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Technical paper

The Bread Making Qualities of Bread Dough Supplemented with Whole Wheat Flour and Treated with Enzymes

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Whole wheat flour (WWF) contains various kinds of functional ingredients, such as dietary fiber, resistant starch, minerals, etc., and bread containing WWF is in great demand in many countries because of its health benefits. Although dough with WWF often results in lower quality bread, previous studies suggested that this could be improved by the addition of suitable enzymes. This study investigated the effects of WWF substitution and enzyme treatments using α-amylase (AM) and hemicellulase (HC) on bread making qualities. Results showed that the addition of WWF produced dough with low gas retention of dough (GRD) and specific loaf volume (SLV). However, AM and HC drastically improved both GRD and SLV of WWF-substituted dough and bread by degrading damaged starch and hemicellulose. Thus, these results indicated that the treatments with suitable enzymes could drastically improve the bread making qualities of dough made with WWF.

Keywords: whole wheat flour, bread making quality, α-amylase, hemicellulase, enzyme

Introduction

The functional ingredients of whole grains, including dietary fiber, resistant starch, vitamins and minerals, have various physiological benefits related to "western diseases", such as coronary heart disease, colon cancer and diabetes. However, products made with whole grains are not as attractive as white wheat flour products because of the higher amounts of bran and germ contained in whole wheat flour (WWF), which reduce the quality and sensory value of the final products (Ozboy and Koksel, 1997; Wang *et al*., 2002). In breadmaking, the presence of bran and germ causes the deterioration of rheological properties of dough, i.e., decrease in bread loaf volume, increase in crumb hardness and darkening of crumb appearance (Lai *et al*., 1989). Moreover, the addition of WWF imparts a different flavor profile to bread as compared with that of white flour bread (Chang and Chambers, 1992).

It is generally accepted that the damaged starch (DS) and fiber in flour influence gluten formation, resulting in decreased bread making quality (Dexter *et al.,* 1994; Santiago *et al*., 2015a; Santiago *et al*., 2015b; Yamauchi *et al.,* 2004a; Yamauchi *et al.,* 2004b). There are various kinds of enzymes used in baking as breadmaking improvers. Among them, amylases and hemicellulases are hydrolases active against DS and insoluble pentosan, respectively. α-Amylase (AM) is an endo-type enzyme that catalyzes the cleavage of α-1, 4-glycosidic bonds in the inner part of the amylose or amylopectin chain. The end products of AM action are oligosaccharides with various lengths and α-limit dextrins, which are branched oligosaccharides; and endogenous β-amylase converts these oligosaccharides and DS into maltose, which is used as fermentable sugar by yeast or sourdough

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microorganisms (Synowiecki, 2007; Goesaert *et al*., 2005). β-Amylase, which exists in wheat flour as an endogenous enzyme, has sufficient enzymatic activity to convert the above oligosaccharides and DS into maltose. On the other hand, insufficient amounts of AM exist in wheat flour as an endogenous enzyme, resulting in inadequate enzymatic activity to degrade the DS. In this regard, supplementation with AM modifies the balance of α- and β-amylases and breaks down DS particles into low molecular weight saccharides during dough fermentation (Martin and Hoseney, 1991). The increased levels of reducing sugars lead to the formation of Maillard reaction products, intensifying bread flavor and crust color. In addition, α- and β-amylases can improve the gas-retention properties of fermented dough and reduce dough viscosity during starch gelatinization, with consequent improvements in bakery product volume and softness (Goesaert *et al*., 2009; Poutanen, 1997).

Total dietary fiber (TDF) is composed of soluble (SDF) and insoluble dietary fiber (IDF). Lignin, cellulose and hemicellulose are classified as IDF and are important factors in breadmaking. Excess amounts of IDF, especially hemicellulose, weaken formation of the gluten network (Pomeranz, 1977). A hemicellulase (HC), such as xylanase, is a hydrolytic enzyme that degrades water insoluble hemicelluloses (mainly insoluble pentosan) in bread dough into water soluble forms. Addition of this enzyme results in bread dough that is slacker, softer and more viscous, and the bread obtained has greater bread volume, finer and more uniform crumb, longer shelf life, and low staling rate (Jiang *et al.,* 2005). Moreover, it is expected that the addition of xylanases to dough processing will increase the concentration of arabinoxylo-oligosaccharides in bread, which have beneficial effects on human health, such as improving the enteral environment, due to its prebiotic effect (Courtin and Delcour, 2001; Bhat, 2000).

