Technical paper

Bread Making Improvement of Mashed Potato-supplemented Dough by Treating with Optimal Bakery Enzymes

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Bread substituted with mashed potato (MP) instead of wheat flour has been attracting attention because of added components, such as gelatinized starch (GS), dietary fibers (DFs), vitamins, and minerals, etc., that have a beneficial effect on the nutritional value, texture, flavor, and taste of the bread. On the other hand, excess amounts of GS and DFs in MP inhibits gluten network formation in dough and greatly deteriorates the bread making quality (BMQ). In this study, we investigated the optimal addition of two types of bakery enzymes, α-amylase (AM) and hemicellulase (HC), to improve the BMQ of MP-added dough. Optimal amounts of added enzymes were determined using the response surface methodology (RSM) and optimization technique (OT). As the results, BMQ, such as specific loaf volume (SLV), gas retention of dough, and bread staling, of MP dough and bread with optimal concentrations of AM and HC were remarkably improved compared to those without enzymes. These results showed that RSM and OT were effective methods to reasonably and easily derive the optimal concentrations of multiple enzymes, resulting in a good quality MP-supplemented bread with high SLV, desirable texture, flavor, and taste, but not crust color.

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

Introduction

Potato is a major crop globally and is widely produced in many countries throughout the world.[i),ii)] In Japan, potato is also a major agricultural crop, and about 2.5 million tons are produced per year. This crop is used for a variety of purposes, such as table food, processing, and starch extraction, etc.[iii)]

However, potato is not widely used in bread making in Japan, and is mainly used for other purposes, such as potato salad for sandwiches. The main reason is that when MP is added to bread dough, the gluten network of the dough deteriorates due to GS and DFs, especially insoluble DFs,

remarkably decreasing the bread making properties. On the other hand, the use of raw materials containing a large amount of gelatinized or swollen starch, such as MP, for bread making results in positive effects, such as a slightly sweet taste, low staling, and sticky texture, as reported by Murayama *et al*., 2015 and Yamauchi *et al*., 2014. In addition, potato starch in various starches has been found to have a very high swelling power and viscosity when heated in water, and its GS retains a large amount of water (Hossen *et al*., 2011; Li and Yeh, 2001). Therefore, the addition of MP to dough is expected to improve the water absorption of dough and bread qualities.

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Table 1. Central composite face-centered design 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	-1.0	-1.0	0.00	0.00
2	-1.0	+1.0	0.00	0.10
3	+1.0	-1.0	0.10	0.00
4	+1.0	+1.0	0.10	0.10
5	+1.0	0.0	0.10	0.05
6	-1.0	0.0	0.00	0.05
7	0.0	+1.0	0.05	0.10
8	0.0	-1.0	0.05	0.00
9	0.0	0.0	0.05	0.05
10	0.0	0.0	0.05	0.05
11	0.0	0.0	0.05	0.05
12	0.0	0.0	0.05	0.05

[1]Scaled values and actual concentrations of AM and HC are shown in above Table. 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-0.05)/0.05, where the actual concentration of HC ranged from 0.00 to 0.10/100 g flour.

Recently, it was reported that although the BMQ of dough containing a large amount of GS or DFs greatly degrades, it can be remarkably improved by using multiple enzymes for bread making, such as AM and HC, etc.(Caballero *et al.*, 2007; Matsushita *et al.*, 2017; Santiago *et al.*, 2015a). However, when multiple enzymes are used to improve BMQ, it is necessary to be able to reasonably establish optimal amounts of enzymes without performing many experiments. This makes it possible to efficiently develop high quality bread.

Therefore, in this study, we adopted a central composite face-centered design (CCF) (Flander *et al.*, 2007) as a reasonable and effective method to acquire the evaluation data to determine the optimal amounts of multiple enzymes for maximum improvement in the BMQ of MP-supplemented dough. A response surface model (RSMd) was obtained using the data acquired based on the CCF, and then the optimal amounts of multiple enzymes were determined by using an OT (Excel add-in software, Solver). Finally, to validate the effectiveness of these methods, a bread making experiment with the optimal amounts of multiple enzymes was conducted, and the effectiveness was verified from the BMQ of the dough and various bread evaluations.

Materials and Methods

Flour, enzymes, and MP used Strong wheat flour, Camellia, (Nisshin Flour Milling Co., Ltd., Tokyo, Japan) was used in this study. Two commercial enzymes were used: AM (Sumizyme AS) containing 1,500 α-amylase U/g and HC (Sumizyme SNX) containing 14,000 xylanase U/g. Both enzymes were manufactured by Shin Nihon Chemical Co., Ltd. (Anjo, Japan). A commercial table potato variety, *Solanum*

tuberosum L. cv. May Queen, was purchased from a local market and used for preparation of MP. The MP prepared as follows was used for bread making. The potato tubers were peeled, boiled for 40 min, cooled for 30 min at room temperature, and then mixed at high speed for 1 min using a food processor (MK-K81, Panasonic Co, Ltd., Osaka, Japan). The prepared MP was placed in an aluminum laminate bag, rapidly cooled at -30℃ for 30 min, and then stored at -20℃ until use. The moisture content of the MP was 81.6%.

