Research and Development of Novel Bread Making Method by Bakery Enzyme

製パン用酵素利用による

新規製パン法の研究・開発

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Chapter 1. Literature Review

1.1. Whole Wheat Flour

Whole grain wheat flour has gained considerable attention as a breadmaking ingredient due to its nutritional and health benefits. Compared to refined wheat flour, whole wheat flour contains higher levels of vitamins, minerals, fibers (e.g., non-starch polysaccharides including arabinoxylans), antioxidants, and other phytochemicals such as carotenoids, flavonoids, and phenolic acids (Jonnalagadda et al. 2011; Slavin 2004; Zhou et al. 2004). Whole grain intake has been linked to health benefits such as decreased risk of chronic diseases including cardiovascular disease, diabetes, cancer, and obesity, and all-cause mortality (Jacobs et al. 1998; Jonnalagadda et al. 2011; Slavin 2004). The 2015-2020 Dietary Guidelines for Americans recommend that at least half of all grain intake comes from whole grains (USDHHS/USDA, 2015). However, in the U.S., the average intake of whole grains is less than 1 oz. equivalent per day (USDHHS/USDA, 2015). Barriers to increasing whole grain consumption are often texture and sensory related, but also include higher cost of whole grain products, confusion in identifying whole grain foods, and lack of knowledge regarding the health benefits of whole grain consumption (Kantor et al. 2001).

Whole wheat flour produces dough and bread with characteristic differences compared to refined wheat flour. Effects associated with whole wheat bread production and their causes have been reviewed (Doblado-Maldonado et al. 2012; Gan et al. 1989; Heiniö et al. 2016) and include low loaf volume, increased crumb hardness, coarse texture, darker color, and distinctive flavor and aroma. These attributes may not be

appealing to consumers accustomed to white bread, which is made from refined flour. Reasons suggested for the effects of non-endosperm components on bread quality are fiber-gluten interactions (Noort et al. 2010); dilution of gluten protein by the bran and non-endosperm protein; competition for water by the water-soluble and water-insoluble fiber constituents leading to insufficient hydration of gluten proteins and starch; physical effects of bran particles, fiber, and arabinoxylans on the gluten network; and higher levels of ferulic acid (Heiniö et al. 2016). The germ contributes reducing compounds such as glutathione which degrade bread making ability (Lai et al. 1989; Every et al. 2006). The germ also contains high levels of non-polar lipids, which have various effects on the dough and bread throughout the entire breadmaking process, and tend to destabilize gas cells and thus decrease loaf volume (Pareyt et al. 2011). The fiber, or non-starch polysaccharide fraction, of whole wheat is composed primarily of arabinoxylans, and also includes arabinogalactans, cellulose, β-glucans, glucomannans, and lignins (Hille and Schooneveld-Bergmans 2004). These compounds, broadly referred to as hemicellulose, are found in plant cell walls. Whole wheat flour contains approximately 4-7% of the hemicellulose fraction, whereas white flour contains roughly 3% (Hille and Schooneveld-Bergmans 2004). Arabinoxylans are classified as either water-extractable or water-unextractable, with the former producing beneficial effects in dough and bread and the latter generally considered detrimental to quality (Goesaert et al. 2005). The interaction of water-unextractable pentosan with wheat gluten changes the rheological properties and network structure of dough (Ma et al. 2009).

The physical and chemical effects of the bran and germ necessitate some degree of formula and process modifications as compared to white bread. Water

absorption must be increased. Vital wheat gluten, dough conditioners such as oxidizing agents, emulsifiers, and enzymes, as well as shortening and mold inhibitors are often added or their concentration is increased compared to white bread formulations (Dubois and Vetter 1987). Phenolic compounds in bran are strongly flavored, so more sucrose is needed to attain a level of perceived sweetness equivalent to that of white bread. If employing the sponge for the sponge and dough process, more water must be used in the sponge (Dubois and Vetter 1987). Whole wheat dough is more susceptible to overmixing due to the physical action of the bran on the gluten. To reduce the likelihood of overmixing, adjustments are made including lowered sponge/dough ratio, longer mixing times at lower speed, shortened total mixing time, and lower dough temperature. Over fermentation is also a greater risk for whole wheat dough compared to white dough. A lower sponge ratio and set temperature and decreased fermentation time help to minimize this problem. Whole wheat dough is stiff. This may cause erratic scaling. Proofing at lower relative humidity (RH) for proofing is often used to prevent excess moisture from condensing on and absorbing into the dough, which would further weaken its structure and contribute to sidewall collapse. Longer baking times and lower baking temperatures are often needed compared with white bread. The higher water activity of whole wheat breads can lead to shorter shelf life and necessitate the addition of mold inhibitors.

Wheat flour mills as well as bread manufacturers may add a variety of amounts of non-endosperm components to refined wheat flours. For example, some products consist of various amounts of bran combined with endosperm but without the germ, thus creating “germ-free and bran-rich flours.” However, in order for the product to be labelled whole grain, it must include all parts of the caryopsis – the endosperm, germ,

and bran – in the same proportions as are present in the intact kernel (AACC International, 1999). The effects of wheat bran presence in bread have been recently reviewed and summarized (Hemdane et al. 2016), and many publications exist on the use of improvers in reconstituted dough systems, where ground bran is added back to refined wheat flour. In contrast, this review focuses mainly on improvers and other functional ingredients in whole wheat dough and bread, rather than the deleterious effects of endogenous wheat components. Furthermore, it covers studies that used whole wheat flour rather than refined flour to which bran was added. Within the whole wheat bread system, there is a need to improve the quality and sensory aspects to increase consumer appeal and therefore increase the intake of whole grain bread. For breads that are inherently firmer, such as whole wheat bread, softer breads achieve higher scores for overall acceptability (Armero and Collar 1996a). With this in mind, this review gives particular attention to crumb hardness and loaf volume, which is a strong contributor to hardness. In this review, a very large space has been given to enzymes, especially amylase, phytase, and xylanase. Other sections introduce specific emulsifiers, hydrocolloids, oxidants, and other functional ingredients such as vital wheat gluten and miscellaneous flours.

1.2. Bakery Enzymes

The use of enzymes in commercial applications has increased in recent years as consumers demand bakery products with more natural-sounding ingredients. Various types of enzymes can be used as alternatives to chemical improving agents, such as some hydrocolloids and emulsifiers, and those types used in bakery applications can all be declared by the single word “enzymes,” a term which many consumers perceive as

natural and clean label compared to additives labeled by their chemical name. Many enzymes occur naturally in flour, but several enzymes are added, specifically for their beneficial effects on dough and bread characteristics. Consequences include increased dough handling and hydration, improved volume and/or crumb texture, reduced rate of staling, or improved nutritional qualities. Enzyme activity is affected by several factors including temperature, pH, water activity, and enzyme concentration. Commonly used exogenous enzymes include xylanase, phytase, and amylases. Table 2.1 presents the major findings that have been published on the uses of enzymes in whole wheat dough and bread.

1.2.1. α-Amylase

α-Amylase is an endo-hydrolosate that catalyzes the hydrolysis of α-1,4-glycosidic bonds of starch polymers, producing low molecular weight polysaccharides and dextrins. β-Amylase decreases the molecular size/weight of these polysaccharides by cleaving the disaccharide maltose from the non-reducing end. Unlike higher glucose polysaccharides, maltose is fermentable by yeast. The resulting increase in fermentable sugars has a positive effect on yeast fermentative activity, which along with gas retention is a fundamental element of bread production. An increase in fermentative gas production, combined with the ability of the dough to retain that gas, leads to an increase in loaf volume. In 60:40 blends of refined flour and whole wheat flour, α-amylase improved the gas retention of dough (GRD), increased specific loaf volume (SLV), and decreased crumb hardness and staling rate (Matsushita et al. 2017). Remarkably, the hardness of the whole wheat-supplemented bread prepared with α-amylase was lower than that of the refined wheat control after 3 d of

storage, demonstrating this enzyme's promise for improving the shelf life of whole wheat bread, which often has a shorter shelf life than refined wheat bread. The decrease in hardness and staling achieved by α-amylase is due to both the increase in low molecular weight saccharides (LMWS) and the increase in specific volume. The low molecular weight products of starch hydrolysis are not available for retrogradation, and these smaller saccharides also delay the retrogradation of gelatinized starch (Matsushita et al. 2017). Furthermore, those saccharides interfere with starch-protein interactions in the aging bread, which decreases firming. α-amylase retains its activity early in baking and is capable of degrading gelatinized starch, and this partially decomposed starch has a low rate of retrogradation.

