**Highlights**

- Effect of bakery enzyme and high-pressure on bread making quality (BMQ) was evaluated.
- The optimal condition was determined by response surface methodology (RSM).
- The combination of bakery enzyme and high-pressure maximally improved the BMQ.

**Abstract**

Various enzymes are added to dough to improve the quality. Two enzymes are α-amylase and hemicellulose (bakery enzymes), whose substrates are damaged starch and insoluble dietary fiber, respectively. They improve the formation of gluten networks in the dough, resulting in a higher specific loaf volume (SLV). The use of high-pressure treatment has also increased as a substitute for heat treatment and various products are being processed utilizing high-pressure treatment. This study investigated the effect of combing bakery enzyme and high-pressure treatment on dough qualities. The optimal concentration of bakery enzymes and high-pressure level were determined using response surface methodology and optimization technique. Bread dough was prepared by the optimal condition, 0.20% of bakery enzyme and 43MPa of high-pressure treatment, and the bread dough was then baked. Optimal combining bakery enzyme and high-pressure treatment drastically improved bread making qualities such as increased SLV, higher concentrations of reducing sugar, and lower concentrations of damaged starch and insoluble dietary fiber compared to the Control and to those that were only treated with bakery enzymes or high-pressure treatment, respectively. In addition, the bread with both bakery enzymes and high-pressure treatment showed improved micro structure in the crumb and maintained freshness longer.

**Key words**: high pressure; bread making quality; enzymes; response surface methodology

## Introduction

Generally, damaged starch (DS) and insoluble dietary fibers (DFs) (especially insoluble pentosan) in wheat flour inhibit the formation of a suitable gluten network in dough, and they are considered as factors reducing bread making quality (BMQ) (Wang et al. 2002; Hung et al. 2007; Santiago et al. 2015a). Various enzymes for bread making are used to improve BMQ; especially α-amylase and hemicellulase, which are hydrolases enzymes, that act on the DS and insoluble DF. The addition of these enzymes results in an increase of low molecular saccharides, the formation of a desirable gluten network, an increased specific loaf volume (SLV) and a retarded bread staling rate during storage (Santiago et al. 2015a; Matsushita et al. 2017).

In recent years, the use of high-pressure processing technology, a new food processing method, has been increasing and is expected to become an alternative to heat treatment (Unni et al 2015). High-pressure treatment is a technique that applies high hydrostatic pressure on food products during the processing to suppress the growth of bacteria and promote the immersion effect (Kim and Han, 2012). Moreover, some enzymes are activated by applying high-pressure treatment and the process effectively distributes the enzymes uniformly throughout the food (Fujiwara et al. 2001).

From previous studies, it appears that high-pressure treatment promotes enzymatic activity on bread dough, and it is our belief that combining high-pressure treatment with additional enzymes can be an effective approach to improving BMQ (Asaka et al. 1991; Fujiwara et al. 2001; Kim and Han, 2012). However, it is necessary to experiment with a large number of combinations in order to determine the optimum conditions for combining additional enzymes and high-pressure treatment for bread making. In this study, we conducted bread making tests according to the central composite plan and determined the regression coefficients from the subsequent data to develop a response

surface model (RSMd). By using the expression from the RSMd and Solver (Excel add-in software), it was possible to derive the optimal combination of additional enzymes and high-pressure treatment level for the maximized SLV of the bread. We evaluated the effect of adding bakery enzyme and using a high-pressure treatment on BMQ by using our derived optimal condition.

## Materials and Methods

### Flour and bakery enzyme used

A commercial strong wheat flour (Camellia) manufactured by Nisshin Flour Milling Co., Ltd. (Tokyo, Japan) and a commercial bakery enzyme, which contains α-amylase and hemicellulase, (IBIS Yellow Clean Label) manufactured by Lesffre Co., Ltd. (Marcq-en-Baroeulnjo, France) were used in this study.

### Optimization of concentration of bakery enzyme and high-pressure level

A central composite face-centered design (CCF), as reported by Flander et al (2007), was used with two variables to determine the optimal condition of concentration of bakery enzyme and high-pressure treatment. This CCF was composed of twelve experiments with four replicates at the center point. The two variables optimized were bakery enzyme (%), based on flour, and high-pressure treatment level (MPa). The concentration of bakery enzyme and high-pressure treatment level ranged from 0.000 to 0.250 % flour base and from 0 to 100 MPa, respectively. Experimental conditions (concentration of added bakery enzyme and high-pressure treatment level) at the center point were 0.125 % flour base and 50 MPa. The conditions at the axial point were 0.250 % and 50 MPa, 0.000 % and 50 MPa, 0.125 % and 0 MPa, and 0.125 % and 100 MPa. The conditions at the factorial point were also 0.000 % and 0 MPa, 0.000 % and