For the above-mentioned reasons, the quality of bread supplemented with WWF may be improved by the addition of enzymes. As a result, it is expected that attractive final products with high nutritional value and functionality may be obtained. In this study, we examined the effect of WWF supplementation on bread making quality and also determined the applicability of AM and HC in improving the bread making quality of WWF-supplemented bread.

Materials and Methods

Flours and enzymes used The commercial strong wheat flour (Camellia) and WWF (Zenryufun Kyoriki) used in this study were manufactured by Nisshin Flour Milling Co., Ltd. (Tokyo, Japan) and Ebetsu Flour Milling Co., Ltd. (Ebetsu, Japan), respectively.

Two commercial enzymes manufactured by Shin Nihon Chemical Co., Ltd. (Anjo, Japan) were used. AM (Sumizyme AS) contains 1500 α-amylase U/g, and HC (Sumizyme SNX) contains 14,000 xylanase U/g.

Dough preparation and bread making The bread-making tests were carried out using the no-time method and following the standard wheat bread formulation as the control, which is prepared with 200 g of wheat flour, 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 salt (The Salt Industry Center of Japan, Tokyo, Japan), 20 mg of ascorbic acid (Wako Pure Chemical Industries, Ltd., Osaka, Japan), and a suitable amount of water, as reported by Yamauchi *et al.* (2001). The optimal water absorption of each test was determined using a Farinograph at 500 BU according to the method used by the AACC (1991).

For the WWF-substituted bread making treatments, 40% of the original white wheat flour content of the control was replaced with WWF, which, as described below, was determined as the maximum concentration at which the bread making qualities could be improved with enzymes. The dough treated with AM and HC contained an optimum amount of 0.025% (0.05 g) and 0.05% (0.10 g), respectively. The dough was mixed with a pin mixer (NATIONAL COMPLETE 100-200 GRAM MIXER, MODEL 100-200A; National Mfg. Co., Lincoln, NE, USA) to just beyond the peak development, as indicated by the electric power curve of the mixing motor. After mixing, the dough was divided into 100 g and 20 g portions, rounded, and incubated for 20 min (bench time) at 30℃ and 75% relative humidity (RH) in a fermentation cabinet. The dough samples (100 g) were molded and rolled using a molding machine with upper 7.9 mm and lower 4.7 mm clearance, respectively. The dough was panned and proofed for 70 min at 38℃ and 85% RH, and then baked at 180℃ for 25 min, as reported by Santiago *et al*. (2015a). Meanwhile, the 20-g dough samples were used for the analysis of gassing power (GP) and gas retention of dough (GRD). The GP and GRD were measured using the doughs of the twice bread making tests

Maximum WWF ratio and optimum amounts of AM and HC determination As a preliminary experiment, we determined the maximum amount of WWF substitution by evaluating the bread making qualities of dough with various amounts of WWF and optimal amounts of both enzymes. As a result, we found that the bread quality of dough substituted with more than 40% WWF was inferior compared with the control, even when the optimum amounts of both enzymes were added. Therefore, the amount of added WWF was set at 40%. We also determined the optimum amount of AM or HC to be added to the 40% WWF-substituted dough by evaluating SLV and dough handling properties. From these experiments, we found that adding an excess amount of AM or HC resulted in lower SLV and poor dough handling properties, such as extremely sticky dough. In the case of >0.15% AM addition, the dough showed rather sticky properties and bread SLV was lower than that of bread with 0.025% AM. Meanwhile, dough with >0.1% HC addition showed extremely sticky properties and SLV of the bread was slightly low compared to bread with 0.05% HC.

