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Original paper

Improvement of Bread Dough Supplemented with Crust Gel and the Addition of Optimal Amounts of Bakery Enzymes

Daisuke Goshima[1], Koki Matsushita[2], Junki Iwata[1], Tadashi Nakamura[1], Kanenori Takata[3] and Hiroaki Yamauchi[1,*]

[1]*Department of Life and Food Sciences, Obihiro University of Agriculture and Veterinary Medicine, 2-11 Nishi, Inada, Obihiro, Hokkaido 080-8555, Japan*
[2]*The United Graduate School of Agricultural Sciences, Iwate University, 3-18-8 Ueda, Morioka, Iwate 020-8550, Japan*
[3]*Western Region Agricultural Research Center, National Agriculture and Food Research Organization, 6-12-1 Nishifukatsu-cho, Fukuyama-shi, Hiroshima 721-8514, Japan*

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A large amount of bread crust is generated in the production of sandwiches and other bread products which, is not sufficiently used as food. If this crust can be reused effectively in the bread making process, it can add value, reduce waste, and improve the flavor and texture of bread. However, an excess amount of gelatinized starch (GS) in crust inhibits gluten network formation in bread dough and greatly decreases bread making qualities (BMQs). In this study, we investigated the optimal amount of two bakery enzymes, α-amylase (AM) and hemicellulase (HC), that can be added to improve the BMQs of crust-added dough. The optimal amounts of the enzymes were determined using response surface methodology and Solver, an Excel add-in software. The results showed that BMQs, specific loaf volume (SLV), gas retention of dough (GRD) and staling of dough and bread with added crust gel (CG) and optimal amounts of AM and HC were drastically improved compared to those without additional enzymes. It was concluded that response surface methodology and Solver are effective methods to easily determine the optimal amounts of the two enzymes to add to CG-supplemented dough to obtain high SLV and desirable texture, flavor and taste. The only negative aspect is poor bread color.

Keywords: bread crust, bread making qualities, α-amylase, hemicellulase, response surface methodology, optimization technique

Introduction

Bread crust is mainly a by-product of making special types of bread products like sandwiches at a bakery. Most of it is reused in fried confectioneries and bread crumbs, etc., or as feed for livestock. [i), ii), iii)] However, most local oven fresh bakeries rarely use bread crust as food and it is just discarded. Crust contains many flavorful components and therefore, it can be reused in bread making to improve the flavor and taste of bread (Kimpe and Keppens, 1996). As such, it can add value and reduce waste. However, when crust is used for bread making, it inhibits gluten network formation at the dough developing stage because of the large amounts of gelatinized and damaged starches (GS and DS) present, so the bread making qualities (BMQs) of the dough deteriorate noticeably (Santiago *et al*., 2015b; Yamauchi *et al*., 2014). Therefore, using crust for bread making is not very common.

Recently, it was reported that dough containing large amounts of GS and DS, which degrade BMQs, can be remarkably improved using multiple bread making enzymes, such as α-amylase (AM) and hemicellulase (HC) (Caballero *et*

*To whom correspondence should be addressed. E-mail: yamauchi@obihiro.ac.jp

Table 1. CCF on scaled values and actual concentrations of AM and HC[1)]

Run		Scaled value (-)[2)]		Actual concentration (g/100 g flour)	
		X_1	X_2	AM	HC
1	Center point	0.0	0.0	0.05	0.10
2		0.0	0.0	0.05	0.10
3		0.0	0.0	0.05	0.10
4		0.0	0.0	0.05	0.10
5	Axial point	+1.0	0.0	0.10	0.10
6		-1.0	0.0	0.00	0.10
7		0.0	-1.0	0.05	0.00
8		0.0	+1.0	0.05	0.20
9	Factorial point	-1.0	-1.0	0.00	0.00
10		-1.0	+1.0	0.00	0.20
11		+1.0	-1.0	0.10	0.00
12		+1.0	+1.0	0.10	0.20

[1)] Scaled values and actual concentrations of AM and HC are shown in above Table.
CCF: central cpmposite face- centered design, AM : α-amylase, HC : hemicellulase.

[2)] X_1= (AM-0.05)/0.05, where the actual concentration of AM ranged from 0.00 to 0.10/100 g flour.
X_2= (HC-0.10)/0.10, where the actual concentration of HC ranged from 0.00 to 0.20/100 g flour.

al., 2007; Santiago *et al*., 2015a). However, when using multiple enzymes to improve the BMQs of dough, it is very important to have an easy method to logically derive the optimal amounts of enzymes to add without performing numerous experiments.

Therefore, in this study, we adopted a central composite face-centered design (CCF) (Flander *et al.*, 2007) as an effective method to acquire evaluation data for determining the optimal amount of enzymes to add to improve the BMQs of bread dough supplemented with crust gel (CG). In this study, a response surface model (RSMd) was derived using data acquired from the CCF, and then the optimal amounts of multiple enzymes were determined using Solver, an Excel add-in software. Finally, to validate the effectiveness of these methods, bread making experiments with CG-supplemented dough using various amounts of the two enzymes were conducted and verified from the BMQs of the dough and various sensory evaluations of the bread.

Materials and Methods

Flour, enzymes and crust used A strong wheat flour, Camellia, (Nisshin Flour Milling Co., Ltd., Tokyo, Japan) along with two commercial enzymes: AM (Sumizyme AS) containing 1500 α-amylase U/g and HC (Sumizyme SNX) containing 14,000 xylanase U/g. (Shin Nihon Chemical Co., Ltd., Anjo, Japan) were used. Bread crust (white bread, Jukuryoku) was purchased from Masuya Shouten Co., Ltd. (Obihiro, Japan). CG was prepared and used for the bread making experiments. Bread crust was ground at high speed for 90 seconds in a food processor (MK-K81, Panasonic, Co., Ltd., Osaka, Japan), and water was added until a 1:3 crust-water ratio was attained. The mixture was then homogenized using the same processor.