α-Amylase increased loaf volume and decrease the crumb hardness of both white and whole wheat bread (Armero and Collar 1996a). Hardness is measured by a compression test, and the two factors that influence the compressibility are the amounf of surface of resistant material and the resistance of that material (Armero and Collar 1996a). The decreased firmness was due to the increase in loaf volume, which decreases the surface of resistant material alone or in combination with a reduction in material resistance. Sensory evaluation by a trained panel using semistructured scales determined that the enzyme increased the elasticity and "eatability" score of whole wheat bread, and improved the crumb grain, typical taste, and overall acceptability of both whole wheat and white breads (Armero and Collar 1996a).

Other researchers have examined the use of malt flour in whole wheat bread rather than adding purified α-amylase. Malt flour is commonly used as an enzyme supplement because it is rich in α-amylase, and it also contains maltose, minerals, proteins, and flavor compounds. These components modify the color, flavor, and

moisture retention of the bread (Boz et al. 2010). However, the effect of malt flour depends on flour quality (Hruskova et al. 2003). The addition of 2% malt flour in whole wheat dough decreased the resistance to extension, suggesting a weaker dough (Boz et al. 2010). Extensibility and water absorption were increased by malt flour. Malt flour also lowered the dough energy as measured by the extensograph. Stickiness, adhesion, and stringiness measured using the SMS/Chen-Hoseney stickiness rig on a texture analyzer were all increased by malt addition, indicating that the dough may be more difficult to handle with this additive. A subsequent study (Boz and Karaoglu 2013) reported that 2% malt flour provided only marginal improvement to the general acceptability sensory score of whole wheat bread. The score for crumb grain decreased, and the aroma was rated no differently than the control bread. Loaf volume showed a small but significant increase compared to the control, but crumb firmness was not significantly improved. Based on this study, malt flour provides only marginal improvement to whole wheat bread, and other improvers may be needed in addition to the malt in order to produce a more acceptable product.

1.2.2. Hemicellulase

Hemicellulases include any enzyme that catalyzes the hydrolysis of non-starch polysaccharides. Of these enzymes, endoxylanases are the most commonly used in breadmaking. Hemicellulases such as xylanase are well known to improve the dough and bread properties of refined wheat bread, with beneficial effects such as softening the dough, increasing loaf volume, improving crumb structure, and decreasing staling rate (Jiang et al. 2005). The effects of hemicellulases are especially relevant for whole wheat bread, which has higher levels of insoluble arabinoxylans than does refined

wheat bread.

The non-starch polysaccharides present in the cells walls of bran and germ are one of the reasons for the poor bread making quality (BMQ) of whole wheat flour (Autio 2006). During dough mixing, arabinoxylans compete with gluten for water (Labat et al. 2002; Li et al. 2012). Xylanases hydrolyze the xylanase backbone of water-unextractable arabinoxylan, reducing their molecular size and water-holding capacity (Gruppen et al. 1993). This allows for greater gluten hydration, which results in better gluten matrix development and breadmaking ability.

1.3. Utilization of Potato in Bread Making

Potato is major crops globally and being produced widely in many countries of the world. In Japan, potato is also major agricultural crop and about 2.5 million tons are produced per year. Those are used for much utilization such as table food, processing and starch extraction, etc. However, potato is not used much in bread making in Japan and mainly used for other purposes, such as potato salad for sandwiches, than bread dough production. The main reason is that when mashed potato (MP) is added to the dough, the gluten network in the dough deteriorates due to DS and DFs, especially insoluble DFs, and the bread making properties remarkably decrease. On the other hand, using raw materials containing a large amount of gelatinized or swollen starch such as MP for bread making results in a positive effect that the bread has slightly sweet taste, low staling, and sticky texture as reported by Murayama et al. (2015) and Yamauchi et al. (2014). In addition, in particular, potato starch in various starch has been found to have very high swelling power and viscosity when heated in water, and its DS retains a large amount of water (Hossen et al. 2011;

Li and Yeh 2001). Therefore, by adding MP to dough, it is expected to improve the water absorption of dough and bread qualities.

1.4. High-Pressure Treatment

Thermal processing is a primary method for food pasteurization and sterilization. However, the application of heat impairs food quality. As an alternative to thermal processing, high-pressure treatment uses elevated pressures, with or without the addition of heat, to achieve microbial inactivation or to alter the food attributes. Because high-pressure treatment does not break covalent bonds, it can retain food quality and natural freshness while extending microbiological shelf-life. The process is also commonly referred to as high hydrostatic pressure processing and ultra high-pressure processing. High-pressure processing has been a topic of interest for several reasons. For one, the technology has been quoted as being one of the best innovations in food processing in fifty years (Dunne 2005). It gives food processors the opportunity to process foods with cleaner ingredients and fewer additives. High-pressure treatment is effective on a wide variety of foods, such as fruits, juices, vegetables, seafood, sauces, and ready-to-eat meats. During the last decade, the technology has been used by the food industry as an intervention technology for killing Escherichia coli, Salmonella, Listeria, and Vibrio pathogens in food products without additional heat processing. The US Department of Defense and NASA are interested in high-pressure processing for preservation of high-quality, shelf-stable low-acid foods. 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). Beyond the food industry, high-pressure technology could lead to processing

of biological pharmaceutical products and specialized intravenous solutions, or lead to development of a human vaccine from pressure-inactivated viruses serving as antigens for inoculation.

1.5. Response Surface Methodology and Central Composite Face-Centered Design

In statistics, response surface methodology (RSM) explores the relationships between several explanatory variables and one or more response variables. The method was introduced by Box and Wilson (1951). The main idea of RSM is to use a sequence of designed experiments to obtain an optimal response. Box and Wilson suggest using a second-degree polynomial model to do this. They acknowledge that this model is only an approximation, but they use it because such a model is easy to estimate and apply, even when little is known about the process. Statistical approaches such as RSM can be employed to maximize the production of a special substance by optimization of operational factors. In contrast to conventional methods, the interaction among process variables can be determined by statistical techniques.

A Design of Experiment is a structured, organized method for determining the relationship between a number of factors affecting a process and the output of that process. Regardless of the domain of application, this methodology is useful for three objectives: screening, optimization, and robustness testing. Employed at the beginning of the investigation of a new application, screening experiments are commonly designed to explore many factors, in order to evaluate their effects on the responses. It also makes it possible to obtain the best possible precision on the modeling of results and thereafter the optimization of the process. A central composite face-centered

design (CCF) from Design of Experiment was employed to determine the optimal conditions for the critical factors. This design is a kind of central composite design, in which the axial points are placed on the face centers of the cube; therefore, each factor has only three levels instead of five in central composite design. With such cubical design, one or two center runs are sufficient to produce a reasonable stability of prediction variance.

1.6. Summary

The utilization of bakery enzymes and high-pressure treatment could improve the BMQ such as GRD, SLV, and staling rate of bread crumb. In addition, it is expected to improve the water absorption of dough and bread qualities by adding MP to dough. On the other hand, it is necessary to experiment with a large number of combinations in order to determine the optimum conditions for the utilization of enzymes, high-pressure treatment, and MP. Thus, in this study, response surface model (RSMd) was created using the data acquired, based on the CCF, and then the optimal conditions 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 determined condition were conducted, and the effectiveness of each combination was verified from the BMQ of the dough and various evaluations of the bread.

Chapter 2. Effect of Whole Wheat Flour Substitution and Enzymatic Treatments on Bread Making Quality

2.1. Introduction

The functional ingredients of whole grains, dietary fiber (DF), resistant starch, vitamins and minerals, have various physiological benefits related to “western diseases” such as coronary heart disease, colon cancer and diabetes. However, the product of whole grains is not attractive as those of white wheat flour because the higher amount of bran and germ contained in whole wheat flour reduce the quality and sensory value of final products (Ozboy and Koksel 1997; Wang et al. 2002). In bread making, the presence of bran and germ causes the deterioration of the dough-rheological properties, decrease in loaf volume of breads, increase in crumb hardness and darkening of crumb appearance (Lai et al. 1989). Moreover, the addition of whole wheat flour gives different flavor profiles for whole wheat flour breads as compared with those of white flour bread (Chang and Chambers 1992).

