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Physicochemical and Thermal Properties of Amaranth (*Amaranthus Hypocondriacus*) Flour

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Abstract:
Amaranth is a major pseudo-cereal used in food industry in the form of flour, starch and grains. In the present investigation flour of amaranth was analyzed for physicochemical properties (chemical composition, water and oil absorption capacity, swelling power and solubility, color parameters), thermal and pasting behaviour. Chemical composition of amaranth flour confirmed good nutritional value of amaranth having 14% protein, 5% crude fibre and 5% crude fat content. Swelling power (12 g/g), solubility (41%), water absorption capacity (132%) and oil absorption capacity (144%) of flour indicated its wide applicability in functional foods. Paste clarity of flour decreased progressively during storage at refrigeration temperature. High luminosity of flour makes it suitable for consumer's preference. DSC showed wide range of gelatinization temperature and RVA demonstrated change in viscosity of paste during heating and cooling. Enhanced understanding of these properties aids in selection of processing conditions and advances utilization of amaranth flour.

Keywords: *Amaranth, flour, functional, RVA, DSC*

1. Introduction

Amaranthus, a major pseudo-cereal, is an ancient plant belonging to family Amaranthaceae, which is believed to have originated from central and southern America. Although it includes more than 60 species, of which only three species (*Amaranthus caudatus, A. cruentus and A. hypochondriacus*) present interesting agricultural characteristics, such as rapid establishment, tolerance to water deficits, biomass production, nutrient cycling and use as both human and animal food. (Spehar et al, 2003). At the present time it is also called a third millennium crop plant (Rastogi and Shukla, 2013). Today amaranth is considered as an alternative crop and researchers in many parts of world have focused on improving agronomic features of the plant, the nutritional quality, and processing technology of the seed. Amaranth is a hardy, wild, fast growing plant with very broad leaves that produces very small spherical shape seeds (approximately 1mm diameter) vary in color from creamish yellow to reddish (Singh et al, 2014) Amaranth have higher protein content (12% to 18%) than most of the cereals with a significant higher content of lysine and acceptable level of tryptophan and methionine, which are found in low concentration in cereal and legume grains (Mendoza and Bressani, 1987). It has lipid content of 10 to 17% with a comparatively high amount of unsaturated fatty acids and it is also an excellent source of vitamins and minerals (Mburu et al, 2014). Owing to their significant starch, protein, and lipid content, amaranth seeds can be considered promising raw materials for the production of flour, starch and protein. High-protein and high-carbohydrate flour have been obtained from amaranth seeds by an enzymatic process, which might find application as a dry milk extender and sweetener, respectively. Flour and starch of amaranth is becoming an important constituent of different food products, such as gravies, sauces, breakfast cereals, muffins, cookies, snacks, pastas, and health foods because of its unique composition (Teli et al, 1996). The utilization of amaranth flour in food system greatly depends on its functional properties. Water and fat absorption, emulsification and viscosity are some important functional properties of flour that can decide their utilization for different food application (Shevkani et al, 2014). Even though it's nutritional and health importance, amaranth flour has not gained sufficient research attention to its physicochemical, functional and thermal properties. Studies investigating the properties of amaranth flour are scarce. The present investigation was undertaken to examine the physicochemical, functional, pasting and thermal properties of flour and starch of amaranth in order to identify their potential application as food ingredients.

2. Materials and Methods

2.1. Materials

Grains of amaranth (Amaranthus hypocondriacus) of cultivar VL-44 used in this study were procured from National Bureau of Plant Genetic Resources Regional Station, Shimla, India. The grains were screened to remove foreign matter and stored in sealed container at room temperature previous to their use. The flour was prepared by grinding seeds on laboratory mill and stored in polyethylene bags at 10ºC.

2.2. Chemical Composition

Flour was estimated for moisture, crude fat, crude fibre, ash and protein (N x 6.25) content by employing the standard methods (AOAC, 1990). The amylose level was determined according to modified methods of Williams et al. (1970). The standard curve used for amylose was Y = 0.0089X + 0.0528 (r = 0.99), where X = amylose content (%), and Y = absorbance at 680 nm, based on fractionation of rice starch by Montgomery and Senti (1958).

