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Effect of heat-moisture-treated cassava starch and amaranth malt on the quality of sorghum-cassava-amaranth bread

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Gluten-free batter and bread was prepared from modified cassava starch, sorghum and amaranth flour. Heat-moisture treatment (80°C, 18, 24 or 30% moisture content, and 8, 16 or 24 h incubation) of cassava starch increased its crystallinity, onset pasting temperature and water absorption index; and decreased its swelling power, water solubility index and peak viscosity. Heat-moisture treated cassava starch was made into batter with sorghum and amaranth in the ratio of 50:40:10. The other ingredients, weighed on flour-weight-basis, were water (75%), sugar (6.7%), fat (2%), salt (1.7%), and amaranth malt at 0, 1 or 2.5%. The interaction effect (that is, starch modification x amaranth malt) significantly ($P \leq 0.05$) affected batter consistency. Breads made from heat-moisture treated starch were softer than those containing native starch. The interaction effect (that is, starch modification x amaranth malt) was significant ($P \leq 0.05$) only for the texture profile analysis property of cohesiveness. Further investigation of the effect of heat-moisture treatment showed that the interaction effect (that is, moisture content x incubation time) significantly ($P \leq 0.05$) affected only the Texture Profile Analysis properties of hardness and chewiness.

Key words: Amaranth, cassava, gluten-free bread, heat-moisture treatment, malt, sorghum.

INTRODUCTION

As a continent, Africa is the largest producer of sorghum, with sorghum representing around 70% of the cereals produced in West Africa, 30% in East Africa, and 10% in Southern Africa (Dendy, 1995). More than 252 million metric tons of cassava was produced worldwide in 2011, of which Africa accounted for 56% (FAO, 2011). The prevalence of these crops in Africa is because they are well adapted to hot semi-arid regions, and their ability to resist drought makes them especially valuable foods in these areas which suffers from chronic food shortages. Thus, nutritional intervention through development of processed products, such as bread, from these crops can reach out to millions of people in the region. Viewed from a health perspective, the lack of gluten in these crops

makes them safe for people suffering from coeliac disease (Schober, 2009; Schoenlechner et al., 2008).

The manufacture of gluten-free bread is technologically challenging due to the lack of gluten in the gluten-free flours. Consequently, starch is the most important structure-forming ingredient in gluten-free breads (Onyango et al., 2011a; Onyango et al., 2011b; Schober, 2009). Addition of pure starch to gluten-free sorghum bread causes gelatinization to occur more readily and completely, resulting in an early increase in batter viscosity, and facilitating the development of a cohesive and consistent crumb network that traps gas bubbles and prevents loss of carbon dioxide and crust collapse (Schober et al., 2007; Taylor et al., 2006).

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It also dilutes the endosperm and bran particles of sorghum which disturb the homogeneity of the starch gel and obstruct uniform gas cell formation in the batter. The amount of starch and its botanical origin (Onyango et al., 2011a) affects the crumb texture of gluten-free bread. Starch modification can also influence crumb quality. Onyango et al. (2011b) found that incorporation of pre-gelatinised starch in sorghum-based gluten-free bread increases crumb adhesiveness: an undesirable crumb property. It is acknowledged that processed foods have better functional properties when appropriately modified starches are incorporated in them (Miyazaki et al., 2006). In this study, we hypothesized that other starch modification techniques, such as heat-moisture treatment (HMT), can improve the quality of gluten-free bread.

Starch modification can be physical, chemical or enzymatic (Miyazaki et al., 2006), but in view of the increasing demand by consumers for additive-free foods, there is growing interest for physically modified starch because it alters starch functionality without introducing foreign substances (Jacobs and Delcour, 1998). Heat-moisture treatment is a form of physical modification of starch, without gelatinization or damage to granular integrity with respect to size, shape or birefringence, through controlled application of heat and moisture (Jacobs and Delcour, 1998). It refers to the exposure of starch to high temperatures, commonly above the gelatinization temperature, at restricted moisture content of about 18 to 27% (Collado and Corke, 1999). Heat-moisture treatment causes molecular reordering of starch polymers leading to changes in its physicochemical properties. For instance, swelling of starch granules decreases (Adebowale and Lawal, 2003; Gunaratne and Hoover, 2002); and onset pasting temperature increases (Gunaratne and Hoover, 2002) whereas the peak viscosity decreases (Singh et al., 2005; Adebowale and Lawal, 2003). The first aim of this work was to determine the functional effect of HMT cassava starch on the rheological properties and crumb texture of sorghum-cassava-amaranth batter and bread, respectively. Amaranth was added to the composite flour mixture because of its good nutritional properties. The seeds are rich in protein and have higher levels of essential amino acids, and better protein digestibility and bioavailability than cereals, such as sorghum (Schoenlechner et al., 2008).