Optimization of concentrations of added enzymes A CCF, as reported by Flander *et al*. (2007), was used with two variables to determine the optimal concentrations of enzymes. This CCF was composed of 12 runs with four replicates at the center point (Table 1). The two variables optimized were AM (g/100 g flour) and HC (g/100 g flour). Experimental conditions (amounts of added enzymes) at the center point were 0.05 and 0.10 (g/100 g flour) for AM and HC, respectively. Each concentration of AM and HC ranged from 0.00 to 0.10 and 0.00 to 0.20 (g/100 g flour), respectively. The minimum and maximum concentrations of these enzymes were determined using data from preliminary bread making tests that used various amounts of these enzymes. The bread making tests using CCF were performed randomly. In this study, specific loaf volume (SLV) and amounts of added enzymes (AM and HC) were adopted as a response and factors for analysis of RSMd, respectively. The reason SLV was selected as a response was that it is a representative index of BMQs. From the results of 12 runs based on CCF, an RSMd between a response and factors was derived using multiple regression analysis. The selection of the explanatory variables of the RSMd was determined using a stepwise back selection method for the variable with an F-value of 2.0 as an index. The effectiveness of this model was assessed by verifying the RSMd effect and lack of fit of the model with analysis of variance (ANOVA). Optimal amounts of the added enzymes were also determined with this model using Solver, an Excel add-in software. After the CCF experiments, bread making tests were performed with a variety of doughs: Control (no enzymes, no CG), CG-supplemented dough only (CG dough), and doughs supplemented with CG and optimal concentrations

of enzymes (AM and/or HC) (CG+AM, CG+HC and CG+AM+HC doughs). The BMQs for each dough type were evaluated in detail.

Dough preparation and bread making Bread making tests were performed using the no-time method and standard white bread formulation as previously reported by Yamauchi *et al*. (2001). The optimal amount of water for bread making was determined using a Farinograph at 500 BU according to the AACC method (1991). Ten percent of the wheat flour in the standard white bread formulation that was used for the Control was replaced with CG for the CG, CG+AM, CG+HC and CG+AM+HC doughs on a dry weight basis. In the preliminary bread making test, it was found that replacing 10 % of the wheat flour with CG was the optimal percentage: less had little impact on flavor and more reduced dough handling qualities.

Evaluation of BMQs The gas retention of dough (GRD) was evaluated by measuring the maximum expansion volume of 20 g of dough proofed for 70 min at 38 ℃ and 85 % relative humidity in a cylinder subjected to low pressure (Yamauchi *et al.,* 2000). The gassing power of 20 g of dough after bench time was measured at 30 ℃ for 1, 2 and 3 h using a Fermograph II (ATTO Co., Ltd., Tokyo, Japan) as reported by Santiago *et al*. (2015a). 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). Photographs and images of the breads and bread crumbs were also recorded using the method reported by Santiago *et al*. (2015a). The color of the bread top crust and crumbs was measured with a colorimeter (Matsushita *et al*., 2017). The moisture content of bread crumbs taken from breads stored for 1 day in polyethylene bags at 20 ℃ and 70 % relative humidity was measured using the method of Santiago *et al*. (2015a).

Analysis and evaluation of doughs and breads Soluble sugar contents, such as total, reducing, mono- and di-saccharides, were also analyzed using the method reported by Santiago *et al.* (2015b).

Dough sample preparation and storage before GS and DS, and dietary fiber (DF) analysis were carried out according to the method reported by Santiago *et al*. (2015a). The GS and DS contents in doughs were measured with the Megazyme starch damage assay kit (Megazyme International Ireland Ltd., Wicklow, Ireland) based on the method of Gibson *et al*. (1992). Neutral detergent fiber (NDF), the amounts of the hemicellulose, cellulose and lignin, and acid detergent fiber (ADF), the amounts of cellulose and lignin, were measured using the official AOAC method (AOAC, 2000). Subsequently, the approximate hemicellulose content was calculated as the difference between NDF and ADF. GS and DS, DF values are shown on a dry weight basis.

The temporal change of crumb hardness (bread staling) was measured at 1, 2 and 3 days of storage as described in Yamauchi *et al*. (2001). The loaves were sliced into 2 cm-thick slices and a 3×3 cm square was cut from the center. Using a rheometer (RE2-33005C, Yamaden Co., Ltd., Tokyo, Japan), the temporal hardness change was measured by compressing the sample up to a 50 % strain rate with a special cube plunger (6 cm length × 6 cm width × 2 cm height).

Sensory evaluation of breads Sensory evaluation of breads was conducted using samples stored in polyethylene bags for 1 day at 20 ℃ and 70 % relative humidity. Quantitative descriptive analysis of CG-supplemented and CG+AM+HC-supplemented breads (CG and CG+AM+HC breads) were evaluated and compared with the Control. Appearance was evaluated based on volume, shape and color. The crumb evaluation included crumb grain, color, texture, flavor and taste. The rating scale for volume ranged from 0 (small) to 30 (large). The scale for shape, crust color and crumb color was from 0 (poor) to 10 (excellent), that for crumb texture was from 0 (hard) to 10 (soft), and those for flavor and taste were from 0 (dislike) to 10 (like). The combined score for all attributes was on a scale of 100. Volume, other sensory attributes, and overall total points of the Control were set at 15, 5 and 50, respectively. The evaluations of CG and CG+AM+HC breads were carried out by a two-sample comparison method with the Control by nine panelists who were undergraduate and graduate students of Obihiro University of Agriculture and Veterinary Medicine. The panelists were trained and frequently assessed on their sensory evaluation of bread.

Statistical analysis Significant differences of all data measurements except for water absorption were evaluated by ANOVA at a 5 % significance level with Tukey's multiple range test using Excel statistical add-in software 2012 (SSRI Corporation, Tokyo, Japan).

Results and Discussion

Optimization of concentrations of added enzymes The results of 12 bread making runs with CCF are shown in Table 2. The RSMd between a response (SLV) and factors (AM and HC) is shown below, which was obtained using multiple regression analyses based on the results shown in Table 2.

$$Y=13.6778X_1+9.3287X_2-94.6387X_1^2-26.5918X_2^2 -27.6675X_1X_2+4.0951 \quad \cdots\cdots \text{Eq. 1}$$

Where Y is SLV (mL/g); X_1 is the concentration of AM (g/100 g flour); and X_2 is the concentration of HC (g/100 g flour). R^2 and corrected R^2 in the model showed high values, 0.8620 and 0.7469, respectively. The results of the ANOVA are shown in Table 3. Using Table 3, the effectiveness and lack of fit for the model were also assessed and were significant at a 5 % level and not significant at a 5 % level, respectively. In addition, correlation analysis between explanatory variables was performed, and there was no major problem concerning multicollinearity with respect to explanatory variables in this model.

