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Chemical, physicochemical, pasting and microstructural properties of amaranth (*Amaranthus hypochondriacus*) flour as affected by different processing treatments

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RESEARCH ARTICLE

Abstract

The effect of cooking, germination, and fermentation on the proximate composition, physicochemical, thermal, pasting and microstructural properties of amaranth flour was investigated. The data revealed that protein and amylose content of native and processed amaranth flours ranged from 14.86 to 16.19% and 2.34 to 7.05% respectively. The protein content increased significantly ($P \leq 0.05$) after germination and fermentation. However, a significant ($P \leq 0.05$) decrease in amylose content was observed after various processing treatments. Processing treatments (cooking, germination, and fermentation) significantly ($P \leq 0.05$) reduced the tannin and phytate content and significantly ($P \leq 0.05$) increased the total phenol content. Also, given processing treatments had varied effect on water absorption capacity, oil absorption capacity, swelling power and solubility, thermal properties and pasting properties. Shape and size of granules were determined by scanning electron microscopy and size of granules ranged from 0.5 to 1μm. The cooked amaranth showed the lowest value whereas fermented amaranth showed the highest value for peak viscosity, hot paste viscosity and cold paste viscosity.

Keywords: germination, cooking, fermentation, thermal properties, pasting properties

1. Introduction

Pseudocereals include starchy food grains – mainly buckwheat, amaranth and quinoa – and excluding the currently defined cereals, legumes or nuts (Fletcher, 2004). Higher content of lysine and sulphur containing amino acids like methionine (Becker *et al.*, 1981; Bressani *et al.*, 1987), higher content of minerals like calcium, magnesium, iron and zinc (Alvarez-Jubete *et al.*, 2010) and higher antioxidant activity (Escudero *et al.*, 2011; Klimczak *et al.*, 2002) make amaranth grains nutritionally superior to conventional cereal grains like wheat. The last 20 years have witnessed a growing number of systematic research on pseudocereals based gluten-free products and other composite flour-based product development. A variety of processing treatments like cooking, germination and fermentation are applied to pseudocereals to enhance their nutritional value by suppressing the antinutritional factors. Amaranth grains are traditionally germinated for sprouts and malted for the production of traditional *chicha* beer in Peru and fermented by lactic acid bacteria to produce *ogi* in Africa. Grain can be milled into flour to use in different mixtures for pancakes, bread, muffins, crackers, cookies, puddings, etc. (Bejosano and Corke 1998; Berghofer and Schoenlecher, 2002). These treatments not only improve the nutritional quality and consumption of pseudocereals but also affect their techno-functionality in the processed foods.

Amaranth has been extensively studied and a voluminous literature exist, particularly on the nutritional qualities, crop breeding and production and processing methods, development and commercialisation of new amaranth products. This includes many studies on the impact of the processing treatments like germination (Afify *et al.*, 2012; Chauhan and Singh, 2013; Colmenares de Ruiz and Bressani, 1990; Perales-Sanchez, 2014), cooking (Afify *et al.*, 2012; Amare *et al.*, 2016) and fermentation (Afify *et al.*, 2012; Amare *et al.*, 2016) on nutritional quality characteristics of amaranth grain, but only few systematic studies have been carried out to assess the impact of processing treatments,

particularly germination, fermentation and autoclaving on the functional, thermal and pasting properties of amaranth flour. Effect of extrusion cooking on the physicochemical and other functional properties of amaranth flour has been studied and higher water absorption, water retention and lower final viscosity have been reported for the extruded amaranth flour (Choi *et al.*, 2004; Mendoza and Bressani, 1987; Menegassi *et al.*, 2011). Flour and starch obtained from dry heat treatments like roasting and popping of the amaranth grains have been reported to exhibit significantly higher peak viscosity (Choi *et al.*, 2004; Muyonga *et al.*, 2014). The physicochemical parameters like water absorption index, particle size index and water solubility index of the nixtamalized amaranth flour were reported to be dependent on the cooking temperature with first two parameters being positively correlated with the temperature (Valdez-Niebla *et al.*, 1993). The effect of germination on the nutritional and functional properties of amaranth flour has been studied by Chauhan and Singh (2013), Chauhan *et al.* (2015), Choi *et al.* (2004) and Olawoye and Gbadamosi (2017). These studies have shown contradictory results regarding the impact of germination on water absorption and solubility index of amaranth flour. The fermentation of the amaranth grains has been reported to significantly affect/improve the protein digestibility, antioxidant activity and mineral content of amaranth grains (Olawoye and Gbadamosi, 2017). The physicochemical properties, functional properties and starch digestibility of amaranth based millet blends have been reported to be greatly improved by fermentation (Simwaka *et al.*, 2017). Since, the studies regarding the effect of cooking, germination and fermentation on the physicochemical, pasting and microstructural properties of amaranth flour are still inconclusive, the present investigation aimed to study the effect of these conventional processing treatments on chemical composition, physicochemical properties, thermal properties, pasting properties and microstructural properties of amaranth flour.

2. Materials and methods

Amaranth (*Amaranthus hypochondriacus*) grains were purchased from Vivekanand Parvatiya Krishi Anusandhan Sansthan (VPKAS), Almora, Uttarakhand, India.