It is generally accepted as truth that the damaged starch (DS) and DF in flour have an influence on the gluten formation, resulting in decrease of BMQ (Dexter et al. 1994; Santiago et al. 2015a; Santiago et al. 2015b; Yamauchi et al. 2004a; Yamauchi et al. 2004b). There are various kinds of enzymes used in baking as bread making improvers. Among them, α-amylase (AM) and hemicellulase (HC) are hydrolases having activities for DS and insoluble pentosan, respectively. AM is endo-type enzymes that catalyze the cleavage of α-1,4-glycosidic bonds in the inner part of the amylose or amylopectin chain. The end products of AM action are oligosaccharides

with various lengths and α-limit dextrins, which are branched oligosaccharides, while endogenous β-amylase converts mainly these oligosaccharides and DS into maltose which is used as fermentable sugar by the yeast or sourdough microorganisms (Synowiecki 2007; Goesaert et al. 2005). β-Amylase, which exists in wheat four as an endogenous enzyme, has enough enzymatic activity to convert the above oligosaccharides and DS into maltose. On the other hand, the insufficient amount of AM exists in wheat flour as an endogenous enzyme and doesn't have enough enzymatic activity to degrade the DS. In this regard, the supplemented AM modify the balance of α-and β-amylase activities and break down DS particles into LMWS during the dough preparing stage (Martin and Hoseney 1991). The increased levels of reducing sugars lead to the formation of Maillard reaction products, intensifying bread flavor and crust color. In addition, α-and β-amylase can improve the gas-retention properties of fermented dough and reduce dough viscosity during starch gelatinization, with consequent improvements in bakery product volume and softness. (Goesaert, et al. 2009; Poutanen 1997)

The total DF is composed of soluble and insoluble DF. Lignin, cellulose and hemicellulose are classified in insoluble DF and they work as important factors in breadmaking. The excess amount of insoluble DF, especially hemicellulose, causes the weakening on the formation of gluten network (Pomeranz 1977). HCs like xylanase are hydrolytic enzymes that degrade water insoluble hemicelluloses (mainly insoluble pentosan) in bread dough into water soluble forms. By adding this enzyme to bread dough, the dough changes slacker, softer, and more viscous properties and the bread obtained from this dough has greater bread volume, finer and more uniform crumb, longer shelf life, and low staling rate (Jiang et al. 2005). Moreover, it is expected that

the addition of xylanases on dough processing make to increase the concentration of arabinoxylo-oligosaccharides in bread, which have beneficial effects on human health such as improving the enteral environment as prebiotic effect (Courtin and Delcour 2001; Bhat 2000).

For the above-mentioned reasons, the quality of bread supplemented whole wheat flour may be improved by addition of enzymes. As the results, it is expected that good final products with higher nutritional value and functionality may be obtained. In this study, it is the purpose to examine the effect of whole wheat flour supplementation on BMQ and determine the applicability of AM and HC in improving the BMQ of bread with whole wheat flour

2.2. Materials and Method

2.2.1. Flour and Enzymes used

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

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

2.2.2. Dough Preparation and Bread Making

The bread-making tests were carried out using the no-time method and following the standard wheat bread formulation as the Control, which is prepared from

200 g of wheat flour, 10 g of sugar (Nippon Beet Sugar Mfg. Co. Ltd., Tokyo, Japan), 10 g of shortening (Snowlight, Kaneka Corp., Osaka, Japan), 4 g of wet yeast (Regular yeast, Nippon Beet Sugar Mfg. Co. Ltd., Tokyo, Japan), 4 g of salt (The Salt Industry Center of Japan, Tokyo, Japan), 20 mg of ascorbic acid (Wako Pure Chemical Industries, Ltd., Osaka, Japan), and suitable amount of water, as presented by Yamauchi et al. (2001). The optimal water absorption of each test was determined using a Farinograph at 500 BU according to the method used by the AACC (1991).

For the whole wheat flour -substituted bread making treatments, 40 percent of the original wheat flour content of the Control was replaced with whole wheat flour which is the maximum concentration that the BMQ can be improved with enzymes. The dough treated with AM and HC contained optimum amount of 0.025 % (0.05 g) and 0.05 % (0.10 g), respectively, which were determined from preliminary testing of the enzymes (data not shown). The dough was mixed to just beyond the peak development, as indicated by the electric power curve of the mixing motor. After mixing, the doughs were divided into 100 g and 20 g, rounded, and incubated for 20 min (bench time) at 30℃ and 70 % RH in a fermentation cabinet. The dough samples (100 g) were molded and rolled using a molding machine with upper 7.9 mm and lower 4.7 mm clearance, respectively. The doughs were panned and proofed for 70 min at 38℃ and 85 % RH, and then baked at 180℃ for 25 min as reported by Santiago et al. (2015a). Meanwhile, 20 g dough samples were used for the analysis of gassing power (GP) and GRD.

2.2.3. Evaluation of BMQ

GRD was evaluated by measuring the maximum expansion volume of 20 g dough proofed at 38℃ and 85 % RH in a cylinder subjected to 0 to 75 cmHg as

presented by Yamauchi et al. (2000). GP with 20 g of dough after bench time was measured at 30℃ for 1, 2, and 3 h using Fermograph II (ATTO Co. Ltd., Tokyo, Japan).

SLV of bread cooled at room temperature for 1 h after baking was measured by the rapeseed-replacement method. The images of bread and bread crumbs were recorded by a digital camera (model EX-H15; Casio Computer Co., Ltd., Tokyo, Japan) and a scanner (model GT-S640; Seiko Epson Co., Ltd., Nagano, Japan), respectively. Color of the bread crust and crumb were determined using a colorimeter (CR-400, Konica Minolta Sensing, Inc., Tokyo, Japan). The moisture content of the bread crumb was measured based on the AOAC official method (AOAC, 2000).

2.2.4. DFs and DS Analysis

One hundred gram of dough samples after final proofing for 70 min at 38℃ and 85 % RH were frozen at -30℃ for 30 min using a blast freezer and stored in a freezer at -20℃ until used for analysis of DFs and DS content.

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

On the other hand, before DS analysis, water soluble sugars were removed by mixing 100 mg of the dough with 8 ml of distilled water in a vortex mixer for 1 min and centrifugation at 2200 g for 10 min at 20℃ Mixing and centrifugation were repeated twice, and the resulting precipitate was used for DS analysis using the Megazyme

assay kit (Megazyme International Ireland Ltd., Wicklow, Ireland) based on the method of Gibson et al. (1991).

2.2.5. Hardness of Bread Crumb

Texture properties of bread crumb during storage were analyzed using the method as presented by Yamauchi et al. (2001). Temporal hardness changes of bread crumbs during storage were evaluated using a creep meter (RE2-33005C, Yamaden Co., Ltd., Tokyo, Japan). Loaves cooled for 1 h at room temperature after baking were packed into polyethylene bags and stored at 20℃ and 70 % RH. After storage for 24, 48 and 72 h, the loaves were sliced at 2 cm thickness and 3 cm × 3 cm square crumbs were cut from the center of each slice by using ultrasonic cutter (USC-3305; Yamaden Co. Ltd., Tokyo, Japan). The maximum stress as hardness was measured by compressing the crumbs from 2 cm thickness to 1 cm thickness with 1 mm/sec of compression rate using a special cube plunger (D: 6 cm × W: 6 cm × H: 2 cm) with a creep meter (RE2-33005C; Yamaden Co., Ltd., Tokyo, Japan).

2.2.6. 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.

2.3. Results and Discussion

2.3.1. Evaluation of BMQ

The BMQ of Control dough and dough supplemented with whole wheat flour (WWF), and doughs supplemented with WWF and treated with enzymes, such as AM and HC, are presented in Table 2.1. Results showed that the addition of whole wheat flour significantly lowered GRD compared with the others, whereas the doughs with WWF+AM or WWF+HC have significantly higher GRD compared to the Control. On the other hand, the dough supplemented with WWF+AM+HC has the significantly highest GRD among the all samples.

On the doughs containing whole wheat flour, an increasing tendency of GP during fermentation of each dough varied among bread making treatments, depending on fermentation time. Initially at 1 h of fermentation, the GP of the all doughs with whole wheat flour had significantly higher value than the Control. At 2 h of fermentation, the GP of the WWF and WWF+AM doughs had significantly higher value than the Control. At 3 h of fermentation, the GP differences of the doughs with WWF or WWF+AM and the other dough became bigger and the values of WWF and WWF+AM resulted in the significantly highest value among all samples. The dough with WWF+HC had the higher value than the Control and the dough with WWF+AM+HC at 2h of fermentation period. Therefore, the GP after 2h of fermentation on the Control and the dough with WWF+AM+HC had the lower values than the other samples.

In terms of SLV, the bread with WWF was significantly lower than the all other breads, while though it was no significant differences, the bread supplemented with WWF+AM+HC has the highest SLV among the all breads.

There was no significant difference on the moisture content among all samples.

The low GRD and SLV of bread supplemented with whole wheat flour can be

attributed to the relatively higher fiber and DS content as shown in Table 2.1 and 2.3. The excessive DF of whole wheat flour disrupts the gluten network formation resulting in a weaker gluten network (Lai et al. 1989; Ozboy and Koksel 1997; Wang et al. 2002). The improved GRD and SLV of bread treated with WWF+AM compared with only WWF can be explained by the enzymatic activity of AM which leads to the hydrolysis of damaged and gelatinized starch to maltose, dextrin, etc.. These results agree with the report of Kim et al. (2006) wherein SLV decreased when the bread was supplemented with polished wheat flour that has high fiber and DS content, and SLV increased upon the addition of AM. Similar observation was also reported by Patel et al. (2012) on the improvement in SLV of chemically leavened bread treated with fungal AM.