These results showed that this RSMd is sufficiently effective for estimating SLV in cases using varying amounts of

Table 2. Experimental data of SLV as response of RSMd[1)]

Run		Actual concentration (g/100 g flour)		SLV (ml/g)
		AM	HC	
1	Center point	0.05	0.10	4.91
2		0.05	0.10	5.11
3		0.05	0.10	5.10
4		0.05	0.10	5.08
5	Axial point	0.10	0.10	4.77
6		0.00	0.10	4.98
7		0.05	0.00	4.69
8		0.05	0.20	5.00
9	Factorial point	0.00	0.00	3.93
10		0.00	0.20	4.84
11		0.10	0.00	4.53
12		0.10	0.20	4.89

[1)] Actual concentrations of AM and HC are shown in above Table. Each SLV value is the mean (n=3). SLV: specific loaf volume, RSMd: response surface model, AM : α-amylase, HC : hemicellulase.

Table 3. Result of ANOVA of RSMd[1)]

	SS	DF	MS	F	p	SL
ROM	1.028	5	0.206	7.494	0.015	*
Residual	0.165	6	0.027			
LOF	0.139	3	0.046	5.360	0.101	
Pure error	0.026	3	0.009			

[1)] The result of ANOVA of RSMd was obtained using the data of acquired based on the CCF. ANOVA: analysis of variance, RSMd: response surface model, SS: sum of squares, DF: degree of freedom, MS: mean of square, F: F-value, p: probability, SL: significant level (*, $p < 0.05$), ROM: regression of model, LOF: lack of fit.

AM and HC. Furthermore, the partial regression coefficients of X_1^2, X_2^2 and $X_1 X_2$ explanatory variables in the RSMd were negative values. Therefore, when both enzymes were added to the doughs in large amounts, they lowered BMQs (SLV). Since the magnitude of the partial regression coefficients on these explanatory variables followed the order of X_1^2, X_1X_2 and X_2^2, this showed that when the amounts of enzymes added were too large, there was a drastic decrease in SLV in the order of addition of AM, both enzymes and HC.

The optimal amounts of AM and HC calculated using Solver were 0.0576 and 0.1000 g/100 g flour, respectively. In dough with CG, the SLV increased with the addition of HC, but the improvements plateaued at 0.1000 g/100 g flour. When excessive HC was added, the dough became very sticky, which made handling extremely difficult. Therefore, in calculating the optimum concentrations of these enzymes, HC was limited to 0.1000 g/100 g flour as an upper concentration.

Evaluation of BMQs BMQs of the Control, CG, CG+AM, CG+HC and CG+AM+HC (optimal condition) doughs are shown in Table 4. The doughs with CG showed higher water absorption than the Control. The main reason is believed to be the high-water absorption capability of GS in CG. The dough with only CG showed a significantly lower GRD compared to the others, while the dough with CG+AM+HC showed the highest GRD among all the samples.

The gassing power of doughs with CG significantly decreased compared to the Control at 1 and 2 h fermentation. On the other hand, the gassing power of doughs with CG was lower or significantly lower than the Control at 3 h fermentation.

The bread with only CG had significantly lower SLV than the others, while the other breads with CG had similar SLV compared with the Control. The actual SLV, 5.04 mL/g, of CG+AM+HC bread showed a value very close to the estimated one, 5.07 mL/g, calculated using the RSMd. The actual bread making experiment verified the effectiveness of this model.

From Table 4, the moisture content of bread crumbs did not differ greatly among all samples, but CG bread showed the highest value, while CG+AM+HC bread showed the lowest value. These results seem to be related to the SLV of each bread and the water absorption of the dough. It is believed that an increase in SLV of bread promotes water loss from dough at

the baking stage due to the increase in the bread surface area. These results also corresponded to reports that breads with a smaller SLV or made from high water absorbing dough will produce bread with high moisture content (Santiago *et al*., 2015b; Tsai *et al*., 2012; Yamauchi *et al*., 2014).

Lower GRD and SLV of dough and bread with only CG may be due to the significantly large amounts of GS and DS. It has been suggested that excessive GS and DS, and DF inhibits gluten network formation in dough, resulting in a weaker gluten network (Lai *et al*., 1989; Özboy and Köksel, 1997; Wang *et al*., 2002).

In terms of GRD and SLV, the dough and bread with CG and enzymes (AM and/or HC) were similar compared to the Control. However, those of CG+AM+HC showed the highest values among all samples. Moreover, the GRD and SLV of CG+AM, CG+HC and CG+AM+HC, especially CG+AM+HC, were significantly greater than those of CG samples, which might be attributed to the catalytic activities of AM and HC. Goesaert *et al*. (2009) and Jiang *et al*. (2005) found that AM and HC decompose GS, DS and pentosan (equivalent to NDF-ADF) into monosugars in dough, which promotes yeast fermentation and improves the gassing power during fermentation. However, the gassing power of the four doughs with CG was significantly lower or lower compared with the Control (Table 4). These results are different from earlier studies. High concentrations of various components (monosaccharides and disaccharides) in CG dough are known to reduce yeast activity. It is also believed that the high water absorption of dough with CG reduces the yeast concentration in dough, which decreases yeast fermentation.

Endogenous AM and β-amylase of flour hydrolyze GS and DS, and change GS and DS to dextrin and maltose in dough without added enzymes. However, Barrera *et al*. (2016), Kim *et al*. (2006) and Yamauchi *et al*. (2004) reported that high amounts of GS, DS and DF decreased the SLV of bread with wheat flour, but the decreased SLV was greatly improved with the addition of AM and other enzymes. Patel *et al*. (2012) also had a similar finding that the addition of fungal AM increased the SLV of chemically leavened bread. Likewise, Jiang *et al*. (2005) and Rouau *et al*. (1994) reported that HC catalyzes the degradation of polysaccharides (mainly hemicellulose) into monosugars and short chain saccharides, resulting in superior gluten network formation. The catalytic activity of HC may have led to higher GRD and SLV in dough and bread with CG+HC and CG+AM+HC compared to those with only CG. The addition of xylanase, a kind of HC enzyme, also improved the SLV of whole wheat bread including high DF and millet/wheat composite bread as reported by Shah *et al*. (2006) and Schoenlechner *et al*. (2013), respectively.