2.3. Hunter Color Parameters

Color of flour was measured using Ultra Scan VIS Hunter Lab (Hunter Associated Laboratory Inc., Raston Va., U. S. A.). The system determines the L^*, a^* and b^*values,where L^* represents lightness and darkness; a^* represents the opposition between green and red color ranging from positive (red) to negative (green) values; and b^* is the yellow/blue opposition also ranging from positive (yellow) to negative (blue) values.

2.4. Water and Oil Absorption Capacity

Water absorption capacity (WAC) and oil absorption capacity (OAC) of flour were determined by using the method of Ige et al (1984). A suspension of 1.5g of flour sample in 10 ml distilled water was agitated 4 times allowing 10 min resting periods between each mixing and centrifuged at 3250 rpm for 25 min. The supernatant was decanted and tubes were air dried and weighed. For determination of oil absorption capacity (OAC), 3ml refined groundnut oil was added to 0.5g of flour sample and stirred for 1 min. After resting period of 30 min at room temperature, the tubes were centrifuged at 3200 rpm for 25 min. The volume of unabsorbed oil was determined.

2.5. Swelling Power and Solubility

Flour sample (4 g) was heated for 1 hour with 40 ml of water at 90°C. Constant stirring was done to avoid lump formation. The dispersion was centrifuged at 5,000 rpm for 10 min. Sediment was weighed and supernatant was carefully taken in pre-weighed petri dish and dried to constant weight in drying oven at 100°C. The residue obtained after drying of supernatant represented the amount of flour solubilized in water (Subramanian et al, 1994; Raina et al, 2006). Swelling power of flour was calculated by using following formula-

$$\text{Swelling Power} = \frac{\text{Wt of sediment paste x 100}}{\text{Wt of sample on dry basis x } (100 - \text{ \% solubility})}$$

2.6. Paste Clarity

Light transmittance (%) of pastes from flour was measured by method of Perera and Hoover (1999) with slight modifications. Aqueous suspension (1%) of flour was heated in water bath at 90ºC for 1 hour with constant stirring. The suspension was cooled to room temperature. Samples were stored for a period of 5 days at refrigeration temperature and transmittance was measured at an interval of 24 hours at 640 nm against a water blank using GENESYS 10S UV–VIS Spectrophotometer (Thermo Fisher Scientific, 81 Wyman Street Waltham, MA USA).

2.7. Bulk Density and Least Gelation Concentration

Bulk density of flour was determined following the method as described by Balandran Quintana et al (1998). Flour sample (10g) was put in measuring cylinder, tapped 8-10 times from a particular height and volume of sample was noticed. Bulk density was measured as weight of sample per unit volume. For determination of least gelation concentration, the method described by Mishra and Rai (2006) was followed with slight modifications. Solutions (5ml) of different concentration of flour (8-30 % w/v) in test tubes were heated at 90°C in water bath for 1hour, cooled immediately in ice chilled water bath and kept overnight at 4°C. The gelation was confirmed by inverting the test tubes.

2.8. Pasting Properties

Pasting properties of amaranth flour were determined using a Rapid Visco Analyser (Perten Instruments, Australia). Flour sample (3 g, 14% moisture basis) was mixed with calculated amount of double distilled water in the RVA sample canister. The slurry was manually homogenized using plastic paddle to stay away from lump formation before RVA run. A programmed heating and cooling cycle was used where the samples were held at 50°C for1 min, heated to 95°C in 3.30 min, held at 95°C for 3 min before cooling to 50°C in 3.30 min and holding at 50°C for 2 min. The mixture was stirred at a constant speed of 160 rpm during the test. A RVA plot of viscosity (cP) versus time (s) was used to determine peak viscosity (PV), trough viscosity (T), breakdown viscosity(BD), final viscosity (FV), set back (SB), peak time (P time) and pasting temperature (P temp) of flour.