Optimization of concentrations of added enzymes CCF as reported by Flander *et al.* (2007) was used with two variables to determine the optimal concentrations of enzymes. This CCF comprises twelve 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 (g/100 g flour) for both AM and HC. Both concentrations of these enzymes ranged from 0.00 to 0.100 (g/100 g flour). These minimum and maximum concentrations of enzymes were determined using the data of bread making tests in which various amount of these enzymes were added, and the bread making tests determined by using CCF were randomly conducted. In this study, SLV and amounts of added enzymes (AM and HC) were respectively adopted as the response and factors in the analysis of RSMd. The reason for choosing SLV as the response is that it is a representative index of BMQ. From the results of twelve runs based on CCF, a RSMd between the response and factors was derived by using multiple regression analysis. Selection of the explanatory variables of the RSMd was determined by the stepwise back selection method for variables with an F value of 2.0 as an index. The

effectiveness of this model was assessed by verification of the factor effect and lack of fit with analysis of variance. Optimal amounts of added enzymes were also determined with this model by using the Excel add-in software Solver. After CCF experiments, bread making tests of dough with added MP and optimal concentrations of enzymes (M+E dough) were conducted with Control and MP supplemented dough (MP dough), and the improving effects were evaluated in detail.

Dough preparation and bread making   Bread making tests were conducted by 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 method used by the AACC (1991). Five percent of the wheat flour in the standard white bread formulation for the Control was replaced with MP for the MP substituted and MP+E added bread making treatments on a dry weight basis. The 5 % of MP replacement instead of wheat flour was the maximum percentage at which the BMQ could be improved with optimal enzymes.

Evaluation of BMQ   The gas retention of dough (GRD) was evaluated by measuring the maximum expansion volume of 20 g dough proofed for 70 min at 38 ℃ and 85 % relative humidity (RH) in a cylinder subjected to 0 to 75 cmHg, following the report of Yamauchi *et al.* (2000). The gassing power (GP) of 20 g dough after bench time was measured at 30 ℃ for 1, 2, and 3 h using a Fermograph II (ATTO Co., Ltd.) 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 were also recorded using the method reported by Santiago *et al.* (2015a). The color of the bread top crust and crumb was measured with a colorimeter as reported by Matsushita *et al.* (2017).

Analysis and evaluation of MP, doughs, and breads The moisture contents of MP just after preparation and bread stored for 3 days in polyethylene bags at 20 ℃ and 70 % RH were measured with homogenized MP and bread crumbs according to the method of Santiago *et al.* (2015a). Soluble sugar contents, such as total, reducing, mono- and di-saccharides, were also analyzed using the method reported by Santiago *et al.* (2015b).

The sample preparation and storage before damaged starch (DS) and DFs analysis were carried out according to the method reported by Santiago *et al.* (2015a). The DS content in doughs was measured using a Megazyme Assay Kit (Megazyme International Ireland Ltd., Wicklow, Ireland), based on the method of Gibson *et al.* (1991). Neutral detergent fiber (NDF), the amounts of hemicellulose, cellulose, and lignin, and acid detergent fiber (ADF), the amounts of cellulose and lignin, were measured using the AOAC official method (AOAC, 2000). Subsequently, the approximate hemicellulose content was calculated as the difference between NDF and ADF.

The temporal changes of crumb hardness (bread staling) were measured at 1, 2, and 3 days of storage as described in the literature (Yamauchi *et al.*, 2001). In short, the loaves were sliced into 2 cm-thick slices and a square of crumb (3 x 3 cm) was cut from the central part. Using a rheometer (RE2-33005C; Yamaden Co., Ltd.), the temporal hardness changes of bread crumbs were measured by compressing up to 50 % strain rate with a special cube plunger (6 cm length x 6 cm width x 2 cm height) as the textural properties.

Sensory evaluation of breads   Sensory evaluation of breads was performed using the samples stored in polyethylene bags for 1 day at 20 ℃ and 70 % RH. Quantitative descriptive analysis of MP supplemented and MP+E supplemented breads (MP and MP+E breads) was evaluated and compared with the Control by 12 panelists, comprised of undergraduate and graduate students of Obihiro University of Agriculture and Veterinary Medicine. Evaluation of appearance was comprised of three items: volume, shape, and color. The crumb evaluation also included crumb grain, color, texture, flavor, and taste. The full points of volume are 30. Those of other items and the overall total full points are 10 and 100, respectively. The volume, those of other items, and overall total points of the Control are 15, 5, and 50, respectively. The rating scale for volume ranges from 0 (small) to 30 (large). The scale for shape, crust color, and crumb color are from 0 (poor) to 10 (excellent), that for crumb texture is from 0 (hard) to 10 (soft), and those for flavor and taste are from 0 (dislike) to 10 (like). Evaluation of the above breads was carried out by a two-sample comparison method.

Statistical analysis   Significant differences of all data measurements except for water absorption were evaluated by analysis of variance at a 5 % significance level with Tukey's multiple range test using Excel statistical software 2012.