Cooking

The method of Jood *et al.* (1998) was used for cooking of amaranth seeds. The seeds were soaked for 12 h in water at room temperature (1:3, w/v), cooked (1:2, w/v) in an autoclave (10 min at 15 psi pressure) and then dried for 14 h at 40 °C. After drying, seeds were ground, packed and stored in air-tight containers.

Fermentation

The method of Hassan *et al.* (2006) was used for fermentation of amaranth seeds. The amaranth seeds were milled and flour was dispersed into distilled water (1:3, w/v). The dispersion was incubated (37 °C, 16 h), followed by drying in a hot air oven (40 °C, 14 h), milled, packed and stored in an airtight container.

Germination

The germination of seeds was done using the method explained by Colmenares de Ruiz and Bressani (1990). The amaranth seeds were soaked at ambient temperature in distilled water (1:5, w/v) for 12 h and kept at 28 °C for 48 h for germination. Then germinated seeds were dried in hot air oven (40 °C, 14 h), milled, packed and stored in airtight containers.

Chemical composition

The moisture, fat, ash and crude fibre content were determined using the standard AOAC methods (1990). The crude protein was measured using the standard micro-Kjeldahl method. The apparent amylose content was estimated using the method explained by Williams *et al.* (1970).

Total phenols

The total phenol content of samples was measured using the Folin-ciocaltu reagent method (Zhou and Yu, 2006). The flour was defatted using n-hexane solvent for 6 h with continuous shaking. The defatted flour sample (200 mg) was dispersed in 100 ml of 1% HCl. The suspension was refluxed for 2 h, cooled, filtered and volume was made up to 200 ml with acid-methanol. The distilled water (7.5 ml) and Folin-ciocaltu reagent (0.5 ml) was added to the extract (0.2 ml) and mixed. Then saturated sodium carbonate solution (1 ml) was also added and volume was made up to 10 ml with distilled water. After 30 min, the absorbance of the solution was measured at 760 nm.

Phytate content

Phytate content was measured according to the procedure by Haug and Lantzsch (1983). Sample (0.06 g) was dispersed in 0.2 N HCl (10 ml) solution and kept for half an hour with intimate shaking. The 2 ml of ferric solution was added to 1 ml of extract into the test tube and covered with a stopper. The solution was heated for 30 min in a water bath, cooled to room temperature. To the content, 4 ml of 2, 2-bipyridine solution was added, mixed and the absorbance of the sample was measured using spectrophotometer at 519 nm.

Tannin content

Tannin content of samples was analysed using the method of Prince *et al.* (1978) with slight modifications. 1 ml of methanolic extract was added to 5 ml vanillin reagent, mixed and kept at 30 °C in a thermostat controlled water bath. Blank was prepared using 4% HCl in methanol, instead of vanillin reagent. The contents were kept at 30 °C for 20 min and absorbance was measured at 500 nm.

Physicochemical properties

Water and oil absorption capacity of flour samples were measured using the method of Sosulski *et al.* (1976). The least gelation concentration (LGC) was measured according to the method of Adebowale and Lawal (2003). The bulk density of flour samples was measured using the method of Chau and Huang (2003). The method of Waliszewski *et al.* (2003) was used for estimation of swelling power and solubility. The swelling power and solubility of all the samples were measured at 55, 65, 75, 85, and 95 °C.

Thermal properties

Differential scanning calorimeter (DSC 200, TA Instruments, DE, USA) was used to analyse the thermal properties of native and processed amaranth flour. Flour (3.5 mg, dry weight basis) was poured into an aluminium pan and distilled water was added to form a suspension containing 70% water. Samples pans were hermetically sealed and kept at room temperature for 1 h before heating in the DSC. Sample pans were heated at a rate of 10 °C/min from 20 to 120 °C.

Pasting properties

Pasting properties of flour samples were determined using a rapid visco-analyser (RVA) (Starch Master TM, Newport Scientific, Warriewood, Australia) according to the method of Yadav *et al.* (2012). The slurry was prepared by dispersing a weighed amount of sample (3.0 g) in distilled water (25.0 ml) and stirred in container initially at 960 rpm for 10 s and finally at 160 rpm for the remaining test.

Scanning electron microscopy

Flour samples were sprinkled over double-sided silver tape attached to the stub and coated with gold-palladium. Scanning electron micrographs were viewed using a scanning electron microscope (Zeiss EVO 50, Carl Zeiss SMT Ltd., Cambridge, UK) operated at an accelerating voltage of 20 kV and at 5,000× magnification.

Statistical analysis

All observations were taken in triplicate and analysed statistically using one and two way ANOVA (SPSS 19.0, IBM Corporation, Armonk, NY, USA) followed by Tukey's HSD post hoc comparison test at $P<0.05$.

