Optimization of enzymes addition to improve whole wheat bread making quality by response surface methodology and optimization technique

Koki Matsushita[1,3], Ayaka Terayama[1], Daisuke Goshima[1], Dennis Marvin Santiago[2], Takao Myoda[4], Hiroaki Yamauchi[1]

[1] Department of Food Science, Obihiro University of Agriculture and Veterinary Medicine, 2-11 West, Inada, Obihiro, Hokkaido 080-8555, Japan

[2] Institute of Food Science and Technology, College of Agriculture and Food Science, University of the Philippines Los Ban˜os, College, Batong Malake, 4031 Los Ban˜os, Laguna, Philippines

[3] The United Graduate School of Agricultural Science, Iwate University, 3-18-8 Ueda, Morioka, Iwate 020-8550, Japan

[4] Faculty of Bio-Industry, Tokyo University of Agriculture, 196 Yasaka, Abashiri, Hokkaido 099-2493, Japan

Highlights

Optimal amounts of enzymes required to improve bread making qualities (BMQ) were determined by response surface methodology (RSM).

Adding optimal amounts of enzymes maximized the BMQ of WWF dough and bread.

Combining RSM and optimization technique is an effective method to calculate the optimal amounts of enzymes to add.

Abstract

The functional ingredients in whole wheat flour, such as dietary fiber, vitamins, and minerals, have beneficial health effects. However, the excessive amount of dietary fiber in whole wheat flour inhibits gluten network formation and diminishes bread making qualities (BMQ). Adding appropriate amounts of enzymes, α-amylase (AM) and hemicellulase (HC), could be a solution to these problems. In this study, response surface methodology (RSM) created a RS model and Solver (Excel add-in software) calculated the optimal amounts of the enzymes. Adding optimum concentrations of AM and HC drastically improved BMQ (gas retention of dough, specific loaf volume, and bread staling) of whole wheat flour dough and bread compared to whole wheat flour dough and bread without the enzymes. These results show that combining RSM and Solver is an effective and reasonably easy method to determine optimal concentrations of enzymes to obtain the highest quality bread when using whole wheat flour.

Key words: whole wheat flour, bread making quality, enzymes, response surface methodology

Introduction

Whole wheat flour is derived by milling or grinding whole grain of wheat, which contains several functional compounds, such as dietary fiber (DF), vitamins, and minerals. These functional compounds have various positive effects on health such as reduced risk of cardiovascular diseases (Tucker et al. 2010), diabetes (Murtaugh et al. 2003), and some cancers (Schatzkin et al. 2008; Nimptsch et al. 2011). In order to enhance the functionality of bread, whole wheat flour has been used for bread making. As the characteristics of whole wheat flour are substantially different from those of white wheat flour, whole wheat flour bread exhibits increased crumb firmness, dark crumb appearance and, in some cases, alters the taste of the bread (Bruckner et al. 2001; Hung et al. 2007). In addition, an excessive amount of dietary fiber (DF), especially insoluble DF, inhibits the formation of the gluten network and decreases loaf volume (Lai et al. 1989). Thus, it is necessary to modify the bread making method or use certain additives to offset the disadvantages of making bread with whole wheat flour.

In this study, two kinds of enzymes, α-amylase (AM) and hemicellulase (HC), were used as improvers (Caballero et al. 2007). These enzymes act on the damaged starch (DS) and insoluble DF. DS is generated by the physical damage that occurs during the milling process, which has a negative effect on the final bread quality. The most evident effects are the reduction of loaf volume and an increase in bread staling rate. On the other hand, it is possible to improve the bread making quality (BMQ) by adding α-amylase, which decomposes DS. In addition, insoluble DF plays a role in disrupting the formation of the gluten network, which diminishes BMQ, resulting in smaller and firmer bread (Wang et al. 2002). Hemicellulase decomposes insoluble DF, which also inhibits the formation of the gluten network, thus improving BMQ (Santiago et al. 2015a).

A combination of several enzymes that specifically counteract each negative factor is more effective in improving BMQ compared to using an individual enzyme (Caballero et al. 2007; Santiago et al. 2015a; Santiago et al. 2015b; Matsushita et al. 2017). However, determining the optimal concentration of each enzyme is difficult and a time and labor intensive task due to the complex nature of the interactions among multiple enzymes. It requires the comparison of an enormous amount of data obtained for bread making quality parameters using various enzyme combinations.

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 optimum amounts of multiple enzymes that would maximum the BMQ of dough with whole wheat flour. A response surface model (RSMd) was created using the data acquired, based on the CCF, and then the optimal amounts of multiple enzymes were determined by using an optimization technique (OT) with Solver (Excel add-in software). Finally, in order to validate the effectiveness of these methods, bread making experiments, with the optimal amounts of multiple enzymes, were conducted, and the effectiveness of each combination was verified from the bread making qualities of the dough and various evaluations of the bread.