From these findings, it is believed that drastic improvements of GRD and SLV in dough and bread with CG and optimal amounts of enzymes (AM and HC) can be expected.

Analysis and evaluation of doughs and breads The colors

Table 4. BMQs of various doughs[1)]

Bread making treatments	Water absorption (%)	GRD (ml)	Gassing power (ml)			SLV (ml/g)	Moisture content of crumbs (%)
			1h	2h	3h		
Control	66.5	102.6 ± 2.3 a	27.6 ± 0.2 a	61.0 ± 0.7 a	89.8 ± 2.5 a	5.03 ± 0.14 a	40.42 ± 0.67 bc
CG	71.0	82.6 ± 1.4 b	25.1 ± 0.8 b	56.1 ± 1.4 b	83.8 ± 2.3 b	4.09 ± 0.15 b	41.89 ± 0.62 a
CG+AM	71.0	103.5 ± 4.5 a	23.7 ± 0.4 b	54.2 ± 0.5 b	82.5 ± 1.4 b	4.79 ± 0.18 a	40.36 ± 0.44 bc
CG+HC	71.0	106.1 ± 5.6 a	23.7 ± 0.9 b	55.1 ± 2.0 b	87.5 ± 1.4 ab	4.99 ± 0.14 a	41.27 ± 0.54 ab
CG+AM+HC	71.0	110.6 ± 1.5 a	23.8 ± 0.8 b	55.2 ± 1.7 b	86.7 ± 2.0 ab	5.04 ± 0.03 a	39.79 ± 0.19 c

[1)] BMQs: bread making qualities, GRD: gas retention of dough, GP: gassing power of dough, SLV: specific loaf volume, CG: crust gel, AM : α-amylase, HC : hemicellulase. Optimal amounts of enzymes, α-amylase and hemicellulase, were added in dough of CG+AM+HC. In the doughs of CG+AM and CG+HC, the amount of AM or HC equal to CG+AM+HC was added in each dough. Each value except for water absorption is the mean ± SD (GRD, GP, and SLV: n=3, Moisture content of crumbs: n=4). The values followed by different letters within column are significantly different ($p<0.05$).

Table 5. Colors of various breads[1)]

Bread making treatments	Bread crust color (-)			Bread crumb color (-)		
	L*	a*	b*	L*	a*	b*
Control	53.14 ± 2.36 a	15.79 ± 0.86 a	32.79 ± 1.85 a	82.71 ± 1.67 a	-2.06 ± 0.05 c	9.02 ± 0.45 d
CG	50.37 ± 1.92 a	16.21 ± 0.06 a	31.63 ± 1.52 a	77.19 ± 1.71 b	-1.12 ± 0.12 a	14.88 ± 0.66 a
CG+AM	43.04 ± 0.73 b	15.91 ± 0.33 a	26.69 ± 0.55 b	70.48 ± 2.25 c	-1.36 ± 0.05 b	13.01 ± 0.48 b
CG+HC	43.52 ± 1.66 b	15.98 ± 0.41 a	26.47 ± 1.92 b	71.02 ± 0.78 c	-1.36 ± 0.13 b	12.98 ± 0.31 b
CG+AM+HC	40.67 ± 2.83 b	14.57 ± 0.29 b	22.20 ± 2.70 b	65.05 ± 1.55 d	-1.20 ± 0.09 a	11.79 ± 0.44 c

[1)] L*: level of lightness, a*: level of redness, b*: level of yellowness, CG: crust gel, AM : α-amylase, HC : hemicellulase. Optimal amounts of enzymes, α-amylase and hemicellulase, were added in dough of CG+AM+HC. In the doughs of CG+AM and CG+HC, the amount of AM or HC equal to CG+AM+HC was added in each dough. Each value is the mean ± SD (Bread crust color: n=5, Bread crumb color: N=8). The values followed by different letters within column are significantly different ($p<0.05$).

of the various breads measured with a colorimeter are shown in Table 5. In terms of crust color, the Control bread had the highest values of L* and b* among all the samples and a similar a* value compared to the others except for CG+AM+HC bread. CG addition also basically resulted in decreases in the L* and b* values, and an increase in the a* value, except for CG+AM+HC bread. In addition, all crust colors (L*, a*, and b*) of CG+AM+HC bread showed the lowest values among all samples and were significantly lower than the Control and CG breads. All crust colors (L*, a* and b*) of breads with enzymes (AM and /or HC) were also lower than the CG bread. These results show that the addition of CG decreased the L* and b* values, and increased the a* value. On the other hand, the addition of enzymes (AM and/or HC) to CG dough decreased all color values of bread crust.

In terms of crumb color, the Control bread had significantly the highest value of L* and the lowest values of a* and b* among all samples. The addition of CG significantly decreased the L* value, while it significantly increased the values of a* and b*. All crumb colors (L*, a* and b*) of breads with CG+enzymes (AM and /or HC) were also lower than the CG bread. In addition, the L* value of CG+AM+HC bread had significantly the lowest value among all samples and the a* and b* values of the breads were similar to CG bread and significantly lower compared to the others with CG, respectively. These results show that the addition of CG decreased the L* value and increased the a* and b* values, while the addition of enzymes (AM and/or HC) to CG dough decreased all color values of bread crumbs as well as the crust.

The color data of the crust and crumbs of CG bread and breads with CG and enzymes (AM and/or HC) seem to be basically related to the high reducing sugar content of doughs with CG and high red and yellow color (brown color) of CG.

Figure 1 shows the bread appearances and crumb images. The breads with CG, especially CG+AM+HC bread, exhibited darker external brown color compared to the Control. The top crust of breads with CG, especially CG+AM+HC bread, was also a darker brown than the Control. In addition, the crumbs of breads with CG were rather dark compared with the Control crumbs, which mainly relates to the brown color of CG. The results of the sensory evaluation of the coloring of the crust and crumbs of the various breads basically corresponded to the color data measured with the colorimeter (Table 5).