3. Results and discussion

Chemical composition

The chemical composition of the native and processed amaranth flours is given in Table 1. The ash content of native amaranth flour was 2.93% and germination and cooking did not affect the ash content significantly. However, fermentation reduced the ash content significantly to 1.66% ($P\leq0.05$). The fat content of the native amaranth grains was 7.33%, which decreased slightly after processing of the grains. Amaranth grain has higher lipid content than most cereal grains (Silva *et al.*, 2009). The protein content of native amaranth flour was 15.27%. Burgos and Armada (2015) also reported a similar value for the protein content of native amaranth flour. A significant ($P\leq0.05$) increase in protein content was observed after germination (16.19%) and fermentation (15.75%). The increase of protein content in germinated and fermented amaranth might be due to the synthesis of amino acids by the biochemical activity of enzymes in sprouted seeds and microorganism activity during fermentation. The activity of protease enzyme increases during germination which causes the breakdown of peptide component to amino acids and resulting into increase in protein content (Narsih *et al.*, 2012). A similar increase in protein content after germination and fermentation was reported for amaranth flour (Chauhan and Singh, 2013). Several other researchers also reported the increase in protein content after germination (Inyang and Zakari, 2008; Mostafa and Rahma, 1987; Mubarak 2005).

Table 1. Chemical composition of native and processed amaranth flour.

Amaranth flour	Moisture (%)	Ash (%)	Fat (%)	Crude fibre (%)	Protein (%)	Amylose (%)
Native	9.51±0.29^{b}	2.93±0.18^{b}	7.33±0.23^{b}	2.04±0.04^{b}	15.27±0.28^{ab}	7.05±0.10^{c}
Cooked	8.82±0.14^{a}	2.06±0.10^{b}	6.42±0.34^{a}	2.01±0.19^{b}	14.86±0.18^{a}	2.34±0.04^{a}
Fermented	9.03±0.06^{a}	1.66±0.11^{a}	7.20±0.34^{b}	1.84±0.12^{a}	15.75±0.46^{bd}	6.32±0.25^{b}
Germinated	9.05±0.05^{a}	2.20±0.25^{b}	6.51±0.09^{a}	2.19±0.07^{b}	16.19±0.22^{cd}	5.99±0.02^{b}

However, the cooked amaranth flour had the lowest value for protein content. This decrease in the protein content of cooked amaranth flour might be due to the leaching out of the nitrogenous substances into the cooking water (Osman, 2004). Sharma *et al.* (2013) and Hefnawy (2011) also reported the decrease in protein content after cooking. The amylose content of native and processed amaranth flours ranged from 2.34 to 7.05%. The results are in agreement with the reported results of Kong *et al.* (2009). Amaranth starch mostly contains amylopectin (88.9 to 99.9%) making it 'waxy type' starch characterised by high viscosity and high gelatinisation temperature in contrast to normal starches (Silva *et al.*, 2009). A significant ($P \leq 0.05$) decrease in amylose content was observed after processing treatments. This decrease in amylose content after germination might be due to fact that α-amylase activity is increased during germination of grains. This increased activity of enzyme hydrolyses amylose and amylopectin to dextrins and maltose. The decrease in amylose content of starch in the present study was in agreement with the previous studies on various starches obtained from germinated grains/seeds (Frias *et al.*, 1998; Li *et al.*, 2017). However, decrease in amylose content in cooked amaranth flour might be due to the increased solubility of amylose with heating and leaching of soluble amylose in cooking water (Bibi *et al.*, 2011). A similar decrease in amylose content after various processing treatments including soaking, cooking, germination and fermentation was reported in sorghum varieties (Afify *et al.*, 2012). The decrease in amylose content in the starch obtained from germinated grains can improve the digestibility of starch (Frias *et al.*, 1998).

Total phenol, phytate and tannin content

The data pertaining to total phenol, phytate and tannin content of native and processed amaranth flours are presented in Table 2. The total phenol content of native and processed amaranth flour varied from 2.59 to 5.76 mg/g and it was in the range as reported in the earlier study by Muyonga *et al.* (2014). A significant ($P \leq 0.05$) increase in total phenol content was observed after germination (26.7%) and fermentation (45.4%). The fermented amaranth had highest total phenol content (5.76 mg/g) whereas cooked amaranth had lowest phenol content (2.59 mg/g). The increase in phenol content after fermentation might be due to the formation of new bioactive compounds by the metabolic activity of microbes (Dordevic *et al.,* 2010). The liberation or synthesis of various bioactive compounds also occurred due to rupture of cell wall during fermentation (Katina *et al.,* 2007). The increase in phenolic content after germination might be due to activation of endogenous enzymes and the biochemical metabolism during germination (Duenas *et al.,* 2009). A similar increase in phenolic content after germination was reported for lupines (Duenas *et al.,* 2009). The phytate content of native and processed amaranth flour ranged from 1.85 to 2.91 mg/g. A significant ($P \leq 0.05$) reduction in phytate content was observed after processing treatments. The germinated amaranth flour had the lowest value of phytate content (1.85 mg/g) followed by fermented (2.48 mg/g) and cooked (2.65 mg/g) amaranth flour. The decrease in phytate content during germination and fermentation might be due to the hydrolytic activity of the phytase enzyme. The hydrolysis of phytate phosphorus into inositol monophosphate due to the activity of phytase causes the decrease in phytic acid.