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

The dough with WWF+AM+HC has the highest value of GRD compared with other treatments including the Control. It seemed that it was caused by the decreased content of DS and insoluble hemicellulose with the combined catalytic activity of AM and HC as shown in Table 2.1 and 2.3. Goesaert et al. (2009) and Jiang et al. (2005)

reported that the hydrolytic activity of AM and HC added to bread dough caused the increase of mono-sugars, which were mainly produced from DS and pentosan. From those reports, it seems that the significant improvements of GP with AM or HC in early fermentation stage relate to the increasing of mono-sugars produced by the above enzymes, which generally promote the yeast fermentation. However, from the results shown in Table 2.1, the effect concerning the increase of GP was large on the addition of whole wheat flour compared to those of the addition of enzymes. This may be related to that whole wheat flour contains much amount of various nutrients to promote the fermentation of yeast.

2.3.2. Bread Color and Appearance

Table 2.2 summarizes the color of breads. The crust of the Control bread had the significantly highest values of L*, a* and b*among all samples. The values of L*, a* and b* was decreased by the addition of whole wheat flour. Those of breads supplemented with enzymes had significant lower values than those of WWF. In terms of the crumb color, the L* of the Control had significantly higher than those of the others and the others had no significant differences. On the other hand, the Control showed significantly lower values of a* than those of the others. In regard to b* values, the Control were also significantly lower than all other bread crumbs except of the sample with WWF+AM+HC and the bread supplemented with WWF+AM showed the highest values.

The bread appearance and crumb images are shown in Figure 2.1. Darker external color of bread was observed in the breads substituted with whole wheat flour compared to the Control. Similarly, the crumbs of WWF had the darker color than the

white crumbs of the Control. The appearance of bread supplemented with whole wheat flour alone was smaller than the Control, whereas the breads with whole wheat flour and enzymes treatments were either of the same size or larger than the Control. These results corresponded to those of the SLV in Table 2.1.

The addition of whole wheat flour resulted in darker color of the bread crust compared with the Control. Likewise, the individual and combined treatments of AM and HC also caused darker color compared with the Control and WWF, which was evidenced by their significantly lower L* value and photograph in Table 2.2 and Figure 2.1. The addition of enzymes basically also decreased the values of redness and yellowness, indicated by the lower a* and b* value as shown in Table 2.2. These color changes can be attributed to the increase in the concentration of reducing sugars like glucose, fructose, etc., which promote the Maillard reaction (data not shown). These sugars also lead to intensify the bread flavor and browning as reported by Goesaert et al. (2009).

The darker bread crumb color with the addition of whole wheat flour can be attributed to the natural dark brown color of wheat bran. Similarly, this brawn color of whole wheat flour also influences the change in color of crumb to brown one which is the characteristic of whole wheat flour bread, resulting in the decrease in L* value and increase in redness and yellowness as shown in Table 2.2.

2.3.3. DFs and DS Contents of Dough

Table 2.3 shows DFs and DS content of doughs from different treatments. Results showed that the NDF, ADF, and crude hemicellulose (NDF-ADF) content of doughs supplemented with whole wheat flour and, WWF and enzymes treatments were

significantly higher than the dough of the Control. With the enzyme treatments, the values were rather lower than those of WWF alone. In the term of DS, the value of the Control was the highest among all samples and those of WWF were slightly lower than the Control. The amount of DS of the dough with the enzyme treatment was significantly decreased, especially, the value of WWF+AM+HC was the lowest as compared with all other samples.

The dough supplemented with whole wheat flour has higher percentage of fiber (NDF and ADF), because whole wheat flour contains much DFs. Generally, the excess contents of fiber cause the inhibition of optimal gluten formation, resulting in the decrease of SLV and GRD. On the other hand, the doughs with the enzyme treatments show lower content of fiber compared with the dough of WWF and especially, the dough with WWF+HC has the lowest content of fiber. The xylanase activity of the HC, which catalyzes hydrolysis of hemicellulose such as xylan, arabinoxylan to xylobiose, xylose, etc. may have resulted in low NDF as well as crude hemicellulose (NDF-ADF) content of doughs with WWF+HC and WWF+AM+HC (Stojceska and Ainsworth 2008; Jiang et al. 2005).

DS content of the dough without added AM can be basically associated with DS contained in wheat flour after milling. The excess amount of DS causes undesirable effects on BMQ. The Control and dough of WWF have higher values of DS compared to the others. In contrast, the lower DS of the doughs with enzymes seems to be the effects of AM activity, especially, the doughs with AM have a significantly lower DS than other doughs. These decreases in the contents of DS and fiber (mainly insoluble hemicellulose (pentosan)) improve GP, GRD and SLV of the bread treated with AM and HC.

2.3.4. Hardness of Bread Crumb

The temporal hardness changes of bread crumbs during storage of 3 days is shown in Figure 2.2. After storage of 1 day, the bread of WWF had significantly higher value than other samples. Control and those with enzymes have similar value after 1 day. After storage of 2 days, the Control and the bread of WWF had similar high value, while those with enzymes remained significantly lower value than the other samples. The value of bread of WWF drastically increased after storage of 3 days, resulting in the significantly highest value among all samples. On the other hand, the values of breads with enzymes remained low and especially, the bread added AM had the lowest value compared with all other samples.

The hardness changes of bread crumbs relate to various factors. Among those, it is considered that the retrogradation rate of gelatinized starch gel (GSG) in bread, DS and insoluble pentosan contents in dough, and SLV are the main factors on the staling of WWF bread. AM mainly break down the DS in dough and GSG during dough baking into low molecular weight dextrins, oligo- sacchrides, etc., while endogenous β-amylase converts above saccharides into maltose. The AM and β-amylase have different but complementary functions during bread making process, bringing the increase of LMWS contents on the breads with AM. It is reported that those LMWS decrease the amount of available starch for retrogradation and retard the retrogradation of GSG in bread crumb (Duran et al. 2001; Palacios et al. 2004; Goesaert et al. 2009). Moreover, these saccharides products of AM hydrolysis interfere with starch-protein interactions, resulting in few and weak crosslinks and thus reducing the hardening rate of bread (Martin and Hoseney 1991; Martin et al. 1991). From Table 2.1 and Figure 2.1, the SLV of breads with AM were also lager than those of the Control. Maleki et al.

(1980) have reported that the staling rate of large SLV bread clearly becomes slow. From these findings, it seems that the low hardening rate of bread with AM is mainly caused by the anti-staling effects due to the increase of these saccharides and the high SLV on breads with WWF+AM.

HC mainly attacks the insoluble pentosan in dough which interferes the gluten network formation in dough, resulting in the improvements of BMQ such as the high SLV and the increase of LMWS in breads with HC as well as AM (Caballero et al. 2007; Ghoshal et al. 2013). These previous findings show that the staling of the breads with HC is retarded by the effects similar to the AM addition. As the data to support this discussion, the crude hemicellulose contents of the doughs with HC in Table 2.3 indicate lower values than that of the dough with only whole wheat flour.

The above discussions showed that the main causes of staling retarding effects on the breads added AM or HC is nearly same. However, in the results of Figure 2.3, it seems that the staling retarding effects of AM addition is slightly larger than that of HC. As the main factor, AM attacks the GSG and decompose that to low molecular weight starch in early stage of baking process, which is generally considered to be the greatest factor on the low bread staling. It is reported by Martin and Hoseney (1991) and Palacios et al. (2004) that the retrogradation rate of the starch gel decomposed partially is low. About the anti-staling effect of bread by AM addition, Caballero et al. (2007) and Palacios et al. (2004) reported the similar results. From the above findings, the higher anti-staling effect of AM compared to HC chiefly seems to be due to the decomposition ability of GSG in bread.

2.4. Conclusion

Whole wheat flour has much amount of DFs and high functionality, while it causes negative effects on the breadmaking property by excess DFs contents. Among DFs, especially, insoluble pentosan interfere the gluten network formation, resulting in the decrease of GRD and SLV and acceleration of the staling rate. On the other hand, these problems are basically solved by the addition of some enzymes for bread making such as AM and HC, resulting in the desirable formation of gluten network and improved dough property. These improvements were mainly brought about by the degradation of DS and hemicellulose (mainly insoluble pentosan) into soluble LMWS, which do not interfere with the gluten network formation during bread dough development. In the results, the preferable bread supplemented with 40 % of whole wheat flour is obtained by addition of suitable enzymes for bread making. This bread has high amount of DFs and desirable properties such as increased GRD and SLV and retarded staling rate.