Table 1. Bread making qualities of dough supplemented with WWF, AM and HC[1)]

Bread Making Treatments	Water absorption (%)	GRD	GP (mL)			SLV	Moisture Content of crumb[2)]
		(mL)	1h	2h	3h	(cm^3/g)	(%)
Control	68	98.3 ± 2.9 cd	22.6 ± 0.8 b	55.7 ± 0.7 b	88.7 ± 0.7 c	4.85 ± 0.05 c	41.6 ± 1.0 a
WWF	69	90.0 ± 0.0 d	23.8 ± 0.8 ab	58.0 ± 0.8 a	94.8 ± 0.8 a	4.43 ± 0.16 d	42.1 ± 0.4 a
WWF+AM	69	110.0 ± 0.0 ab	24.0 ± 0.5 ab	57.7 ± 0.6 a	94.0 ± 0.6 a	5.08 ± 0.10 ab	41.8 ± 0.3 a
WWF+HC	69	106.7 ± 5.7 bc	24.8 ± 0.5 a	57.1 ± 1.0 ab	91.9 ± 1.4 b	4.91 ± 0.04 bc	41.5 ± 0.6 a
WWF+AM+HC	69	118.0 ± 2.9 a	24.4 ± 0.4 a	55.8 ± 0.6 b	89.7 ± 0.7 c	5.19 ± 0.08 a	41.6 ± 0.4 a

Abbreviations : GRD, gas retention of dough; GP, gassing power of dough; SLV, specific loaf volume; WWF, whole wheat flour; AM, α-amylase; HC, hemicellulase

[1)]Each value, except for water absorption is the mean ± SD. The values followed by different letters within column are significantly different ($p < 0.05$).

[2)]Moisture content of crumb was meas ured with the samples stored 1 day after baking.

Dough properties and bread evaluation GRD was evaluated by measuring the maximum expansion volume of 20 g dough proofed at 38℃ and 85% RH in a cylinder subjected to 0 to 75 cmHg, following the report of Yamauchi *et al*. (2000). GP of 20 g of dough after bench time was measured at 30℃ for 1, 2, and 3 hrs using a Fermograph II (ATTO Co., Ltd., Tokyo, Japan).

The SLV of bread cooled at room temperature for 1 h after baking was measured by the rapeseed-displacement method according to the AACCI (2000). Two loaves were made by a single bread making test, and the test in all conditions was also performed twice. The SLV of bread was measured using a total of four loaves. Images of the bread and bread crumb were recorded using a digital camera (model EX-H15; Casio Computer Co., Ltd., Tokyo, Japan) and scanner (model GT-S640; Seiko Epson Co., Ltd., Nagano, Japan), respectively. Color of the bread crust and crumb were determined using a colorimeter (CR-400; Konica Minolta Sensing, Inc., Tokyo, Japan). The moisture content of the bread crumb was measured based on the AOAC official method (AOAC, 2000).

Fiber and DS analysis Dough samples (100 g) after final proofing for 70 min at 38℃ and 85% RH were frozen at −30℃ for 30 min using a blast freezer and stored in a freezer at −20℃ until used for analysis of dough fiber and DS content.

The dough samples were lyophilized and ground prior to fiber analysis. Neutral detergent fiber (NDF), an estimate of the cellulose, hemicellulose and lignin content, and acid detergent fiber (ADF), which is equivalent to the amount of cellulose and lignin, were analyzed using AOAC official methods (AOAC, 2000). Subsequently, the crude hemicellulose content was estimated as the difference between NDF and ADF.

On the other hand, water soluble sugars were removed before DS analysis, by mixing 100 mg of the dough with 8 mL of distilled water in a vortex mixer for 1 min and centrifugation at 2200 g for 10 min at 20℃. Mixing and centrifugation were repeated twice, and the resulting precipitate was used for DS analysis using the Megazyme assay kit (Megazyme International Ireland Ltd., Wicklow, Ireland) based on the method of Gibson *et al.* (1991).

