Technical paper

Texture and Structure of Bread Supplemented with Purple Sweet Potato Powder and Treated with Enzymes

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Sweet potato a nutritious and abundantly available food crop in many developing countries has been explored for baking to increase its use in food processing. Though it often results in lower quality bread it can be improved with enzyme treatments. Thus, this study evaluated the effect of purple sweet potato powder (PSPP) supplementation, and α -amylase (AM) and hemicellulase (HC) treatments on the texture and retrogradation during storage, and structure of bread. Results showed that PSPP supplementation gave rise to bread with higher firming rate related with greater starch-gluten interaction. On the other hand, AM and HC treatment resulted in lower firming rate, amylose content, enthalpy of retrogradation, rupture properties, and moisture loss during storage of PSPP supplemented bread. These improvements in texture properties and structure indicate more acceptable bread that may lead in increased utilization of purple sweet potato.

Keywords: purple sweet potato, bread texture, bread structure, α -amylase, hemicellulase

Introduction

Bread is one of the most widely consumed foods worldwide, and is a staple food in many developed and developing countries (Abdelghafor *et al.*, 2011; Rosell, 2011). In developing countries, the wheat flour of baked products is supplemented with locally grown starchy crops in order to improve its nutritional value and reduce costs related to wheat imports (Olaoye *et al.*, 2006; Olaoye and Ade-Omowaye, 2011). Moreover, many studies have focused on non-wheat flour supplementation with the goal of developing specialty breads with added nutritional value, flavor and color (Hathorn *et al.*, 2008). Sweet potato is an abundantly available, inexpensive food crop in developing countries, and is of significant socio-economic importance because of its high nutrient, carotenoid and anthocyanin contents (Antonio *et al.*, 2011; Ray and Tomlins,

2010; Lu and Gao, 2011). However, despite its abundance, low cost and high nutrient content sweet potato remains an underutilized food resource (Hathorn *et al.*, 2008). To enhance utilization, sweet potato supplementation to baked products has been explored in many developing countries, and has been commercialized on a limited scale in Peru and Japan (Woolfe, 1992). However, lower bread quality is an issue due to the lack of gluten protein, and high fiber and damage starch content of non-wheat flour (Hathorn *et al.*, 2008). Thus, the use of enzymes, *e.g.*, α -amylase and hemicellulase, has been explored to improve loaf volume, crumb texture and staling properties of bread, important considerations for both bakers and consumers (Scanlon and Zghal 2001; Jiang *et al.*, 2005; Caballero *et al.*, 2007; Rozylo and Laskowski, 2011; Wang *et al.*, 2013). Loaf volume and texture

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dictates the quality and acceptability of bread, whereas staling serves as a measure of freshness and can be associated with changes in crumb moisture, hydration capacity and firmness during storage (Brady and Mayer, 1985; Greene and Bovell-Benjamin, 2004; Lai and Lin, 2006; Gomes-Ruffi *et al.*, 2012).

In Japan, many sweet potato cultivars with white, yellow, orange and purple flesh are available. Although the yellow-fleshed variety is the most common, purple sweet potato has received much attention because of its nutritional value and heat stable color, attributed to its anthocyanin content (Terahara *et al.*, 2000; Oki *et al.*, 2002; Kim *et al.*, 2012; Bovell-Benjamin, 2007). In this regard, purple sweet potato has been employed as a natural food colorant in noodles, jam, chips, confectionery, juice, alcoholic drinks and bread (Oki *et al.*, 2002; Choi *et al.*, 2011). However, despite its use in bread making, its effect on the texture, structure and staling of bread has not yet been fully explored.

Therefore, this study evaluated the effect of purple sweet potato powder (PSPP) supplementation and α -amylase (AM) and hemicellulase (HC) treatment on the texture and structure of doughs and breads. Changes in crumb hardness, cohesiveness and moisture were also determined to evaluate the effect of PSPP supplementation and enzyme treatment on bread staling.