Materials and Methods

Flour and enzymes used

Camellia (Nisshin Flour Milling Co., Ltd., Tokyo, Japan) and Zenryufun Kyoriki (Ebetsu Flour Milling Co., Ltd., Ebetsu, Japan) were used in this study. Two commercial enzymes were used: AM (Sumizyme AS) containing 1500 α-amylase U/g, and HC (Sumizyme SNX) containing 14,000 xylanase U/g. Both were manufactured by Shin Nihon Chemical Co., Ltd. (Anjo, Japan).

Optimal concentrations of added enzymes

A central composite face-centered design (CCF), as reported by Flander et al. (2007), was used with two variables to determine optimal concentrations of enzymes. This CCF was composed of twelve experiments 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

0.1 g/100 g flour for both AM and HC. Concentrations of both enzymes ranged from 0 to 0.2 g/ 100 g flour. Then random bread making tests were done using various combinations of the amounts of the enzymes. In this study, specific loaf volume (SLV) was adopted as the response and the amounts of added enzymes (AM and HC) were the factors in analysis of RSM. The reason for choosing SLV as a response trait is that it is representative of BMQ. From the results of twelve CCF experiments, a RSMd, for a response and factors, was derived by multiple regression analysis. Selection of the explanatory variables of the RSMd was determined by the stepwise back selection method with a 2.0 F value as an index. Effectiveness of the model was assessed by verifying the factor effect and lack of fit with the analysis of variance (ANOVA). Optimal amounts of added enzymes were also determined with the model by using the Excel add-in software Solver. After the CCF experiments, bread making tests were conducted using a Control and whole wheat flour doughs with and without enzymes, and the effects on BMQ were evaluated in detail.

Dough preparation and bread making

The Control and whole wheat flour doughs were prepared according to the formula described by Matsushita et al. 2017. The optimal amount of water was determined using a Farinograph at 500 BU according to the method used by the AACC (1991). Forty percent of the standard white wheat flour formulation used for the Control was replaced with whole wheat flour because it is the maximum percentage at which the BMQ can be improved with enzymes (Matsushita et al. 2017). The no-time method and the standard wheat bread formulation were employed (Yamauchi et al. 2001).

Dough properties and bread evaluation

The gas retention of dough (GRD) was evaluated by measuring the maximum expansion volume of 20 g of dough proofed at 38℃ and 85% relative humidity (RH) in a cylinder subjected to 0 to 75 cmHg (Yamauchi et al. 2000). The gassing power (GP) 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.) (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 were prepared in a single bread making test to measure the GRD, GP and SLV, respectively. Photographs and images of the breads were recorded using the method reported by Santiago et al. (2015a). The color of the top bread crust and crumb was measured with a colorimeter (CR-400, Konica Minolta Sensing, Inc., Tokyo, Japan). Moisture content of the bread crumb samples, stored for 1 day in polyethylene bags, was measured using the official method of the AOAC (2000). The color values and moisture content of bread crumbs were measured from eight and ten slices of bread, respectively, from two loaves of the same replicate.

DS and DF analysis

Sample preparation, before DS and DF analysis, was done according to the method reported by Santiago et al. (2015a). The DS content in dough was measured with a Megazyme assay kit (Megazyme International Ireland Ltd., Wicklow, Ireland) based on 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 (2000). The difference between NDF and ADF was calculated and used as a rough number for hemicellulose content. The DS and DF of doughs, after final proofing, were measured using eight and four samples, respectively, of the same replicate.

Bread staling evaluation

The temporal changes of crumb hardness were measured at 1, 2, and 3 days of storage (Yamauchi et al. 2001). The loaves were cut into 2 cm thick slices and a 3 x 3 cm square crumb was cut from the center. Using a creep meter (RE2-33005C; Yamaden Co., Ltd., Tokyo, Japan), the changes in temporal hardness of the bread crumbs were measured by compressing them with a special cube plunger (6 cm length x 6 cm width x 2 cm height).

Statistical analysis

The samples were prepared from the replicated bread making tests for all data measurements except, for water absorption. Significant differences, except for water absorption, were evaluated using Tukey's multiple range test at 5% significance level with Excel 2012.