Bread staling rate evaluation Texture properties of bread crumb during storage were analyzed using the method presented by Yamauchi *et al*. (2001). Temporal changes in hardness of bread crumb during storage were evaluated using a rheometer (RHEONER RE2-33005C; Yamaden Co., Ltd., Tokyo, Japan). Loaves cooled for 1 h at room temperature after baking were packed into polyethylene bags and stored at 20℃ and 70% RH. The hardness of breads was measured using two loaves after storage for 1, 2 and 3 days. The two loaves were sliced at 2-cm thickness and three sample slices in the central portion, for a total of six samples, were used for measurements. The 3 cm × 3 cm square crumbs were cut from the center of each slice by using an ultrasonic cutter (USC-3305; Yamaden Co. Ltd.). The maximum stress as hardness was measured by compressing the crumbs from 2 cm thickness to 1 cm thickness at a 1-mm/sec compression rate using a special cube plunger (D: 6 cm × W: 6 cm × H: 2 cm) on a rheometer (RHEONER RE2-33005C; Yamaden Co., Ltd.).

Statistical analysis All data except for water absorption, SLV, color values of bread and hardness of bread were measured 6 times by using the samples prepared from the two bread making tests. The SLV, color values of bread and hardness of bread were performed 4, 10 and 6 times, respectively. Significant differences in the data presented in Table 1 to Table 3 and Fig. 2, except for water absorption, for bread making were evaluated by analysis of variance at 5% significance level with Tukey's multiple range test using Excel statistical software 2012.

Results

Bread making quality evaluation The bread making qualities of the control, WWF-supplemented, and enzyme-treated (AM and HC) and WWF-supplemented doughs are presented in Table 1. Results showed that the addition of WWF significantly lowered the GRD compared with the other treatments, whereas doughs with WWF+AM or WWF+HC had significantly higher GRD compared with the control. On the other hand, the dough supplemented with WWF+AM+HC showed significantly higher GRD than the other

Table 2. Color of bread crusts and crumbs[1)]

Bread Making Treatments	Bread crust color			Bread crumb color		
	L*	a*	b*	L*	a*	b*
Control	46.00 ± 0.52 a	15.50 ± 0.10 a	29.00 ± 0.36 a	75.21 ± 0.72 a	-2.19 ± 0.09 b	9.67 ± 0.60 c
WWF	43.79 ± 0.64 b	13.97 ± 0.21 b	25.21 ± 0.91 b	69.82 ± 1.62 b	-0.37 ± 0.19 a	10.94 ± 0.55 ab
WWF+AM	40.56 ± 1.02 c	13.76 ± 0.33 b	21.65 ± 1.29 c	69.02 ± 1.25 b	-0.17 ± 0.29 a	11.19 ± 0.79 a
WWF+HC	41.31 ± 1.26 c	14.03 ± 0.12 b	23.09 ± 1.24 c	68.35 ± 1.43 b	-0.11 ± 0.29 a	10.78 ± 0.83 ab
WWF+AM+HC	39.80 ± 1.08 c	13.68 ± 0.26 b	21.23 ± 1.13 c	69.28 ± 0.89 b	-0.38 ± 0.30 a	10.35 ± 0.58 abc

Abbreviations : WWF, whole wheat flour; AM, α-amylase; HC, hemicellulase; L*, level of lightness or darkness ; a*, level of redness or greeness ; b*, level of yellowness or blueness

[1)]Each value is the mean ± SD. The values followed by different letters within column are significantly different (p <0.05).

Table 3. Fiber and damaged starch contents of doughs[1)]

Bread Making Treatments	NDF (%)	ADF (%)	NDF-ADF[2)] (%)	DS (%)
Control	0.37 ± 0.11 b	0.22 ± 0.03 b	0.15 ± 0.08 b	3.54 ± 0.07 a
WWF	2.31 ± 0.36 a	0.90 ± 0.01 a	1.41 ± 0.25 a	3.26 ± 0.04 b
WWF+AM	2.21 ± 0.31 a	0.89 ± 0.03 a	1.31 ± 0.28 a	2.26 ± 0.05 d
WWF+HC	1.91 ± 0.09 a	0.85 ± 0.02 a	1.06 ± 0.08 a	2.39 ± 0.02 c
WWF+AM+HC	2.14 ± 0.01 a	0.84 ± 0.05 a	1.30 ± 0.04 a	2.25 ± 0.08 d

Abbreviations : NDF, neutral detergent fiber; ADF, acid detergent fiber; DS, damaged starch; WWF, whole wheat flour; AM, α-amylase; HC, hemicellulase

[1)]Each value is the mean ± SD. The values followed by different letters within column are significantly different (p <0.05).