100 MPa, 0.250 % and 0 MPa, and 0.250 % and 100 MPa. Then random bread making tests were done using the various combinations of the concentration of the bakery enzyme and high-pressure level. In this study, SLV was adopted as a response, and concentration of bakery enzyme and high-pressure treatment level were factors for analysis with RSMd. The reason for choosing SLV as a response is that it is a representative index of BMQ. From the results of twelve CCF experiments, a RSMd for a response and factors was derived using multiple regression analyses. Selection of the explanatory variables of the RSMd was determined by a stepwise back selection method with a 2.0 of F value as an index. Effectiveness of the model was assessed by verifying the factor effect with analysis of variance (ANOVA). The optimal concentration of bakery enzyme and the high-pressure treatment level were also determined with the model by using the Excel add-in software Solver. After the CCF experiments, bread making tests were conducted using the Control, bakery enzyme supplemented (BE), high-pressure treated (HP), and optimal bakery enzyme supplemented and high-pressure treated (BE/HP) doughs, and the BMQ of the doughs were evaluated in detail.

**Dough preparation and bread making**

The bread making tests were carried out using the remix-straight method. The optimal amount of water absorption was determined using a Farinograph at 500 BU according to the AACC (1991). All materials, except the yeast, were put into a pin mixer (National Complete 100-200 Gram Mixer, Model 100-200A, National Mfg. Co., Lincoln, USA) with Versa-Logger (ATTO Co., Ltd., Tokyo, Japan) and mixed for 3 min at 25Hz. The Control dough and the doughs with bakery enzyme was incubated for 10 min at 20 ℃ and 70% relative humidity in a fermentation cabinet and then mixed again after the addition of yeast to just beyond peak development, as indicated by the electric power

curve of the mixing motor. The doughs with high-pressure treatment for 10 min at 20 ℃ or both treatments were tested under various high-pressure treatment levels, and then the treatments of the dough were made in same way. After remixing, dough and bread were made by the standard no-time method (Yamauchi et al. 2001).

**DS and DFs analysis**

Samples for DS and DFs analysis were prepared according to the method reported by Santiago et al. (2015a) using dough after proofing. DS content in dough was measured with a Megazyme assay kit (Megazyme International Ireland Ltd., Wicklow, Ireland) according to the method of Gibson et al. (1991). Neutral detergent fiber (NDF), which are cellulose, hemicellulose and lignin content, and acid detergent fiber (ADF), which are cellulose and lignin content, were measured using the official AOAC method (2000). The difference between NDF and ADF was calculated to approximate hemicellulose content.

**Soluble sugar analysis**

Water-soluble fractions of the bread crumb after baking were extracted for the measurement of sugar content and composition. The measurements of total and reducing saccharide content and the HPLC analysis of glucose, fructose, sucrose, and maltose contents were carried out using the same methods as reported by Santiago et al. (2015b).

**Dough properties and bread evaluation**

The gas retention of dough and the gassing power of dough were measured by the same method 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). Replicates of three doughs and loaves, respectively, were prepared in a single bread making test to measure the gas retention of dough, gassing power and SLV. Photographs and images of the breads were taken with a digital camera and scanner and crust color was recorded with a colorimeter (CR-400, Konica Minolta Sensing, Inc., Tokyo, Japan), according to the method described by Santiago et al. (2015a).

**Scanning electron microscopy observation**

The images of gluten structure in bread crumb were obtained using a Scanning Electron Microscopy according to the same method reported by Santiago et al. (2015b). To observe clearly the gluten network structure of bread crumb, the bread crumb samples were washed with deionized distilled water in a sonicator for 10 min to elute the starch in the crumb.

**Evaluation of bread staling rate**

The temporal change of crumb hardness was measured at 1, 2, and 3 days of storage according to the same method reported by Yamauchi et al. (2001). The loaves were sliced into 2 cm-thick pieces and a square of crumb (3 x 3 cm) was cut from the central part. Using a creep meter (RE2-33005C; Yamaden Co., Ltd., Tokyo, Japan), the temporal hardness changes of the bread crumb were measured by compressing each square with a special cube plunger (6 cm length x 6 cm width x 2 cm height).

**Statistical analysis**

Significant differences in the data presented in Table 1 to Table 3 and Fig. 3 were

evaluated using ANOVA at a 5% level of significance with Tukey's multiple range test (Excel statistical software 2012).

## Results and Discussion

### Optimization of concentration of bakery enzyme and high-pressure level

The RSMd with SLV as the response and the concentration of bakery enzyme and high-pressure treatment level as the factors shown below was derived by using multiple regression analysis based on the results of twelve bread making CCF experiments.