Table 2.1 BMQ of doughs supplemented with WWF and treated with enzymes [1)]

Bread Making Treatments	Water absorption (%)	GRD (ml)	GP (ml)			SLV (ml/g)	Moisture Content of crumb (%) [2)]
			1h	2h	3h		
Control	68	98.33 ± 2.90 [cb]	22.56 ± 0.79 [b]	55.71 ± 0.72 [b]	88.74 ± 0.74 [c]	4.82 ± 0.03 [bc]	41.62 ± 1.02 [a]
WWF	69	90.00 ± 0.00 [d]	237.8 ± 0.85 [ab]	58.00 ± 0.77 [a]	94.79 ± 0.79 [a]	4.56 ± 0.01 [c]	42.12 ± 0.42 [a]
WWF +AM	69	110.00 ± 0.00 [ab]	24.01 ± 0.61 [ab]	57.66 ± 0.56 [a]	93.97 ± 0.56 [a]	5.05 ± 0.16 [ab]	41.81 ± 0.31 [a]
WWF +HC	69	106.67 ± 5.70 [bc]	24.77 ± 0.46 [a]	57.14 ± 0.96 [ab]	91.92 ± 1.38 [b]	4.93 ± 0.03 [b]	41.53 ± 0.62 [a]
WWF + AM + HC	69	117.50 ± 2.90 [a]	24.44 ± 0.39 [a]	55.78 ± 0.60 [b]	89.75 ± 0.69 [c]	5.26 ± 0.07 [a]	41.62 ± 0.42 [a]

[1)] GRD: gas retention of dough, GP: gassing power of dough, SLV: specific loaf volume, WWF: whole wheat flour, AM: α-amylase, HC: hemicellulase. Each value, except for water absorption is the mean ± SD (GRD, GP, and moisture content of crumb: n=6, SLV: n=4). The values followed by different letters within column are significantly different ($p<0.05$).

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

Table 2.2 Color of bread crusts and crumbs made from doughs supplemented with WWF and treated with enzymes [1)]

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

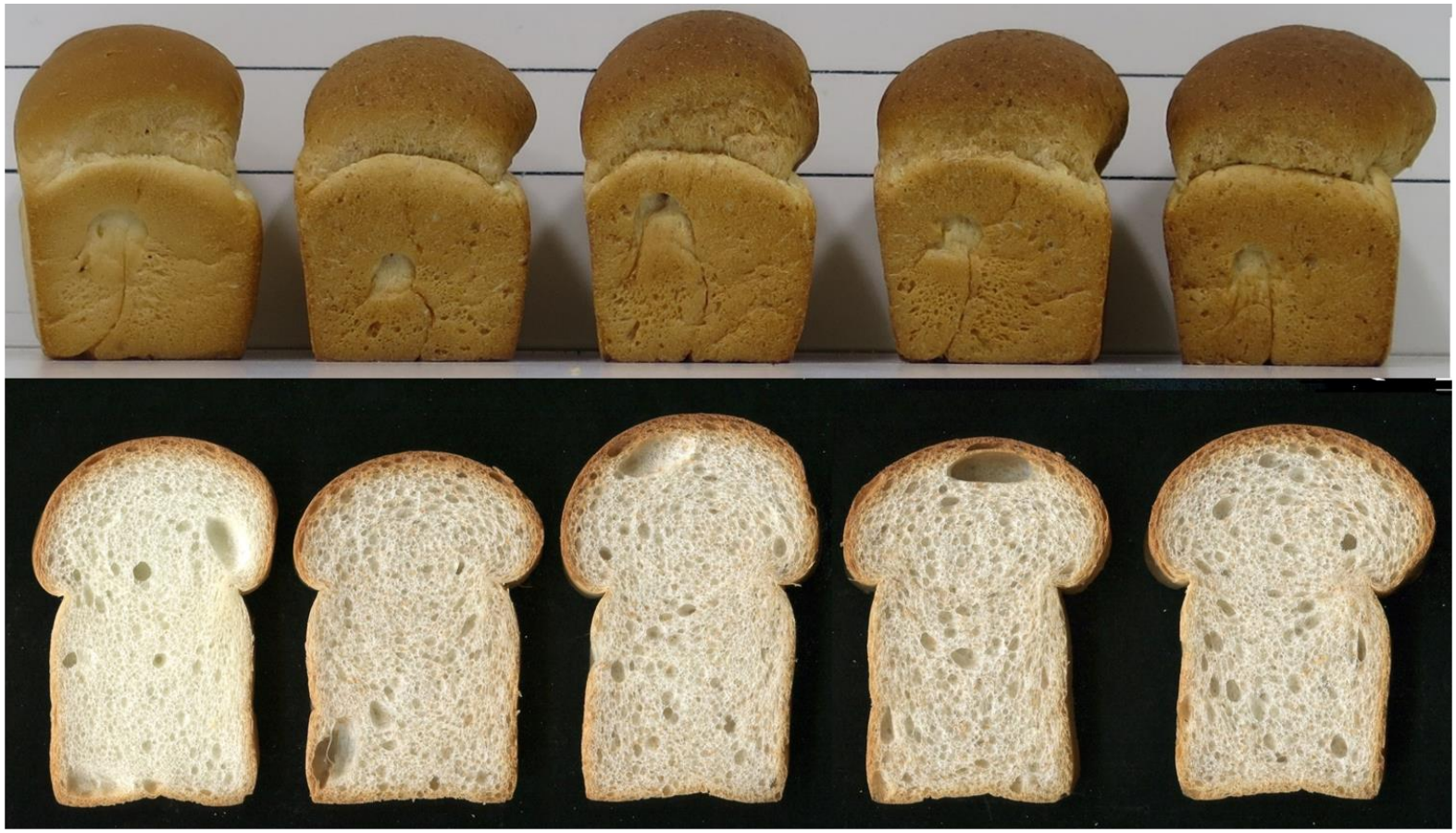
[1)] WWF: whole wheat flour, AM: α-amylase, HC: hemicellulase, L*: level of lightness or darkness, a*: level of redness, b*: level of yellowness. Each value is the mean ± SD (n=10). The values followed by different letters within column are significantly different ($p<0.05$).

Table 2.3 DFs and DS contents of doughs supplemented with WWF and treated with enzymes [1)]

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

[1)] DFs: dietary fibers, DS: damaged starch, NDF: neutral detergent fiber, ADF: acid detergent fiber, WWF: whole wheat flour, AM: α-amylase, HC: hemicellulase. Each value is the mean ± SD (n=6). The values followed by different letters within column are significantly different ($p<0.05$).

[2)] NDF-ADF: crude hemicellulose content.



Control WWF WWF+AM WFF+HC WWF+AM+HC

Figure 2.1 Photographs of appearance and scanned crumb images of breads made from doughs supplemented with WWF and treated with enzymes [1)]

[1)] WWF: whole wheat flour, AM: α-amylase, HC: hemicellulase.