Results and Discussion

Optimization of concentrations of added enzymes   RSMd between the response (SLV) and factors (AM and HC) is shown below, which was derived by using multiple regression analysis based on the results of twelve bread making runs on CCF.

$$Y = 11.8667X_1 + 9.5000X_2 - 61.5000X_1^2 - 35.5000X_2^2 - 93.000X_1X_2 + 4.4763$$

where Y is SLV (mL/g); X_1 is the concentration of AM (g/100 g flour); X_2 is the concentration of HC (g/100 g flour). R^2 and adjusted R^2 of the above model showed high values, 0.9125 and 0.8396, respectively. By using analysis of variance, the effectiveness and lack of fit of the above model were also assessed, which were significant at a 1 % significance level and not significant at a 5 % significance level, respectively. From these results, it was clarified that this RSMd is sufficiently effective as an equation for estimating SLV using two kinds of added enzyme concentrations. Furthermore, the partial

Table 2. BMQ of doughs of Control, MP, and MP+E [1]

Bread making treatments	Water absorption (%)	GRD (mL)	GP (mL)			SLV (mL/g)
			1h	2h	3h	
Control	66.5	100.6 ± 1.7 b	26.8 ± 0.3 a	61.5 ± 0.4 a	92.8 ± 0.3 b	5.32 ± 0.05 a
MP	70.7	82.8 ± 5.1 c	25.4 ± 0.3 b	59.6 ± 0.5 b	100.2 ± 0.7 a	4.62 ± 0.06 b
MP+E	70.7	117.8 ± 2.6 a	25.6 ± 0.3 b	59.3 ± 0.5 b	99.7 ± 0.8 a	5.20 ± 0.28 a

[1] BMQ: bread making qualities, MP: mashed potato, E: enzymes, GRD: gas retention of dough, GP: gassing power of dough, SLV: specific loaf volume, Water absorption (%): Optimal amount of water for bread making (%).
Optimal amounts of enzymes, α-amylase and hemicellulase, were added in dough of MP+E. Each value except for water absorption is the mean ± SD (n=3). The values followed by different letters within column are significantly different ($p < 0.05$).

regression coefficients of the X_1^2, X_2^2, and X_1X_2 explanatory variables in RSMd show negative values. Therefore, when both enzymes are added to the doughs in large excess, these explanatory variables have the effect of obviously lowering the BMQ (SLV).

Since the magnitude of the partial regression coefficient on these explanatory variables is in the order of X_1X_2, X_1^2, and X_2^2, this shows that when the enzymes are added in excess, the effect of decreasing SLV is large in the order of addition of both enzymes, AM, and HC.

The optimal concentrations of AM and HC calculated with the optimization method, using the Excel add-in software Solver, were 0.059 and 0.05 g/100 g flour, respectively. In dough with MP, the SLV increased with the amount of added HC; however, the improving effect plateaued. When excessive HC was added, the dough became very sticky and handling was extremely difficult. Therefore, in calculating the optimum concentrations of these enzymes, the concentration of added HC was limited to 0.05 g/100 g flour as the upper concentration.

BMQ evaluation BMQ of Control, MP, and MP+E doughs are presented in Table 2. Incidentally, Table 2 does not show the data of Control+E, in which an optimum amount of enzyme was added to the Control. The reasons are as follows: results of the bread making test with the Control+E dough indicated that as the enzymes reaction proceeded more than necessary, the SLV decreased to 5.00 mL/g and the bread after baking had a tendency to cave-in and showed overall inadequate quality with a very rough crumb grain and poor bread appearance (data not shown in detail).

The MP and MP+E doughs showed higher water absorption than the Control. The main reason for this is speculated to be due to the high-water absorption of starch and DFs in MP. The MP dough showed a significantly lower GRD compared to the others, while the MP+E dough showed a significantly higher GRD among all samples.

The MP and MP+E doughs showed a significant decrease in GP compared to the Control at 1 and 2 h fermentation. On the other hand, GP of these doughs was significantly higher than the Control at 3 h fermentation.

The MP bread had a significantly lower SLV than the others. Meanwhile, the Control and MP+E breads had a similar SLV. The actual SLV, 5.20 mL/g, of MP+E bread showed a very close value to the estimated value, 5.08 mL/g, calculated by the above RSMd. Thus, the effectiveness of this model was verified by the actual bread making experiment.

In terms of GRD and SLV, the dough and bread with MP were significantly lower than the others, conversely, those with MP+E were significantly higher or similar compared to the Control, respectively. Moreover, GRD and SLV of M+E were significantly greater than those of MP, which might be attributed to the optimal combined catalytic activities of AM and HC. Goesaert *et al.* (2009) and Jiang *et al.* (2005) suggested that AM and HC decompose DS and pentosan (equivalent to NDF-ADF) into mono-sugars in dough, which consequently promotes yeast fermentation and improves GP during fermentation. However, the GP of two doughs with MP before 3 h fermentation was significantly lower compared with the Control, as shown in Table 2. Meanwhile, the GP of the doughs with MP at 3 h fermentation showed a significantly higher value, similar to the above reference, compared to the Control. High concentrations of various components like mono- and di-saccharides in MP seemed to promote yeast fermentation at the final stage of fermentation.