The volume of the CG bread shown in Figure 1 is smaller than the Control, while the volumes of breads with CG and enzymes (AM and /or HC), especially CG+AM+HC bread, were nearly the same compared with the Control. These results were congruent with those of the SLV shown in Table 4.

Goesaert *et al*. (2009) reported that the addition of AM increased reducing sugars, such as glucose, fructose and maltose, in dough, which promotes the Maillard reaction. Santiago *et al*. (2015b) and Matsushita *et al*. (2017) also reported that when enzymes (AM and/or HC) are added to dough with added sweet potato powder or whole wheat flour, the crust color of the breads becomes remarkably brown compared with each of the Control breads without the enzymes.

From previous findings, it is believed that the dark browning of the crust of breads with CG+enzymes (AM and/or HC), especially CG+AM+HC bread, (shown in Table 5 and Fig. 1) is mainly the result of the large production of reducing sugars from CG reacting to the added enzymes, which promotes browning reactions such as the Maillard reaction and caramelization.

Table 5 shows that the L* values of bread crumbs with CG, especially CG+AM+HC bread crumbs, are significantly lower than the Control and, conversely, the a* and b* values of bread crumbs with CG are significantly high compared with the Control. It seems that these results are related to the brown color of the added CG and the differences in crumb structure that accompany changes in SLV, and that the Maillard reaction and caramelization have almost no effect.

Figure 1 shows that the addition of enzymes (AM and/or HC) obviously increased the volume of the bread, which increased the porosity of the crumb grain of the bread and

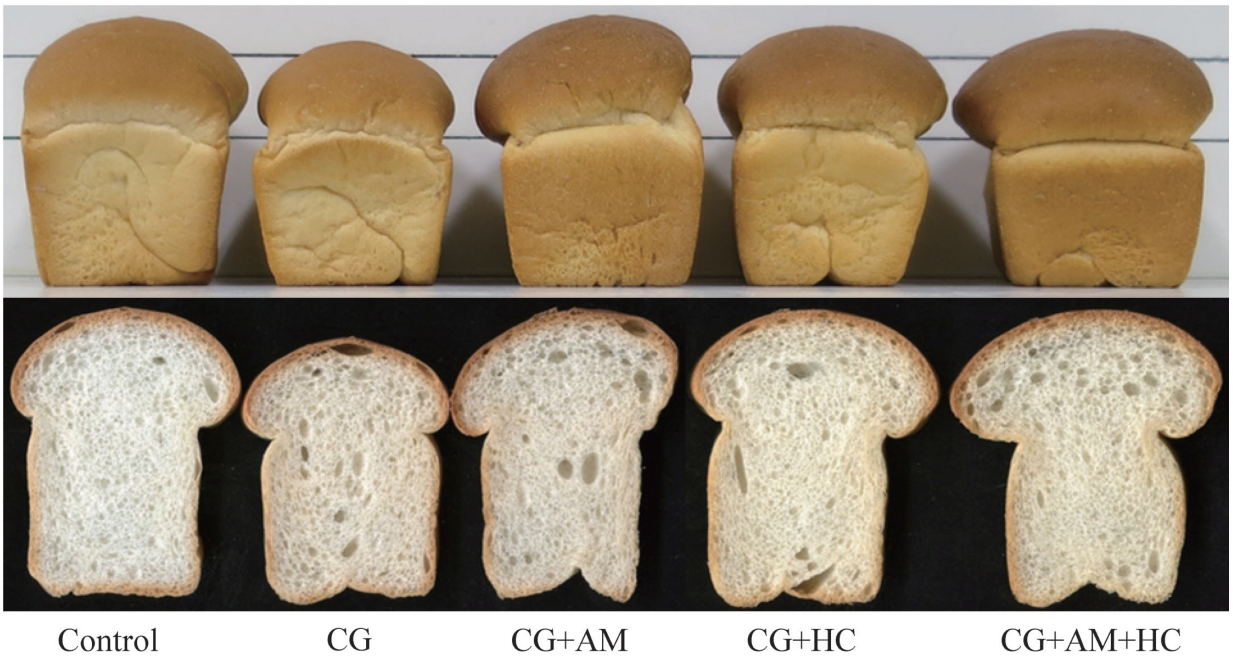

Fig. 1. Photographs of appearances and scanned crumb images of various breads[1)]
[1)]CG: crust gel, AM : α-amylase, HC : hemicellulase. Optimal amounts of enzymes, α-amylase and hemicellulase, were added in dough of CG+AM+HC. In the doughs of CG+AM and CG+HC, the amount of AM or HC equal to CG+AM+HC was added in each dough.

changed the crumb structure. From these results, it seems that there is a possibility that the difference in crumb structure affects the crumb color.

Generally, it is well known that the temperature of the bread crumb during baking does not rise above 100℃. Actually, Therdthai *et al.* (2002) reported that the internal crumb temperature did not exceed 100℃ when the dough was baked at 180 to 210℃ for 22 to 30 min. Purlis and Salvadori (2009) also reported that the browning reaction, such as the Maillard reaction and caramelization of dough during the baking process, rarely begins at temperatures below 120℃.

From previous findings, it seems that the browning reaction hardly affects the changes of bread crumb color with the addition of CG and enzymes (AM and/or HC).

The saccharide contents in the water soluble fraction of bread crumbs are shown in Table 6. Since the sucrose content of all samples was nearly zero, the data were omitted. All saccharide contents in CG+AM+HC bread except for fructose and maltose were the highest values among all samples. In addition, all saccharide contents of breads with CG were basically high and, in particular, total saccharide, reducing saccharide and maltose were significantly higher compared with the Control. These results basically agreed with what has been reported in previous papers concerning Yudane bread produced with gelatinized and swollen flour paste and bread made by adding gelatinized sweet potato powder (Santiago *et al*., 2015b; Yamada *et al.*, 2004; Yamauchi *et al.*, 2014). These studies also reported that when materials containing gelatinized, swollen starch and various other polysaccharides, such as Yudane dough and gelatinized sweet potato powder, are added to dough, they produce more total and reducing saccharides and maltose in the dough compared to the Control without these materials. It has been reported that GS and swollen starch added to dough are broken down by various endogenous amylases in wheat flour but adding AM and HC has a greater effect (Santiago *et al*., 2015b).