[2)]NDF-ADF: crude hemicellulose content

bread making treatments.

In WWF–supplemented doughs, GP showed a tendency to increase during dough fermentation, which varied among bread making treatments depending on the fermentation time. Initially, at 1-h fermentation, the GP of all WWF doughs were significantly higher than the control. At 2-h fermentation, the GP of the WWF and WWF+AM doughs were significantly higher than the control. At 3-h fermentation, differences in GP between the doughs with WWF or WWF+AM and the other doughs increased, and WWF and WWF+AM showed significantly higher values among all samples. The WWF+HC dough had a higher value than the control and WWF+AM+HC doughs at 2-h fermentation. Therefore, the GP after 2 h of fermentation in the control and WWF+AM+HC doughs was lower than the other samples.

In terms of SLV, the WWF bread was significantly lower than the other breads. WWF+AM+HC bread had the highest SLV among the treatments, although the value was not significantly different compared to the WWF+AM bread.

There was no significant difference in terms of moisture content among all samples.

Evaluation of bread crust and crumb Table 2 summarizes the color of breads. The crust of the control bread showed significantly higher values of L*, a* and b* than the other samples. The values of L*, a* and b* decreased with the addition of WWF. On the other hand, the crust of enzyme-treated breads had significant lower L* and b* values than the WWF-supplemented bread. In terms of crumb color, L* of the control was significantly higher than the other treatments, and the WWF-supplemented treatments with or without enzymes were not significantly different. On the other hand, the crumb of the control showed significantly lower a* value than those of the others. In terms of b* value, the control was also significantly lower than all other bread crumbs except for WWF+AM+HC, while the WWF+AM bread showed the highest value.

The bread appearance and crumb images are shown in Fig. 1. Darker external color of bread was observed in the breads substituted with WWF compared to the control. Similarly, the crumbs of breads with WWF were darker in color than the control crumb. The WWF bread was smaller in appearance than the control, whereas the breads with WWF and enzyme treatments were either of the same size or larger than the control. These results correspond to those for SLV presented in Table 1.

Fiber and DS content of dough Table 3 shows the fiber composition and DS content of doughs from different treatments. Results showed that the NDF, ADF and crude hemicellulose (NDF-ADF) contents of doughs supplemented with WWF or WWF +