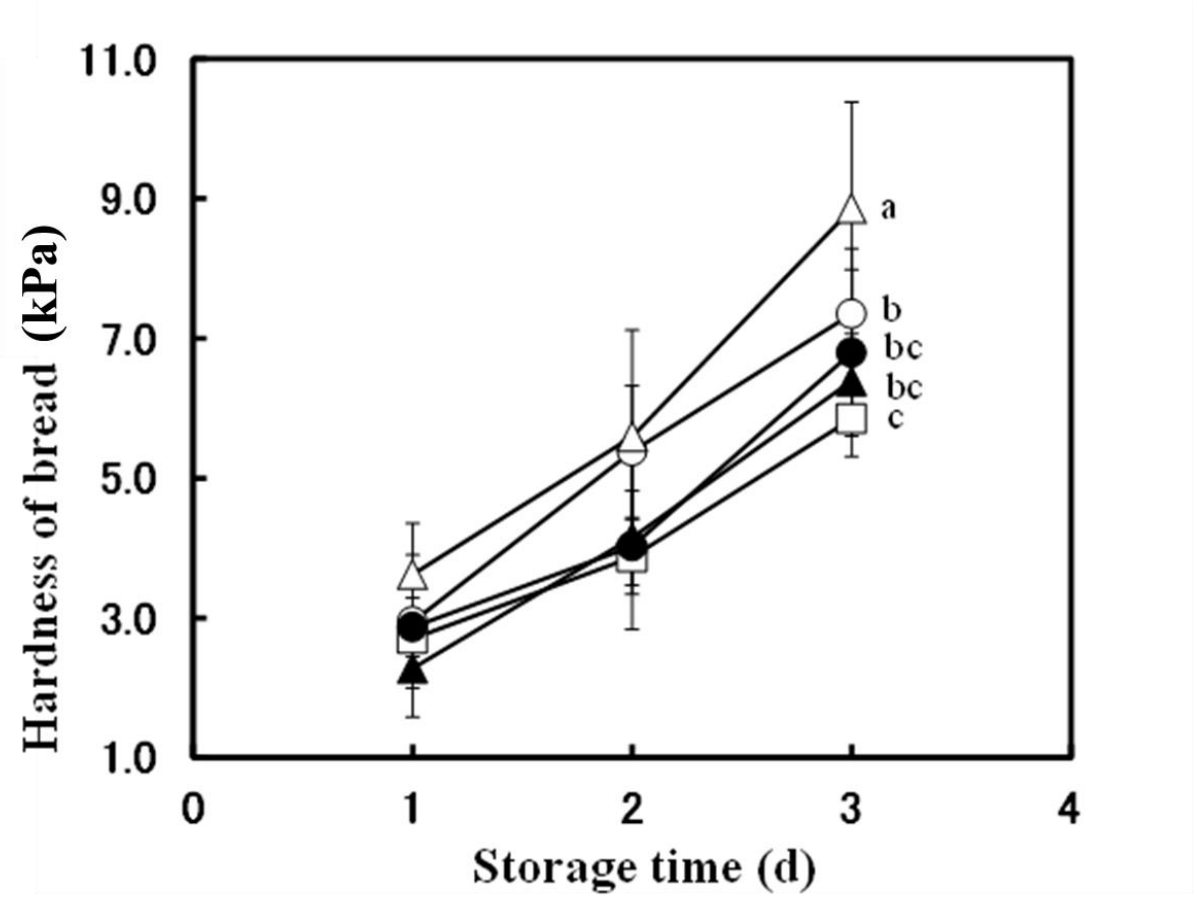


Figure 2.2 Temporal hardness changes of bread crumbs made fom doughs supplemented with WWF and treated with enzymes [1)]

[1)] WWF: whole wheat flour, AM: α-amylase, HC: hemicellulase. The vertical bar is the standard deviation of each value (n=6). The symbols followed by different letters are significantly different ($p<0.05$). ○: Control, △: WWF, □: WWF+AM, ●: WWF+HC, ▲: WWF+AM+HC.

Chapter 3. Optimization of Enzymes Addition to Improve Whole Wheat Bread Making Quality by Response Surface Methodology and Optimization Technique

3.1. Introduction

Whole wheat flour is derived by milling or grinding whole grain of wheat, which contains several functional compounds, such as 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 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 Chapter, two kinds of enzymes (AM and HC) were used as improvers (Caballero et al. 2007). These enzymes act on the 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 BMQ by adding AM, 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).

In Chapter 2, the 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 BMQ parameters using various enzyme combinations.

Therefore, in this Chapter, we adopted a 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 RSMd was created using the data acquired, based on the CCF, and then the optimal amounts of multiple enzymes were determined by using an 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 BMQ of the dough and various evaluations of the bread.

3.2. Materials and Methods

3.2.1. Flour and Enzymes used

The commercial strong wheat flour, whole wheat flour, and two commercial enzymes were same with those used in Chapter 2.

3.2.2. Optimization of Concentrations of added Enzymes

A CCF was used with two variables to determine optimal concentrations of enzymes (Flander et al. 2007). This CCF was composed of twelve experiments with four replicates at the center point (Table 3.1). 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, 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 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.

3.2.3. 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).

3.2.4. Evaluation of BMQ

The GRD was evaluated by measuring the maximum expansion volume of 20 g of dough proofed at 38°C and 85% RH in a cylinder subjected to 0 to 75 cmHg (Yamauchi et al. 2000). The GP of 20 g of dough after bench time was measured at 30°C 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.

3.2.5. DFs and DS Analysis

Sample preparation, before DFs and DS analysis, was done according to the method reported by Santiago et al. (2015a). NDF, which are cellulose, hemicellulose and lignin content and 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 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). The DFs and DS of doughs after final proofing were measured.

3.2.6. Hardness of Bread Crumb

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).

3.2.7. Statistical Analysis

Significant differences were evaluated using same method with that used in Chapter 2.

3.3. Results and Discussion

3.3.1. Optimization of 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.

$Y=5.60X_1+4.35X_2-15.62X_1^2-16.07X_1X_2+4.85$

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 p values of the effectiveness in this model was 0.00627, which assessed that the effectiveness was significant at 1% significance level. 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 variables on RSMd shown minus values. Therefore, especially, when both enzymes were added to the dough in a large excess, these explanatory variables had the effect of largely lowing the BMQ (SLV). Since the magnitude of the partial regression coefficient on these explanatory variables was nearly same and X_2^2 explanatory variable was not included in this RSMd, it shown that when the enzymes are added excessively, the effect of decreasing SLV of AM was large compared to the HC.

The optimal concentrations of AM and HC calculated using Solver 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 maximum concentration of HC was limited 0.1 g/100 g flour.

3.3.2. Evaluation of BMQ

BMQ of the Control dough, 40% of whole wheat flour (WWF) dough, and 40% of whole wheat flour with enzyme (WWF+E) dough are shown in Table 3.2. 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 3.4). 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 3.4). The Control dough and bread had higher GRD and SLV values despite having high DS content (4.58%) 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 whole wheat flour 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 from 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.

3.3.3. Bread Color and Appearance

Table 3.3 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 whole wheat flour decreased the values of L*, a* and b*. In addition, all values of the WWF+E were significantly lower than those of the Control.

In terms of crumb color, the addition of whole wheat flour 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 3.1 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 3.2.

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 (Figure 3.1), which corresponded with its lower L* values (Table 3.3). 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 3.3). 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 3.3). 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.

3.3.4. DFs and DS Contents of Dough

Table 3.4 shows the DFs and DS contents of doughs from different treatments. 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 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.

As shown in Table 3.4, 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). 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 cause 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. 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).

3.3.5. Hardness of Bread Crumb

Figure 3.2 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 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 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 3.2 and Figure 3.1). 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 3.4).

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.

3.3.6. 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 3.2 and Figure 3.2). 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 3.3). 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 almost 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.

3.4. 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 LMWSs 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.

Table 3.1 Central composite face-centered design on scaled values and actual concentration of AM and HC [1)]

Run	Scaled value [2)]		Actual concentration (g/100 g flour)	
	X_1	X_2	AM	HC
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

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

[2)] X_1= (AM-0.1)/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 3.2 BMQ of doughs supplemented with WWF and treated with optimal concentration of enzymes [1)]

Bread making treatments	Water absorption (%)	GRD (ml)	GP (ml)			SLV (ml/g)	Moisture content of crumb (%) [2)]
			1h	2h	3h		
Control	68	100.00 ± 13.23 [ab]	28.98 ± 0.03 [a]	63.95 ± 0.31 [b]	93.53 ± 0.45 [b]	4.95 ± 0.14 [b]	41.87 ± 1.13 [a]
WWF	69	91.67 ± 5.77 [b]	28.70 ± 0.26 [a]	65.09 ± 0.51 [a]	102.19 ± 0.59 [a]	4.59 ± 0.11 [c]	42.09 ± 0.44 [a]
WWF+E	69	117.22 ± 2.55 [a]	27.89 ± 0.25 [b]	64.05 ± 0.50 [ab]	101.86 ± 0.51 [a]	5.66 ± 0.24 [a]	41.64 ± 0.40 [a]

[1)] 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 in dough of WWF+E. Each value, except for water absorption, is the mean ± SD (GRD, GP, and SLV: n=8, moisture content of crumb: n=10). The values followed by different letters within column are significantly different ($p<0.05$).

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

Table 3.3 Color of bread crusts and crumbs made from doughs supplemented with WWF and treated with optimal concentration of enzymes [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]

[1)] 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 in dough of WWF+E. Each value is the mean ± SD (n=8). The values followed by different letters within column are significantly different ($p<0.05$).

Table 3.4 DFs and DS contents of doughs supplemented with WWF and treated with optimal concentration of enzymes [1)]

Bread making treatments	NDF (%)	ADF (%)	NDF-ADF (%) [2)]	DS (%)
Control	0.82 ± 0.44 [b]	0.60 ± 0.18 [b]	0.34 ± 0.16 [b]	4.58 ± 0.25 [a]
WWF	2.29 ± 0.28 [a]	1.19 ± 0.08 [a]	1.10 ± 0.21 [a]	4.09 ± 0.05 [b]
WWF+E	1.85 ± 0.28 [a]	1.24 ± 0.08 [a]	0.62 ± 0.22 [b]	2.21 ± 0.10 [c]

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

[2)] NDF-ADF: crude hemicellulose content.

