Characterization of Amaranth (Amaranthus Hypocondriacus) Starch

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Abstract - **Grain amaranth (*Amaranthus hypocondriacus*) is a pseudo- cereal and starch is its major component which plays an important role in the functional and technological properties of end-use food products. In the present study starch was isolated from the grain amaranth and analysed for its physicochemical, thermal and pasting properties. Chemical composition of starch included very small level of protein, fibre, fat and ash content which indicated high purity of extracted starch. SEM observation revealed that granules were polygonal shaped, extremely small in size and surface was smooth with no fissures. Results of color starch meet consumers demand due to high luminosity. Decreasing trend was noticed in paste clarity during storage. High oil absorption capacity was observed than water absorption capacity of starch. DSC demonstrated a wide gelatinization temperature range. High viscosity, swelling power and solubility of starch indicated its wide applicability in food industry as functional ingredient.**

Keywords- Amaranth; starch; functional;SEM; DSC; RVA

1. INTRODUCTION

Starch is the major reserve polysaccharide in plants and is a valuable functional ingredient in the food industry. The physicochemical and functional characteristics that are imparted by starch to the aqueous system in various food applications vary with the biological origin (Svegmark and Hermansson, 1993). Cereal grains such as maize, rice and wheat and tubers are the significant sources of starch. Due to its growing demand current starch research is focussed on searching the new starch sources with diverse physicochemical and functional characteristics that give them a broad range of potential industrial uses. Amaranth (*Amarnathus* spp.) is a promising new starch source as it is rich in carbohydrates and identified as a major potential crop by National Academy of Sciences, US (1985). . Many potential food applications such as stabilizers, thickeners, gelling agents, etc. and non food applications like biodegradable plastics, dusting powders and cosmetics of amaranth starches have been noticed (Breene, 1991; Choi et al, 2004). 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). Starch, mainly located in perisperm, is the major component in amaranth grain accounting for 48-69 g/100g of its weight, depending on the species, the properties of starch will dominate the characteristics of their products (Teutonico and Knorr, 1985). Due to its extremely small granules of 0.75–3µm in diameter, amaranth starch has gained attention for food applications. It is characterized as low amylose starch and consists of 92–95% amylopectin, which in turn provide excellent freeze/thaw stability. Swelling power and solubility of starch has been used to demonstrate differences between various types of starches (Crosbie, 1991). High clarity and good gel strength of starch are important properties increasing utilization of starch in processed food products. The process of gelatinization is energy absorbing than can be monitored through Differential Scanning Calorietry (DSC) which has been used for analysis of starch (Freitas et al, 2004). Analysis of thermal properties of starch can provide the important information to direct the processing and the utilization of starch. It is generally accepted that swelling of granules increase the viscosity during heating of starch in water. The properties of the swollen granules and the soluble materials leached out from the granules are cooperatively ruling the viscosity parameters during pasting (Doublier et al, 1987). Enhanced understanding of the properties of starch can present the important information to direct the processing and the application of starch. Although lots of studies have been carried out on starch systems, few studies have been done on starch of amaranth. Thus, the present investigation focuses on physicochemical, thermal, pasting behaviour analysis of amaranth starch in order to identify its 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 were prepared by grinding seeds on laboratory mill and stored in polyethylene bags at 10ºC.

2.2 Starch isolation

Starch was isolated from amaranth grains by following the alkaline steeping method (Choi et al, 2000) and stored in polythethylene bags at room temperature till further analysis. Grains were steeped in 0.25% aqueous NaOH solution for 18 hours at room temperature and stirred 3-4 times during this period. After steeping, the grains were washed 2-3 times with distilled water and ground in a blender at full speed for 2 min. Slurry was filtered step wise through 100 mash (150µm), 270 mesh (53µm) and 400 mesh (38 µm) sieves. The starch was isolated from the filtrate by centrifugation at 25,000g for 20 min. The supernatant was discarded, and the top yellowish layer of protein was removed. This step was repeated to obtain a white starch layer. The starch layer was resuspended in distilled water, shaken and centrifuged as described above. Thereafter, the isolated starch was dried in hot air oven at below 40ºC for 8- 10 hours and stored at room temperature in sealed container.