Results and Discussion

Optimal concentrations of added enzymes

The RSMd with SLV as the response and AM and HC as the factors, shown below was derived by multiple regression analysis based on the results of the twelve, bread making CCF experiments.

$$Y=5.60X_1+4.35X_1-15.62X_1^2-16.07X_1X_2+4.85$$

where Y is SLV (ml/g); X_1 is concentration of AM (g/100 g flour); X_2 is concentration of HC (g/100 g flour). R^2 and adjusted R^2 in the model showed high values, 0.841 and 0.751, respectively. Using ANOVA, the effectiveness and lack of fit were also assessed and those were significant at 1% significance level and not significant at 5% significance level, respectively. These results clarified that this RSMd sufficiently estimates SLV when using two levels of added enzyme concentrations. Furthermore, the partial regression coefficients of X_1^2 and X_1X_2 explanatory variable on RSMd show minus values. Therefore, especially, when both enzymes are added to the dough in a large excess, these explanatory variables have the effect of largely lowing the BMQ (SLV). Since the magnitude of the partial regression coefficient on these explanatory variables is nearly same, it shows that when the enzymes are added excessively, the effect of decreasing SLV of both enzymes and AM is large.

The optimal concentrations of AM and HC, calculated using Solver, an Excel add-in software, were 0.128 and 0.1 g/100 g flour, respectively. In whole wheat flour dough, SLV increased with the amount of HC added, but the dough became very sticky and extremely difficult to handle, and the improving effect plateaued when added HC exceeded 0.1 g/100 g flour. Therefore, the optimum concentration of HC is 0.1 g/100 g flour.

BMQ evaluation

BMQ of the Control dough, 40% of whole wheat flour (WWF) dough, and 40% of whole wheat flour with enzyme (WWF+E) dough are presented in Table 2. Although the WWF dough showed a lower GRD compared to the others, the GRD of the WWF+E dough was significantly the highest among all the samples.

Initially, GP of WWF and WWF+E doughs were lower than the Control at 1 h fermentation. At more than 2 h fermentation, the GP of these doughs were nearly same or significantly higher compared to the Control, respectively.

The WWF bread had significantly lower SLV than the others. On the other hand, the

SLV of the WWF+E bread was significantly the highest among all breads. The 5.66 value of SLV of the WWF+E bread was very close to the 5.54 value calculated using the RSMd. The experiments verified the effectiveness of this model.

In terms of moisture content, there was no large difference among the samples, but WWF+E bread was significantly lower than the others. The main reason seems to be the large reduction in weight when baking WWF+E dough, which is related to the dough's significant expansion from the addition of enzymes.

Lower GRD and SLV of dough and WWF bread can be due to the higher amounts of DS and DF compared to the WWF+E (Table 4). It suggests that the excessive DF in whole wheat flour disrupts the gluten network formation in dough, resulting in a weaker gluten network (Lai et al. 1989; Wang et al. 2002; Ozboy and Koksel 1997).

In terms of GRD and SLV, the WWF+E dough and bread had significantly the highest values among all the samples. This might be attributed to the combined catalytic activities of AM and HC that decreases DS and insoluble hemicellulose (equivalent to NDF-ADF) in the dough (Table 4). The Control dough and bread had higher GRD and SLV values despite having high DS content (4.16%) which might be attributed to the lower values of total DF (equivalent to NDF), especially hemicellulose (equivalent to NDF-ADF) compared to the WWF dough. The GP of WWF and WWF+E doughs were significantly higher than the Control at 3 h fermentation. This may be related to that high concentrations of various nutrients in WWF promote fermentation of yeast.

Regarding the effect of each added enzyme, AM hydrolyzes damaged and gelatinized starch in to maltose and dextrin in dough. Kim et al. (2006) reported that the high amounts of DS and DF decreased SLV of bread made with polished wheat flour, but SLV was increased by the addition of AM. Patel et al. (2012) also had a similar observation that the addition of fungal AM increased SLV in chemically leavened bread. Likewise, Jiang et al. (2005) reported that HC catalyzes the degradation of polysaccharides 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 WWF+E dough and bread compared to those with WWF. The addition of xylanase, a kind of HC enzyme, improved SLV of whole wheat flour bread (Shah et al. 2006), and a millet/wheat composite bread (Schoenlechner et al. 2013).

From these findings, it is reasonable to expect drastic improvements of GRD and SLV in WWF+E dough and bread.

Bread color and appearance

Table 3 shows the results of the bread color measurement. In terms of crust color, the Control bread had the highest values of L*, a* and b*among all samples. The addition of WWF decreased the values of L*, a* and b*. In addition, all values of the WWF+E were significantly lower than the Control.

In terms of crumb color, the addition of WWF significantly decreased the value of L*, while it significantly increased the values of a* and b*. L* values of crumb significantly decreased in descending order of the Control, WWF, and WWF+E. The a* value of the Control crumb was significantly lower compared to WWF and WWF+E breads. The b* values significantly increased in the order of Control, WWF+E, and WWF.