2.9. Thermal Properties

Thermal properties were analyzed using a Differential Scanning Calorimeter 2920 (TA Instruments, New Castle, DE, USA) according to Bao et al (2004) with some medications. Flour sample (6.0 mg, db) was weighed into an aluminium pan and 6μl distilled water was added. The pan was hermetically sealed and equilibrated at room temperature for 1 h, then heated at the rate of10°C/min from 30°C to 120°C with an empty sealed pan as a reference. Parameters such as onset (To), peak (Tp), conclusion (Tc) temperature and enthalpy (ΔH) of gelatinization were determined by software provided in the system.

3. Results and Discussion

Moisture Content (%)	9.75±0.47	**Swelling Power (g/g)**	12.05±0.45
Protein (%)	14.38±0.37	**Solubility (%)**	41.52±1.26
Crude fibre (%)	5.73±0.70	**Water Absorption Capacity (%)**	132.0±0.85
Crude fat (%)	5.37±0.15	**Oil Absorption Capacity (%)**	144±0
Ash Content (%)	2.90±0.01	**Bulk Density (g/ml)**	0.53±0.0
Amylose Content (%)	5.40±0.65	**Least Gelation concentration (%)**	24±0

Table 1: Chemical composition and functional properties of flour
Values expressed as mean ± SD (n=3).

The chemical composition and functional properties of amaranth flour is presented in Table 1. The values of protein and ash content of flour were 14.38% and 2.90% respectively and found to be in range reported by Sevkani et al (2014). Similar results of protein and fat were obtained by Chauhan and Singh (2013) for amaranth flour. Flour had crude fat content 5.37% which was in agreement with the results obtained by Choi et al (2004). However, Colla et al (2006) reported 10.8% lipid in amaranth flour. In present study fibre content was found to be slightly higher (5.73%) than recorded by Chauhan and Singh (2013). Slight difference in composition of flour from previous record might be due to difference in climatic conditions of crops. Amylose content is an important factor affecting functional properties like swelling power and solubility of flour and starch. The fraction of amylose in amaranth flour is 5.40%, which was comparable with the corresponding results of 7.5% for amaranth flour reported by Tapia - Blacido (2010). However, in present study of amaranth flour amylose content was very low as noticed by Singh et al (2005) in different pea's flour. The water absorption capacity is the ability of the flour to hold water against gravity wherein proteins and carbohydrates enhance the water absorption capacity of flour by providing hydrophilic parts like polar and charged side chains (Pomeranz, 1985). Water absorption capacity of amaranth flour was 132% which was higher than results of Aseniyi and Obatolu (2014) reported 107% for *A. Hypochondricus* while Shevkani et al (2014) observed water absorption capacity of flour in the range of 209%-243% for same variety of amaranth. The water absorption capacity of amaranth flour in present investigation was comparable with the soybean (130%) and chick pea (133 to 147%), and lower than dry bean (223 to 265%) reported in literature (Oshodi and Ekperigia, 1989; Kaur and Singh, 2005; Siddiq et al, 2010). Oil absorption capacity of flour is due to interactions between the nonpolar amino acid side chains and hydrocarbon chains of lipid determine mouthfeel and flavour retention of products. In this study oil absorption capacity of flour was 144%, that was comparable with the results of Pachelo de Delahaye (1987) reported 150% for amaranth flour but higher than that reported by Asenity and Obatolu (2014) for flour of amaranth. Overall trend of WAS and OAC in present study was similar to the trend noticed by Shimelis et al (2006) for bean flour showing higher values of OAC than WAC.