Ibrahim *et al.* (2005) also reported a decrease in phytate content for sorghum after fermentation. The tannin content of native and processed amaranth flour ranged from 0.27 to 0.80 mg/g. A significant ($P \leq 0.05$) decrease in tannin content was observed after processing treatments. Cooking was found to be most effective in reducing the tannin content (66.2%), which was in conformation with reports of earlier studies on pseudocereals like amaranth, buckwheat and quinoa (Olawoye and Gabadamosi, 2017) and legumes (Khattab and Arntfield, 2009). This decrease in tannin content in the cooked amaranth flour can be ascribed to leaching out of the water-soluble compounds (Kumar *et al.*, 2010), heat degradation of the heat labile compounds (Olawoye and Gabadamosi, 2017). Germination and fermentation of amaranth grain reduced its tannin content by 36.2 and 57.5% respectively. The decrease in tannin content might be due to the formation of a hydrophobic association of tannins with seed proteins and enzymes after germination (Megat Rusydi and Azrina, 2012) and due to the leaching of tannins into the soak water (Shimelis and Rakshit, 2007). Megat Rusydi and Azrina (2012) also observed a decrease in tannin content for germinated soybean and peanut. The decrease in tannin content after fermentation may be due to their binding ability with cotyledon endosperm (Emambux and Taylor, 2003). Germination reduced the phytate content of amaranth

Table 2. Total phenols, phytate content and tannin content of native and processed amaranth flour.

Amaranth flour	Total phenol content (mg/g)	Phytate content (mg/g)	Tannin content (mg/g)
Native	3.96 ± 0.01^{b}	2.91 ± 0.01^{d}	0.80 ± 0.03^{c}
Cooked	2.59 ± 0.02^{a}	2.65 ± 0.01^{c}	0.27 ± 0.01^{a}
Fermented	5.76 ± 0.03^{d}	2.48 ± 0.00^{b}	0.34 ± 0.02^{a}
Germinated	5.02 ± 0.03^{c}	1.85 ± 0.01^{a}	0.51 ± 0.03^{b}

grain most significantly (36.4%) followed by fermentation (14.7%) and cooking (9.0%). Germination and fermentation also affected the total phenolic compounds of amaranth grain more significantly in comparison to cooking and total phenolic content was increased by germination and fermentation to an extent of about 45%. The biosynthesis of phenolic compounds caused by enzyme hydrolysis during germination or fermentation could be ascribed to the higher total phenolic content of germinated and fermented amaranth flours compared to non-germinated amaranth flours (He *et al.*, 2011). However, cooking decreased the total phenol content by 34.5%. A similar decrease in total phenolic content after cooking has been reported by Muyonga *et al.* (2014) for amaranth grain. The reduction in total phenol of cooked amaranth grains might be due to degradation of these components by heat or leaching into the cooking medium (Dykes and Rooney, 2006; Khandelwal *et al.,* 2010).

Physicochemical properties

Physicochemical properties of native and processed amaranth flour are presented in Table 3. Water absorption capacity (WAC) varied significantly ($P<0.05$) among different flours and it ranged from 2.09 to 2.73 g/g. A significant ($P\leq0.05$) increase in WAC was observed after germination and cooking. However, a decrease in WAC was observed after fermentation treatment. A similar decrease in WAC has been reported for fermented sorghum, pearl millet and maize (Singh *et al.*, 2012). The increase in WAC of cooked amaranth was probably due to the unfolding of the protein structures during heating, resulting in the exposure of more hydration sites, thereby making them available to interact with water. Increase in WAC in germinated amaranth might be due to the modification of macromolecules and production of new degradation products like simple soluble sugars having good water absorption capacity (Adedeji *et al.*, 2014). Germination and cooking have been reported to increase the water absorption capacity of flours (Chauhan and Singh, 2013; Gamel *et al.*, 2006). Since flour with high WAC is preferred as an ingredient in bakery products like bread, the cooked amaranth flour can be used in bread formulation for promoting dough handling features and freshness of the bread. Oil absorption capacity (OAC) of native and processed flours ranged from 2.02 to 2.52 g/g. A significant ($P\leq0.05$) increase in OAC was observed after processing treatments. The germinated amaranth had highest (2.52 g/g) whereas native amaranth had lowest (2.02 g/g) value for OAC. The increase in OAC after germination and fermentation might be due to the entrapment of oil related to the non-polar side chains of proteins.