Figure 1 shows the bread and crumb images. The addition of whole wheat flour made the external color darker; especially the color of WWF+E bread was darker compared to the Control. The crumbs of WWF and WWF+E breads were darker compared to the Control crumb. The loaf size of WWF bread was smaller than the Control, while the WWF+E bread was obviously larger than the Control. These results were congruent with the SLV data presented in Table 2.

The crust color of WWF bread was darker than the Control. In addition, the WWF+E bread was darker compared to the Control and WWF breads (Fig. 1), which corresponded with its lower L* values (Table 3). The values of redness and yellowness in crust were also significantly decreased by the addition of enzymes compared to WWF bread, which is evidenced by the lower a* and b* values of crust (Table 3). These results show that bread with WWF+E was inferior in regard to excessive darkness of the crust. Goesaert et al. (2009) reported that the addition of AM increased concentrations of reducing sugars, such as glucose and fructose, resulting in the enhancement of the Maillard reaction.

The natural dark brown color of wheat bran makes bread crumb color darker in WWF and WWF+E breads, which results in the reduction in the L* value and the increase in a* and b* (Table 3). However, the L* value of WWF+E bread crumb was significantly lower compared to that of WWF bread crumb. These results show that WWF+E has decreased L* values of the bread crumb which makes it slightly inferior to the WWF bread crumb.

DS and DF contents of dough

Table 4 shows the DS content and DF composition of doughs from different treatments. The Control had significantly higher DS content than the others. The WWF dough had a lower value than the Control but significantly higher than the WWF+E dough. The addition of an optimal amount of enzymes decreased the amounts of DS in dough, therefore the WWF+E dough had significantly lower DS content than those of other samples.

Table 4 also shows the DF content of the various doughs. The WWF and WWF+E doughs had significantly higher values than the Control dough except for the

NDF-ADF of WWF+E dough. Furthermore, the values of WWF+E dough were lower than that of WWF dough except for ADF. In addition, NDF-ADF of WWF+E dough was significantly lower compared to that of WWF dough.

The higher DS contents of dough without the enzymes can be associated with the amounts of DS generated due to the physical damages during the milling process. Excess amounts of DS causes undesirable effects on BMQ (Santiago et al. 2015a; Yamauchi et al. 2014). The WWF+E dough had significantly lower DS than the others, which can be related to the enzymatic activity of AM.

From Table 4, WWF dough had higher DF content (NDF, ADF, and NDF-ADF), since whole wheat flour contains high amounts of DF. Generally, excess DF negatively effects the formation of the optimal gluten network, resulting in the reduction of GRD and SLV. Conversely, WWF+E dough showed lower DF content, except for ADF, which was attributable to the xylanase activity of HC, compared to WWF dough. HC hydrolyzes DF, such as xylan and arabinoxylan, resulting in low NDF content and crude hemicellulose (NDF-ADF) in the WWF+E dough (Stojceska and Ainsworth 2008; Jiang et al. 2005).

Ultimately, the improvement of GRD and SLV of bread treated with the optimal amount of enzymes can be associated with the reduction of the amounts of DS and DF (mainly pentosan, an insoluble hemicellulose).

Hardness of bread

Figure 2 shows staling of breads from different treatments during 3-day storage. The WWF bread showed a significantly higher value than that of WWF+E bread at 1-day storage. The Control and WWF+E breads showed similar values. The hardness of the Control and the WWF breads had similar values and were significantly higher than

WWF+E bread at 2-day storage. WWF bread had significantly the highest value of hardness among all samples at 3-day storage, while the WWF+E bread had a significantly lower value than the others.

There are various factors which relate to the temporal changes in crumb hardness during the storage: retrogradation rate of gelatinized starch gel (GSG), the contents of DS and insoluble pentosan, and SLV.

The AM mainly breaks down DS and GSG in dough into low molecular weight dextrins, and oligo-saccharides during bread making. In addition, the endogenous β-amylase in wheat flour converts the saccharides into maltose. These complementary functions during the bread making process bring about partial decompositions of DS and GSG. As a result, AM increases the content of low molecular weight saccharides (LMWSs) in bread. It was reported that these LMWSs retard the retrogradation of GSG and reduce the amount of available starch for the retrogradation in bread (Duran et al. 2001; Palacios et al. 2004; Goesaert et al. 2009). Caballero et al. (2007) and Palacios et al. (2004) also reported that the AM has an anti-staling effect on bread during the storage. Martin and Hoseney (1991) and Palacios et al. (2004) suggested that the partially decomposed starch gel has a lower retrogradation rate. Moreover, the LMWSs produced by the AM hydrolysis in the dough interfere with the starch- protein interactions, resulting in few and weak crosslinks between the starch and protein, and a reduction of hardening rate of the bread (Martin and Hoseney 1991; Martin et al. 1991).