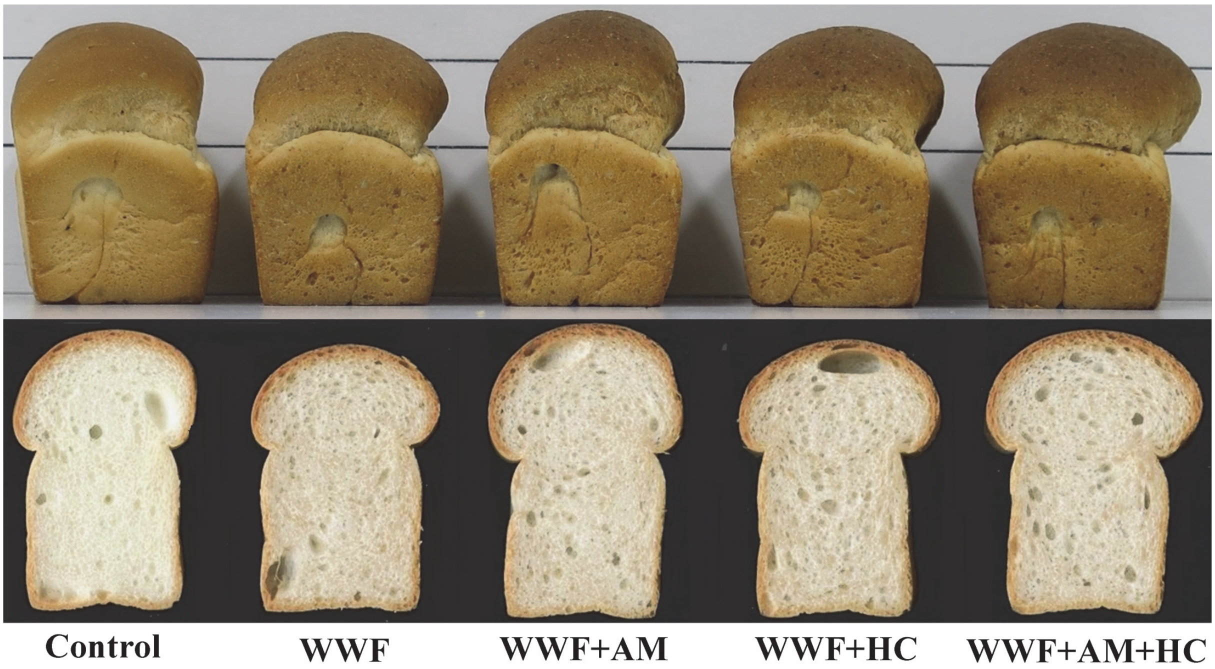

Fig. 1. Photocopy and scanned images of breads with WWF, etc.
Abbreviations: WWF, whole wheat flour; AM, α-amylase; HC, hemicellulose

enzymes were significantly higher than the control dough. Further, these values were lower in enzyme-treated doughs than for WWF alone.

In terms of DS, the control value was significantly higher than those for all other treatments. DS of the WWF-supplemented dough was significantly lower than the control but higher than all enzyme-treated doughs. The amount of DS in doughs treated with enzymes was significantly decreased, with the WWF+AM+HC dough showing the lowest value, compared to the other samples.

Hardness of bread Temporal changes in the hardness of bread crumbs during storage for 3 days is shown in Fig. 2. After storage for 1 day, the WWF bread showed a significantly higher value than the other samples. The control bread and those with enzymes showed similar values after 1-day storage. After storage for 2 days, the control and WWF breads had a similar high value, while those with enzymes remained significantly lower than the other samples. The hardness of WWF bread increased drastically after 3-day storage, resulting in a significantly higher value among all samples. On the other hand, the hardness of breads with enzymes remained low; the WWF bread treated with AM had the lowest value, followed by the WWF+AM+HC and WWF+HC breads. In addition, the hardness of breads with added enzymes after 3-day storage was significantly lower than that of WWF bread and showed lower values compared to the control; although, except for the WWF+AM bread, those values did not significantly differ from the control.

Discussion

Evaluation of bread making quality The low GRD and SLV of bread supplemented with WWF can be attributed to the relatively higher fiber and DS contents as shown in Tables 1 and 3. The excessive DF of WWF disrupts formation of the gluten network, resulting in a weaker gluten network (Lai *et al.,* 1989; Ozboy and Koksel, 1997; Wang *et al*., 2002). The improved GRD and SLV of WWF+AM bread compared to WWF bread can be explained by the enzymatic activity of AM, which leads to the hydrolysis of damaged and gelatinized starch to maltose, dextrin, etc. These results agree with the report of Kim *et al*. (2006), who demonstrated that SLV decreased when the bread was supplemented with polished wheat flour high in fiber and DS content, and which increased upon the addition of AM. A similar observation was also reported by Patel *et al*. (2012) on the improvement in the specific volume of chemically leavened bread treated with fungal AM.

Likewise, HC catalyzes the degradation of polysaccharides, such as glucans, galactans, mannans, pentosans, xylans, etc., into mono-sugars and short chain saccharides, including glucose, galactose, mannose, arabinose, xylose, xylobiose and xylotriose, which do not disturb formation of the gluten network as reported by Jiang *et al.* (2005). This catalytic activity may have caused the higher GRD and SLV of WWF+HC bread compared to WWF bread. The same improvements in SLV after adding xylanase, a kind of HC enzyme, to whole wheat and millet/wheat composite bread were observed by Shah *et al.* (2006) and Schoenlechner *et al.* (2013), respectively.