During germination (malting) of grains, hydrolytic enzymes progressively degrade the starch and the protein in the seed endosperm into dextrin, glucose, and amino acids. In gluten-free bread making, malt addition decreases batter viscosities at both proofing temperature and during heating leading to a decrease in bread density and formation of more open crumb thus improved bread volume (Makinen et al., 2013). However an excessive malt addition will result in excessive liquefaction and dextrinization, yielding bread with a wet sticky crumb (Khalil et al., 2000). In an attempt to further improve the textural properties of bread, this study also focused on evaluating the suitability for use of malted amaranth flour in gluten-

free baking.

MATERIALS AND METHODS

Modification of cassava starch by heat-moisture treatment

Cassava starch [11% moisture content (MC), 87.6% starch] was purchased from Universal Starch Public Company Ltd., Bangkok, Thailand. Its MC was adjusted to 18, 24 or 30% w/w using distilled water. The samples were transferred to sealed containers and equilibrated at 4°C for 12 h. Thereafter, they were incubated at 80°C for 8, 16 or 24 h then dried in a cabinet drier at 4°C to about 10% MC.

Physical characteristics heat-moisture treated cassava starch

Damaged starch was determined using the Megazyme Starch Damage Assay kit, K-SDAM 05/2008, (Megazyme International Ireland Ltd., Co. Wicklow, Ireland) and expressed on dry weight basis. Water solubility index (WSI) was determined by adding 1 g into 20 ml distilled water in test tubes and heated in a water bath at 60°C for 30 min. At the end of heating, the mixture was centrifuged at 1,200 x g for 20 min and 10 ml of the supernatant decanted and dried to constant weight at 110°C. Water solubility index was expressed as the percent of dried solid weight based on weight of the sample.

Water absorption index (WAI) was determined by weighing 5 g into pre-weighed 50 ml centrifuge tubes. Distilled water (25 ml) was added to each tube, the cap secured and the tubes vigorously hand-shaken for 5 s to suspend the flour. The suspension was allowed to solvate and swell for 20 min with intermittent shaking at 5, 10, 15 and 20 min. The samples were centrifuged at 1,000 x g for 15 min after which the supernatant was decanted and pellet drained by touching with an adsorbent-paper towel. Water absorption index was determined as the difference between the weight of the drained tube and the weight of the original empty tube/weight of dry sample.

Swelling power was determined by weighing 0.1 g into pre-weighed test tubes containing 10 ml distilled water. The tubes were heated in a water-bath at 60°C for 30 min with intermittent hand-shaking for 5 s after 5, 15 and 25 min. The tubes were centrifuged at 1,000 x g for 15 min and the supernatant decanted and the weight of the starch paste determined. Swelling power was calculated as weight of starch paste divided by the weight of dry starch.

A viscograph-E (Brabender GmbH and Co. KG, Duisburg, Germany) was used to evaluate the pasting profile of the starch. Samples (ca. 27 g) were transferred into a canister and water (ca. 433 ml) was added. They were then heated from 30 to 90°C at 1.5°C/min and held at 95°C for 10 min. The onset pasting temperature (°C) and peak viscosity in Brabender units (BU) were determined.