The SLV of WWF+E bread was significantly larger than the others (Table 2 and Fig. 2). It has also been reported that the staling rate clearly decreases when there is a large SLV (Maleki et al. 1980).

The insoluble pentosan in dough interferes with the formation of a desirable gluten network, and HC attacks the insoluble pentosan, resulting in the improvement of BMQ.

It was reported that the addition of HC improved SLV and increased LMWSs in dough (Caballero et al. 2007; Matsushita et al. 2017; Ghoshal et al. 2013). The WWF+E dough had significantly lower amounts of crude hemicellulose (NDF-ADF) than WWF dough (Table 4).

From these findings, it seems the main factors concerning the suppression of staling in the WWF+E bread is that the enzymes decompose DS and insoluble pentosan and strengthen the gluten network , which promote high SLV, and the enzymes produce the LMWSs that retard starch gel retrogradation in the bread.

Overall BMQ

This study established that a treatment with an optimal amount of AM and HC drastically improves BMQ of whole wheat flour dough and bread. The most improved properties of BMQ were GRD and SLV, which increased, and the suppression of bread staling (Table 2 and Fig. 2). These WWF+E dough and bread properties were significantly improved compared to those of WWF dough and bread, which were also significantly better than the Control. On the other hand, a negative effect of the treatment was a reduction in the bread color evaluation, especially a decrease in L* value of crust, (Table 3). In the WWF bread, the decrease in L* value of the bread was comparable to the Control and was considered to be an acceptable characteristic. However, the addition of enzymes resulted in increased browning of the bread crust, an effect of promoting the Maillard reaction during the baking process, and lowering the bread color evaluation. This seems to be a negative effect of adding enzymes. In this study, the optimal amounts of enzymes (AM and HC) were derived using SLV as a response in an RSM and OT. The optimal value calculated for SLV using the RSMd was 5.54, which corresponded to the actual experimental value of 5.66, which validates this model to some extent. There is a limit to optimizing bread making conditions using SLV as an index of optimum bread quality because degradation in crust color, a negative trait, was obtained when using enzymes in this study. Based the findings, combining RSM and OT is effective method for the optimizing bread making conditions. To more effectively use this method in the future, it will be necessary to create an overall index that integrates SLV, bread color, and staling suppression as indicators of BMQ.

Conclusion

Although the high amounts of DF in whole wheat flour have good functionality, it decreases bread making properties. The insoluble pentosan of DF interferes with the formation of the gluten network, resulting in the reduction of GRD and SLV, and the acceleration of staling rate during the storage. The addition of optimal amounts of enzymes (AM and HC) solved these problems. These changes can be attributed to the degradation of DS and hemicellulose (mainly insoluble pentosan) into soluble low molecular weight saccharides which do not negatively influence the formation of the gluten network. As a result, the addition of optimal amounts of enzymes enables the production of satisfactory whole wheat flour bread which has a large amount of DF and several desirable BMQ, such as high GRD and SLV, and a suppressed staling rate. The findings suggest that the combination of RSM and OT (Solver) are an effective method for establishing optimum conditions for bread making with whole wheat flour.

References

AACC (1991) “Approved Methods of the AACC”, 8th ed. Method 08-01. The American Association of Cereal Chemists, St. Paul, MN

AACCI (2000) “Approved Methods of the AACCI”, 11th ed. Method 10- 05.01. The American Association of Cereal Chemists International, St. Paul, MN

AOAC (2000) “Official methods of analysis”, 17th ed. AOAC International, Gaithersburg, MD

Bruckner P, Habernicht D, Carlson G, Wichman D, Talbert L (2001) Comparative bread quality of white flour and whole grain flour for hard red spring and winter wheat. Crop Sci 41:1917-1920

Caballero PA, Gomez M, Rosell CM (2007) Improvement of dough rheology, bread quality and bread shelf-life by enzymes combination. J Food Eng 81:42-53

Duran E, Leon A, Barber B, De Barber CB (2001) Effect of low molecular weight dextrins on gelatinization and retrogradation of starch. Eur Food Res Technol 212:203-207

Flander L, Salmenkallio-Marttila T, Suortti T, Autio K (2007) Optimization of ingredients and baking process for improved wholemeal oat bread quality. LWT 40:860-870

Ghoshal G, Shivhare US, Banerjee UC (2013) Effect of xylanase on quality attributes of whole-wheat bread. J of Food Quality 36:172-180

Gibson TS, Al Qalla H, McCleary BV (1991) An improved enzymatic method for the measurement of starch damage in wheat flour. J Cereal Sci 15:15-27

Goesaert H, Slade L, Levine H, Delcour JA (2009) Amylases and bread firming – an integrated view. J Cereal Sci 50:345-352

Hung PV, Maeda T, Morita N (2007) Dough and bread qualities of flours with whole waxy wheat flour substitution. Food Res Int 40:273-279

Jiang Z, Li X, Yang S, Li L, Tan S (2005) Improvement of the bread-making quality of wheat flour by the hyperthermophilic xylanase B from Thermotoga maritime.