Materials and Methods

Bread making treatments Bread making tests were performed following the no-time method and a standard wheat bread formulation was employed as the control following the method of Yamauchi *et al.* (2001). Control bread was prepared from 200 g of Camellia wheat flour (Nisshin Flour Milling Co., Ltd., Tokyo, Japan), 10 g of sugar (Nippon Beet Sugar Mfg. Co., Ltd., Tokyo, Japan), 10 g of shortening (Snowlight; Kaneka Corp., Osaka, Japan), 4 g of wet yeast (Regular yeast; Nippon Beet Sugar Mfg. Co. Ltd.), 4 g of deionized salt (The Salt Industry Center of Japan, Tokyo, Japan), and 20 mg of L-ascorbic acid (Wako Pure Chemical Industries, Ltd., Osaka, Japan). A suitable amount of water was added based on the optimal water absorption of dough at 500 BU and determined using Farinograph analysis as presented by AACC (1991). For the PSPP-added treatments, 4 percent of the original wheat flour content of the control was replaced with PSPP (Kumamoto Flour Milling Co., Ltd., Kumamoto, Japan). PSPP was prepared by heat-treating the raw purple sweet potato variety, Ayamurasaki, resulting in almost completely gelatinized starch. The amount of added PSPP, 4%, was determined as the minimum concentration that resulted in a clear crumb color change according to a previous report (Santiago *et al.*, 2015). For the enzyme treatment, optimum amounts of 0.025 g AM and 0.05 g HC (Shinnihon Chemical Co., Ltd., Anjo, Japan) were added to the formulation. AM and HC are crude products for food processing applications, each of which contain some other enzymes. Optimum amounts of these enzymes were determined according to a previous report (Santiago *et al.*, 2015).

Bread making and evaluation The dough was mixed to just beyond peak development, as indicated by the electric power curve of the mixing motor. Pieces of dough (100 g and 20 g) were weighed, rounded, and incubated for 20 min (bench time) at 30°C and 75% relative humidity (RH) in a fermentation cabinet, panned and proofed for 70 min at 38°C and 85% RH.

Gas retention of dough (GRD) and gassing power (GP) of 20 g proofed dough were evaluated by measuring the maximum expansion volume at 0 to 75 cmHg, and gas production at 30°C for 1, 2, and 3 h using a Fermograph II (ATTO Co., Ltd., Tokyo, Japan), respectively. Meanwhile, the 100 g proofed dough was baked at 180°C for 25 min and specific loaf volume (SLV) of the bread was measured by the rapeseed displacement method 1 h after baking in accordance with Yamauchi *et al.* (2000). Photographs of bread and scanned images of bread crumbs were recorded using a digital camera and scanner.

Texture and rupture measurements Textural properties of bread crumb during storage were analyzed using the method as presented by Yamauchi *et al.* (2001). Loaves were stored in a polyethylene bag at 20°C and 70% RH for 3 days. At each storage day, 3 loaves were cut into 2 cm-thick slices and a square of crumb (3 x 3 cm) was cut from the center of the slices using an ultrasonic cutter (USC-3305; Yamaden Co., Ltd., Tokyo, Japan). Textural properties were measured by compressing the whole crumb twice from 2-cm to 1-cm thickness at a speed of 1 mm/s using a special cube plunger (6 cm length x 6 cm width x 2 cm height), up to 50% strain rate with a creep meter (RE2-33005C; Yamaden Co., Ltd.). From the resulting stress-strain curve, the hardness, cohesiveness, springiness, gumminess and chewiness of breads were calculated. Moreover, the firmness rate was calculated based on changes in bread hardness during storage.

Rupture force (RF), rupture deformation (RD) and rupture energy (RE) were measured using the same size of crumb sample as for the textural analysis. Crumb samples were placed in the center of a 5 x 5 cm measuring table with a 1.5 x 1.0 cm square hole in the center of the table, and then ruptured at a speed of 5 mm/s up to 150% strain rate using the No. 64 wedge plunger of the creep meter (RE2-33005C; Yamaden Co., Ltd.).

Amylose content and enthalpy of retrogradation of bread Using the same sample as in the textural analysis, bread crumbs were air-dried after 99.5% ethanol and acetone treatment. Dried bread crumbs were ground, stored in polyethylene bags and used for determinations of amylose content and enthalpy of retrogradation. Amylose content of bread crumbs was analyzed using a Megazyme assay kit (Megazyme International Ireland Ltd., Wicklow, Ireland) based on the method of Gibson *et al.* (1996). On the other hand, the enthalpy of retrogradation was determined using a differential scanning calorimeter (DSC) (Micro DSC II, Setaram, Inc., Caluire, France). A sample of 200 mg dry weight basis (dwb) was weighed in a DSC pan and distilled water was added to give a suspension of 30% dwb. The pan was sealed and allowed to stand

overnight at 20°C. The scanning temperature range was set at 30 to 95°C and the heating rate was 1.5°C /min. Water (400 mg) was used as a reference.