2.3 Chemical composition
Starch 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).

2.4 Hunter color parameters
Color of starch 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.

2.5 Functional properties
Water and oil absorption capacity

Method of Ige et al (1984) was used for determination of water absorption capacity (WAC) and oil absorption capacity (OAC) of starch. A suspension of 1.5g of 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 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.

Swelling power and solubility
Starch sample (4 g) was heated for 1 hour with 40 ml of water at 90°C. Lump formation was prevented by stirring. The dispersion was centrifuged at 5,000 rpm for 10 min. Starch 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 starch solubilised in water (Subramanian et al, 1994; Raina et al, 2006). Swelling power was calculated by using following formula-

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

Transmittance of paste (%)

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

Bulk density and Least gelation concentration
Bulk density of starch was determined as per the method described by Balandran Quintana et al (1998). 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 describe by Mishra and Rai (2006) was followed with slight modifications. Solutions (5ml) of different concentration of starch (1-10 % 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.

2.6 Scanning electron microscopy
The granule morphology of starch sample was observed in a scanning electron microscope (SEM). The sample was mounted on an aluminium stub using a double sided copper tape and coated with gold in sputter coater. The samples were examined at magnification of 2000X at an accelerated voltage of 10kV.

2.7 Pasting properties
Pasting properties of starch were determined using a Rapid Visco Analyser (Perten Instruments, Australia). Starch (3 g, 14% moisture basis) was mixed with calculated amount of double deionised 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).

2.8 Thermal properties
Thermal properties were analysed using a Differential Scanning Calorimeter 2920 (TA Instruments, New Castle, DE, USA) according to Bao et al (2004) with some

medications. Starch (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 gelatinisation were determined by software provided in the system.

3. RESULTS AND DISCUSSION

The chemical composition and functional properties of amaranth starch are presented in Table 1. Levels of components such as protein, fat, fibre and ash were very less in starch and which are in consistent with the observation of earlier studies (Mundigler, 1998; Choi et al, 2004). Proteins and fibres are removed during starch isolation process. Only starch bound fat could not be removed during isolation process. Additionally the presence of polar lipids interacted with proteins cannot be ruled out (Kikugawa et al, 1981). Minerals were lost during washing of starch pallets. A smaller amount of moisture content was noticed in starch, which might be due to longer period of drying after extraction, and that was in ranges generally accepted for dry products in order to obtain desirable shelf life and other conventional starches (Sriroth et al, 2000). Amylose content is an important factor affecting functional properties like swelling power and solubility of starch. The fraction of amylose in starch was 7.24% , comparable with the corresponding result of 7.8% for amaranth starch reported in by Qian et al, (1999); and much lower than the amylose content of cereal, root, tuber and legume starches. But Lorenz (1981) and Menegassi et al (2011) even reported the amylose content in amaranth starch to be as low as 0.2% and 0.3%, therefore referred amaranth starch to waxy starch. The chemical composition is a simple and convenient way of illustrating the purity of the starch extracts whereby lower contents of other components (protein, fat, ash, fiber) are highly desirable and which could be noticed in present study.

TABLE 1. Chemical composition and functional properties of amaranth starch

Moisture Content (%)	3.7±0.6	Swelling Power (g/g)	13.6±1.54
Protein (%)	0.86±0.28	Solubility (%)	76.33±0.41
Crude fibre (%)	0.07±0.01	Water Absorption Capacity (%)	119.66±13.50
Crude fat (%)	0.64±0.04	Oil Absorption Capacity (%)	146±18.33
Ash Content (%)	0.33±0.03	Bulk Density (g/ml)	0.69±0.01
Amylose Content (%)	7.24±0.22	Least Gelation concentration (%)	24±0

Values expressed as mean ± SD (n=3).