The dough and bread with WWF+AM+HC showed the highest values of GRD and SLV of all treatments including the control. This might be attributed to the decreased content of DS and insoluble hemicellulose following the combined catalytic activity of AM and HC, as shown in Tables 1 and 3. Despite this, the control bread showed a high DS content, 3.54%, and rather high values of GRD and SLV. The main reason seemed to be related to the very low value of TDF, especially hemicellulose, in the control dough compared to those of the other WWF-substituted doughs. Goesaert *et al.* (2009) and Jiang *et al.* (2005) reported that the hydrolytic activity of AM and HC added to bread dough caused an increase in mono-sugars, mainly produced from DS and pentosan, which consequently resulted in elevated yeast fermentation and significant improvements of GP in the early fermentation stage. However, from the results shown in Table 1, large increases in GP were observed with WWF addition compared to samples with added enzymes. This may be related to the high concentration of various nutrients in WWF that promote fermentation by yeast.

Bread color and appearance The addition of WWF resulted in a darker crust color compared with the control. Likewise, the individual and combined treatments of AM and HC also caused darkening of color compared with the control and WWF breads, evidenced by their significantly lower L* values and the images shown in Table 2 and Fig. 1. Enzyme addition also decreased the values of redness and yellowness, indicated by the lower a* and b* values, as shown in Table 2. These color changes can be attributed to the increase in the concentration of reducing sugars like glucose, fructose, etc., which promote the Maillard reaction (data not shown). These sugars also intensify bread flavor and browning as reported by Goesaert *et al*. (2009).

The darker bread crumb color with added WWF can be attributed to the natural dark brown color of wheat bran. Similarly, the brown color of WWF influences the color of the crumb of WWF-supplemented bread to brown, thus, resulting in the decrease in L* value and increase in redness and yellowness, as shown in Table 2.

Fiber and DS contents of dough The fiber and DS contents of dough after proofing are shown in Table 3. The WWF-supplemented dough has a higher percentage of fiber (NDF and ADF), because WWF is high in dietary fiber. Generally, excess fiber causes an inhibition of optimal gluten formation, resulting in decreased SLV and GRD. On the other hand, the enzyme-treated doughs showed lower fiber content compared with the WWF-supplemented dough. The WWF+HC dough had the lowest fiber content, attributable to the xylanase activity of HC, which catalyzes the hydrolysis of hemicelluloses such as xylan and arabinoxylan to xylobiose, xylose, etc. This activity may have resulted in the low contents of NDF and crude hemicellulose (NDF-ADF) of WWF+HC and WWF+AM+HC doughs (Stojceska and Ainsworth, 2008; Jiang *et al.,* 2005).

The DS content of the dough without added AM can be basically associated with the amount of DS contained in the wheat flour after milling. Excess amounts of DS cause undesirable effects on bread making qualities. The control and WWF doughs showed higher DS values compared with the enzyme treated doughs. WWF+AM and WWF+AM+HC had significantly lower DS than the other treatments, which can be related to the effects of AM activity. Ultimately, these decreases in the contents of DS and fiber (mainly insoluble hemicellulose (pentosan)) improved the GP, GRD and SLV of the bread treated with AM and HC.

Hardness of bread Changes in the hardness of bread crumbs relate to various factors: such as the retrogradation rate of the gelatinized starch gel in bread, DS and insoluble pentosan contents in dough, and SLV, and which are the main factors related to the staling of WWF-supplemented bread. AM mainly breaks down the DS in dough and gelatinized starch gel during dough baking into low molecular weight dextrins, oligo-saccharides, etc., while endogenous β-amylase converts the above saccharides into maltose. AM and β-amylase have different but complementary functions during the bread making process, bringing about an increase in low molecular weight saccharides in the breads with AM. It is reported that these low molecular weight saccharides decrease the amount of available starch for retrogradation and retard the retrogradation of gelatinized starch gel in bread (Duran *et al*., 2001; Palacios *et al.,* 2004; Goesaert *et al.,* 2009). Moreover, these saccharide products of AM hydrolysis interfere with starch-protein interactions, resulting in few and weak crosslinks, and thus reduce the hardening rate of bread (Martin and Hoseney, 1991; Martin *et al*., 1991). From Table 1 and Fig. 1, the SLV values of breads with AM were also larger than those of the control. Maleki *et al*. (1980) reported that the staling rate of large SLV bread clearly decreases. From these findings, it appears that the low hardening rate of bread with AM is mainly caused by the anti-staling effects resulting from increases in these saccharides and the high SLV in WWF+AM breads.