Moisture content and soluble sugar analysis Changes in the moisture content (MC) of bread stored for 3 days were determined using 2 x 3 x 3 cm bread crumbs according to the AOAC official method (AOAC, 2000).

Water-soluble fractions of the breads were extracted for determinations of sugar content and composition. Total and reducing saccharide contents were determined using the phenol-sulfuric acid and dinitrosalicylic acid methods as reported by Dubois *et al.* (1956) and Luchsinger and Cornesky (1962), respectively. Glucose, fructose, and maltose contents were analyzed using 1 mL of the water extract, diluted with an equal volume of acetonitrile and filtered through a 0.45-µm membrane filter (Millipore Japan Co., Ltd., Tokyo, Japan). HPLC analyses of soluble sugars were performed using a Shodex Asahipak NH2P-50 4E (4.6 mm ID x 250 mm) column and RI-930 Intelligent RI detector (JASCO Corporation, Tokyo, Japan).

Scanning Electron Microscopy Pieces of dough just after mixing and proofing were blast frozen at -40°C for 30 min and stored at -30°C until used for analysis. Dough samples were cut into 1 cm-thick slices using an ultrasonic cutter and blast frozen at -40°C for 30 min. A representative portion of the frozen sample was cut and viewed using a scanning electron microscope (JCM-6000; JEOL Ltd., Akishima, Japan). On the other hand, for the elution treatment, dough samples were washed with deionized distilled water in a sonicator for 10 min and blast frozen as described above. Likewise, representative samples were cut and viewed using a scanning electron microscope (SEM).

For bread just after baking, loaves were cut into 1 cm-thick slices and a square of crumb (2 x 2 cm) was cut from the center of the slices using an ultrasonic cutter. The sample was blast frozen, crushed with a hammer, and a representative sample was observed using SEM. For the elution treatment, 1 x 2 x 2 cm bread samples were washed with deionized distilled water in a sonicator for 10 min, blast frozen, crushed, and representative samples were viewed by SEM. All bread dough samples were scanned to observe the bread structures at 500x magnification.

Statistical analysis All data except for water absorption, SLV and physical properties of bread were measured in triplicate. The SLV and physical properties of bread were performed 4 and 12 times, respectively. All data were statistically analyzed using SPSS for Windows (ver. 17.0). ANOVA and Tukey's multiple range test were performed to compare means at a 5% significance level. Pearson's bivariate test was used to evaluate the correlation of parameters.

Results

Bread making quality The bread making qualities of doughs supplemented with PSPP, treated with AM and HC and control are shown in Table 1. Results showed that the PSPP dough has significantly lower GRD than the control and PSPP+AM+HC. On the other hand, the PSPP+AM+HC dough showed improved GRD, but did not significantly differ from the control. Table 1 also shows that the GP of PSPP+AM+HC dough was significantly higher than the doughs of PSPP and control at all incubation periods ($p < 0.05$). In terms of SLV, PSPP bread had the lowest value, not significantly different from the control, whereas the PSPP+AM+HC bread had a significantly higher SLV than the control and PSPP bread ($p < 0.05$). The difference in loaf volume is illustrated in Fig. 1, wherein the PSPP bread was smaller than the control. On the other hand, the PSPP+AM+HC bread appeared larger than the control and PSPP. In regards to bread crumb color, PSPP and PSPP+AM+HC had a light purple appearance, while the control was white (Fig. 1).

Textural properties, amylose content and enthalpy of retrogradation of breads during storage Fig. 2 and Table 2 shows the typical stress-time plots and textural properties of bread just after baking, respectively. Fig. 2 shows that PSPP bread had a clearly higher peak than the control and PSPP+AM+HC. Correspondingly, PSPP bread had significantly higher hardness, gumminess and chewiness than the control and PSPP+AM+HC breads, as shown in Table 2 ($p < 0.05$). On the other hand, cohesiveness of PSPP+AM+HC bread was significantly lower than the control and PSPP bread ($p < 0.05$). Moreover, Fig. 3 illustrates the increase in hardness, gumminess and chewiness, and decrease in cohesiveness and springiness of breads during storage. It was observed that the bread treatments showed significantly different