The water absorption capacity (WAC) is the ability of starch is to hold water against gravity wherein proteins and carbohydrates enhance the WAC of starch by providing hydrophilic parts like polar and charged side chains (Pomeranz, 1985). Water absorption capacity of starch was 119.66%, which was lower than the findings of Choi et al, (2004) recorded 130.7% for amaranth starch. Higher value of water absorption capacity (145.6%) of amaranth starch was observed by Lorenz and Collins (1990). OAC is due to interactions between the non-polar amino acid side chains and hydrocarbon chains of lipid determine mouth-feel and flavour retention of products. Value of OAC for amaranth starch was 146%, which was comparable with the results of Pachelo de Delahaye (1987) noticed 150% but lower than that reported (35%) by Adeniyi and Obatolu (2014) for amaranth starch. Overall trend of lower value of water absorption than oil absorption capacity in present study was similar to the trend noticed by Shimelis et al (2006) for bean starches. 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 starch was 13.6g/g which was found to be higher than starches of other sources like banana, lotus stem and sweet potato but comparable to wheat and some varieties of corn (Kaur et al, 2011; Sandhu and Singh, 2006); it might be due to higher amylopectine in amaranth starch comparing with other starches. Solubility of starch was 76.33% which was in agreement with results of Kong et al (2009) observed 85-91% for different cultivars of *A. Hypochondricus* starch. Least gelation concentration is the index of gelation properties which depends on the amount of starch and pasting properties of starch. Least gelatiton capacity was found to be 24% for starch. Gelation properties are interrelated to water absorption capacities hence the good water absorption capacity recorded for starch could explain the good gel formation capacity. Bulk density of starch was 0.69g/ml which indicated that starch would serve as good thickeners in food products. The effect of storage on paste clarity of amaranth starch is represented in Table 2. Transmittance of starch paste was found to decrease with progressive storage at refrigeration temperature. 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).

TABLE 2. Pasting properties of starch and effect of storage on paste clarity of amaranth starch

RVA parameters		Paste clarity	
Peak Viscosity (cP)	1212.66±187.44	Storage period (Days)	Transmittance (%)
Trough Viscosity (cP)	846±101.20	0	11.31±0.2
Breakdown viscosity (cP)	371.66±88.28	1	10.12±0.15
Final Viscosity (cP)	1032.33±124.50	2	9.89±0.31
Setback Viscosity (cP)	186.33±23.45	3	9.38±0.7
Peak Time (min)	3.9±0.03	4	9.15±0.23
Pasting temperature (°C)	75.15±0.05	5	8.92±0.12

Values expressed as mean ± SD (n=3).

Pasting properties of starch of amaranth are depicted in Table 2. Starch is widely applied in the food industry partially due to viscosity change of starch paste during heating and cooling. The pasting curve represents changes in behaviour of paste viscosity of starch with change in temperature and mainly varies with characteristics of starch. 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 temperature of starch was 75.15ºC, that was in consistent with the reported data, which were 75.7ºC and 75.1ºC for amaranth starch determined by Kong et al (2009) and Lee et al (1999) respectively. The point of maximum swelling of starch granules is indicated by peak viscosity. Break down viscosity is the measure of resistance of gel to disintegrate at high temperature. Peak viscosity (1212.66cP) and break down viscosity (371.66cP) of starch were lower than that reported (1662Cp and 870cP) by Qian and Kuhn (1999) for amaranth starch. Lower break down visosity of starch represents greater resistance to shear thinning and high stability of paste. Final viscosity represents the ability of starch to form a viscous paste, was found to 1032.33cP for starch. Increase in final viscosity might be due to the aggregation of amylose molecules (Miles et al, 1985). Set Back viscosity of starch is the measure of syneresis upon cooling of cooked paste and it was 186.33cP in present investigation. Comparatively higher SB was noticed in earlier studies (Choi et al, 2004; Kong et al, 2009) and) for amaranth starch.

TABLE 3. Thermal properties, color parameters of starch and size of amaranth starch granules

Thermal properties		Color parameters	
Onset temperature (ºC)	64.10±1.20	L*	101.94±0.26
Peak temperature (ºC)	75.9±0.4	a*	-0.06±0.0
Conclusion temperature (ºC)	89.1±1.0	b*	1.43±0.02
Δ H (J/g)	15.1±0.5	Diameter(µm)	1.33±0.26

Values expressed as mean ± SD (n=3).