Starch crystallinity was determined using fourier transform infrared spectroscopy (FT-IR). The FT-IR spectra were obtained on a Nicolet iS10 FT-IR spectrometer (Thermo Fisher Scientific Inc., Massachusetts, USA). About 0.8 mg sample was mixed with potassium bromide (80 mg), homogenized and compressed with a manual press. For each sample, 16 scans were recorded at 25±1°C in the wave number interval of 400-4000 cm⁻¹ with a spectral resolution of 4 cm⁻¹ and co-added. A background spectrum was recorded and subtracted from the sample spectrum. Spectra were collected and processed using OMNIC 8.2.388 software (Thermo Fisher Scientific Inc., Massachusetts, USA). The spectral region 1200-800 cm⁻¹, which is sensitive to the structural state of starch (van Soest et al., 1995) was truncated and baseline corrected by subtracting a two point linear function touching 1200 and 880 cm⁻¹ and the resulting spectra normalised to unit area. Voigt function peaks were fitted at 1047 and 1022 cm⁻¹ prior to calculating the ratios of peak heights at 1047/1022.

Preparation and characterization of native and malted amaranth flours

Amaranth (*Amaranthus cruentus*) flour and grains were purchased from Allgrain Company Ltd., Nairobi, Kenya. The grains were soaked in excess water for 6 h after which they were spread thinly on a wire tray and allowed to drain for 6 h at 25°C. Hereupon they were covered with a fine cotton cloth and left to germinate for 30 h. The germinated grains were placed on drying racks and sun-dried to 10% moisture content. The dried malt (together with external roots and shoots) was finely milled to pass through a 0.8 mm sieve.

Moisture content, protein (N x 5.85), fiber, ash, and lipid contents of native and malted amaranth flours were determined according to AACC Method 44-15A, 46-11A, 32-10, 08-01, and 32-25 (AACC, 1995), respectively. Total carbohydrate was determined as the difference between 100 and the sum of values for moisture, lipid, protein, fiber and ash. Starch content was determined using the megazyme total starch assay kit (Megazyme International Ireland Ltd., Co. Wicklow, Ireland). Total soluble sugars were determined by the phenol-sulphuric acid method (Dubois et al., 1956). Reducing sugars were determined using the Nelson-Somogyi alkaline copper reduction method (Somogyi, 1952). Diastatic power (that is, joint α - and β -amylase activity) was determined according to the procedure of American Society of Brewing Chemists (ASBC, 1958). Free amino nitrogen (FAN) was determined by adding 1 g sample to 40 ml 5% trichloroacetic acid at 30°C and extraction carried out for 1 h at 30°C. The tubes were swirled at 15 min inter-vals to suspend the contents. The extract (10 ml) was centrifuged at 4,500 x g for 10 min and 1 ml of clear supernatant diluted to 25 ml with distilled water. The samples were subjected to ninhydrin assay according to AOAC Method 10.180 (AOAC, 1980).

Effect of heat-moisture treated cassava starch and amaranth on rheology of gluten-free batter

Composite flour was prepared from modified cassava starch, sorghum (gadam variety; its composition, on dry-weight basis, was: protein, 13.2%; fiber, 2.0%; ash, 1.6%; lipid, 3.6%; and carbohydrates, 79.6%) and amaranth flour at a ratio of 50:40:10. The other ingredients, weighed on flour-weight-basis (fwb), were water (75%), sugar (6.7%), baker's fat (2%), salt (1.7%) and amaranth malt at 0, 1 or 2.5%. The dry ingredients were manually mixed in a wide bowl before adding to the mixing bowl containing water and baker's fat. The ingredients were mixed using a Kenwood KM264 kitchen mixer (Kenwood Limited, Hampshire, England) for 3 min to obtain homogenous batter. The batters were incubated at 30°C for 1 h before measuring their consistencies using an HDP/FE forward extrusion cell of a TA.XT.plus Texture Analyzer (Stable Microsystems, Surrey, United Kingdom) equipped with a 50 kg load cell (Onyango et al., 2011a).