$$Y=1.56952X_1+0.00468X_2-1.72571X_1^2-0.00004X_2^2-0.00360X_1X_2+4.23071$$

where Y is SLV (ml/g); $X_1$ is concentration of bakery enzyme (%) based on flour; $X_2$ is high-pressure treatment level (MPa). $R^2$ and adjusted $R^2$ showed high values, 0.9554 and 0.8996, respectively, and F value of all explanatory variables were more than 2.0. Using ANOVA, the p value of the effectiveness in this model was 0.0083, which assessed that the effectiveness was significant at 1% significance level. The value of $R^2$ can explain 95.54% of the total variation of SLV values by this model and its standard error is very small at 0.044 ml/g. From these results, it was clarified that this RSMd is a sufficiently effective equation for estimating SLV using the concentration of bakery enzyme and high-pressure treatment level. Therefore, optimal concentration of bakery enzyme and high-pressure treatment level for maximum SLV were calculated with the above equation using Solver. The optimal combination of added bakery enzyme and high-pressure treatment level were 0.20 % flour and 43 MPa, respectively. In other words, BE were supplemented with bakery enzyme at 0.20 %, HP were treated with high-pressure at 43 MPa, and BE/HP were supplemented and treated with bakery enzyme and high-pressure at 0.20 % and 43 MPa, respectively.

**Contents of DS in dough**

Table 1 shows the contents of DS in the Control, BE, HP, and BE/HP doughs. BE and BE/HP doughs had significantly lower values than the Control dough; especially BE/HP dough had the lowest value among all samples. On the other hand, HP dough had a somewhat lower value compared to the Control.

The higher DS content of doughs without bakery enzyme are associated with insufficient decomposition of DS, which is generated by the physical damage during the milling process (Santiago et al. 2015b). Douzals et al. (1998) reported that the gelatinization of wheat starch begins above 300 MPa and finishes at 600 MPa. Hence, the starch in HP dough was not gelatinized and damaged under 43 MPa in this study. Since the activity of endogenous α-amylase in dough was also low, it seems that DS degradation does not proceed sufficiently even with HP treatment at 43 MPa. In addition, it suggests that the DS content in BE/HP dough was lower than those in BE dough because high-pressure treatments enhance the amylase activity, especially α-amylase, on the dough.

**Contents of DFs in dough**

Table 1 shows the contents of DFs in the Control, BE, HP, and BE/HP doughs. The NDF content in BE/HP dough were significantly lower than the Control. Those in BE and HP doughs were not significantly different from those in the Control and BE/HP doughs, and there was no significant difference in all the samples among the means of ADF. The NDF-ADF contents of BE and BE/HP doughs were lower or significantly lower than those of the Control and HP doughs. In comparison, HP dough had similar values to the Control dough.

Generally, DFs negatively affect the formation of an optimal gluten network, resulting

in the reduction of gas retention of dough and SLV (Hung et al. 2007; Matsushita et al. 2017). BE and BE/HP doughs showed lower DFs except for ADF content, especially NDF-ADF, than the Control which was attributed to the xylanase activity of the hemicellulase in the bakery enzyme. In addition, enhancement of the enzymatic activity was also observed in this result since the DS and NDF contents of BE/HP dough had lower values than those of BE dough. The hemicellulase hydrolyzes the DFs, such as xylan and arabinoxylan, resulting in low NDF content and approximate hemicellulose (NDF-ADF) content in the dough (Jiang et al. 2005; Stojceska and Ainsworth, 2008). Ultimately, the improvement of gas retention of dough and SLV of BE and BE/HP doughs and bread can be associated with the reduction in the amounts of DS and DFs (mainly insoluble hemicellulose (pentosan)) as shown in Table 1.

**Soluble sugar contents of bread**

Table 2 shows the sugar contents of the water-soluble fractions in various breads. The maltose content of the Control was significantly lower compared with that of the BE/HP bread. The BE/HP bread showed the highest maltose content among all samples, 12.83 ± 2.13 mg/g dough, whereas the BE and HP doughs had higher values compared with the Control, but there was no significant difference among those doughs. Glucose, fructose, and sucrose contents were not also significantly different among all samples.

In terms of reducing sugar, the Control had a significantly lower content of 23.41 ± 0.99 mg/g bread than all others. The HP bread had 24.84 ± 0.67 mg/g bread, which is significantly higher than the Control but significantly lower than the BE and BE/HP breads. The BE and BE/HP breads had high values, 26.89 ± 0.45 and 28.64 ± 0.38 mg/g bread, respectively. The BE/HP bread showed the significantly highest reducing sugar content among all samples.

Regarding the total sugars, the BE and BE/HP breads had significantly higher content than the Control and HP. The BE/HP bread had a higher total sugar content than the BE bread but there was no significant difference between these samples.

The α-amylase mainly breaks down DS (including gelatinized starch) in dough into low molecular weight dextrins and oligo-saccharides during the bread making process, and the endogenous β-amylase in wheat flour converts the saccharides into maltose (Hidalgo et al. 2013). In addition, the hemicellulase catalyzes the degradation of polysaccharides (mainly hemicellulose) into mono sugars and short chain saccharides, resulting in the increased soluble sugar contents in BE and BE/HP breads as shown in Table 2. Santiago et al. (2015b) also reported that the addition of α-amylase and hemicellulase increases the soluble sugar contents in bread.