The increase in OAC of the fermented amaranth flour is in line with the findings of Singh *et al.* (2012) and Oloyede *et al.* (2016) who reported that OAC of pearl millet and maize flour and moringa flour increased with fermentation. Surface availability of hydrophilic amino acids and other non-polar amino acid chains in the processed amaranth are the major causes for the OAC of flour. The LGC is the index of gelation properties which depends on the amount of starch and pasting properties of starch. A significant ($P\leq0.05$) increase ranging from 57.1 to 85.7% was observed in the LGC of processed amaranth flours. Cooked amaranth had highest LGC followed by fermented and germinated amaranth flour. The increase of LGC during fermentation might be due to the enzymatic hydrolysis of the carbohydrates and proteins. A similar increase in LGC after fermentation was reported for millet and pigeon pea (Onweluzo and Nwabugwu, 2009) and moringa flour (Oloyede *et al.*, 2016). The amylase enzyme released during germination interacted with the starch component of flour, resulting in to increase of gelation property. A similar increase in LGC was observed in germinated pearl millet (Fasasi, 2009). The bulk density of native amaranth flour was 0.54 g/ml and it compared favourably with the result as reported by Chauhan and Singh (2013). A significant ($P\leq0.05$) increase in the bulk density up to an extent of 37% was observed after cooking and fermentation treatments, which is in line with the decreased bulk density of the fermented samples as reported by Simwaka *et al.* (2017) but in contradiction to the findings of El-Khalifa *et al.* (2005) where bulk density decreased after fermentation. However, it decreased with the germination of amaranth grains. Chauhan and Singh (2013) also reported a decrease in bulk density after germination for amaranth grains.

Swelling power and solubility

The swelling power of native and processed amaranth flours was determined over a temperature range of 50-90 °C at an interval of 10 °C (Figure 1A). The swelling power and

Table 3. Physicochemical properties of native and processed amaranth flour.

Amaranth flour	Water absorption capacity (g/g)	Oil absorption capacity (g/g)	Least gelation concentration (%)	Bulk density (g/ml)
Native	2.26±0.01^{a}	2.02±0.07^{a}	14±0.00^{a}	0.54±0.02^{b}
Cooked	2.70±0.14^{b}	2.30±0.02^{b}	26±0.00^{d}	0.67±0.01^{c}
Fermented	2.09±0.03^{a}	2.20±0.09^{b}	24±0.00^{c}	0.74±0.01^{d}
Germinated	2.73±0.13^{b}	2.52±0.01^{c}	22±0.00^{b}	0.50±0.01^{a}

solubility describe the interactions of the polymeric chains made up of amorphous and crystalline granule fraction. The swelling power of all the samples increased with a rise in temperature with maximum swelling at 90 °C. The increase in temperature and vigorous vibration break the intermolecular bonds of the starch and thereby allowing the hydrogen bonding sites to accommodate more water molecules (Claver *et al.,* 2010). The swelling power of native and processed amaranth flour ranged from 3.58 to 7.55 g/g (at 90 °C). A significant ($P \leq 0.05$) reduction in swelling power was observed after processing. The lowest swelling power was observed for cooked amaranth (3.58 g/g, at 90 °C) followed by fermented (4.88 g/g, at 90 °C) and germinated amaranth (4.20 g/g, at 90 °C). A similar decrease in swelling power after fermentation was reported for sorghum, pearl millet and maize (Singh *et al.*, 2012). Germination decreases the swelling power probably due to disruption of hydrogen bonds by amylases and proteases into sugars and amino acids.

The effect of temperature on the solubility of native and processed amaranth flours are presented in Figure 1B. The solubility of native and processed amaranth flour varied from 1.62 to 3.58 g/10 g (at 90 °C). A significant ($P \leq 0.05$) increase in solubility was observed in cooked (3.58 g/10 g, at 90 °C), fermented (2.93 g/10 g, at 90 °C) and germinated (1.92 g/10 g, at 90 °C) amaranth. The increase in solubility after cooking treatment might be due to the heating effect that degraded the starch and produced more soluble molecules. A similar increase in solubility after fermentation was reported for *Moringa* seeds (Oloyede *et al.*, 2016).

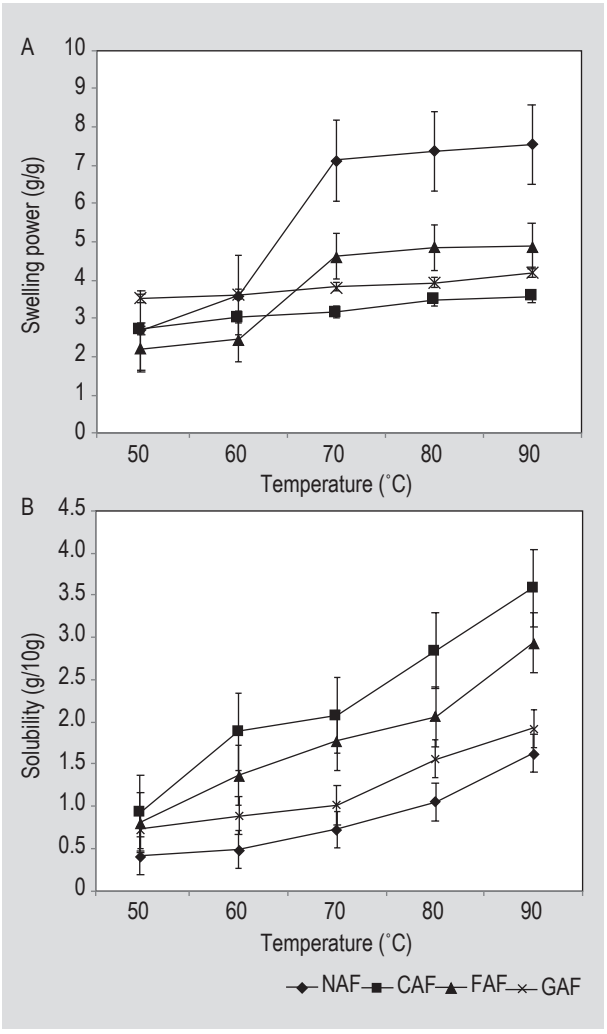

Figure 1. Swelling power (g/g) (A) and solubility (g/10 g) (B) of native and processed amaranth flour as affected by temperature. (CAF = cooked amaranth flour; FAF = fermented amaranth flour; GAF = germinated amaranth flour; NAF = native amaranth flour).