Table 1. Bread making quality of bread dough¹⁾

Bread making treatments	Water absorption (%)	GRD (mL)	GP (mL)			SLV (mL/g)
			1h	2h	3h	
Control	68	100.0 ± 0.0 b	26.1 ± 0.2 b	59.5 ± 0.3 a	90.9 ± 0.7 a	4.95 ± 0.07 a
+PSPP	69	90.0 ± 0.0 a	25.7 ± 0.1 a	59.4 ± 0.3 a	91.9 ± 0.5 a	4.82 ± 0.04 a
+PSPP+AM+HC	69	103.3 ± 2.9 b	26.4 ± 0.1 c	61.0 ± 0.0 b	95.1 ± 0.1 b	5.28 ± 0.16 b

Abbreviations: GRD, gas retention of dough; GP, gassing power of dough; SLV, specific loaf volume; PSPP, purple sweet potato powder; AM, α-amylase; HC, hemicellulase

¹⁾Each value except of water absorption for bread making is the mean ± SD. The values followed by different letters within columns are significantly different ($p < 0.05$).

Fig. 1. Photograph and scanned images of bread and bread crumbs
Abbreviations: PSPP, purple sweet potato powder; AM, α -amylase; HC, hemicellulase

Fig. 2. Typical stress-strain curves of breads
Abbreviations: PSPP, purple sweet potato powder; AM, α -amylase; HC, hemicellulase

hardness, cohesiveness, gumminess and chewiness after 3 days of storage, with PSPP+AM+HC revealed to have the lowest values ($p < 0.05$). On the other hand, the PSPP bread had the highest hardness, gumminess and chewiness after 3 days of storage ($p < 0.05$).

Table 3 shows that the PSPP bread had a significantly higher firming rate at $2625.0 \pm 105.5 \text{ N/m}^2$ per day than the control at $2185.0 \pm 95.7 \text{ N/m}^2$ per day ($p < 0.05$). On the other hand, PSPP+AM+HC bread had the lowest firming rate at $1993.2 \pm 74.8 \text{ N/m}^2$ per day ($p < 0.05$). Likewise, PSPP+AM+HC had the lowest amylose content ($p < 0.05$) among the bread

treatments, which did not change during storage (data not shown). In addition, bread treatments showed significantly different enthalpy of retrogradation just after baking, with PSPP+AM+HC as the lowest value ($p < 0.05$). In Fig. 4, the enthalpy of retrogradation increased during bread storage. However, a significantly lower change of retrogradation enthalpy was observed for PSPP+AM+HC after 3 days of storage.

Rupture properties of breads Table 4 presents the rupture properties of bread crumbs for each treatment. Results showed that after 1 day of storage, the rupture force (RF) of bread crumbs for each treatment differed significantly, with the PSPP bread showing the highest value and PSPP +AM+HC exhibiting the lowest ($p < 0.05$). Decreases in RF, RD and RE were observed during storage. After 3 days of storage, PSPP+AM+HC had significantly lower RF than the control and PSPP ($p < 0.05$). Similarly, the RE of PSPP+AM+HC was significantly lower than the other bread treatments under all incubation periods ($p < 0.05$).

Moisture and soluble sugar content of bread Table 5 shows the decrease in moisture content of bread during storage. Table 5 showed that the moisture loss of PSPP+AM+HC after 3 days of storage was significantly lower than the control and PSPP ($p < 0.05$). On the other hand, Table 6 shows that the PSPP bread had significantly higher water-soluble glucose, maltose, reducing and total saccharide content compared with the control ($p < 0.05$). Moreover, PSPP+AM+HC had the highest water-soluble glucose,

Table 2. Texture properties of breads with PSPP¹⁾

Bread making treatments	Hardness N/m ²	Cohesiveness (-)	Springiness (-)	Gumminess N/m ²	Chewiness (-)
Control	1063.9 ± 51.4 a	0.84 ± 0.01 b	0.96 ± 0.00 a	897.8 ± 45.0 a	866.7 ± 52.6 a
+PSPP	1480.6 ± 69.7 b	0.85 ± 0.01 b	0.97 ± 0.00 a	1252.6 ± 55.7 b	1210.0 ± 56.1 b
+PSPP+AM+HC	1056.9 ± 49.9 a	0.82 ± 0.00 a	0.97 ± 0.00 a	861.5 ± 41.00 a	832.2 ± 41.5 a

Abbreviations: PSPP, purple sweet potato powder; AM, α -amylase; HC, hemicellulase

¹⁾Each value is the mean $\pm$ SD. The values followed by different letters within columns are significantly different ($p < 0.05$).