**BMQ evaluation**

Table 3 shows the BMQ of various doughs. The BE, HP and BE/HP doughs had decreased gassing power compared with the Control dough. The gassing power of BE and HP doughs were significantly lower than that of the Control at 1 h fermentation in particular. There was no significant difference observed after 2 h and 3 h fermentation.

Although there was no significant difference among treatments in the gas retention of dough, the bakery enzyme heightened the gas retention of dough, and as a result, the BE and BE/HP doughs had higher values compared with the Control. The BE/HP dough especially had a higher value compared with the others. On the other hand, the HP dough had similar gas retention of dough compared with the Control.

Regarding SLV, the BE/HP bread had significantly the highest values, and there was no significant difference among the others. The SLV (4.60) of BE/HP bread showed a very close value to the estimated value (4.65) calculated using the RSMd described earlier.

This indicates that effectiveness of this model was verified by the experiments.

Goesaert et al. (2009) and Jiang et al. (2005) suggested that α-amylase and hemicellulase decompose DS and pentosan (equivalent to NDF-ADF) into mono-sugars in dough, which consequently promotes yeast fermentation and improves gassing power during the fermentation. However, in this study, the gassing power of two doughs with BE during fermentation was significantly lower or lower compared with the Control (Table 3). It seems that the high concentrations of various components, like mono- and di-saccharides, produced by the addition of bakery enzyme suppresses yeast fermentation (Matsushita et al. 2019). This may be the reason the gassing power of BE and BE/HP doughs were slightly suppressed compared to the Control. While it seems that the increased gas retention of dough of BE and BE/HP doughs is related to the improvement of SLV. Patel et al. (2012) also reported a similar result that the addition of fungal α-amylase increased the SLV of chemically leavened bread. Likewise, Jiang et al. (2005) and Shah et al (2006) reported that hemicellulase catalyzes the degradation of polysaccharides (mainly hemicellulose) into mono sugars and short chain saccharides, resulting in superior gluten network formation. The catalytic activity of α-amylase and hemicellulase may have led to the dough and bread of BE and BE/HP having higher gas retention of dough and SLV compared to those of the Control. These results show that BE/HP had the most improved SLV compared to others, which indicates that high-pressure treatment enhances enzymatic activity and the combination of bakery enzyme and high-pressure treatment has a greater impact than individual treatments of bakery enzyme and high-pressure. Asaka et al (1991) also investigated the effects of high-pressure on the enzymatic activity and suggested that the enhancement in activity was from pressure induced changes in the interactions with other constituents or from the release of membrane-bound enzymes. In addition, RSMd and the optimization

technique using Solver were effective in determining the optimal combination of bakery enzyme and high-pressure because the predicted value of SLV (4.65 ml/g) from this model was very close with the measured value (4.60 ml/g).

**Bread color and appearance**

Table 3 shows crust color of various breads. In terms of values of L* and b*, BE and BE/HP breads had lower or significantly lower values than the Control and HP breads. Regarding the values of a*, BE and BE/HP breads had higher or significantly higher values than the Control HP breads. There was no significant difference between the Control and HP breads in the mean of L*, a*, and b*. The crust color of BE and BE/HP breads became darker than the Control, which corresponded to their lower values of L* and b*, shown in Table 3 and Fig. 1, respectively. Fig. 1 shows the bread appearances and crumb images. The crust redness of BE/HP bread in Fig. 1 was also significantly stronger compared to the Control, which is evidenced by the significantly higher a* values of crust (Table 3). The loaf sizes of BE and BE/HP breads were larger than the Control and BE/HP bread was obviously larger than others. These results were congruent with their SLV (Table 3). In terms of crumb, BE and BE/HP bread crumbs had larger vertical bubbles compared with the Control and HP, which related to the larger SLV of BE and BE/HP breads. These results show that breads with bakery enzyme has an excessively dark crust color, which related to the significantly higher reducing sugar contents of BE and BE/HP breads. Goesaert et al. (2009) reported that the addition of α-amylase increased concentrations of reducing sugars such as glucose, fructose, resulting in the enhancement of the Maillard reaction.

**Bread crumb structure**

Figure 2 shows images of the various bread crumbs just after baking, which illustrates the gluten network and the crosslinks between starch gel and gluten because the bread crumbs were eluted to almost completely remove the swollen starch. The Control and HP bread crumbs (Fig. 2a and c) had the crosslink between gelatinized starch gel and the gluten network, which was almost not present in the BE and BE/HP bread crumbs and the crosslink parts are shown with arrows (Fig. 2b and d). The Control and HP bread crumbs (Fig. 2a and c) likely have weak gluten networks with more gelatinized starch-gluten crosslinks. The residual gelatinized starch was not completely decomposed by the intrinsic enzymes in wheat flour, and subsequently was cross-linked to the gluten network during the baking process, resulting in a weak gluten network in the bread crumb. On the other hand, BE and BE/HP bread crumbs (Fig. 2b and d) had lesser starch-gluten crosslinks and fine and uniform gluten networks compared to the Control and HP bread crumbs. This improvement by using optimal bakery enzyme and high-pressure treatment is associated with the increased gas retention of dough and SLV (Table 3). Santiago et al. (2015b) also reported that the addition of α-amylase and hemicellulase improved the crumb structure (gluten network and crosslink starch gel and gluten) of bread made from the dough supplemented with sweet potato powder.