Control WWF WWF+E

Figure 3.1 Photographs of appearance and scanned crumb images of breads made from dough supplemented with WWF and treated with optimal concentration of enzymes [1)]

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

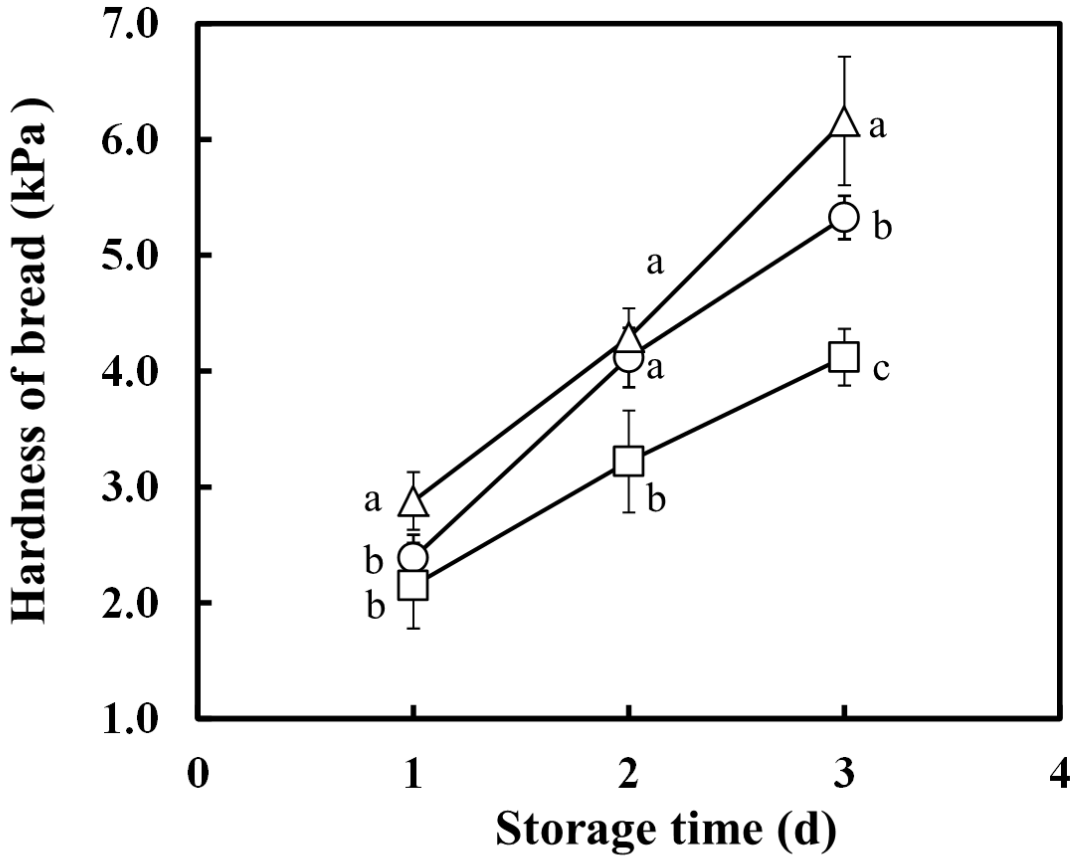


Figure 3.2 Temporal hardness changes of bread crumbs made from doughs supplemented with WWF and treated with enzymes[1)]

[1)] WWF: whole wheat flour, E: enzymes. The optimal amounts of enzymes, α-amylase and hemicellulase, were added. 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.

Chapter 4. Influence of the Addition of Whole Wheat Flour and Optimum Enzymes on Pullman-Type White Bread Qualities

4.1. Introduction

The physical properties of bread have a great relationship with the acceptability and they are very important for evaluating BMQ. Bread physical property evaluation methods have been established mainly by Wang et al. (2002). The hardness evaluation, fracture analysis, and creep analysis have been performed as physical property evaluations (Yiping et al. 1992a-d). In evaluating bread hardness, the maximum stress is usually measured by the compression at a constant speed and used as an indicator of crumb hardness. In the fracture analysis, the rupture force and deformation are measured by breaking test with a wedge-shaped plunger and they are mainly used as an index of texture. In the creep analysis, the viscoelastic coefficient is measured from the strain change when a certain load is applied to the bread crumb.

Previous studies have revealed changes in crumb hardness during storage, the relationship between crumb grains shape and viscoelasticity, and the impact of rice flour on bread viscoelasticity (Tsuboi et al. 2017; Shibata et al. 2010; Shibata et al. 2011). In addition, the bread making properties of bread dough and the physical properties of bread were changed by substituting a portion of wheat flour for other crop flours (barley flour, oat flour, sweet potato flour, potato flour, etc. (Santiago et al. 2015a; Matsushita et al. 2017; Lai et al. 1989; Wang et al. 2002). DS is generated by the physical damage that occurs during the milling process, which has a negative effect on the final bread quality (Barrera et al. 2007; Naito et al. 2005; Yamada et al. 2015).

In addition, the excessive amounts of DFs also decrease the BMQ because DFs inhibit the desirable gluten network formation (Lai et al. 1989).

On the other hand, previous studies have shown that the addition of enzymes significantly reduces bread hardness and staling rate (Matsushita et al. 2017). However, there are few studies that comprehensively evaluated the effects of storage time, using whole wheat flour, and enzyme addition on staling rate, rupture property, and viscoelasticity of bread. Therefore, in this study, we conducted a pullman scale bread making test for analysis of physical properties in detail.

4.2. Materials and Methods

4.2.1. Flour and Enzymes used

The commercial strong wheat flour, whole wheat flour, and two commercial enzymes were same with those used in Chapter 2 and 3.

4.2.2. Dough Preparation and Bread Making

The Control and whole wheat flour doughs were prepared at 2100 g flour basis according to the formula described in Chapter 3. 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). The optimal mixing conditions for individual doughs were set as the dough mixing time at which the dough showed maximum gas retention, which was measured

using the method of Yamauchi et al. (2000). The bread making tests (2100 g scale on a flour basis) were then conducted. The dough was mixed for the optimal time and then divided into 230 g pieces, rounded by hand, and allowed to rest for 20 min (bench time) in a fermentation cabinet (QBX-232DCST2; Fukushima Industries Corp., Osaka, Japan) at 30℃ and 70% RH. Each 4 pieces dough to form a U-shape were panned each of the two pan cases for the Pullman-type white bread after shaping using a molding machine (MMR230-2; Aicohsha Manufacturing Co., Ltd.). The panned doughs were proofed for 60 or 65 min at 38℃ and 85% RH and then baked in an oven (MOC-GGH- 11S; Sankokikai Co., Ltd., Tokyo, Japan) at 190℃ (top) and 210℃ (bottom) for 35 min.

4.2.3. Evaluation of BMQ

The GRD was evaluated by measuring the maximum expansion volume of 20 g of dough just after mixing and proofed at 38℃ and 85% RH in a cylinder subjected to 0 to 75 cmHg (Yamauchi et al. 2000). The 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). Replicates of three doughs and three loaves were prepared in a single bread making test to measure the GRD and GP. 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).

4.2.4. Enthalpy of Starch Retrogradation in Dough

Using the same sample as in the textural analysis, bread crumbs were air-dried after 99.5% ethanol and acetone treatment. Dried bread crumbs were ground, stored in polyethylene bags and used for determinations of amylose content and enthalpy of retrogradation. The enthalpy of retrogradation was determined using a differential scanning calorimeter (Micro DSC II, Setaram, Inc., Caluire**,** France). A sample of 250 mg dry weight basis (dwb) was weighed in a differential scanning calorimeter pan and distilled water was added to give a suspension of 30% dwb. The pan was sealed and allowed to set the scanning calorimeter. The scanning temperature range was set at 30 to 95℃ and the heating rate was 0.8℃ /min. The distilled water (700 mg) was used as a reference.

4.2.5. Hardness of Bread Crumb

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).

4.2.6. Rupture Analysis of Bread Crumb

Rupture force (RF), rupture deformation (RD) and rupture energy (RE) were measured using the same size of crumb sample as for the textural analysis. Crumb samples were placed in the center of a 5 x 5 cm measuring table with a 1.5 x 1.0 cm

square hole in the center of the table, and then ruptured at a speed of 5 mm/s up to 150% strain rate using the No. 64 wedge plunger of the creep meter (RE2-33005C; Yamaden Co., Ltd.).