Table 7 shows the GS and DS contents and DF compositions of the final proofing doughs from different treatments. The Control and CG doughs had higher values for GS and DS contents than the others. The doughs with CG and enzymes (AM and/or HC) had lower values than the Control and values were significantly lower than the dough with only CG. The addition of AM decomposed large amounts of GS and DS in the dough; therefore, the doughs with AM had the lowest GS and DS contents among all samples.

Table 7 also shows the DF contents of the doughs. There was no significant difference in ADF of all samples and the addition of CG had almost no effect in increasing ADF. The NDF of doughs with HC also had low or significantly low values compared with the others. Furthermore, the NDF-ADF (approximate hemicellulose contents) of doughs with HC (CG+HC and CG+AM+HC) were the lowest values among all samples.

The higher GS and DS contents of doughs without enzymes can be associated with the amount of GS in CG and DS generated by physical damage during the milling process. Excessive amounts of GS and DS negatively affect BMQs (Murayama *et al*., 2015; Santiago *et al*., 2015a; Yamauchi *et*

Table 6. Saccharide contents in water soluble fraction of various bread crumbs[1)]

Bread making treatments	Saccharide contents in water soluble fraction of bread crumbs (mg/bread crumb)				
	Total saccharide	Reducing saccharide	Glucose	Fructose	Maltose
Control	92.62 ± 4.62 d	56.16 ± 3.00 c	8.72 ± 0.22 b	16.78 ± 1.63 a	33.13 ± 0.54 d
CG	117.27 ± 6.97 c	75.30 ± 4.58 b	9.83 ± 0.31 b	18.00 ± 1.86 a	51.39 ± 064 c
CG+AM	226.15 ± 23.33 a	115.86 ± 9.47 a	10.53 ± 1.01 b	17.76 ± 2.10 a	79.75 ± 4.24 a
CG+HC	183.27 ± 6.55 b	102.83 ± 6.24 a	10.57 ± 0.22 b	17.49 ± 1.60 a	69.55 ± 4.42 b
CG+AM+HC	237.15 ± 16.60 a	117.22 ± 6.97 a	12.59 ± 1.20 a	17.39 ± 1.65 a	75.91 ± 9.41 ab

[1)] CG: crust gel, AM : α-amylase, HC : hemicellulase. Optimal amounts of enzymes, α-amylase and hemicellulase, were added in dough of CG+AM+HC. In the doughs of CG+AM and CG+HC, the amount of AM or HC equal to CG+AM+HC was added in each dough.
Each value is the mean ± SD (n=3). The values followed by different letters within column are significantly different ($p<0.05$).

Table 7. GS and DS, and DF contents of various doughs[1)]

Bread making treatments	GS and DS (%)	NDF (%)	ADF (%)	NDF-ADF (%)
Control	4.01 ± 0.34 b	0.60 ± 0.15 a	0.29 ± 0.06 a	0.32 ± 0.10 a
CG	6.20 ± 0.29 a	0.51 ± 0.03 ab	0.34 ± 0.05 a	0.16 ± 0.03 b
CG+AM	2.74 ± 0.75 b	0.53 ± 0.05 ab	0.39 ± 0.07 a	0.15 ± 0.05 b
CG+HC	3.19 ± 0.58 b	0.38 ± 0.07 b	0.28 ± 0.08 a	0.09 ± 0.04 b
CG+AM+HC	2.90 ± 0.14 b	0.38 ± 0.07 b	0.29 ± 0.10 a	0.09 ± 0.03 b

[1)] GS and DS: gelatinized and damaged starches, DF: dietary fiber, NDF: neutral detergent fiber, ADF: acid detergent fiber, NDF-ADF: approximate hemicellulose contents,) , CG: crust gel, AM : α-amylase, HC : hemicellulase. The concentration of GS and DS, NDF, ADF and NDF-ADF are shown on dry basis. Optimal amounts of enzymes, α-amylase and hemicellulase, were added in dough of CG+AM+HC. In the doughs of CG+AM and CG+HC, the amount of AM or HC equal to CG+AM+HC was added in each dough. Each value is the mean ± SD (n=4) . The values followed by different letters within column are significantly different ($p<0.05$).

al., 2014). The doughs with CG and enzymes (AM and/or HC) had lower or significantly lower GS and DS than the others, which may be related to the enzymatic decomposition of GS and DS by the added AM and the AM included in the crude HC.

Generally, DF has a negative effect on the formation of an optimal gluten network, resulting in the reduction of GRD and SLV (Lai *et al*., 1989; Matsushita *et al*., 2017). The doughs with HC showed lower DF content except for ADF, especially NDF-ADF, compared to the CG dough, which was attributed to the xylanase activity of HC. HC hydrolyzes DFs such as xylan and arabinoxylan, resulting in low NDF content and approximate hemicellulose (NDF-ADF) in the doughs with HC (Jiang *et al*., 2005; Stojceska and Ainsworth, 2008).

Ultimately, the significant improvement in GRD and SLV of the CG+AM+HC dough and bread can be associated with the reduction of the amounts of GS, DS and DF (mainly insoluble hemicellulose (pentosan)).

Figure 2 shows the temporal changes in bread hardness during 3 days of storage (bread staling). The Control and CG breads showed higher or significantly higher values than the others during all storage times. CG bread also had the highest value of hardness during all storage times among all samples, while the breads with CG and enzymes (AM and/or HC), especially CG+AM+HC bread, had the lowest values during all storage times among all samples.