**Hardness of bread**

Figure 3 shows the temporal hardness changes of the various bread crumbs during storage. The Control bread had the significantly highest value among all samples at 1 day. While BE/HP bread had the significantly lowest value among all samples. There was no significant difference between BE and HP bread. The hardness of bread at 2 days showed significantly high values in the following order: Control, HP, BE, and BE/HP. BE and BE/HP breads especially had very lower values compared to the others.

The values of the Control and HP breads drastically increased at 3 days and, especially, the former showed the significantly highest value among all samples. While the values of BE and BE/HP breads remained low with the BE/HP bread having the lowest value among all samples.

There are various factors which relate to the temporal changes in bread crumb hardness during storage: retrogradation rate of gelatinized starch gel in bread, SLV, low molecular saccharides content, and bread moisture. It was reported that α-amylase decomposes DS and gelatinized starch to low molecular weight saccharides which retards the retrogradation of gelatinized starch gel and reduces 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 the α-amylase has an anti-staling effect on bread during storage. Martin and Hoseney (1991) and Palacios et al. (2004) suggested that the partially decomposed starch gel has a lower retrogradation rate. Moreover, the starch-protein interactions are interfered with by these low molecular weight saccharides, which are produced by α-amylase hydrolysis in dough, resulting in a few and weak crosslinks between the starch gel and protein (Fig. 2), and reduces the hardening rate of the bread (Martin and Hoseney 1991; Martin et al. 1991). The SLV of BE/HP bread (Table 3 and Fig. 1) with the optimum concentration of bakery enzyme and high-pressure treatment was significantly the largest among all samples. Clarke et al. (2002) also reported that the staling rate of bread clearly decreased with a large SLV. The staling suppression of bread with the accompanying increase of SLV is considered to be mainly due to the increased porosity of the crumb. In addition, the insoluble hemicellulose (mainly insoluble pentosan) interferes with the formation of a desirable gluten network, while hemicellulase mainly attacks the insoluble pentosan and changes it to low molecular weight saccharides, resulting in the

improvement of BMQ. It was reported that the addition of hemicellulase improved SLV and increased low molecular weight saccharides in dough (Caballero et al. 2007; Ghoshal et al. 2013; Matsushita et al. 2017). In this study, the BE and BE/HP doughs actually had significantly lower amounts of approximate hemicellulose (NDF-ADF) than the Control dough (Table 1).

From these findings, it seems that the main factors suppressing bread staling in BE/HP bread is high SLV because of the fine gluten network structure that accompanies the degradation of DS and insoluble pentosan and retards starch gel retrogradation in bread with low molecular weight saccharides. This improvement is caused by a larger increase in some saccharides with decompositions of DS and DFs in BE/HP compared with the Control and HP dough. These results (Table 2) support the conclusions reported by Caballero et al. (2007), Matsushita et al. (2017), Ghoshal et al. (2013), and Goesaert et al. (2009).

## Conclusion

This study investigated the effects of combining bakery enzyme and high-pressure treatment on BMQ. In addition, the optimum concentration of bakery enzyme and high-pressure treatment level (0.20 % and 43 MPa) were established using RSMd and the optimization technique by Solver. The high-pressure treatment enhances enzymatic activity (degradation of damaged starch and hemicellulose, mainly insoluble pentosan, into soluble low molecular saccharides), resulting in the formation of desirable gluten networks and dough properties. Ultimately, the bread treated at the optimal condition by both bakery enzyme and high-pressure had desirable properties such as increased gas retention of dough and SLV, desirable gluten network development, and retarded bread staling rate during storage compared with doughs treated by bakery enzyme or

high-pressure individually. In addition, the combination of bakery enzyme and high-pressure treatment increased maltose, reducing sugar, and total sugar content in dough. These findings suggest that the optimal combination of bakery enzyme and high-pressure treatment drastically improves BMQ. RSMd and the optimization technique are an effective method to establish the optimum conditions for combining bakery enzyme and high-pressure treatment in bread making because the value of $R^2$ can explain 95.54% of the total variation of SLV values by this model and its standard error is very small at 0.044 ml/g.

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**Figure Caption**

**Figure 1.** Photographs and scanned images of various breads and their crumbs

BE: bakery enzyme-supplemented bread, HP: high-pressure treated bread, BE/HP: optimal bakery enzyme-supplemented and high-pressure treated bread.

**Figure 2.** Electron microscope photographs of various bread crumbs

The bread crumb samples were washed with deionized distilled water in a sonicator for 10 min to elute the starch in the crumb. BE: bakery enzymes-supplemented bread, HP: high-pressure treated bread, BE/HP: optimal bakery enzymes-supplemented and high-pressure treated bread. The arrows indicate the crosslinks between gelatinized starch gel and gluten.