Swelling power and solubility can be utilized to measure the extent of interaction between starch chains, within the amorphous and crystalline domains of the starch granule (Ratnayake et al, 2002). Furthermore, it is influenced by amylose and amylopectin characteristics (Chan et al, 2009). Swelling power of flour was 12.05g/g which was slightly lower than swelling power of starch of amaranth reported in literature (Kong et al, 2009) which might be due to higher amount of protein and lipid in flour than starch. According to Pomeranz (1991), formation of protein-amylose complex in native starches and flours may be the cause of decrease in swelling power. Solubility is the leaching out of linear molecules of amylose or linear portion of long chain of amylopectine at or above gelatinization temperature. Solubility of amaranth flour was 41.52%, which was lower than the value of 90-91% solubility of amaranth starch reported by Kong et al (2009) which might be due to low level of amylopectine in flour than starch of amaranth. Leach et al (1959) reported solubility value of 82% for potato starch and 19.1% for wrinkled pea starch. Yang et al (1988) noticed that the solubility decreased as the amylose content of the variety decreased, which might be responsible for lower swelling power of flour than starch of amaranth reported in literature. Factors that may influence solubility of starches are source, swelling power, inter-associative forces within the amorphous and crystalline domains and presence of other components like phosphorous. Ong et al (1995) inferred that long chains of amylopectine interact with the amylose to form double helix structures that lowers the swelling and leaching of materials on cooking. This might also be responsible for low swelling and solubility of amaranth flour. The least gelation concentration of amaranth flour was 24%, which is the index of gelation properties that depends on the amount of starch and pasting properties of starch present in starch. Gelation properties are interrelated to water absorption capacities hence the low water absorption capacity recorded for the flours could explain the deficient gel formation capacity. Bulk Density of flour was 0.53g/ml which was in agreement with the finding of Chauhan and Singh (2013) for amaranth flour. Bhavsar et al (2013) reported higher bulk density 0.86 g/ml and 0.74 g/ml for buckwheat flour and refined wheat flour.

RVA parameters		Paste clarity	
Peak Viscosity (cP)	733.33±15.14	Storage period (Days)	Transmittance (%)
Trough Viscosity (cP)	681.33±12.66	0	3.63±0.3
Breakdown viscosity (cP)	56±2.64	1	2.89±0.1
Final Viscosity (cP)	800.66±13.05	2	2.55±0.21
Setback Viscosity (cP)	119.33±23.45	3	2.13±0.5
Peak Time (min)	6.02±0.1	4	1.98±0.22
Pasting temperature (°C)	77.45±0.05	5	1.77±0.11

Table 2: Pasting properties and effect of storage on paste clarity of amaranth flour
Values expressed as mean ± SD (n=3).

Pasting properties of flour of amaranth are depicted in Table 2. The pasting curve represents changes in behaviour of paste viscosity of flour with change in temperature and mainly varies with composition of flour and characteristics of starch present in it. Increase in viscosity during heating may be attributed to the swelling of granules, as a result of loss of crystalline order and absorption of water (Bao and Bergman, 2004). Pasting properties of flour were similar to the result of amaranth starch reported by Kong et al (2009), who studied fifteen cultivars of amaranth grain. Pasting temperature of amaranth flour was 77.45ºC which was higher than the range (71.3ºC-72.1ºC) reported by Shevkani et al (2014) for amaranth flour. The high pasting temperature of flour indicates its higher resistance towards swelling. The point of maximum swelling of starch granules is indicated by peak viscosity. Peak viscosity of flour was lower than the range (1050cP-1459cP) reported by Shevkani et al (2014) for flour. Break down viscosity, is the measure of resistance of gel to disintegrate at high temperature, was found to be higher in previous studies (Choi et al, 2004; Shevkani et al, 2014). Lower BV of flour (56cP) represents greater resistance to shear thinning and high stability of paste. Final viscosity represents the ability of starch/flour to form a viscous paste, was found to be 800cP for flour. Increase in final viscosity might be due to the aggregation of amylose molecules (Miles et al, 1985). Set Back viscosity is the measure of syneresis upon cooling of cooked paste. The pasting properties of flour depend mainly on starch present in it. However, other components present in flour affects the pasting properties (Ragaee and Abdel-Aal, 2006) such as higher amount of lipid present in flour lowers viscosity. The role of lipids on the pasting properties was observed as positive relation with pasting temperature while negative relation with viscosity (Shevkani et al, 2011; Singh et al, 2014).