Thermal properties

The gelatinisation thermograms of native and processed flour are shown in Figure 2A. The peak temperature (T_p) for native and processed amaranth flour ranged from 68.13 to 70.67 °C (Table 4). The fermented amaranth showed highest T_p followed by germinated and native amaranth flour. The higher T_p of fermented and germinated samples coincided with their higher pasting temperatures obtained by RVA. The peak temperature for the native amaranth flour was comparable to the results of previous studies, which have reported the gelatinisation temperature of amaranth starch in the range from 68 to 78 °C, higher than other cereal grains (Baker and Rayas-Duarte, 1998; Kong *et al.*, 2009). Onset (T_o), peak (T_p) and conclusion (T_c) temperature of fermented amaranth were significantly. The enthalpy of gelatinisation (ΔH_{gel}) was observed to be highest (1.22 J/g) for native amaranth flour, whereas germinated amaranth showed the lowest value (0.86 J/g). The increase in T_p after fermentation might be due to the growth of natural bacteria releasing more proteolytic enzymes which cause the disruption of grain cell walls releasing more starch hence resulting in more crystalline structural proportions. A similar increase in T_p after fermentation was reported for foxtail millet flour (Amadou *et al.*, 2014) and a decrease in ΔH_{gel} for fermented rice flour (Lu *et al.*, 2005). No endothermic peak of starch gelatinisation in the cooked amaranth flour was observed, indicating that the starch was fully gelatinised during cooking.

Pasting properties

The data pertaining to pasting properties of native and processed amaranth flour analysed by RVA is presented in Table 5 and Figure 2B. The peak viscosity ranged from 56 to 477 cp, lowest for cooked amaranth flour and highest for fermented amaranth. Peak viscosity is the ability of starch to swell freely before their physical break down. The increase in viscosity after fermentation could be attributed to the increase in protein content

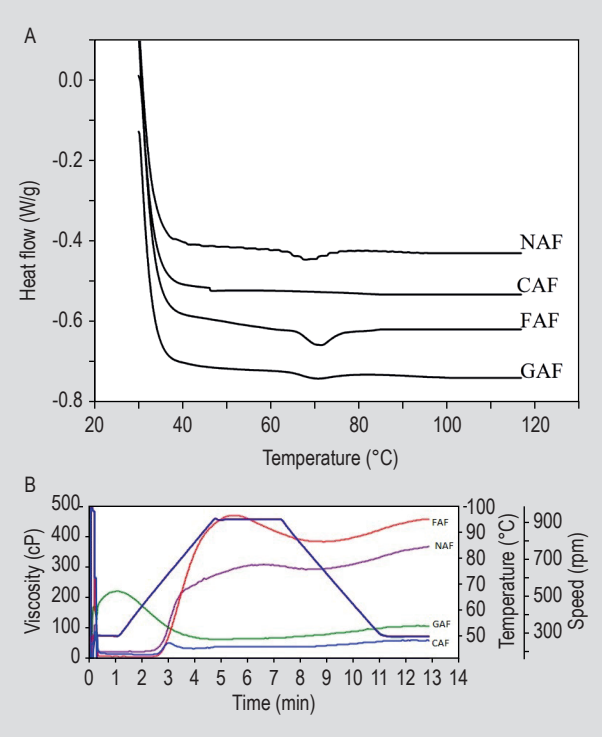

Figure 2. Differential scanning calorimeter thermograms (A) and pasting profiles (B) of native and processed amaranth flour. (CAF = cooked amaranth flour; FAF = fermented amaranth flour; GAF = germinated amaranth flour; NAF = native amaranth flour).

of amaranth flour after fermentation (Amadou *et al.*, 2014). Zhu *et al.* (2010) reported that due to an increase in protein content, some proteins could protect starch granules from being broken and resulted in increased pasting viscosity. Oloyede *et al.* (2016) also observed an increase in pasting viscosity of *Moringa* seed flour after fermentation. The HPV ranged from 42 cp for cooked amaranth to 390 cp for fermented amaranth. The CPV varied from 64 cp to 465 cp. The fermented amaranth flour showed significantly ($P \leq 0.05$) higher value for HPV and CPV while cooking and germination resulted in a significant ($P \leq 0.05$) reduction of HPV and CPV. This might be because of the high temperature during cooking could complete the gelatinisation of the starch in seeds. The reduction in paste viscosities of germinated amaranth flour might be due to the enzymatic degradation of starch during the germination process (Jan *et al.,* 2016). The results of reduced viscosity in germinated seeds are in agreement with the previously reported results for wheat (Juhasz *et al.*, 2005) and brown rice (Xu *et al.,* 2012). Also, the decrease in viscosity of germinated amaranth flour may be desirable for the preparation of weaning foods.