Effect of heat-moisture treated cassava starch and amaranth on crumb texture of gluten-free bread

Batter was prepared as described above with the addition of instant active dry yeast (3% fwb). Initially, we investigated the effect of HMT cassava starch and amaranth malt (0, 1 and 2.5% fwb) on the crumb quality of gluten-free bread. Thereafter, we investigated the effect of only HMT cassava starch (that is, incubation time and moisture content) on crumb quality of gluten-free bread and therefore did not add amaranth malt to the formulation. The batters (400 g) were weighed into baking pans and proofed for 15 min at 33°C and 85% relative humidity. The batters were covered with a lid and baked at 210°C for 30 min. The loaves were unmold and cooled for 2 h, packed in moisture-permeable polythene bags, closed with a twist tie and stored for 22 h at 25°C. The loaves were

sliced into 10 mm thick slices using a bread slicer (MacAdams Baking Systems, Cape Town, South Africa). Two slices were taken from the centre after 24 h. A ring (30 mm diameter) was punched out from each slice. Texture profile analysis was determined using a TA.XT.plus texture analyzer (Stable Microsystems, Surrey, United Kingdom), equipped with a 50 kg load cell (Onyango et al., 2011a).

Experimental design

Experiments on cassava starch modification, batter consistency and crumb texture were designed as multiple factorial designs with three replicates. The data was analyzed using MINITAB 16 software (Minitab Inc, Pennsylvania, USA). Analysis of variance was carried out and the P-value used to determine significance of interaction between variables at $\alpha \leq 0.05$, $\alpha \leq 0.01$ or $\alpha \leq 0.001$. Amaranth flour and malt characterization experiments were done in triplicate. The data was analysed using *t*-test to evaluate the differences in means between native and malted flours. The P-value was used to determine the level of significance at $\alpha \leq 0.05$.

RESULTS AND DISCUSSION

Physical properties of heat-moisture treated cassava starch

The amount of damaged starch in native cassava starch was 0.34%. Damaged starch contents in HMT cassava starch ranged from 0.11% (80°C, 18% MC, 24 h) to 4.27% (80°C, 30% MC, 16 h). Generally, the mean damaged starch content increased with increasing MC and incubation time. Adjustment of MC to 18, 24 and 30% gave HMT starches with 0.11 to 0.24, 0.54 to 0.66 and 2.78 to 4.27% damaged starch, respectively. The interaction effect of % MC x incubation time significantly affected ($P \leq 0.001$) the amount of damaged starch in HMT starch (Table 1). Damaged starch refers to small particles of starch broken away from the main starch granules. Ideally starch damage does not occur during HMT (Jacobs and Delcour, 1998), however, Kawabata et al. (1994) found that in industrially produced HMT starch, the polarized cross of starch granules becomes slightly unclear and the centric hila of the granules are directly damaged due to non-homogeneity of starch. We also speculated that some starch may have been damaged during the post-HMT operations such as drying and milling). Damaged starches hydrate more easily during dough preparation. The level of starch damage therefore improves the water absorption and dough mixing properties of flour and is of technological significance. Damaged starch has much greater water retention capacity; however, too much starch damage leads to sticky dough, strong proofing, and undesirable red crust colour (Bettge et al., 1995).

Water solubility index, WAI and swelling power of native cassava starch were 1.24%, 1.87 g and 9.76 g/g, respectively. Table 1 shows the statistical summary of the effect of HMT of cassava starch on WSI, WAI and swelling power. The interaction effect was significant for WSI ($P \leq 0.001$), WAI ($P \leq 0.001$) and swelling power ($P \leq 0.01$). Water solubility index of HMT starch ranged from

Table 1. F-values of physical properties of heat-moisture treated cassava starch.

Variable	DS (%)	WSI (%)	WAI (g)	SP (g/g)	Onset pasting temperature (°C)	Peak viscosity (BU)
MC (%)	3012.82***	17.09***	968.75***	6.00**	4543.38***	1378.13***
Time (h)	63.11***	13.58***	128.91***	5.10**	529.31***	1125.19***
MC (%) x time (h)	59.47***	6.83***	60.91***	2.92**	175.86***	236.66***
r ²	0.99	0.83	0.99	0.65	0.99	0.99

Significant at $P \leq 0.01$; *significant at $P \leq 0.001$. DS, Damaged starch; WSI, water solubility index; WAI: water absorption index; SP, swelling power; MC, moisture content.