The GP of the MP+E dough is slightly suppressed compared to that of the MP dough after 2 h fermentation. It seems that this is related to the slight suppression of fermentation by low molecular weight saccharides (LMWSs), etc. produced by the added enzymes.

Regarding the addition effect of each enzyme, endogenous AM and β-amylase of flour firstly hydrolyzes damaged and gelatinized starch to maltose, and dextrin, etc. in dough without added enzymes. Barrera *et al.* (2016), Kim *et al.* (2006), and Yamauchi *et al.* (2004) reported that the high amounts of DS and DFs decreased the SLV of bread with wheat flour, and the decreased SLV was greatly improved by the addition of AM and other enzymes. Patel *et al.* (2012) reported a similar observation, in which 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

Fig. 1. Photographs of appearance and scanned crumb images of breads of Control, MP, and MP+E[1]

[1] MP: mashed potato, E, enzymes. Optimal amounts of enzymes, α-amylase and hemicellulase, were added in dough of MP+E.

degradation of polysaccharides (mainly hemicellulose) into mono sugars 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 MP+E compared to those with MP. The addition of xylanase, a kind of HC enzyme, also improved the SLV of whole wheat bread containing high DFs and millet/wheat composite bread, as reported by Shah *et al*. (2006) and Schoenlechner *et al*. (2013), respectively.

From the above findings, it is thought that the drastic improvements of GRD and SLV in the dough and bread with MP and optimal enzymes (AM and HC) is reasonable. The analysis results and discussion concerning the DS and DFs contents, which are the main factors in the improvement of BMQ in the MP+E dough, are also described in detail later.

Fig. 1 shows the bread appearance and crumb images. The addition of MP made the external bread color darker, especially the color of MP+E bread, and the crusts of MP and MP+E breads were dark compared to the Control. The crumbs of MP and MP+E breads were somewhat yellow compared with the Control crumb.

The appearance of MP bread was significantly smaller than the Control, while the MP+E bread was nearly the same as the Control. These results are congruent with their SLV presented in Table 2. The crust color of MP bread was darker than the Control. In addition, the MP+E bread showed remarkably darker color than the Control and MP breads.

Table 3 shows the results of bread color measurements. In terms of crust color, the Control bread had the highest values of L*, a*, and b* among all samples. However, the addition of MP decreased the values of L*, a*, and b*. Further, all values of the MP+E bread were significantly lower than the Control. In terms of crumb color, the addition of MP significantly decreased the value of a*, while it significantly increased the value of b*. These results show that the addition of MP increases the greenness and yellowness of the bread crumb color. L* and a* of the crumb of MP+E bread were significantly lower, while the b* value was significantly higher compared to the Control. These seem to be related to the high reducing sugar content of dough with MP+E and the high green and yellow color of MP. The above results are consistent with the color data of bread appearance and crumb images in Fig. 1.

Table 3. Color of breads of Control, MP, and MP+E[1]

Bread making treatments	Bread crust color (-)			Bread crumb color (-)		
	L*	a*	b*	L*	a*	b*
Control	52.96 ± 2.65 a	16.25 ± 0.35 a	32.94 ± 2.00 a	84.07 ± 1.34 a	-2.21 ± 0.06 a	9.43 ± 0.34 b
MP	48.90 ± 0.76 b	15.57 ± 0.15 a	27.49 ± 0.63 b	84.23 ± 0.58 a	-2.43 ± 0.05 b	10.73 ± 0.40 a
MP+E	41.87 ± 1.62 c	14.25 ± 0.77 b	19.55 ± 2.05 c	82.55 ± 1.19 b	-2.50 ± 0.12 b	10.71 ± 0.54 a

[1] MP: mashed potato , E: enzymes, L*: level of lightness, a*: level of redness and greenness, b*: level of yellowness and blueness. Optimal amounts of enzymes, α-amylase and hemicellulase, were added in dough of MP+E. Each value is the mean ± SD (Bread crust color: n=5, Bread cumb color: n=8).
The values followed by different letters within column are significantly different ($p < 0.05$).

Table 4. Temporal moisture contents of bread crumbs of Control, MP, and MP+E[1]

Bread making treatments	Moisture contents of bread crumbs (%)			Difference (1day - 3days)of moisture contents of bread crumbs during storage (%)
	1 day after baking	2 days after baking	3 days after baking	
Control	39.25 ± 0.38 a	36.10 ± 0.97 b	34.76 ± 0.66 b	4.48 ± 0.98 a
MP	40.66 ± 0.19 a	38.23 ± 0.61 a	36.83 ± 0.73 a	3.83 ± 0.90 a
MP+E	40.61 ± 1.42 a	37.19 ± 0.34 ab	35.77 ± 0.59 ab	4.85 ± 0.91 a

[1] MP: mashed potato , E: enzymes. Optimal amounts of enzymes, α-amylase and hemicellulase, were added in dough of MP+E.
Each value is the mean ± SD (n=3). The values followed by different letters within column are significantly different ($p < 0.05$).