Fermented amaranth flour paste exhibited a significant ($P<0.05$) increase in its PV, HPV and CPV. The cooked amaranth showed the lowest value whereas fermented amaranth showed the highest value for peak viscosity, HPV

Table 4. Thermal properties of native and processed amaranth flour.[1]

Amaranth flour	T_o (°C)	T_p (°C)	T_c (°C)	ΔH_{gel} (J/g)	PHI	R
Native	63.73 ±0.20^a	68.13±0.10^a	76.37±0.17^a	1.22±0.12^b	0.28	12.64
Cooked	–	–	–	–	–	–
Fermented	65.58±0.10^c	70.67±0.26^b	78.37±0.33^b	1.16±0.10^b	0.22	12.79
Germinated	64.47±0.26^b	70.54±0.20^b	76.68±0.12^a	0.86±0.09^a	0.13	12.21

[1] T_o, T_P, and T_c = onset, peak and conclusion temperature, respectively; ΔH_{gel} = enthalpy of gelatinisation; PHI = peak height index = ΔH_{gel} / ($T_p - T_o$); R = gelatinisation range ($T_c - T_o$).

Table 5. Pasting properties of native and processed amaranth flour.[1]

Amaranth flour	PV (cp)	HPV (cp)	CPV (cp)	BD (cp)	SB (cp)	P_{time} (min)	P_{Temp} (°C)
Native	312±16.52^c	293±13.01^c	366±15.63^c	18±3.78^b	73±2.88^c	6.49±0.10^d	72.53±0.16^a
Cooked	56±3.60^a	42±1.52^a	64±3.21^a	13±2.08^a	22±1.73^a	1.10±0.04^a	-
Fermented	477±6.35^d	390±5.77^d	465±5.77^d	86±0.56^c	75±0.01^d	5.04±0.01^b	74.28±0.02^b
Germinated	228±8.66^b	67±2.30^b	109±3.46^b	156±3.46^d	44±3.46^b	2.95±0.04^b	74.15±0.06^b

[1] BD = breakdown; CPV = cold paste viscosity; HPV = hot paste viscosity; $P_{Temp.}$ = pasting temperature; P_{time} = pasting time; PV = peak viscosity; SB = setback.

and CPV. This might be because of the high temperature during cooking could complete the gelatinisation of the starch in amaranth. PV and other viscosity parameters were not very pronounced for cooked flour, which is indicative of the molecular and structural degradation of the starch granules during cooking (Ilo *et al.*, 1999). This was also found in several other studies (Gutkoski and El-Dash, 1999; Menegassi *et al.*, 2007). The breakdown viscosity of native and processed amaranth flour ranged from 13 to 156 cp. Breakdown viscosity value is the measure of the resistance of swollen granules to disintegrate at high temperature and hence an indicator of the stability to shear thinning of the flour product (Yadav *et al.*, 2011). A significant (P<0.05) increase in breakdown viscosity was observed after fermentation and germination. The increased breakdown value in fermented amaranth would enable easy cooking but, susceptible to stress when processed into the solid form. Thus, germination and fermentation make the amaranth flour more susceptible to disintegration while cooking and shearing making it unsuitable to be used for the products that are likely to be processed under severe cooking conditions. The temperature at which viscosity of starch begins to develop is called pasting temperature. The pasting temperature of the flour samples varied between 72.53 to 74.28 °C. Kong *et al.* (2009) reported values for pasting temperature in the range of 63.4 to 74 °C for amaranth flour. A significant increase (P<0.05) in pasting temperature of amaranth was observed after fermentation and germination. The higher pasting temperature of the flours prepared from germinated and fermented amaranth can be attributed to the reduced swelling power of germinated and fermented flours. A similar increase in pasting temperature was reported for *Moringa oleifera* seed flour (Oloyede *et al.*, 2016).

Scanning electron microscopy

Scanning electron micrographs of native and processed amaranth flour are shown in Figure 3. Morphological characteristics like size, shape and distribution of granules