Food Res Int 38:37-43

Kim JH, Maeda T, Morita N (2006) Effect of fungal α-amylase on the dough properties and bread quality of wheat flour substituted with polished flours. Food Res Int 39:117-126

Lai CS, Hoseney RC, Davis AB (1989) Effects of wheat bran in bread-making. Cereal Chem 66:217–219

Maleki M, Hoseney RC, Mattern PJ (1980) Effect of loaf volume, moisture content, and protein quality on the softness and staling rate of bread. Cereal Chem 857:138-140

Martin ML, Zeleznak KJ, Hoseney RC (1991) A mechanism of bread firming. I. Role of starch swelling. Cereal Chem 68:498-502

Martin ML, Hoseney RC (1991) A mechanism of bread firming. II. Role of starch hydrolyzing enzymes. Cereal Chem 68:503-507

Matsushita K, Santiago DM, Noda T, Tsuboi K, Kawakami S, Yamauchi H (2017) The bread making qualities of bread dough supplemented with whole wheat flour and treated with enzymes. Food Sci Technol Res 23:403-410

Murtaugh MA, Jacobs DR, Jacob B, Steffen LM, Marquart L (2003) Epidemiological support for the protection of whole grain against diabetes. Proc Nutr Soc 62:143-150

Nimptsch K, Kenfield S, Jensen M, Stampfer M, Franz M, Sampson L, Brand-Miller J, Willett W, Giovannucci E (2011) Dietary glycemic index, glycemic load, insulin index, fiber and whole-grain intake in relation to risk of prostate cancer. Cancer Causes Control 22:51–61

Ozboy O, Koksel H (1997) Unexpected strengthening effects of a coarse wheat bran on dough rheological properties and baking quality. J Cereal Sci 25:77–82

Palacios HR, Schwarz PB, D’Appolonia BL (2004) Effect of alpha-amylases from different sources on firming of concentrated wheat starch gels: relationship to bread staling. J Agr Food Chem 52:5987- 5994

Patel MJ, Ng JHY, Hawkins WE, Pitts KF, Chakrabarti-Bell S (2012) Effects of fungal α-amylase on chemically leavened wheat flour dough. J Food Sci 56:644-651

Santiago DM, Matsushita K, Noda T, Tsuboi K, Yamada D, Murayama D, Koaze H, Yamauchi H (2015a) Effect of purple sweet potato powder substitution and enzymatic treatments on bread making quality. Food Sci Technol Res 21:159-165

Santiago DM, Matsushita K, Noda T, Tsuboi K, Yamada D, Murayama D, Kawakami S, Shimada K, Koaze H, Yamauchi H (2015b) Texture and structure of bread supplemented with purple sweet potato powder and treated with enzymes. Food Sci Technol Res 21:537-548

Schatzkin A, Park Y, Leitzmann MF, Hollenbeck AR, Cross AJ (2008) Prospective study of dietary fiber, whole grain foods, and small intestinal cancer. Gastroenterology 135:1163-1167

Schoenlechner R, Szatmari M, Bagdi A, Tomoskozi S (2013) Optimisation of bread quality produced from wheat and proso millet (*Panicum miliaceum* L.) by adding emulsifiers, transglutaminase and xylanase. LWT 51:361-366

Shah AR, Shah RK, Madamwar D (2006) Improvement of the quality of whole bread by supplementation of xylanase from *Aspergillus foetidus*. Bioresour Technol 97:2047-2053

Stojceska V, Ainsworth P (2008) The effect of different enzymes on the quality of high-fibre enriched brewer's spent grain breads. Food Chem 110:865-872

Tucker AJ, MacKay KA, Robinson LE, Graham TE, Bakovic M, Duncan AM (2010) The effect of whole grain wheat sourdough bread consumption on serum lipids in healthy normoglycemic / normoinsulinemic and hyperglycemic / hyperinsulinemic adults depends on presence of the APOE E3/E3 genotype: a randomized controlled trial. Nutr Metab 7:37