0.33% (80°C, 30% MC, 16 h) to 1.33% (80°C, 18% MC, 8 h). Water absorption index ranged from 1.93 g (80°C, 18% MC, 16 h) to 2.37 g (80°C, 30% MC, 24 h). Starch whose MC were adjusted to 30% exhibited higher WAI (2.12 to 2.37 g) than those with 18 or 24% MC (1.93 to 2.02 g), irrespective of incubation time. Swelling power ranged from 4.60 g/g (80°C, 30% MC, 24 h) to 8.13 g/g, (80°C, 18% MC, 8 h). These swelling power values represented the extremes of the experimental design, whereby the treatment with the lowest MC and shortest incubation time had the highest swelling power, and that with highest MC and longest incubation time had the lowest swelling power. The decrease in swelling power and WSI is attributed to changes in the packing arrangement of starch crystallites and interactions between or among starch components in the amorphous region of the granule (Hoover and Vasanthan, 1994). The properties of the swollen granules and the soluble materials leached out from the granules cooperatively control viscosity parameters during pasting which in turn affects bread density and crumb properties. On the other hand, the increase in WAI in HMT starch implies that hydrophilic character is increased by starch modification. Damaged starch may also be responsible for the increase in WAI in HMT starch. Damaged starch granules absorb about four times as much water as intact starch granules (Stauffer, 2007) and thus an increase in damaged starch increases WAI.

The onset pasting temperature and peak viscosity of native cassava starch was 66°C and 576 BU, respectively. Heat-moisture treatment of cassava starch increased the onset pasting temperature (66.90-75.15°C) and decreased the peak viscosity (175 to 452 BU). The interaction effect significantly ($P \leq 0.001$) affected the onset pasting temperature and peak viscosity (Table 1). These findings are similar to other studies in which starches from other botanical origins were investigated (Watcharatewinkul et al., 2009; Olayinka et al., 2008). The increase in onset pasting temperature and decrease in peak viscosity reflect molecular reorganization as a result of HMT. As shown in the FT-IR data, HMT enhances perfection of starch crystallites. This is possibly initiated by incipient swelling and the resulting mobility of amorphous α -glucans which facilitate ordering of double helices (that is, increased inter- and intramolecular hydrogen bonding) (Lawal, 2005). Consequently, HMT starch requires more heat

before structural disintegration and paste formation occurs. Furthermore, the enhancement of crystallinity after HMT limits starch swelling and structural disintegration during heating in the batter which causes difficulty in gelatinizing (Miyazaki et al., 2006), both of which contribute significantly to batter viscosity and bread density.

Fourier-transform infrared spectroscopy was used to probe the molecular order of cassava starch. Band peaks at 1047 cm^{-1} relate to molecular order and crystallinity of starch polymers whereas peaks at 1022 cm^{-1} correspond to the disordered or amorphous phase (van Soest et al., 1995). The ratios of the heights of the bands at 1047 and 1022 cm^{-1} express the amount of ordered starch to amorphous starch (van Soest et al., 1995). The height ratio for native starch at 1047/1022 was 0.631. Heat-moisture treatment of starch increased the degree of crystallinity to mean ratios of 0.653 to 0.682 (Table 2). Crystallinity was highest in starch treated at 80°C, 30% MC and 16 h and lowest in starch treated at 80°C, 18% MC and 8 h. Incubation time had no significant effect ($P > 0.05$) on starch crystallinity, whereas increasing MC increased starch crystallinity ($P \leq 0.05$). Exposure of starch to high temperatures and restricted MC elevates the glass transition temperature, the trigger for polymeric reorganization within granules (Tester and Debon, 2000). Thus, HMT promotes interaction of polymer chains by initially disrupting the crystalline structure and dissociating the double helical structure in the amorphous region followed by rearrangement of the disrupted crystals (Gunaratne and Hoover, 2002).

Composition of amaranth flour and malt

Table 3 shows the proximate composition, on dry-weight basis, of amaranth flour and malt. The protein (N x 5.85), lipid and ash contents were close to values that have been previously reported for the same species (Menegassi et al., 2011). However, Menegassi et al. (2011) reported higher starch (69%) and fiber contents (13%) for this species. Protein, FAN and diastatic power were higher ($P \leq 0.05$) in malted flours, whereas ash and starch contents were higher ($P \leq 0.05$) in native flours (Table 3). The biochemical and physiological changes that take place during malting lead to reduction in the levels of protein, fiber, lipid, ash and carbohydrates (Elmaki et al., 1999). These

Table 2. Effect of heat-moisture treatment on cassava starch crystallinity.