Especially, the respectively dark and remarkably dark crust colors of MP and MP+E breads in Fig. 1 also corresponded with the greatly lower values of L*, a*, and b* of crust color shown in Table 3.

MP addition also resulted in the decrease of all crust color values compared to the Control. Especially, these values were significantly decreased by the addition of optimum amounts of enzymes compared to the MP bread, evidenced by the significantly lower L*, a* , and b* values of MP+E crust in Table 3. These results corresponded with the observation that the bread with MP+E has an inferior color in the appearance of sensory evaluation described later, which resulted in an excessively dark crust color. It seems that, as one factor, the high reducing sugar and maltose contents of the dough with MP+E is related to this result. Goesaert *et al*. (2009) reported that the addition of AM increased concentrations of reducing sugars such as glucose, fructose, and maltose, etc., and it is also reported that the content of free amino acids in potato is higher than wheat[iv], which results in enhancement of the Maillard reaction. From these previous findings, it is thought that the remarkably dark crust color of MP+E bread is greatly affected by the increases of reducing sugars by enzyme reactions and free amino acids with MP addition.

The L* and a* of crumbs of MP and MP+E breads, especially the latter, were lower than the Control, conversely, the b* of crumbs of MP and MP+E breads showed significantly higher values compared with the Control, as shown in Table 3. In particular, the significantly higher b* values of MP and MP+E bread crumbs are in good agreement with the crumb color of breads with MP (MP and MP+E breads), which

showed a somewhat yellow color compared with the Control, as shown in Fig. 1. These may also be related to the fact that MP shows a more yellow color as compared to wheat flour. The obviously dark color of the crust and more dark, green, and yellow color of the crumb of MP+E bread seemed to result from the high reducing sugars in the dough, which facilitated the browning, and the enhanced green and yellow color of MP.

Analysis and evaluation of doughs and breads Table 4 shows the temporal moisture contents of bread crumbs during storage. The moisture contents of MP and MP+E breads were higher than the Control during the storage period. This is thought to be mainly related to the high water absorption of the doughs with MP compared to the Control, as shown in Table 2. In regards to the difference (1 day-3 days) in moisture contents of bread crumbs during storage, MP bread also showed a low value compared to the others. This seems to be related to the fact that the SLV of MP bread is significantly lower than the others, and that the bread's surface area is small and the moisture evaporation during storage is suppressed. These results also correspond to reports that breads with a smaller SLV or with gelatinized starch have less moisture evaporation during baking and storage (Santiago *et al*., 2015b; Tsai *et al*., 2012; Yamauchi *et al*., 2014).

The saccharide contents in the water soluble fraction of bread crumbs are shown in Table 5. As the sucrose contents of all samples were nearly zero, the data were omitted. All saccharide contents in MP+E bread, except for glucose and fructose, were significantly higher than those of the others. The reducing sugar, glucose, fructose, and especially maltose of MP and MP+E breads also showed significantly high values

Table 5. Saccharide contents in water soluble fraction of bread crumbs of Control, MP, and MP+E[1)]

Bread making treatments	Saccharide contents in water soluble fraction of bread crumbs (mg/g bread crumb)				
	Total saccharide	Reducing saccharide	Glucose	Fructose	Maltose
Control	73.24 ± 2.72 b	66.09 ± 0.96 c	10.14 ± 0.41 b	19.18 ± 0.63 b	26.46 ± 0.79 c
MP	81.09 ± 0.92 b	69.45 ± 0.62 b	12.99 ± 0.90 a	22.20 ± 1.03 a	33.76 ± 1.68 b
MP+E	135.35 ± 5.30 a	100.84 ± 1.29 a	14.55 ± 1.76 a	21.10 ± 0.57 a	49.75 ± 1.33 a

[1)] MP: mashed potato , E: enzymes. Optimal amounts of enzymes, α-amylase and hemicellulase, were added in dough of MP+E. Each value is the mean ± SD (n=3). The values followed by different letters within column are significantly different ($p < 0.05$).

Table 6. DS and DFs contents of doughs of Control, MP, and MP+E[1)]

Bread making treatments	DS (%)	NDF (%)	ADF (%)	NDF-ADF (%)
Control	3.63 ± 0.32 ab	0.86 ± 0.09 b	0.42 ± 0.03 b	0.45 ± 0.13 a
MP	4.76 ± 0.42 a	1.28 ± 0.21 a	0.74 ± 0.08 a	0.54 ± 0.14 a
MP+E	2.65 ± 0.08 b	0.80 ± 0.03 b	0.69 ± 0.08 a	0.11 ± 0.04 b

[1)] DS: damaged starch, DFs: dietary fibers, MP: mashed potato, E: enzymes, NDF: neutral detergent fiber, ADF: acid detergent fiber, NDF-ADF: approximate hemicellulose content. The DS and DFs contents are percentage based on the dry base weight of samples.
Optimal amounts of enzymes, α-amylase and hemicellulase, were added in dough of MP+E. Each value is the mean ± SD (n=3).
The values followed by different letters within column are significantly different ($p < 0.05$).