Fig. 3. Changes in texture properties of breads during storage¹⁾

¹⁾The vertical bar is the standard deviation of each value. The data points followed by different letters are significantly different ($p < 0.05$). Abbreviations: PSPP, purple sweet potato powder; AM, α -amylase; HC, hemicellulase

Table 3. Firming rate, enthalpy of retrogradation, and amylose content of bread¹⁾

Bread making treatments	Firming rate (N/m ² per day)	Enthalpy of retrogradation ²⁾ (J/g)	Amylose content ²⁾ (%)
Control	2185.0 ± 95.7 b	1.23 ± 0.02 c	31.81 ± 0.37 b
+PSPP	2625.0 ± 105.5 c	1.13 ± 0.02 b	31.88 ± 1.07 b
+PSPP+AM+HC	1993.2 ± 74.8 a	1.04 ± 0.02 a	28.94 ± 0.62 a

Abbreviations: PSPP, purple sweet potato powder; AM, α -amylase; HC, hemicellulase¹⁾Each value is the mean ± SD. The values followed by different letters within columns are significantly different ($p < 0.05$).²⁾Enthalpy of retrogradation and amylose content of breads just after baking**Table 4.** Rupture properties of breads¹⁾

Bread making treatments	RF (N)		RD (mm)		RE (Jx10 ⁻³)	
	1d	3d	1d	3d	1d	3d
Control	3.66 ± 0.05 b	3.05 ± 0.23 b	21.46 ± 0.19 a	15.67 ± 0.54 a	3.03 ± 0.10 b	2.90 ± 0.48 ab
+PSPP	3.83 ± 0.05 c	2.95 ± 0.10 b	21.54 ± 0.10 a	15.75 ± 0.59 a	3.11 ± 0.09 b	3.02 ± 0.09 b
+PSPP+AM+HC	3.30 ± 0.08 a	2.39 ± 0.15 a	21.54 ± 0.46 a	15.29 ± 0.43 a	2.76 ± 0.12 a	2.46 ± 0.16 a

Abbreviations: RF, rupture force; RD, rupture deformation; RE, rupture energy; PSPP, purple sweet potato powder; AM, α -amylase; HC, hemicellulase¹⁾Each value is the mean ± SD. The values followed by different letters within columns are significantly different ($p < 0.05$).**Table 5.** Changes in moisture content of breads during storage¹⁾

Bread making treatments	Moisture content of crumb (%)				Difference in moisture content of crumb (%)
	Day 0	Day 1	Day 2	Day 3	Day 3-Day 0
Control	43.14 ± 0.42 a	40.43 ± 0.61 ab	38.68 ± 0.73 ab	36.87 ± 0.13 a	6.27 ± 0.29 b
+PSPP	43.58 ± 0.18 a	40.47 ± 0.50 b	39.23 ± 0.33 b	37.28 ± 0.12 b	6.30 ± 0.08 b
+PSPP+AM+HC	43.14 ± 0.24 a	39.66 ± 0.45 a	38.15 ± 0.57 a	37.40 ± 0.28 b	5.74 ± 0.17 a

Abbreviations: PSPP, purple sweet potato powder; AM, α -amylase; HC, hemicellulase¹⁾Each value is the mean ± SD. The values followed by different letters within columns are significantly different ($p < 0.05$). **Fig. 4.** Changes in enthalpy of retrogradation of breads during storage¹⁾¹⁾The vertical bar is the standard deviation of each value. The data points followed by different letters are significantly different ($p < 0.05$).Abbreviations: PSPP, purple sweet potato powder; AM, α -amylase; HC, hemicellulase

fructose, maltose, reducing and total sugar contents ($p < 0.05$). In terms of fructose content, PSPP+AM+HC had the highest content at 11.81 ± 0.12 mg/g bread, which was significantly higher than that of the control (11.35 ± 0.15 mg/g bread).