Moisture content (%)	Peaks fitted at 1047/1022			Mean
	8 h	16 h	24 h	
18	0.609	0.639	0.655	0.634±0.02 ^a
24	0.669	0.655	0.672	0.665±0.01 ^{ab}
30	0.680	0.684	0.682	0.682±0.00 ^c
Mean	0.653±0.04 ^x	0.659±0.02 ^x	0.670±0.01 ^x	

Values followed by the same superscript letter (a-c) in the same column and x in the same row are not significantly different at $P \leq 0.05$.

Table 3. Composition of amaranth flour and malt.

Amaranth	Protein*	Fibre	Ash	Lipid	CHO	Starch	TSS	RS	DP	FAN
Flour	13.37±0.41 ^a	5.53±1.92 ^a	2.78±0.05 ^b	6.93±0.70 ^a	71.39 ^a	53.14±0.68 ^b	59.88±10.42 ^a	0.06±12.22 ^a	8.93±2.81 ^a	9.07±8.69 ^a
Malt	15.98±0.29 ^b	4.96±0.51 ^a	2.37±0.04 ^a	6.65±0.18 ^a	70.04 ^a	49.12±0.15 ^a	69.19±11.74 ^a	0.09±30.36 ^a	60.64±19.03 ^b	83.17±19.74 ^b

All values are given as g/100 g dry-matter-basis, except DP as (dry-matter-basis) and FAN as mg/l. *N x 5.85. CHO, Carbohydrate; TSS, total soluble carbohydrates; RS, reducing sugars; DP, diastatic power; FAN, free amino nitrogen. Values followed by the same superscript letter in the same column are not significantly different at $P \leq 0.05$.

changes were not evident in our study probably because malt was prepared from grains that came from a different batch than that used to prepare native flour. Nonetheless, since malt quality is defined primarily in terms of diastatic power and FAN (Dewar et al., 1997), the different values ($P \leq 0.05$) of these parameters were reliable indicators of different flour qualities.

Effect of heat-moisture treated cassava starch and amaranth on rheology of gluten-free batter

Batter was prepared from native or HMT cassava starch, sorghum and amaranth flour in the ratio of 50:40:10. The other ingredients were water (75%), sugar (6.7%), baker's fat (2%), salt (1.7%), and amaranth malt at 0, 1 or 2.5% fwb. Extrusion force of batter containing native starch and 0, 1 or 2.5% fwb amaranth malt was 110.71, 180.21 and 186.53

N, respectively. Extrusion forces of batters containing HMT starches and different levels of amaranth malt ranged between 127.56 and 283.64 N. All batters prepared from cassava starch exposed to HMT at 30% MC gave extrusion forces that exceeded the load cell (that is, > 490 N) of the texture analyser and thus were not included in the analysis. The interaction effect significantly ($P \leq 0.01$) affected the consistency of the batter (Table 4). Batter consistency tended to increase with increasing MC and incubation time of starch modification and amaranth malt content. During HMT, increase in gel firmness has been attributed to the increased cross-linking between starch chains in the particular amylose portion. This allows for the formation of more junction zones in the continuous phase of the gel, resulting in increased gel hardness (Hoover and Manuel, 1996).

Effect of heat-moisture treated cassava starch and amaranth on crumb texture of gluten-free bread

Texture profile analysis properties of hardness, chewiness, cohesiveness and resilience of bread prepared from HMT starch, sorghum, amaranth flour and 0 to 2.5% fwb amaranth malt ranged between 22.71 to 36.11 N, 11.73 to 17.97 N, 0.51 to 0.58 and 0.30-0.36, respectively. None of the independent variables affected crumb springiness ($P > 0.05$). The interaction effect was significant only for the texture profile analysis property of cohesiveness ($P \leq 0.05$, Table 5). Crumb cohesiveness tended to decrease with increasing amaranth malt content at all HMT combinations (data not shown). In order to evaluate the influence of only starch modification on crumb texture, we also baked the bread without amaranth malt. The inter-

Table 4. F-values of extrusion force of sorghum-cassava-amaranth batter.