**Figure 3.** Temporal hardness changes of various breads crumb

The vertical bar is the standard deviation of each value (n=8). The ANOVA between the data was evaluated using Tukey′s multiple range test (Excel statistical software 2012) The symbols followed by a different letter are significantly different ($p<0.05$). ○: Control, △: E, □: HP, ◇: E/HP. BE: bakery enzyme-supplemented bread, HP: high-pressure treated bread, BE/HP: optimal bakery enzyme-supplemented and high-pressure treated bread.

**Table 1.** Results of damaged starch and fiber contents of dough

| Bread making treatments | DS (%) | NDF (%) | ADF (%) | NDF-ADF (%) |
|---|---|---|---|---|
| Control | 3.95 ±0.49 [a] | 1.15 ±0.08 [a] | 0.29 ±0.04 [a] | 0.86 ±0.05 [a] |
| BE | 3.33 ±0.25 [bc] | 0.93 ±0.12 [ab] | 0.23 ±0.14 [a] | 0.69 ±0.06 [bc] |
| HP | 3.67 ±0.24 [ab] | 1.13 ±0.18 [ab] | 0.28 ±0.06 [a] | 0.85 ±0.12 [ab] |
| BE/HP | 3.05 ±0.13 [c] | 0.84 ±0.09 [b] | 0.29 ±0.02 [a] | 0.55 ±0.07 [c] |

Each value is the mean ± SD (Damaged starch: n=8, Others: n=4). The ANOVA between the data was evaluated using Tukey's multiple range test (Excel statistical software 2012). The values followed by the same letter within a column are not significantly different ($p<0.05$).

The DS and DFs contents are percentage based on the dry base weight of samples.

DS: damaged starch, NDF: natural detergent fiber, ADF: acid detergent fiber, NDF-ADF: approximate hemicellulose content, BE: bakery enzyme-supplemented dough, HP: high-pressure treated dough, BE/HP: optimal bakery enzyme-supplemented and high-pressure treated dough.

**Table 2.** Soluble sugar content of bread crumbs

| Bread making treatments | Glucose (mg/g bread) | Fructose (mg/g bread) | Sucrose (mg/g bread) | Maltose (mg/g bread) | Reducing Sugar (mg/g bread) | Total Sugar (mg/g bread) |
|---|---|---|---|---|---|---|
| Control | 9.87 ±1.15 [a] | 10.27 ±0.15 [a] | 4.40 ±0.33 [a] | 9.30 ±0.53 [b] | 23.41 ±0.99 [d] | 46.56 ±2.09 [b] |
| BE | 9.88 ±0.68 [a] | 9.41 ±1.13 [a] | 4.60 ±0.30 [a] | 11.68 ±1.56 [ab] | 26.89 ±0.45 [b] | 52.27 ±0.74 [a] |
| HP | 10.31 ±1.27 [a] | 9.04 ±0.07 [a] | 3.99 ±0.64 [a] | 10.18 ±0.15 [ab] | 24.84 ±0.67 [c] | 47.62 ±1.61 [b] |
| BE/HP | 11.04 ±2.18 [a] | 9.04 ±0.63 [a] | 3.90 ±0.44 [a] | 12.83 ±2.13 [a] | 28.64 ±0.38 [a] | 54.74 ±0.49 [a] |

Each value is the mean ± SD (n=4). The ANOVA between the data was evaluated using Tukey's multiple range test of (Excel statistical software 2012). The values followed by the same letter within a column are not significantly different ($p<0.05$).

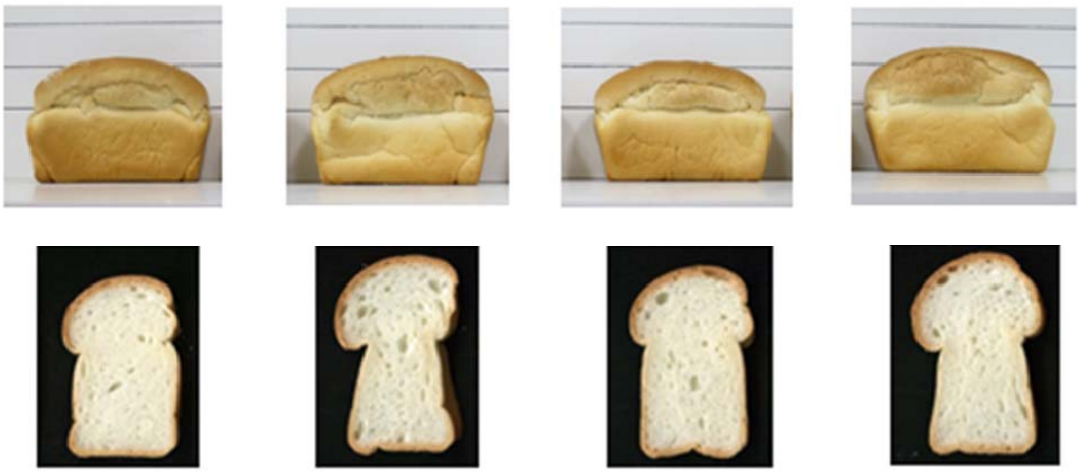
BE: bakery enzyme-supplemented bread, HP: high-pressure treated bread, BE/HP: optimal bakery enzyme-supplemented and high-pressure treated bread.