Dough and bread crumb structure Fig. 5, 6 and 7 show images of the dough just after mixing, proofing, and baking, respectively. Fig. 5a, c and e show the evenly scattered large and small starch granules of the non-eluted control, PSPP and PSPP+AM+HC doughs, respectively. However, no noticeable differences can be observed among non-eluted dough treatments just after mixing. On the other hand, Fig. 5b, d, and f show the eluted control, PSPP and PSPP+AM+HC doughs, respectively, and clearly illustrate the gluten network and crosslinking with starch granules. The interaction of gelatinized starch with the gluten network was observed in the PSPP dough (shown in Fig. 5d), which was not present in the control and PSPP+AM+HC. The areas of SG (starch and gluten crosslinks) are thought to show starch particles that are tightly cross-linked or adhered to the gluten

Fig. 5. Electron microscope photographs of dough just after mixing

a) Control without elution, b) Control with elution, c) +PSPP without elution, d) +PSPP with elution, e) +PSPP+AM+HC without elution, f) +PSPP+AM+HC with elution

Abbreviations: PSPP, purple sweet potato powder; AM, α -amylase; HC, hemicellulase; L, large type starch granules; S, small type starch granules; SG, starch and gluten crosslinks; G, gelatinized starch

network since this dough sample has already undergone elution treatment to sufficiently remove the starch granules.

Similarly, Fig. 6a, c and e show the evenly scattered large and small starch granules of the non-eluted control, PSPP and PSPP+AM+HC doughs, respectively, just after proofing. More swollen starch granules were observed in PSPP+AM+HC (Fig. 6e)

than the other bread treatments. Fig. 6b, d, and f show the gluten network of the eluted control, PSPP and PSPP+AM+HC doughs, respectively, just after proofing. It can be observed in Fig. 6d that the PSPP dough formed a greater number of swollen starch granule-gluten network crosslinks compared with control and PSPP+AM+HC. Moreover, partial interaction of gelatinized starch

Table 6. Saccharide content in water soluble fraction of breads¹⁾

Bread making treatments	Glucose (mg/g bread)	Fructose (mg/g bread)	Maltose (mg/g bread)	Reducing saccharide (mg/g bread)	Total saccharide (mg/g bread)
Control	5.91 ± 0.09 a	11.35 ± 0.15 a	24.21 ± 0.71 a	35.42 ± 0.70 a	70.12 ± 1.08 a
+PSPP	7.01 ± 0.09 b	11.59 ± 0.24 ab	29.22 ± 1.39 b	45.83 ± 0.56 b	82.54 ± 1.24 b
+PSPP+AM+HC	7.57 ± 0.03 c	11.81 ± 0.12 b	42.21 ± 0.65 c	64.03 ± 0.28 c	115.93 ± 0.67 c

Abbreviations: PSPP, purple sweet potato powder; AM, α -amylase; HC, hemicellulase

¹⁾Each value is the mean $\pm$ SD. The values followed by different letters within columns are significantly different ($p < 0.05$).

with the gluten network is observed in PSPP dough in Fig. 6d, which was not present in control and PSPP+AM+HC. On the other hand, lesser starch-gluten crosslinks, smaller pores and greater porosity were observed in PSPP+AM+HC (Fig. 6f) compared to the control and PSPP dough.

Fig. 7a, c and e show images of non-eluted bread just after baking, wherein the swollen starch granules can be respectively observed in control and PSPP bread, while for PSPP+AM+HC the starch granules were ruptured and undistinguishable. Moreover, Fig. 7b, d, and f show the gluten networks for each bread treatment. It was observed that the control (Fig. 7b) had a compact, close gluten network, whereas PSPP+AM+HC (Fig. 7f) had a more open network. On the other hand, a likely weak gluten network with more gelatinized starch-gluten interaction was observed in PSPP, as shown in Fig. 7d. It seems that this weak gluten network in the PSPP bread was caused by the residual gelatinized starch of PSPP not completely decomposed by the intrinsic enzymes of wheat flour, which then cross-linked or adhered to the gluten network during the baking process.

Discussion

Bread making quality The significantly lower GRD of the dough with PSPP compared to control and PSPP+AM+HC can be attributed to the absence of gluten protein, and high fiber and damaged starch content, resulting in a weaker gluten network (Hathorn *et al.*, 2008). Decreased GRD was also observed by Murayama *et al.* (2015) after the addition of potato flour to wheat bread. On the other hand, the improvement in GRD, GP, and SLV of PSPP+AM+HC bread can be attributed to the activities of AM and HC, which resulted in the formation of fermentable sugars that were used by yeast for increased gas production (Goesaert *et al.*, 2009). The same improvement in SLV was reported by Kim *et al.* (2006) and Schoenlechner *et al.* (2013) after adding AM to polished flour supplemented wheat bread and HC in millet/wheat composite bread, respectively. Moreover, as previously reported, the general improvements in the bread making qualities of the dough with PSPP+AM+HC can be mainly explained by the hydrolytic activity of AM and HC, which resulted in the decomposition of damaged starch, including the gelatinized starch of PSPP and hemicellulose such as insoluble pentosans into low molecular weight substances (Santiago *et al.*, 2015).

