying ability of proteins has been reported earlier as well (Mahajan and Dua 1995).

### Viscosity

NaCl and NaHCO<sub>3</sub> significantly ( $P \leq 0.05$ ) enhanced the relative viscosity of amaranth meal protein (Table III). It was higher in NaHCO<sub>3</sub> at all salt concentrations than in NaCl. These observations suggest that increase in viscosity in the presence of salts is due to the unfolding of the proteins, which increases the axial ratios as well as interactions between the protein molecules through hydrogen bonding and hydrophobic and electrostatic interactions. A steady increase in relative viscosity suggests a gradual conformational change in protein due to unfolding or swelling of protein molecules. These types of findings are given for soybean and rapeseed proteins (Mahajan and Dua 1994). The data was significant for all the properties when comparison was made between the two salts using ANOVA.

The pH levels also had a pronounced effect on the relative viscosity of Amaranth meal. It was highest at pH 10 (1.429) and lowest at pH 4 (1.210) (Fig. 2). The observed effects of extreme pH may be attributed to the dissociation of the oligomeric proteins to smaller subunits. Because particle size affects the hydrodynamic volume, the dissociation of protein at extreme pH would be expected to affect the viscosity of protein dispersion.

The present work showed that NaCl and NaHCO<sub>3</sub> improved the WAC, protein solubility, and viscosity, and it may, consequently, improve the functionality of Amaranth meal for the production of meats, soups, and beverages. The different functional properties were moderately good at pH 10. Alkaline pH causes dissociation and gradual unfolding of polypeptides to yield a viscous gel, increases its electrostatic repulsion, and cause scission of disulfide bonds. This may prove useful in enhancing the applicability of amaranth meal at pH 10 in soups and gravies where good viscosity is required and in meats and bakery products where disulphide links in a gel affect the elasticity.

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