Variable	F-value
Starch modification	20.23***
Amaranth malt (%)	189.14***
Starch modification x amaranth malt (%)	4.83**
r ²	0.97

Significant at $P \leq 0.01$; *significant at $P \leq 0.001$.

Table 5. F-values of texture profile analysis parameters of sorghum-cassava-amaranth bread modified with amaranth malt.

Variable	Hardness (N)	Chewiness (N)	Cohesiveness ^a	Resilience ^a
Starch modification	7.23***	3.82**	3.14**	2.64*
Amaranth malt (%)	3.30*	Ns	27.92***	26.96***
Starch modification x amaranth malt (%)	ns	Ns	1.98*	ns
r ²	0.78	0.66	0.81	0.82

^aDimensionless terms. *Significant at $P \leq 0.05$; **significant at $P \leq 0.01$; ***significant at $P \leq 0.001$; ns not significant at $P \leq 0.05$.

Table 6. F-values of texture profile analysis parameters of sorghum-cassava-amaranth bread without amaranth malt addition.

Variable	Hardness (N)	Chewiness (N)
Moisture content (%)	8.95**	ns
Time (h)	21.00***	4.28*
Moisture content (%) x time (h)	6.71**	3.08*
r ²	0.91	0.74

*Significant at $P \leq 0.05$; **significant at $P \leq 0.01$; ***significant at $P \leq 0.001$; ns not significant at $P \leq 0.05$.

interaction effect significantly affected the Texture Profile Analysis properties of hardness ($P \leq 0.01$) and chewiness ($P \leq 0.05$, Table 6). Springiness, cohesiveness and resilience were not affected ($P > 0.05$) by starch modification.

Texture profile analysis is a useful technique for objective assessment of food texture because it measures several parameters (that is, hardness, springiness, cohesiveness, resilience, chewiness, gumminess, fracturability and adhesiveness) from a single test (Bourne, 2002). However, not all parameters may be simultaneously useful. In bread, for example, crumb hardness (or firmness) is the recommended indicator of crumb quality (AACC Method 74-09, 1995) because of its close association with consumer perception of freshness. Hardness of bread increases with storage. This is attributed to the ageing phenomenon or staling which results in an increase in starch crystallinity of the crumb. In this study, hardness was affected ($P \leq 0.01$) by HMT (Table 6) and it tended to decrease with increasing MC and incubation time of starch modification (data not shown). Cohesiveness and resilience showed high levels of significance ($P \leq 0.001$) when amaranth malt was added to the batter (Table 5)

but differences were insignificant ($P > 0.05$) in treatments without amaranth malt. Crumb cohesiveness and resilience of gluten-free bread is caused by the gel network of starch after HMT. This network could be affected by malt addition due to the high amylase activity. Chewiness is classified as a secondary textural parameter because it is the product of the primary textural parameters of hardness, springiness and cohesiveness (Bourne, 2002). It represents the energy required to masticate a solid food product to a state ready for swallowing. Chewiness was not affected ($P > 0.05$) by amaranth malt (Table 5) but it was affected ($P \leq 0.05$) by HMT (Table 6). Similarly to hardness, chewiness tended to decrease with increasing moisture content and incubation time of starch modification. The decrease in bread hardness and chewiness could be due to formation of a more open crumb.

Conclusion

Heat-moisture treatment caused molecular reordering of cassava starch polymers leading to changes in its physicochemical properties. These changes affected the

rheological properties and crumb texture of gluten-free sorghum-cassava-amaranth batter and bread, respectively. Batter consistency tended to increase with increasing moisture content and incubation time of starch modification and increasing amaranth malt content. The most sensitive texture profile analysis parameters were crumb hardness and chewiness, which tended to decrease with increasing moisture content and incubation time of starch modification. Addition of amaranth malt significantly affected crumb cohesiveness and resilience which tended to decrease with increasing level of malt.

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