Changes in textural properties during bread storage The significantly higher hardness, gumminess, chewiness and firming rate (Tables 2 and 3) of PSPP bread can be attributed to the 54% damaged starch content of PSPP (data not shown), which forms cross-links with the protein network during baking and increases in number and strength during storage, causing crumb hardening (Martin *et al.*, 1991). The same increase in firming rate was observed by Yamauchi *et al.* (2004a) for bread supplemented with intact and fermented potato pulps of high decomposed starch and fiber contents. On the other hand, the significantly lower firming rate of PSPP+AM+HC bread corroborates with its significantly lower amylose content and enthalpy of retrogradation (Table 3) and can be related with the anti-staling property of AM and HC ($p < 0.05$). The same lower firming rate of wheat bread with AM and HC was reported by Caballero *et al.* (2007). The activity of AM results in the degradation of mainly damaged starch of wheat flour and gelatinized starch of PSPP into low molecular weight dextrins, oligo-saccharides, maltose and glucose, decreasing the amount of available starch for retrogradation and retarding the retrogradation of gelatinized starch gel in bread (Duran *et al.*, 2001; Palacios *et al.*, 2004; Goesaert *et al.*, 2009; Gomes-Ruff *et al.*, 2012). Moreover, these saccharide products of AM hydrolysis interfere with starch-protein interactions, resulting in few, weak crosslinks, thus reducing the firming rate (Table 3) (Martin *et al.*, 1991; Martin and Hoseney, 1991). The weaker starch-protein interaction may have also resulted in significantly lower cohesiveness of bread with PSPP+AM+HC compared to control and PSPP breads (Table 2). A similar decrease in the cohesiveness of wheat dough resulting from AM addition was reported by Armero and Collar (1997). Likewise, lower hardness and cohesiveness of wheat bread crumb treated with fungal AM compared to the control was reported by Blaszcak *et al.* (2004).

Rupture properties of breads The significantly higher RF of PSPP bread crumb after 1 day of storage compared with the control and PSPP+AM+HC ($p < 0.05$) can be related to its harder texture (Table 2 and Fig. 3), resulting from its low porosity and the high interaction between gelatinized starch and the gluten network (Martin *et al.* 1991). The same increase in RF was observed by Yamauchi *et al.* (2004b) after supplementing wheat bread with 50% rice flour. On the other hand, the lower RF of bread crumb with PSPP+AM+HC than the control and PSPP can be related to

Fig. 6. Electron microscope photographs of dough just after proofing

a) Control without elution, b) Control with elution, c) +PSPP without elution, d) +PSPP with elution, e) +PSPP+AM+HC without elution, f) +PSPP+AM+HC with elution

Abbreviations: PSPP, purple sweet potato powder; AM, α -amylase; HC, hemicellulase; L, large type starch granules; S, small type starch granules; SG, starch and gluten crosslinks; SSG, swollen starch granules; G, gelatinized starch

its softer texture (Table 2 and Fig. 3), which resulted from its high porosity, lesser interaction of gelatinized starch with gluten and the anti-staling effect of AM and HC (Martin and Hosney, 1991; Duran *et al.*, 2001; Caballero *et al.*, 2007). The decrease in RF, RD and RE during storage was caused by starch retrogradation (Fig. 4), making the crumb brittle. The significantly lower RF of

PSPP+AM+HC after 3 days of storage compared to the control and PSPP can be attributed to the collective effect of a softer and brittle bread crumb. Similarly, the significantly lower RE of PSPP+AM+HC compared to the other treatments at all incubation periods can be attributed to its softer texture, and lower firming rate and enthalpy of retrogradation (Figs. 3, 4 and Tables 3, 4).