**Table 3.** Bread making qualities of dough and bread

| Bread making level | Gassing power of dough (ml/20 g dough) | | | Gas retention of dough | SLV | Color of bread crust | | |
|---|---|---|---|---|---|---|---|---|
| | 1h | 2h | 3h | (ml/20 g dough) | (ml/g) | L* (-) | a* (-) | b* (-) |
| Control | 38.73 ±0.56 [a] | 82.07 ±1.77 [a] | 119.25 ±2.46 [a] | 106.11 ±8.43 [a] | 4.21 ±0.06 [b] | 55.19 ±1.47 [a] | 15.84 ±0.38 [b] | 36.34 ±1.33 [a] |
| BE | 36.93 ±0.47 [b] | 79.17 ±1.16 [a] | 116.19 ±1.63 [a] | 111.11 ±1.73 [a] | 4.37 ±0.05 [b] | 52.32 ±1.81 [b] | 15.98 ±0.32 [ab] | 34.71 ±1.34 [b] |
| HP | 37.31 ±0.83 [b] | 79.51 ±2.59 [a] | 116.21 ±3.66 [a] | 106.67 ±0.00 [a] | 4.30 ±0.10 [b] | 54.52 ±1.37 [a] | 15.67 ±0.42 [b] | 35.32 ±1.10 [ab] |
| BE/HP | 37.72 ±0.21 [ab] | 80.35 ±0.66 [a] | 116.85 ±0.96 [a] | 114.17 ±5.00 [a] | 4.60 ±0.17 [a] | 52.33 ±1.06 [b] | 16.37 ±0.52 [a] | 34.25 ±1.16 [b] |

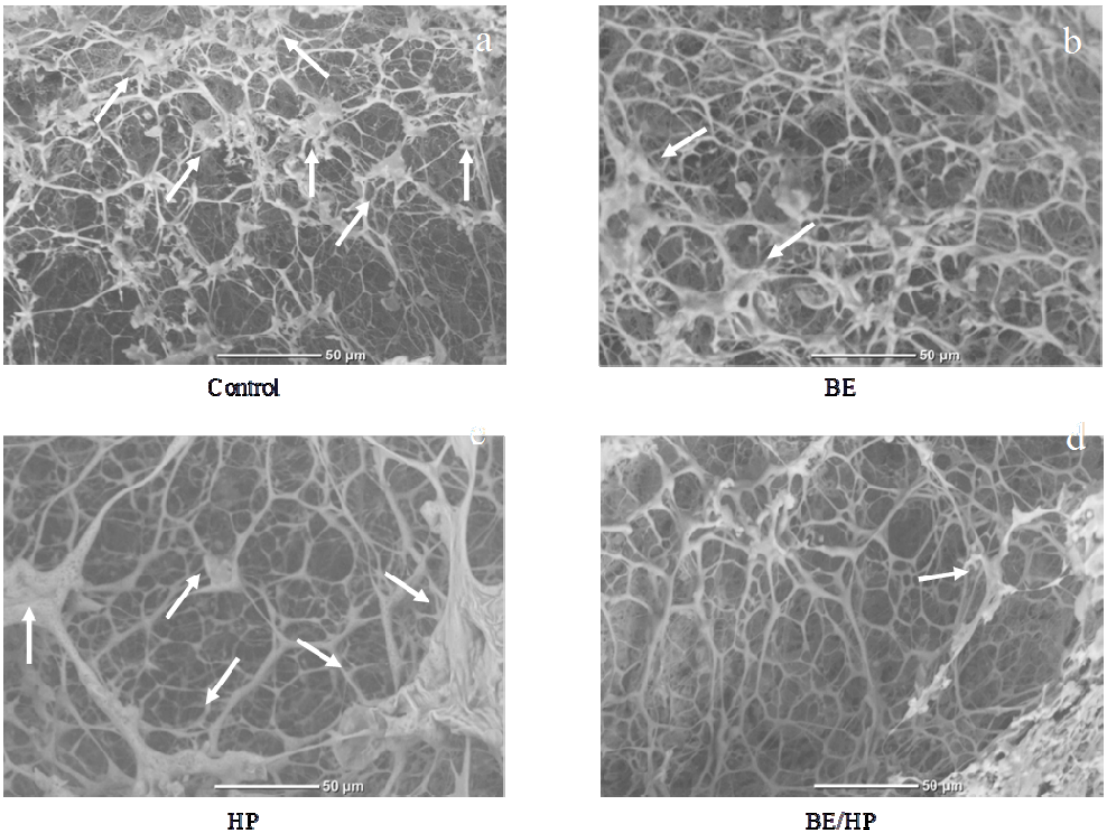
Each value is the mean ±SD (Gassing power of dough: n=3, Gas retention of dough: n=4, Specific loaf volume: n=5, Hue of bread crust: n=15). The ANOVA between the data was evaluated using Tukey's multiple range test (Excel statistical software 2012). The values followed by the same letter within a column are not significantly different ($p<0.05$).