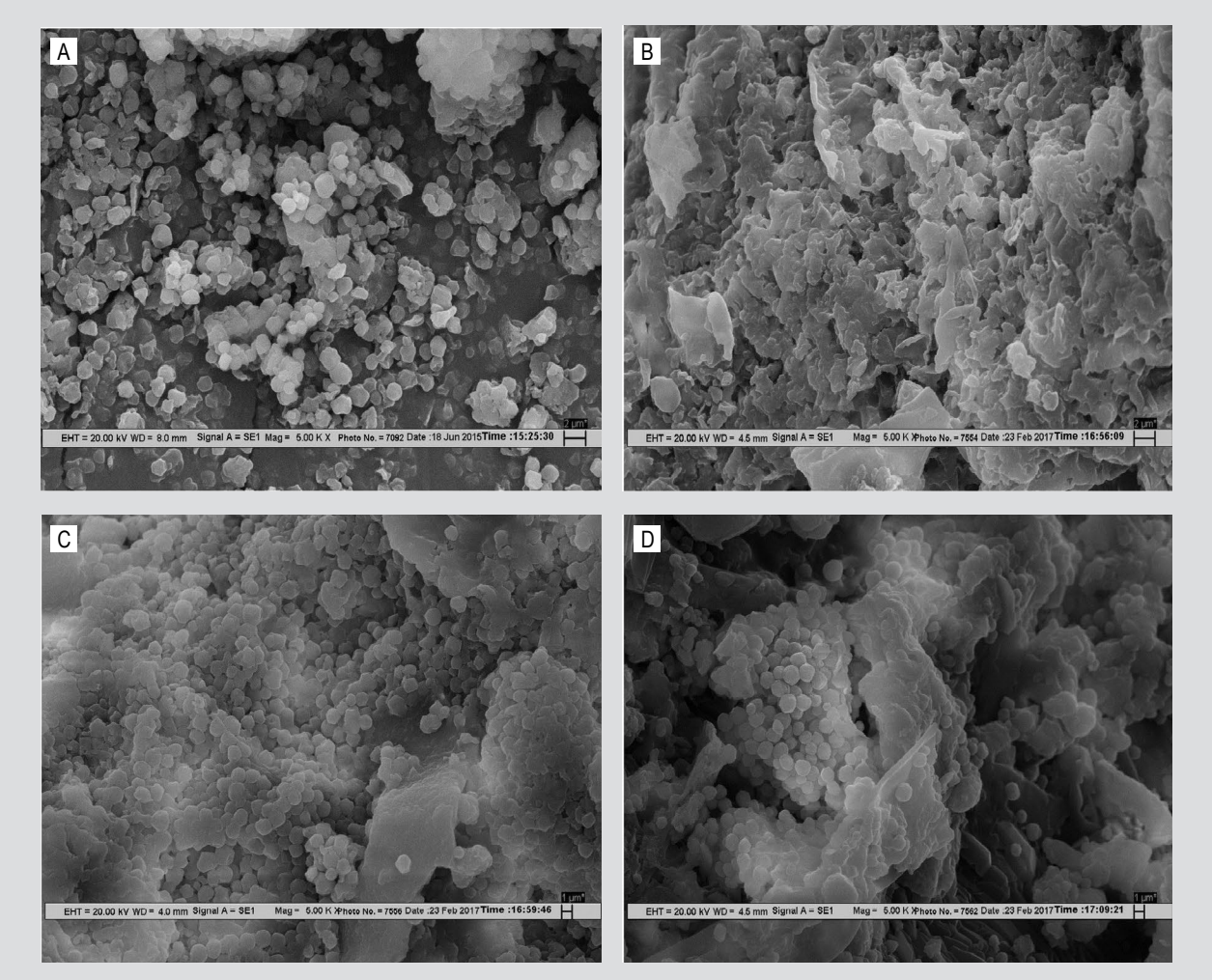

Figure 3. Scanning electron micrographs of native and processed amaranth flour. (A) Native amaranth flour, (B) cooked amaranth flour, (C) fermented amaranth flour, and (D) germinated amaranth flour.

are attributed to the biological origin of the grain. The native flour showed a continuous structure with intact starch granules. The structure revealed the polygonal, oval or spherical shape of granules. As regard to size, the diameter of granules of native amaranth flour ranged from 0.5 to 1 μm. The absence of intact starch granules in the micrograph of cooked amaranth flour might be due to the starch gelatinisation during heat treatment. Uthumporn *et al.* (2016) also reported some structural changes in heat treated samples. The continuous structure was also destroyed in the micrograph of amaranth after germination. This might be due to degradation of starch during germination due to the activity of enzymes (Jan *et al.,* 2016). Scanning electron micrographs of treated flour showed the structural changes in starch granules. These structural differences clearly reflect the influences of processing treatments on amaranth flour that resulted in granule disruptions.

4. Conclusions

The results of the study revealed that the processing treatments like cooking, germination and fermentation considerably affect the chemical composition, physicochemical, functional, pasting and thermal properties of amaranth flour. These processing treatments not only affected the chemical composition of the amaranth flour but also reduced the antinutritional components like tannins and phytates. The results showed that germination of amaranth flour enhances phenolic content and therefore, germinated amaranth grains can be used as an active ingredient in functional foods and pharmaceuticals. Water absorption, oil absorption, LGC, solubility and bulk density of amaranth flour got improved by processing treatments, which increase the suitability of amaranth flour to be used in formulations for processed products like bread and biscuits. Fermentation and germination of amaranth improved its thermal transition temperature with fermentation showing more pronounced effect. The pasting properties of amaranth flour were modified to a great extent by processing as cooking resulted in lowest pasting viscosities and pasting temperature whereas fermentation resulted in the most pronounced increase in all the pasting parameters contrary to the lower pasting viscosities of germinated samples than raw samples. It is therefore concluded that processing treatments like cooking, germination and fermentation could alter the chemical composition, pasting, thermal and microstructural properties of amaranth flour and therefore, these treatments can be exploited to manipulate the chemical composition, physicochemical, pasting and thermal properties of the amaranth flour desirably.

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