compared with the Control. These results agreed with previous reports concerning Yudane bread produced with gelatinized and swollen flour paste and bread made with the addition of gelatinized sweet potato powder (Santiago *et al*., 2015b; Yamada *et al.*, 2004; Yamauchi *et al.*, 2014). They also reported that when the materials containing gelatinized, swollen starch, and various other polysaccharides, such as Yudane dough and gelatinized sweet potato powder, etc., are added to the dough, obviously greater total and reducing saccharides, and maltose were produced in the dough compared to the Control without these materials. It is reported that the added gelatinized and swollen starch to the dough is decomposed by various endogenous amylases in wheat flour and the added AM and HC, with the latter effect being greater (Santiago *et al*., 2015b). From the results in Table 5 and Fig. 1, it was proven that the crust color of MP and especially MP+E breads is greatly influenced by the contents of reducing saccharide and maltose in the dough.

Table 6 shows the DS contents and DFs compositions of the final proofing doughs from different treatments. The Control and MP doughs showed higher values of DS content than the MP+E dough. The MP+E dough had a lower value than the Control and a significantly lower value than the MP dough. The optimal addition of enzymes decomposes the large amounts of DS in the dough; therefore, the dough with MP+E had the lowest DS content among all samples. Table 6 also shows the DFs contents of doughs. The ADF of dough with MP or MP+E had a significantly higher value than the Control

dough, and the NDF of MP dough also had a significantly higher value compared with the others. Furthermore, the NDF-ADF (approximate hemicellulose content) of dough with MP+E was significantly lower than those of the others.

The higher DS contents of dough without enzymes can be associated with the amounts of DS generated by physical damage during the milling process and the gelatinized starch in MP. Excess amounts of DS have negative effects on BMQ (Murayama *et al*., 2015; Santiago *et al*., 2015a; Yamauchi *et al*., 2014). The MP+E dough had lower or significantly lower DS than the others, which can be mainly related to the enzymatic decomposition of DS with added AM. From Table 6, the MP dough also had higher DFs contents (NDF, ADF, and NDF-ADF), which is inherent to the MP added. Generally, excess DFs have a negative effect on optimal gluten network formation, resulting in reduced GRD and SLV (Lai *et al*., 1989; Matsushita *et al*., 2017). Conversely, the MP+E dough showed lower DFs contents, especially NDF-ADF, except for ADF, which was attributable to the xylanase activity of HC, compared to the MP dough. HC hydrolyzes DFs such as xylan and arabinoxylan, etc., resulting in low contents of NDF and approximate hemicellulose (NDF-ADF) in the dough (Jiang *et al*., 2005; Stojceska and Ainsworth, 2008).

Ultimately, from the above findings, the lower GRD and SLV of MP dough and bread (see Table 2) can be attributed to the highest amounts of DS (including GS) and DFs of MP dough among all samples. It was also suggested that the excess DS and DFs of dough disrupted the gluten network (Lai *et al*.,

1989; Ozboy and Koksel, 1997; Wang *et al.*, 2002). Meanwhile, the drastic improvement of GRD and SLV of MP+E dough and bread can be associated with the reduced amounts of DS and DFs (mainly insoluble hemicellulose (pentosan)), which is caused by sufficient decomposition of DS and DFs in the dough from the addition of optimum amounts of both enzymes. In addition, the Control dough and bread in Table 2 also had higher GRD and SLV values despite the high DS and NDF-ADF contents, 3.63 % and 0.45 %, in Table 6, which might be attributed to the rather low values of total DFs (equivalent to NDF), especially ADF, compared to the dough with MP.

Fig. 2 shows the temporal changes of bread hardness during 3 days storage. The Control and MP breads showed significantly higher values than MP+E bread at 1 and 2 days storage. The Control and MP breads also showed similar values until 2 days. The hardness of the Control and MP breads were the highest or significantly higher than that of MP+E bread at 3 days storage. MP bread showed a significantly higher value of hardness at 3 days among all samples, while the MP+E bread was lower or significantly lower than the others at 3 days. A variety of factors relate to the temporal changes in bread crumb hardness during storage, such as retrogradation rate of gelatinized starch gel (GSG) in bread, SLV, LMWSs contents, and bread moisture, etc. The AM mainly breaks down the DS (including GS) in dough into low molecular weight dextrins and oligo-saccharides, etc. during bread making. In addition, endogenous β-amylase in wheat flour converts the above saccharides into maltose. These complementary functions during the bread making process bring about the partial decomposition of DS (including GS). In addition, the added AM increases the contents of LMWSs in bread. It was reported that these LMWSs retard the retrogradation of GSG and reduce the amount of available starch for 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 anti-staling 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, starch-protein interactions are interfered with by these LMWSs, produced by AM hydrolysis in dough, resulting in few and weak crosslinks between starch and protein, and a reduction in the hardening rate of bread (Martin and Hoseney, 1991; Martin *et al.*,1991). From Table 2 and Fig. 2, the SLV of MP+E bread with optimum enzymes including AM was significantly greater than the MP 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. The staling suppression of bread accompanying the increase of SLV is considered to be mainly due to the increase in crumb porosity.