Fig. 7. Electron microscope photographs of bread crumb just after baking

a) Control without elution, b) Control with elution, c) +PSPP without elution, d) +PSPP with elution, e) +PSPP+AM+HC without elution, f) +PSPP+AM+HC with elution

Abbreviations: PSPP, purple sweet potato powder; AM, α -amylase; HC, hemicellulase; SSG, swollen starch granules; GGI, gelatinized starch and gluten interaction

Moisture and soluble sugar content of bread The significantly lower moisture content loss of PSPP+AM+HC compared to control and PSPP may have caused its lower firming rate (Table 3), which conforms to the report of Rogers *et al.* (1988) showing that moisture loss results in higher firming rate. On the other hand, the significantly higher water soluble glucose, maltose, reducing and

total saccharide content of PSPP bread (Table 6) compared to the control may have been affected by the intrinsic sugar content of sweet potato powder and the hydrolytic enzyme products of wheat flour and yeast (Lu and Gao, 2011). Moreover, the significantly higher water-soluble glucose, fructose, maltose, reducing and total sugar contents (Table 6) of PSPP+AM+HC may be due to the

products of AM and HC hydrolytic activities. These higher water-soluble sugar contents may have prevented the loss of water during storage, resulting in the lower firming rate of PSPP+AM+HC (Tables 3, 5 and Fig. 3). Similar observations were reported by Martin and Hosney (1991), *i.e.*, lower firmness of bread with higher maltose content after 5 days of storage. Moreover, Duran *et al.* (2001) reported that sugars and oligosaccharides reduce the retrogradation rate by inhibiting hydrogen bonding among starch chains, which causes a decrease in crumb firmness and staling rate. Furthermore, Yamauchi *et al.* (2014) related the higher moisture and saccharide content of Yudane bread as a reflection of increased water absorption and decomposition of starch, resulting in a softer texture and slower staling.

Dough and bread crumb structure The large and small starch granules detected in all dough treatments just after mixing and proofing were also observed by Blaszcak *et al.* (2004) in the microstructure of wheat dough. The interaction of gelatinized starch with the gluten network observed in Figs. 5d and 6d for PSPP dough may have caused the lower SLV and high firming rate of the resulting bread (Tables 1 and 3).

For dough just after proofing, the greater number of swollen starch granules observed in PSPP+AM+HC (Fig. 6e) can be related to the hydration and swelling pressure caused by AM, as reported by Blaszcak *et al.* (2004). In addition, the lower number of starch-gluten crosslinks, smaller pores and greater porosity of PSPP+AM+HC dough (Fig. 6f) compared to the control and PSPP may have caused its significantly higher SLV and lower firming rate, as shown in Tables 1 and 3. On the other hand, the greater swollen starch granule-gluten network crosslinks can be related to the larger and reduced number of pores (Fig. 6d) caused by PSPP supplementation, which may have resulted in the lower SLV and higher firming rate (Tables 1 and 3).

Ultimately, for bread just after baking, the greater starch granule rupture of PSPP+AM+HC may have been caused by the greater susceptibility of amylose to the action of AM, as also reported by Blaszcak *et al.* (2004). Moreover, the more open network of PSPP+AM+HC (Fig. 7f) compared to the compact, close gluten network of the control (Fig. 7b) may have resulted in the significantly higher SLV and GRD (Table 1). The same result was observed by Blaszcak *et al.* (2004) in wheat bread supplemented with fungal and bacterial AM. On the other hand, the greater gelatinized starch-gluten interaction observed in PSPP bread (Fig. 7d) may have caused the lower SLV and GRD (Table 1).

Conclusion

Our results demonstrated that PSPP supplementation results in bread with a higher firming rate, which can be attributed to the high damaged starch content of PSPP causing greater starch-gluten interaction, as shown by its dough structure. However, moisture loss and rupture force of PSPP bread was the same as the control, which can be attributed to the high water holding capacity of sugars in

PSPP.

On the other hand, treatment with AM and HC resulted in bread with lower firming rate, enthalpy of retrogradation, amylose content, rupture force and energy, and moisture loss during storage. These improvements are related to the anti-staling properties of AM and HC, resulting in lower starch-gluten interaction, as shown by the dough and bread structures. Moreover, the sugar and dextrin products of AM and HC hydrolysis prevents moisture loss and starch retrogradation, resulting in lower firming rate and rupture properties. These enhanced textural properties, enthalpy of retrogradation and structure indicate a more acceptable bread, potentially leading to the increased utilization of purple sweet potato in the baking industry.

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