Wang J, Rosell CM, Barber CB (2002) Effect of the addition of different fibres on wheat dough performance and bread quality. Food Chem 79:221-226

Yamauchi H, Ichinose Y, Takata K, Iriki N, Kuwabara T (2000) Simple estimation of bread-making quality of wheat flour by modified expansion test under reduced pressure. Journal of the Japanese Society for Food Science and Technology 47:46-49

Yamauchi H, Nishio Z, Takata K, Oda Y, Yamaki K, Ishida N, Miura H (2001) The bread-making quality of a domestic flour blended with an extra strong flour and staling of the bread made from the blended flour. Food Sci Technol Res 7:120-125

Yamauchi H, Yamada D, Murayama D, Santiago DM, Orikasa Y, Koaze H, Nakaura Y, Inouchi N, Noda T (2014) The staling and texture of bread made using the Yudane dough method. Food Sci Technol Res 20:1017-1078

Figure Caption

Figure 1. The appearance and scanned crumb images of breads: the Control, WWF, and WWF+E [a]

[a] WWF: whole wheat flour, E, enzymes. Optimal amounts of enzymes, α-amylase and hemicellulase, were added to WWF+E dough.

Figure 2. Temporal hardness changes of bread crumbs: the Control, WWF, and WWF+E during storage [a]

[a] WWF: whole wheat flour, E: enzymes. Optimal amounts of enzymes, α-amylase and

hemicellulase, were added to WWF+E dough. The vertical bar is the standard deviation of each value (n=8). The symbols followed by different letters are significantly different ($p<0.05$). ○: Control, △: WWF, □: WWF+E.

Table 1. Central composite face-centered design on scaled values and actual concentrations of AM and HC [a]

Run"	Scaled value [b]		Actual concentration	
	X_1	X_2	AM (g/100 g flour)	HC (g/100 g flour)
1	0.0	0.0	0.1	0.1
2	0.0	-1.0	0.1	0.0
3	-1.0	-1.0	0.0	0.0
4	0.0	+1.0	0.1	0.2
5	0.0	0.0	0.1	0.1
6	-1.0	0.0	0.0	0.1
7	+1.0	+1.0	0.2	0.2
8	0.0	0.0	0.1	0.1
9	-1.0	+1.0	0.0	0.2
10	0.0	0.0	0.1	0.1
11	+1.0	0.0	0.2	0.1
12	+1.0	-1.0	0.2	0.0

[a] Scaled values and actual concentrations of AM and HC are shown.

AM : α-amylase, HC : hemicellulase.

[b] X_1= (AM-0.1)/0.1, where the actual concentration of AM ranged from 0.0 to 0.2/100 g flour, X_2= (HC-0.1)/0.1, where the actual concentration of HC ranged from 0.0 to 0.2/100 g flour

Table 2. Bread making qualities of doughs: the Control, WWF, and WWF+E [a]

Bread making treatments	Water absorption (%)	GRD (ml)	GP (ml)			SLV (ml/g)	Moisture content of crumb [b] (%)
			1h	2h	3h		
Control	68	105.0 ± 10.0 b	28.4 ± 0.6 a	62.5 ± 1.6 a	92.9 ± 0.8 b	4.95 ± 0.14 b	42.05 ± 1.22 a
WWF	69	95.6 ± 7.7 b	27.1 ± 1.8 a	62.2 ± 3.2 a	99.4 ± 3.1 a	4.59 ± 0.11 c	42.10 ± 0.51 a
WWF+E	69	121.9 ± 6.1 a	26.6 ± 1.4 a	61.2 ± 3.2 a	99.6 ± 2.5 a	5.66 ± 0.24 a	41.56 ± 0.42 b

[a] GRD: gas retention of dough, GP: gassing power of dough, SLV: specific loaf volume, WWF: whole wheat flour, E: enzymes. Optimal amounts of enzymes, α-amylase and hemicellulase, were added to WWF+E dough. Each value, except for water absorption, is the mean ± SD (the others: n=6, moisture content of crumb: n=10). The values followed by different letters within a column are significantly different ($p<0.05$).

[b] Moisture content of crumb was measured with the samples stored 1 day into polyethylene bags after baking.