The staling of CG+AM bread was similar to or slightly slower than that of CG+HC bread (Fig. 2), even though the former bread showed lower SLV and moisture content compared to the latter bread (Table 4). The main factor is considered to be related to the fact that the low molecular weight saccharide (LMWS) contents of CG+AM bread are significantly higher than those of CG+HC bread (Table 6). Namely, it seems that the antistaling effects of high LMWS contents of CG+AM bread are greater compared to the staling promoting effects with the low SLV and moisture content of this bread, whereby CG+AM bread exhibits a slow staling equivalent to that of CG+HC. Although bread with CG+AM+HC also showed lower moisture content than CG+AM bread (Table 4), the staling of this bread was lower than those of CG+AM and CG+HC breads (Fig. 2), which is related to the high antistaling effects of large SLV and

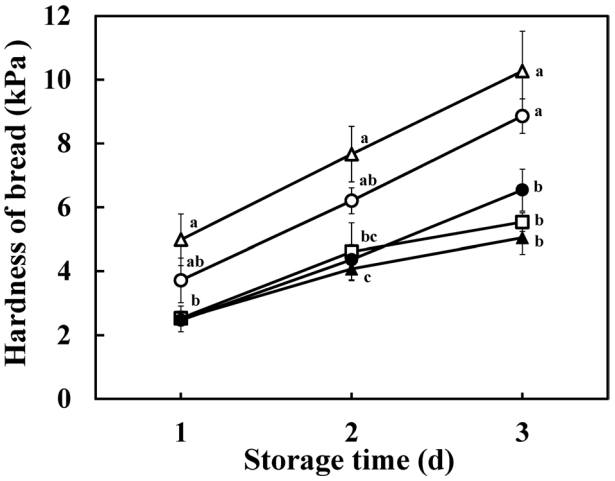

Fig. 2. Temporal hardness changes of various bread crumbs during storage[1)]

[1)]CG: crust gel, AM : α-amylase, HC : hemicellulase. Optimal amounts of enzymes, α-amylase and hemicellulase, were added in dough of CG+AM+HC. In the doughs of CG+AM and CG+HC, the amount of AM or HC equal to CG+AM+HC was added in each dough. The vertical bar is the standard deviation of each value (n=9).

The symbols followed by different letters are significantly different ($p<0.05$). ○: Control, △: CG, □: CG+AM, ●: CG+HC, ▲: CG+AM+HC.

significantly high LMWS contents of this bread compared to those of CG+HC bread (Tables 4 and 6).

There are various factors related to the temporal changes in bread crumb hardness during storage (bread staling), such as the retrogradation rate of GS gel in bread, LMWSs, SLV and bread moisture. The effects of the retrogradation rate of GS gel in bread and LMWSs on bread staling are considered to be largely influenced by the reaction of AM and HC during the bread making process.

The AM mainly breaks down GS and DS in dough into low molecular weight dextrins and oligosaccharides during bread making. In addition, the endogenous β-amylase in wheat flour converts saccharides into maltose. These complementary functions during bread making bring about partial decompositions of GS and DS. In addition, added AM increases the contents of LMWSs in bread. The action of this AM seems to suppress the staling of breads with CG+AM and with CG+AM+HC clearly when compared with those of the Control and CG breads (Fig. 2). It was reported that these LMWSs retard the retrogradation of GS gel and reduce the amount of available starch for the retrogradation (Duran *et al*., 2001; Goesaert *et al*., 2009; Palacios *et al*., 2004). Caballero *et al*. (2007) and Palacios *et al*. (2004) also reported that AM has an antistaling effect on bread during storage. Martin and Hoseney (1991) and Palacios *et al*. (2004) suggested that partially decomposed starch gel has a lower retrogradation rate. Moreover, the starch-protein interactions are interfered with the LMWSs produced by AM hydrolysis in dough, resulting in few and weak crosslinks between the starch and protein, which reduces the hardening rate of the bread (Martin and Hoseney, 1991; Martin *et al*., 1991).

HC mainly attacks insoluble pentosan and changes it to LMWSs, improving the BMQs. It was reported that the addition of HC improved SLV and increased LMWSs in dough (Caballero *et al*., 2007; Ghoshal *et al*., 2013; Matsushita *et al*., 2017). In this study, the doughs with CG+HC and CG+AM+HC actually had significantly lower amounts of approximate hemicellulose (NDF-ADF) and GS and DS than the Control dough, as shown in Table 7. As a result, CG+HC bread showed significantly higher total saccharide and maltose contents than CG bread (Table 6). However, as the NDF value of CG dough was not higher than that of the Control, CG does not contain much DF. Therefore, the effect of HC on CG decomposition is considered to be limited, while the staling of bread with CG+HC was clearly suppressed compared to those of the Control and CG breads (Fig. 2). It is considered that the formation of LMWSs by the action of HC, the improvement of SLV and the high moisture content contribute to slow staling in the bread with GC+HC, which was nearly the same as the bread with GC+AM.

From Table 4 and Figure 1, the SLV of breads with CG and enzymes (AM and/or HC), especially CG+AM+HC bread, was significantly larger than the CG bread and nearly the same as the Control. Maleki *et al*. (1980) also reported that the staling rate of bread is clearly decreased with a large SLV. They showed the large SLV of breads with CG and enzymes (AM and/or HC) considerably contributes to slower staling, which is considered to be mainly due to an increase in the porosity of the crumb accompanying an increase in the SLV.

As shown in Table 4, the moisture content of all breads was in the order of CG, CG+HC, Control, CG+AM and CG+AM+HC bread, with CG+AM+HC having the lowest moisture content among all the breads. However, CG+AM+HC bread showed the slowest staling among all breads. Therefore, although Rogers *et al*. (1998) and Zeleznak *et al*. (1998) reported that high moisture content bread exhibits a low staling rate, it seems that the moisture content of breads in this study did not greatly influence differences in the individual bread staling rate and the differences were mainly caused by other factors described above.

From these findings, it seems that the main factors concerning the suppression of staling in CG+AM+HC bread with the optimum amounts of enzymes were the high SLV, which relates to the strengthened gluten network structure accompanied by the degradation of GS, DS and insoluble pentosan, and the retardation of starch gel retrogradation in the bread by LMWSs.

The results in Figure 2 show low staling rates for breads with various added enzymes and basically agree with data reported by Caballero *et al*. (2007), Matsushita *et al*. (2017), Ghoshal *et al*. (2013) and Goesaert *et al*. (2009).