On the other hand, insoluble hemicellulose (mainly insoluble pentosan) interferes with a desirable gluten network formation, while HC mainly attacks the insoluble pentosan and
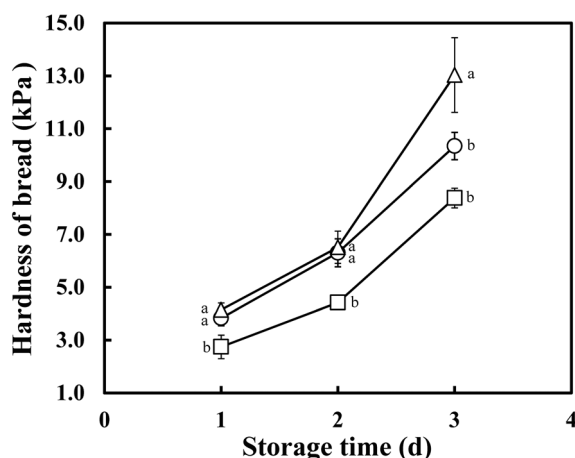

Fig. 2. Temporal hardness changes of bread crumbs of Control, MP, and MP+E during storage [1]
[1]MP: mashed potato, E: enzymes. Optimal amounts of enzymes, α-amylase and hemicellulase, were added in dough of MP+E. The vertical bar is the standard deviation of each value (n=3). The symbols followed by different letters are significantly different ($p < 0.05$). ○: Control, △: MP, □: MP+E.

produces LMWSs, resulting in improvement of BMQ. 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 MP+E dough actually had significantly lower amounts of approximate hemicellulose (NDF-ADF) than the Control and MP doughs in Table 6.

Roger *et al.* (1980) and Zeleznak *et al.* (1986) also reported that high water content bread shows low staling. As shown in Table 4, the breads with MP showed higher moisture contents than the Control during storage. Therefore, in the MP breads, it is considered that the high moisture contents of these breads positively influences the suppression of bread staling.

From the above findings, it seems that common factors concerning the suppressive effect of AM or HC on MP+E bread staling are high SLV through strengthening of the gluten network structure, accompanied by the degradation of DS and insoluble pentosan, as well as the retardation of starch gel retrogradation in the bread by LMWSs and the high moisture content of bread.

Fig. 2 also shows that the optimal addition of enzymes obviously suppressed the staling rate of bread compared to the Control and MP breads. This was caused by the increase of certain saccharides with the decomposition of DS and DFs in bread by optimal addition of AM and HC, as well as the high SLV accompanying decompositions of DS and insoluble DFs, and the high moisture content, which results in the anti-bread staling effects. These results agree with the previous data reported by Caballero *et al.* (2007), Matsushita *et al.* (2017), Ghoshal *et al.* (2013), and Goesaert *et al.* (2009).

Sensory evaluation of breads   Results of sensory evaluation of breads are shown in Table 7. From the results of appearance evaluation, as the volume and color in MP bread

was significantly lower than those of the Control, the total appearance evaluation of the MP bread showed a significantly lower value compared with the Control. On the other hand, in the MP+E bread, the evaluation of color was significantly lower than in the Control, but the evaluation of volume was significantly higher. Therefore, the total appearance was similar to the Control. In the crumb evaluation, MP and MP+E breads showed better results than the Control, except for color. Texture, flavor, and taste were significantly higher than the Control. Furthermore, the texture evaluation (hardness) results of the MP bread were significantly higher than the Control. However, there was no significant difference between the above breads in the hardness of bread at 1 day of storage, as shown in Fig. 2. Sensory evaluation of texture evaluates the hardness of bread and basically estimates the texture as well as the hardness of bread, as shown in Fig 2. Therefore, these results appear to be contradictory. The MP bread showed higher water content of the bread crumb at 1 day of storage than the control, as shown in Table 4. Therefore, from the texture evaluation (hardness) results, the bread (MP bread) with a high moisture content tends to be evaluated as softer than the physical property value by instrument analysis (hardness of bread), shown in Fig. 2.

The total crumb evaluation of these breads showed higher values than the Control; in particular, the MP+E bread showed a significantly higher value. The overall total evaluation of MP bread showed an almost equivalent value with the Control, while that of the MP+E bread showed a significantly higher value, which reflects the above total appearance and crumb evaluation results.

These results are approximately consistent with the above-mentioned BMQ of these bread doughs (Table 2) and various evaluations of these breads (Table 3, Figs. 1 and 2). Namely, although the BMQ of dough greatly decrease due to the addition of MP, the texture, flavor, and taste in the crumb evaluation were greatly improved. In addition, the volume in the appearance evaluation of MP+E bread was greatly improved by the addition of optimal enzymes, and it was found that the crumb grain, texture, and taste of this bread in the crumb evaluation were improved more than the MP bread. However, the crust color of the MP+E bread was much lower than the MP bread, which was the main factor in the total appearance evaluation of MP+E bread being similar to the Control.