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 starch was 101.94 which indicated high luminosity of starch. Negative a^* value of starch was indicative of

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presence of green tint. Positive b^* value indicated presence of yellow components in starch. Thus, in this investigation color of amaranth starch meet consumer preference due to high whiteness and low chroma values which are desired properties of starch. Differential scanning calorimetric studies of amaranth starch provided transition temperatures (T_0, T_P, T_C) and gelatinization enthalpy (ΔH) of starch of amaranth which are shown in Table 3. The gelatinization temperature of starch was (64.10ºC). The peak temperature for starch (75.9ºC) was slightly higher than paste temperature obtained by RVA (75.1ºC). Present investigation agreed with the study of Kong et al (2009) reported peak temperature ranged from 68ºC to 78ºC and onset temperature from 63ºC to 72ºC for fifteen cultivars of amaranth. During DSC analysis the transformed proportion of starch is reflected by the area under the endothermic peak, representing the enthalpy change (ΔH). The enthalpy of gelatinization of starch was (15.1 J/g) in the range registered in literature by other authors (Tapia-Blacido et al, 2010; Kong et al, 2009; Singh et al, 2014), however, it was quite different from other starches like wheat (3.15 J/g), corn (1.08 J/g), potato (2.32 J/g) and pea (3.09 J/g) reported by Li et al (2004). Higher value of enthalpy change of amaranth starch could be due the presence of higher amount of amylopectine than starches of other cereals compared above. Amylopectine is considered as primary participant in crystalline region (Robin et al, 1974). 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).

The microstructure of the starch revealed that the starch granules had a mean diameter of 1.33µm and typical micrographs show polygonal shaped granules (Fig. 1) with no fissures in the surface. The size and shape of granules were in agreement with previous reports (Radosavljevic et al, 1998; Choi et al, 2004; Kong et al, 2009). This is in agreement with Parades- Lopez (1994) who also suggested that amaranth starch can be utilized as excellent dispersion or coating material due to its large surface area per unit weight. The smooth surface of granules indicated that the activity of amylase was low in the seeds and no other isolation factors damaged the particles.

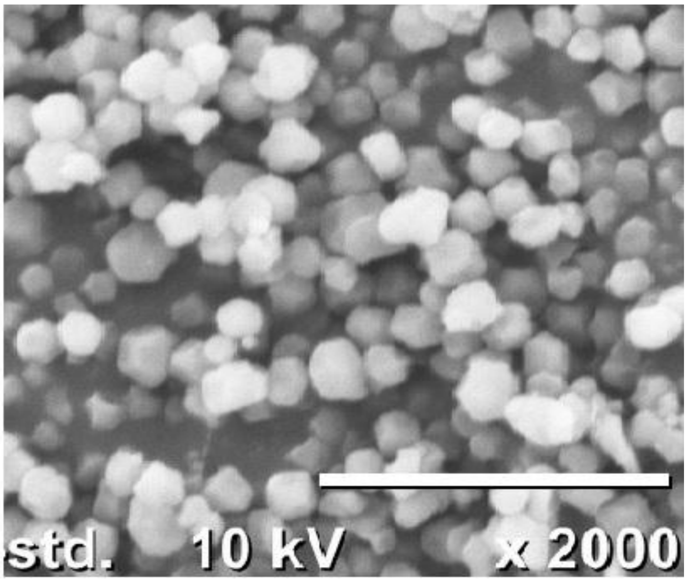

Fig. 1 Scanned image of starch granules of amaranth (X2000)

4. CONCLUSION

The physicochemical properties and functional characteristics of amaranth starch propose that it may have broad possibilities as an ingredient in food systems and other industrial application. Lower level of protein, crude fibre and ash content in starch confirmed the purity of starch. Wide range of gelatinization temperature range, good swelling power, solubility and water absorption capacity noticed for starch which makes it potentially useful in products subject to high temperatures. High clarity of starch paste suggests that it gives shine and opacity to the product. High luminosity noticed in starch is a most desirable property in food industry. Granules of amaranth starch were polygonal in shape and can find wide applications in food and non-food industries due to its extremely small size. Future work is necessary to characterise fine structures of amaranth starch of different origin and analyse their relationships with various properties of starch.

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