The sensory evaluation results for the breads are shown in Table 8. The results show that the volume of CG bread was significantly lower than the others. Therefore, the total appearance evaluation of CG bread had a significantly lower value compared with the others, while in CG+AM+HC bread, the evaluation of color was significantly lower than the others; however, the evaluation of volume was high or significantly high compared with the others. Therefore, the total appearance evaluation was similar to the Control. In the crumb evaluation, CG and CG+AM+HC breads had better or similar results compared with the Control, except for crumb grain and color. The texture, flavor and taste of CG and CG+AM+HC breads were similar to or higher than the Control and the taste of CG+AM+HC bread especially had a significantly high value compared to the others. As such, the total crumb evaluation of bread with CG+AM+HC had a higher value compared to the Control and this bread had significantly the highest value among all samples. The overall total evaluation of CG bread had a significantly lower value than the others. On the other hand, CG+AM+HC bread had the highest value among all samples, which reflects the total appearance and crumb evaluation results.

These results are approximately consistent with the bread making evaluation of these bread doughs (Table 4) and various evaluations of these breads (Table 5, Fig. 1 and 2). Although the BMQs of the dough significantly decreased due to the addition of CG, the texture, flavor and taste in the crumb evaluation were greatly improved by the addition of CG, AM and HC. In addition, the volume evaluation of CG+AM+HC bread was significantly improved by the addition of optimal amounts of enzymes and it was found that all crumb evaluations of this bread are improved more than the CG bread. However, the crust color of CG+AM+HC bread in the appearance evaluation was significantly lower than the CG bread, with the main factor being the bread crust having a very dark brown color. The inferior crust color of CG+AM+HC bread may be caused by excessive production of reducing saccharides with the added enzymes. Therefore, to further improve the overall qualities of this bread, it may be necessary to add crust color as another response to the RSMd or to check if baking CG+AM+HC dough at a low temperature is a suitable and effective means of improving the total evaluation result of CG+AM+HC bread.

Overall BMQs and bread evaluation Overall, this study established that the treatment with optimal amounts of AM and HC drastically improved the BMQs of dough supplemented with 10 % CG. However, since the BMQs of the dough with added CG and optimal enzymes were somewhat better than CG+HC dough and bread, it was found that the main effect of the addition of optimal enzymes was from HC addition. The most improved BMQs were increases in GRD and SLV, suppression of bread staling and high values in the sensory evaluation, especially volume, texture, flavor and taste (Table 4,

Table 8. Results of sensory evaluation of various breads[1)]

Bread-making treatmens	Apearance evaluation (-)				Crumb evaluation (-)						Overall total evaluation (-)
	Volume	Shape	Color	Total	Crumb grain	Color	Texture	Flavor	Taste	Total	
Control	15.00 ± 0.00a	5.00 ± 0.00 a	5.00 ± 0.00 a	25.00 ± 0.00 a	5.00 ± 0.00 a	5.00 ± 0.00 a	5.00 ± 0.00 ab	5.00 ± 0.00 b	5.00 ± 0.00 b	25.00 ± 0.00 b	50.00 ± 0.00 a
CG	8.68 ± 2.43 b	5.07 ± 1.45 a	4.35 ± 0.88 a	18.10 ± 2.71 b	3.66 ± 0.43 b	2.89 ± 1.00 b	4.78 ± 1.56 b	5.83 ± 0.89 ab	5.59 ± 0.69 b	22.76 ± 2.04 b	40.86 ± 3.77 b
CG+AM+HC	16.72 ± 3.00 a	3.91 ± 1.17 a	2.88 ± 1.26 b	23.51 ± 2.98 a	4.49 ± 1.26 ab	3.60 ± 0.88 b	6.37 ± 1.48 a	6.43± 1.79 a	7.02 ± 1.07 a	27.92 ± 3.15 a	51.42 ± 4.68 a

[1)] CG: crust gel, AM : α-amylase, HC : hemicellulase. Optimal amounts of enzymes, α-amylase and hemicellulase, were added in dough of CG+AM+HC. Each value is the mean ± SD (n=12). The values followed by different letters within column are significantly different ($p<0.05$).

Fig. 2 and Table 8). These dough and bread properties were dramatically improved with optimal amounts of enzymes added when compared with those of CG dough and bread, which were better than the Control. On the other hand, a negative effect of this treatment was the drastic reduction in bread color evaluation, especially crust color (Fig. 1 and Table 8). In CG bread, the L* and b* values of the bread crust also decreased compared with the Control, but the color was considered acceptable. However, the crust browning of the CG+AM+HC bread, caused by the Maillard reaction and caramelization during baking, was excessive with the addition of enzymes and the crust color evaluation drastically decreased. As such, the crust color of CG+AM+HC bread was considered unacceptable. Therefore, this is a negative point of adding the optimal amounts of enzymes to CG dough. In this study, the optimal amounts of enzymes were determined to maximize the SLV as a response by using the RSMd and Solver. Since the RSMd calculated the optimal value of SLV to be 5.07 mL/g, which almost corresponded to the actual experimental value of 5.04 mL/g, the validity of this model was verified. Using SLV as an index of optimal bread quality in the model has its limitations because of the large degradation in crust color of bread when the optimal amounts of enzymes are added.

Conclusion

Although the various components in CG, such as GS, had a beneficial effect on bread qualities such as texture, flavor and taste, they decreased general bread making properties. The GS in CG interferes with the formation of the gluten network, resulting in the reduction of GRD and SLV, and the acceleration of the bread staling rate during storage. The optimal amounts of enzymes (AM and HC) to add in order to solve these problems were determined using RSMd and Solver, which resulted in the formation of a desirable gluten network, a remarkable improvement in dough properties, and BMQs such as GRD, SLV and a low staling rate. These changes can be attributed to the degradations of GS and DS (mainly GS) and hemicellulose (mainly insoluble type) into soluble LMWSs, which do not negatively influence gluten network formation. As such, the addition of the optimal amounts of enzymes in CG dough enables the production of satisfactory bread with high GRD and SLV, a retarded bread staling rate and good crumb evaluation, with the exception of bread crust color. These findings suggest that the response surface methodology and Solver are effective methods for establishing optimal conditions in bread making. By using these methods, the optimal conditions can be easily determined. These results suggest the possibility of establishing an effective method to utilize CG for bread making.

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