The transmittance values of paste of flour of amaranth were summarized in Table 2. Transmittance was found to decrease 3.63 to 1.77 with progressive storage at refrigeration temperature for a period of 5 days. The swelling of granules, granule remnants, leached amylose and amylopectine, molecular weight and chain lengths of amylose and amylopectine have been reported to vary with granule size, which ultimately leads to turbidity development and decreased transmittance in starch paste during refrigerated storage (Perera and Hoover, 1999). Decrease in transmittance with refrigeration storage was noticed in paste of corn starch by Sandhu and Singh (2007) and in potato flour paste by Singh et al (2005).

Thermal properties		Colour parameters	
Onset temperature (ºC)	67.20±1.10	L*	85.19±0.30
Peak temperature (ºC)	78.1±0.2	a*	2.87±0.03
Conclusion temperature (ºC)	86.2±1.0	b*	17.24±0.11
Δ H (J/g)	12.1±0.3		

Table 3: Thermal properties and color parameters of amaranth flour
Values expressed as mean ± SD (n=3).

Transition temperatures (T_0, T_P, T_C) and gelatinization enthalpy (ΔH) of flour of amaranth were shown in Table 3. Differential scanning calorimetric studies of amaranth flour showed that the gelatinization temperature of the flour (67.20ºC) was in range reported by Kong et al (2009) for amaranth starch but lower than that (70ºC) reported by Menegassi et al (2011) for amaranth flour. The mucilage present in the flour contain proteins and also some complex polysaccharides which could compete with starch for moisture and result in a higher onset temperature of flour as compare to starch isolate (Taxi et al, 1972). Similar trend of transition temperatures was observed by Jane et al (1992) for taro starches and flours. The peak temperature for flour (78.1ºC) was slightly higher than pasting temperature obtained by RVA. Present investigation agreed with the study of Kong et al (2009) reported T_P which ranged from 68ºC to 78ºC and T_0 from 63ºC to 72ºC for fifteen cultivars of amaranth. The onset, peak and conclusion temperature of flour (67.20ºC, 78.1ºC and 86.2ºC respectively) were found to be comparable with the investigation of Menegassi et al (2011) reported T_O (70.5º C), T_P (76.1ºC) and T_C (82.8ºC) for amaranth flour. During DSC analysis the transformed proportion of starch is reflected by the area under the endothermic peak, representing the enthalpy change (ΔH). The value of ΔH for flour (12.1 J/g) was comparable to the values (10.6J/g, 9.8J/g) reported by Tapia-Blacido et al (2010) while higher than observed (5.5 J/g) by Menegassi et al (2011) for amaranth flour. Amylopectine is considered as primary participant in crystalline region (Robin et at, 1974) which was responsible for higher value of enthalpy change. Because amaranth starch, which is waxy due to rich in amylopectine, granules possess a different crystalline-amorphous structural relationship than normal starch granule. In literature higher values of ΔH for waxy starches than normal starches were reported by other authors (Stevens and Elton, 1971; Inouchi et al, 1984). One of the most important characteristics that can decide successful applications of functional ingredients in different food products is color and clarity. The colour of starch due to the presence of polyphenolic compounds, ascorbic acid and carotene has impact on its quality. Any pigmentation in the starch is carried over to the final product. This reduces the quality, hence acceptability of starch product (Galvez and Resurreccion, 1993). The L^* value of flour (85.19) was in consistent with range (82- 85) reported by Shevkani et al (2014) for amaranth flour. The positive value of a^* was small indicating presence of little red tint in flour. Positive b^* value of flour was 17.24, indicated presence of yellow components in flour. The higher b* value has been reported to be an indication of presence of higher ash content (Kaur and Singh, 2007). Menegassi et al (2011) reported slightly lower b^* value (14.9) for flour of amaranth.

4. Conclusion

The physicochemical properties and functional characteristics of amaranth flour propose that it may have broad possibilities as an ingredient in food systems and other industrial application. High protein content, crude fibre, lipid content and ash content in flour confirmed the good nutritional quality of grain. Wide range of gelatinization temperature range, good swelling power, solubility and water absorption capacity noticed for flour which makes it potentially useful in products subject to high temperatures. High luminosity noticed in flour which is a most desirable property in food industry. Future work is necessary to analyze the amino acid and fatty acid profile, characterize fine structures of starch of amaranth of different origin and analyze their relationships with various properties of flour.

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