HC mainly attacks insoluble pentosan in the dough, which interferes with the formation of the gluten network, resulting in improved dough bread making quality, evidenced by enhanced SLV and increased low molecular weight saccharides in breads with HC as well as AM (Caballero *et al*., 2007; Ghoshal *et al*., 2013). These previous findings show that the staling of breads with added HC is slowed via similar effects observed with AM addition. Supporting this is the data of crude hemicellulose contents of the doughs with HC, shown in Table 3, which indicated lower values than that of the dough with WWF alone.

The above discussion showed that the main causes of slowed bread staling with added AM or HC are nearly the same. However, in the results shown in Fig. 2, it seems that the staling retarding effect of AM addition is slightly larger than that of HC. It is suggested that AM attacks the gelatinized starch gel and decomposes it to low molecular weight saccharides in the early stage of the baking process, which is generally considered to be the greatest factor in low bread staling. Martin and Hoseney (1991) and Palacios *et al*. (2004) reported that the retrogradation rate of the partially decomposed starch gel is low. The anti-staling effect of AM was also reported by Caballero *et al*. (2007) and Palacios *et al*. (2004). From the above findings, the higher anti-staling effect of AM compared to HC can be mainly attributed to its ability to

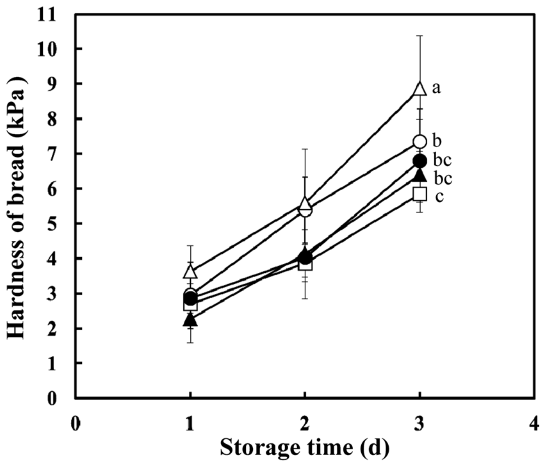

Fig. 2. Temporal hardness changes of bread crumbs with WWF, etc. during storage
Abbreviations: WWF, whole wheat flour; AM, α-amylase; HC, hemicellulase
The vertical bar is the standard deviation of each value. The values followed by different letters are significantly different ($p < 0.05$).
○: Control, △: WWF, □:WWF+AM, ●: WWF+HC, ▲: WWF+AM+HC.

decompose gelatinized starch gel in the bread.

From Fig. 2, it was also found that staling in the breads with added enzymes was obviously suppressed compared to the WWF and control breads, especially the former, and this seemed to be chiefly caused by the anti-staling effects resulting from increases in some saccharides as well as the high SLV. These results basically corresponded to the previous data reported by Caballero *et al*. 2007, Ghoshal *et al*., 2013 and Goesaert *et al*., 2009.

Overall, the results of this study demonstrated that the bread making qualities of the dough supplemented with 40% WWF were drastically improved by the treatment with suitable enzymes.

Conclusion

WWF has good functionality due to its high dietary fiber content and so on; however, its addition to bakery products results in lower bread making properties. Among dietary fibers, insoluble pentosan interferes with gluten network formation, resulting in decreased GRD and SLV, and accelerated staling rate. On the other hand, these problems can be basically solved by the addition of some enzymes for bread making, such as AM and HC, resulting in the desirable formation of the gluten network and improved dough properties. These improvements were mainly brought about by the degradation of DS and hemicellulose (mainly insoluble pentosan) into soluble low molecular weight saccharides, which do not interfere with gluten network formation during bread dough development. Ultimately, satisfactory bread supplemented with 40% WWFs can be obtained by the addition of suitable enzymes for bread making. This bread was characterized by a high amount of dietary fiber and several desirable properties such as increased GRD and SLV, and reduced staling rate.

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