SLV: specific loaf volume, BE: bakery enzyme-supplemented dough and bread, HP: high-pressure treated dough and bread, BE/HP: optimal bakery enzyme-supplemented and high-pressure treated dough and bread.



Control BE HP BE/HP

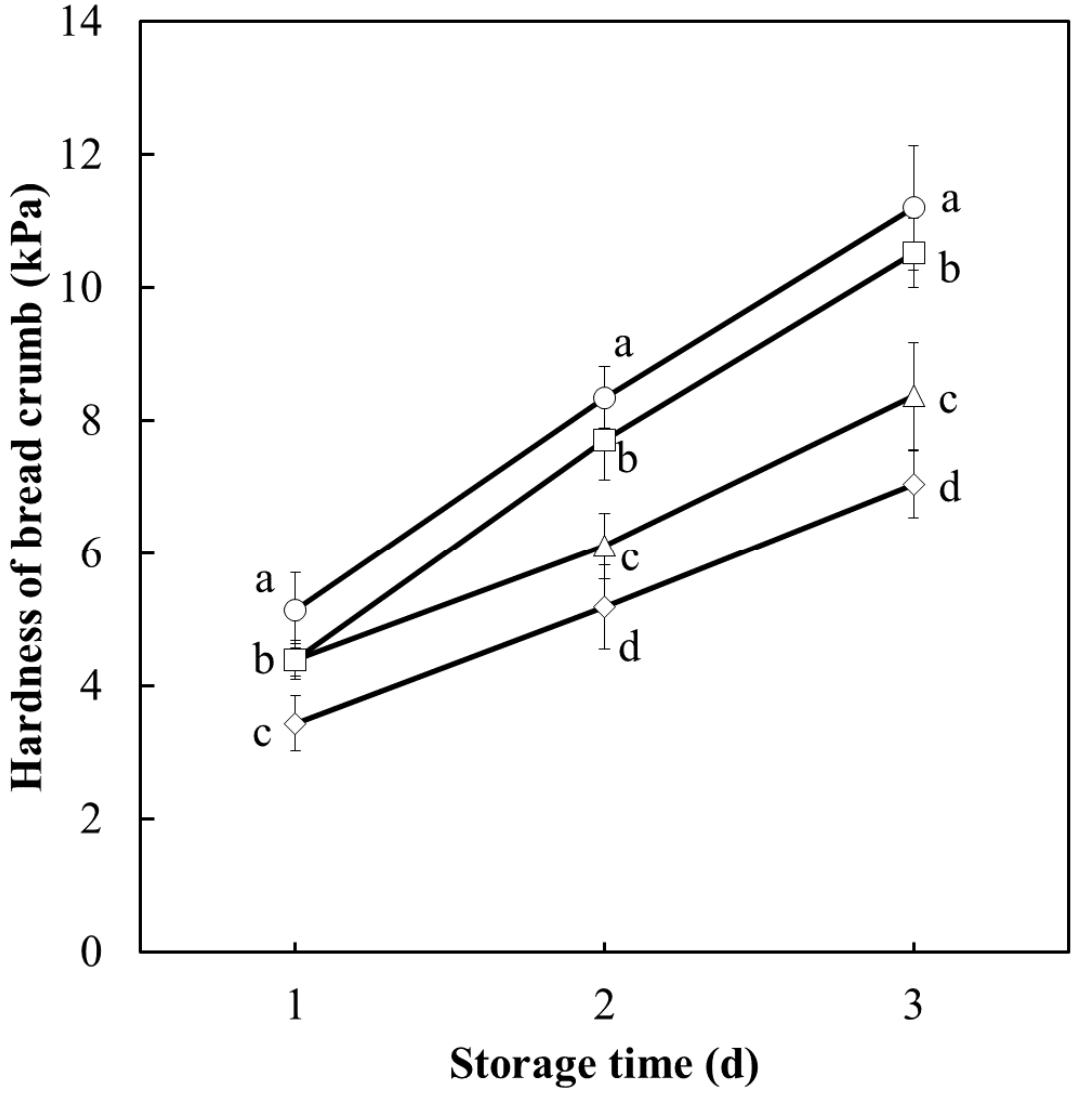
**Figure 1**




1

2

**Figure 2**


Hardness of bread crumb (kPa)
14
12
10
8
6
4
2
0
1
2
3
Storage time (d)
a
b
c
d
a
b
c
d
a
b
c
d

1

**Figure 3**