Although described in the following sections of Overall BMQ and bread evaluation, the inferior crust color of MP+E bread may have been caused by excessive production of reducing saccharides by the added enzymes, as shown in Table 5. Therefore, to further improve the overall qualities of MP+E bread, assessing whether to include crust color in the optimization evaluation index or confirming if baking MP+E dough at low temperatures is suitable is needed. Consequently, the overall total evaluation result of MP+E bread could be further improved.

Overall BMQ and bread evaluation　Overall, this study established that treatment with optimal AM and HC drastically improved the BMQ of dough supplemented with 5 % MP. The BMQ most improved by optimum addition of enzymes are increases of GRD and SLV, suppression of bread staling, and high results of sensory evaluation, especially volume, texture, flavor, and taste, as shown in Table 2, Fig. 2, and Table 7. These properties of dough and bread with optimal enzymes were dramatically and greatly improved compared to MP dough and bread, which were as a whole superior to the Control. On the other hand, as a negative effect of optimal enzymes addition, the reduction in bread color evaluation, especially the large decreases in L* and b* values of the crust, was observed, as shown in Table 3. In MP bread, while the L*, a*, and b* values of the bread also decreased compared with the Control, the color was considered to be acceptable. However, the crust browning of MP+E bread, which reflects the progression of the Maillard reaction, during the baking process proceeded more than ideal with the addition of optimum enzymes, and the crust color evaluation was greatly decreased. As such, the crust color of MP+E bread seemed to be unacceptable. Therefore, this seemed to be a negative point with respect to the addition of optimal amounts of enzymes. In this study, the amounts of optimal enzymes to maximize SLV as a response were determined by using RSMd and OT. As the calculated value (5.08 mL/g) of optimal SLV with RSMd corresponded closely with the actual experimental value (5.20 mL/g); thus, the validity of this model was verified. Meanwhile, this model was limited in optimizing the bread making condition by using SLV as an index of optimum bread quality and, as such, degradation of the crust color of bread obtained with optimal enzyme addition was observed in this study.

Based on the above findings, RSM and OT are basically effective methods for the optimization of bread making conditions. To more effectively apply these methods in the future, the use of an overall index as a response that integrates, for example, SLV, bread color, and staling, etc., to indicate BMQ or the baking of dough at an optimal low temperature condition to prevent deterioration of crust color should be considered.

Conclusion

Although the high amounts of DS (including GS) and DFs in MP had a beneficial effect on bread qualities, such as nutritional value, texture, flavor, and taste, they decreased the general bread making properties. The GS and hemicellulose (mainly insoluble pentosan) among the DFs especially interfered with fine gluten network formation, resulting in the reduction of GRD and SLV, and the acceleration of staling rate during storage. The addition of optimal enzymes (AM and HC) to mitigate these problems was reasonably determined using

Table 7. Results of sensory evaluation of breads of Control, MP, and MP+E[1]

Bread-making treatments	Apearance evaluation (-)				Crumb evaluation (-)						Overall total evaluation (-)
	Volume	Shape	Color	Total	Crumb grain	Color	Texture	Flavor	Taste	Total	
Control	15.00 ± 0.00 b	5.00 ± 0.00 a	5.00 ± 0.00 a	25.00 ± 0.00 a	5.00 ± 0.00 b	5.00 ± 0.00 a	5.00 ± 0.00 b	5.00 ± 0.00 b	5.00 ± 0.00 b	25.00 ± 0.00 b	50.00 ± 0.00 b
MP	10.93 ± 1.85 c	5.42 ± 1.73 a	3.93 ± 0.88 b	20.27 ± 2.81 b	5.36 ± 2.07 a	4.48 ± 1.38 a	5.92 ± 1.08 a	6.17 ± 1.47 a	6.03 ± 0.61 a	27.96 ± 4.67 ab	48.23 ± 5.36 b
MP+E	18.30 ± 3.23 a	4.33 ± 1.24 a	2.53 ± 0.60 c	25.16 ± 3.68 a	5.58 ± 1.66 a	4.58 ± 1.33 a	6.59 ± 1.14 a	6.14 ± 1.38 a	6.73 ± 1.31 a	29.62 ± 3.99 a	54.78 ± 5.67 a

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

RSM and OT, which resulted in the formation of a desirable gluten network and remarkable improvement of dough and bread properties, such as GRD and SLV. This is attributed to the degradation of DS and hemicellulose (mainly insoluble types) into soluble LMWSs, which do not negatively influence the gluten network formation. As such, the addition of optimal enzymes in MP-supplemented dough enabled the production of satisfactory bread supplemented with 5 % MP, and which, with the exception of bread crust color, exhibited several suitable properties, such as high GRD and SLV, retarded staling rate, and good crumb evaluation. The above findings suggest that RSM and OT (Solver) are effective methods to establish optimal conditions in bread making. By using these methods, the optimal conditions can be reasonably and easily determined.

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