Table 3. Color of the breads: the Control, WWF, and WWF+E [a]

Table 3. Color of breads made from doughs of Control, WWF and WWF+E [1)]

Bread making treatments	Bread crust color			Bread crumb color		
	L* (-)	a* (-)	b* (-)	L* (-)	a* (-)	b* (-)
Control	49.31 ± 1.16 a	16.71 ± 0.12 a	31.22 ± 1.53 a	81.12 ± 1.21 a	-2.48 ± 0.09 b	9.32 ± 0.17 c
WWF	48.67 ± 0.79 a	15.26 ± 0.31 b	29.28 ± 1.11 a	75.14 ± 1.33 b	-0.41 ± 0.29 a	11.65 ± 0.51 a
WWF+E	41.75 ± 0.60 b	14.83 ± 0.26 c	22.12 ± 0.70 b	70.11 ± 1.56 c	-0.51 ± 0.13 a	10.76 ± 0.55 b

[a] WWF: whole wheat flour, E: enzymes, L*: level of lightness, a*: level of redness, b*: level of yellowness. Optimal amounts of enzymes, α-amylase and hemicellulase, were added to WWF+E dough. Each value is the mean ± SD (n=8). The values followed by different letters within a column are significantly different ($p<0.05$).

Table 4. DS and DF contents of doughs: the Control, WWF, and WWF+E [a]

Bread making treatments	DS (%)	NDF (%)	ADF (%)	NDF-ADF (%)
Control	4.16 ± 0.48 a	0.59 ± 0.17 b	0.44 ± 0.13 b	0.15 ± 0.09 c
WWF	3.73 ± 0.39 b	2.29 ± 0.28 a	1.19 ± 0.08 a	1.10 ± 0.21 a
WWF+E	2.01 ± 0.07 c	1.85 ± 0.28 a	1.24 ± 0.08 a	0.62 ± 0.22 b

[a] WWF: whole wheat flour, E: enzymes, DS: damaged starch, NDF: neutral detergent fiber, ADF: acid detergent fiber, NDF-ADF: crude hemicellulose content. Optimal amounts of enzymes, α-amylase and hemicellulase, were added to WWF+E dough. Each value is the mean ± SD (DS: n=8, the others: n=4). The values followed by different letters within a column are significantly different ($p<0.05$).

Control WWF WWF+E

Figure 1

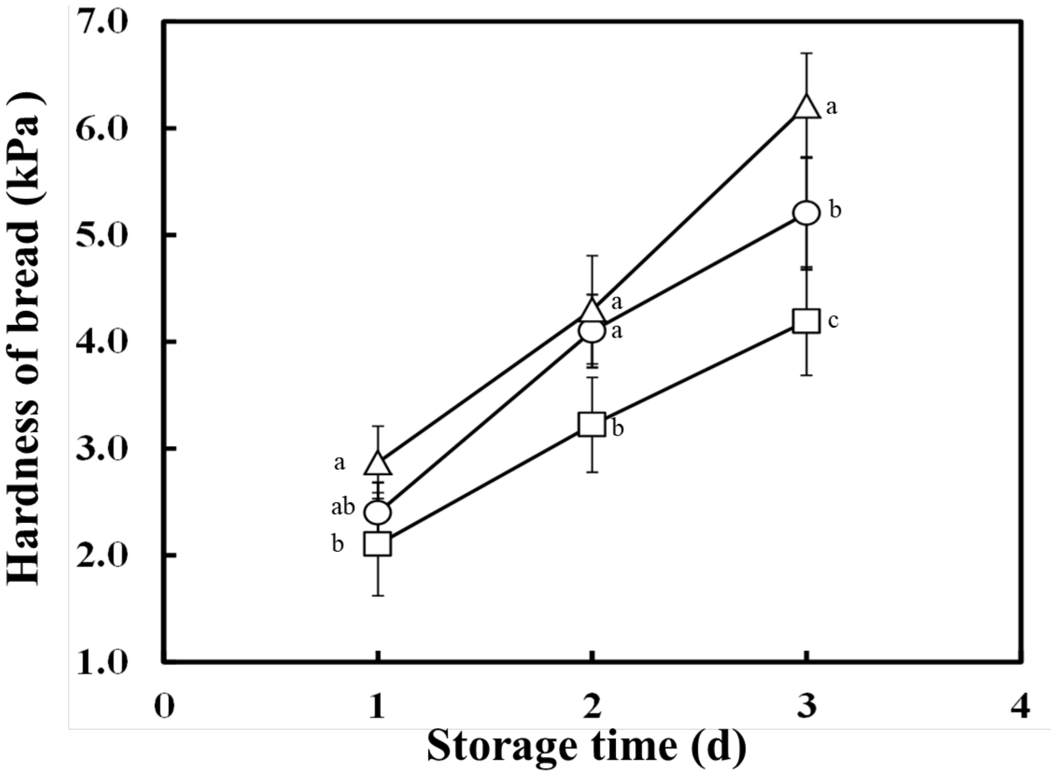
Hardness of bread (kPa)
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
1
2
3
4
Storage time (d)
a
ab
b
a
a
b
a
b
c

Figure 2