4.2.7. Creep Analysis of Bread Crumb

The bread samples were provided to creep tests using creep meter (RE2-33005S, Yamaden Co., Ltd, Tokyo, Japan) with the condition: loading weight 0.25 N, measurement time 1 minute. The compression direction was defined as the directions vertical to the sliced surface. Viscoelastic properties of the bread crumb were obtained by applying the equation below based on the four-element Voigt model to the time-strain curves obtained by the creep test (Yiping et al. 1992d; Shibata et al. 2010; Kawai et al. 2006). The analysis except E_0 was carried out using a statistical software JMP 11 (SAS Institute. Inc., NC, USA). E_0 was determined with the creep analysis software of the creep meter (RE2-33005C; Yamaden Co., Ltd.).

$$\varepsilon(t) = \left(\frac{P_0}{E_0}\right) + \left(\frac{P_0}{E_1}\right)\left\{1 - \exp\left(-\frac{E_1 t}{\eta_1}\right)\right\} + \left(\frac{P_0}{\eta_N}\right) t$$

ε: strain (-), P_0: stress (Pa), E_0: instantaneous elasticity (Pa), E_1: retardation elasticity (Pa), η_1: retardation coefficient of viscosity (Pa・s), η_N: regularity coefficient viscosity (Pa・s), t：time (s)

4.2.8. Statistical Analysis

Significant differences were evaluated using same method with that used in the previous Chapters.

4.3. Results and Discussion

4.3.1. Evaluation of BMQ

BMQ of the Control dough, 40% of whole wheat flour (WWF) dough, and 40% of whole wheat flour with enzymes (WWF+E) dough are shown in Table 4.1. 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 the Control dough was significantly higher than those of WWF and WWF+E at 1 h fermentation. Although there were no significantly difference among all samples at 2 h fermentation, the GP of WWF and WWF+E were higher than that of the Control. The GP of WWF and WWF+E were significantly higher than that of the Control at 3 h fermentation.

Lower GRD of WWF can be due to the higher amounts of DS and DF in whole wheat flour. 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). The WWF+E dough had significantly the highest values among all the samples. Regarding the effect of each added enzyme, AM hydrolyzes damaged and gelatinized starch into maltose and dextrin in dough. Kim et al. (2006) reported that the high amounts of DS and DF decreased SLV of bread made from 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 of WWF+E dough compared to that of

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). 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 whole wheat flour promote fermentation of yeast.

Figure 4.1 shows that the addition of enzymes improved BMQ of WWF+E. Although WWF had the poor bread shape compared to the Control, WWF+E had the fine squared shape similar to the Control. In terms of crumb grain, WWF had the ununiform and poor air bubble while WWF+E had improved crumb grain.

4.3.2. Bread Color and Appearance

Table 4.2 shows the results of the bread color measurement. In terms of crust color, the addition of whole wheat flour significantly decreased the all color values. In addition, WWF+E bread had the significantly lowest values of L* and b* among all samples. a* value of WWF and WWF+E bread were significantly lower than that of the Control. In terms of crumb color, the addition of whole wheat flour significantly decreased the value of L*, while it increased the values of a* and b*. There was no significant difference between WWF and WWF+E on the values of a* and b*.

Figure 4.1 shows that 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, which corresponded with its lower L* values (Table 4.2). 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 4.2). These results show that 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 4.2).

4.3.3. Enthalpy of Starch Retrogradation in Bread

Figure 4.2 shows enthalpy of starch retrogradation in various breads. There was no significant difference among all samples at 0 and 1 day storage. At 2 days storage, WWF+E had significantly lower value compared to the Control, while there was no significant difference between WWF and WWF+E. At 3 days storage, WWF+E had the significantly lowest value among all samples, while there was no significant difference between the Control and WWF.

The main reason that the enthalpy of starch retrogradation of WWF+E decreased was due to that added AM decomposed gelatinized starch in WWF+E bread (Hug-Iten et al. 2001). AM hydrolyzes damaged and gelatinized starch into lower molecular weight saccharides and dextrin in dough. These saccharides reduce the amylopectin retrogradation in gelatinized starch, resulting in slow staling rate as shown in Figure 4.3 (Hug-Iten et al. 2003).

4.3.4. Hardness of Bread Crumb

Figure 4.3 shows staling of breads from different treatments during 3 days storage. The WWF+E bread showed the significantly lowest value among all samples, while there was no significantly difference between the Control and WWF during 3

days storage.

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 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; Gomes-Ruffi et al. 2012). 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 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).

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

4.3.5. Rupture Analysis of Bread Crumb

Table 4.3 shows RF, RD and RE of breads from different treatments at 1 and 3 days storage. In terms of RF, WWF+E had a significantly lower value compared to the Control at 1 day storage. Although WWF had a lower value than the Control, there was no significantly difference between these samples. At 3 days storage, all samples had decreased value than each sample at 1 day storage but there were same tendency on all samples. In terms of RD, WWF+E had a significantly higher value compared to WWF, while there was no significantly difference between WWF+E and the Control at 1 day storage. At 3 days storage, WWF+E had a significantly higher value the Control, while there was no significantly difference between WWF and WWF+E. In terms of RE, although WWF+E had a significantly lower value compared to the Control, there was no significantly difference between the Control and WWF. Though there was no significantly difference among all samples, WWF+E had a lower value compared to the others.

At 1 day storage, the addition of whole wheat flour decreased RF, RD, and RE. This result suggested that the bread crumb texture was changed to fragile texture by the addition of whole wheat flour because the DF in whole wheat flour had influence on the gluten network, resulting in decreased rupture tolerance of bread crumb. On the other hand, WWF+E had lower value of RF and RE compared to the others, suggesting that WWF+E had softer texture. This result was related to that WWF+E had lower value compared to the others on the hardness during 3 days storage as shown in Figure 4.3. In addition, it is supposed that the bread crumb texture of WWF+E was modified to high viscosity by the enzyme addition because RD of WWF+E was higher than the others. Yiping et al. (1992c) previously suggested that the moisture content of bread

crumb was highly related to RF, RD, and RE on the rupture analysis. However, there was no difference among all samples on the moisture content during storage (data was not shown). From these results, it seems that the value of rupture analysis is related to the enthalpy of starch retrogradation and bread crumb hardness during storage on Figure 4.2 and 4.3.

4.3.6. Creep Analysis of Bread Crumb

Table 4.4 shows the results of creep analysis during storage. In terms of E_0, there was no significantly difference among all samples at 1 day storage. While, although there was no significantly difference between the Control and WWF, WWF+E had significantly lower value compared to the others at 3 days storage. In terms of E_1, WWF and WWF+E had significantly lower value than the Control, while there was no significantly difference between WWF and WWF+E at 1 day storage. WWF+E at 3 days storage also had significantly lower value compared to the others. In terms of η_1, WWF and WWF+E had significantly lower value compared to the Control at 1 day storage. Although there was no significantly difference between the Control and WWF, WWF+E had significantly lower value than the Control at 3 days storage. In terms of η_N, WWF and WWF+E had lower value than the Control, while there was no significantly difference between WWF and WWF+E at 1 day storage. On the other hand, although there was no significantly difference between the Control and WWF, WWF+E had significantly lower value than the others. In terms of E_1, η_1, and η_N, WWF and WWF+E had significantly lower value than the Control, while there was no significantly difference between WWF and WWF+E at 1 day storage. From these results, it was suggested that the addition of whole wheat flour had larger effect

on the viscoelasticity of bread crumb than enzymes addition at early storage. On the other hand, WWF+E had significantly lower value compared to the others at 3 days storage, suggesting that the effect of enzymes addition on the viscoelasticity was larger at long storage time. Yiping et al. (1992d) reported that the viscoelasticity of bread crumb was related to the retrogradation of starch in bread during storage. WWF+E had lower enthalpy, especially at long storage time, compared to the others as shown in Figure 4.2. From these results, the optimal enzymes addition decreased starch retrogradation rate in bread during storage, resulting in lower viscoelasticity.

4.4. Conclusion

In this study, we investigated the effects of storage on the various properties of the following bread crumbs: 1) made from white wheat flour as the Control, 2) white flour combined with whole wheat flour (WWF) at a final ratio of 3:2 white to whole wheat flour (C+W), and 3) WWF with enzymes added at optimal concentrations determined in Chapter 3 (WWF+E) using pullman scale bread making. Rupture force, rupture deformation and rupture energy were decreased using whole wheat flour, as the higher amount of insoluble DF in whole wheat flour disturbs the fine gluten network formation in dough, resulting in a weakened bread crumb structure. In comparison, WWF+E had a lower RF, a higher RD and a lower RE values compared with the others, since AM and HC digest DS, GS, and insoluble DFs, which decrease BMQ on bread making, resulting in improved bread crumb texture. This study elucidated that the effects of using whole wheat flour and the addition of enzymes on the mechanical properties of bread crumb during storage. The addition of enzymes made it possible to obtain high quality pullman bread using whole wheat flour.

Table 4.1 BMQ of doughs supplemented with WWF and treated with optimal concentration of enzymes at Pullman-scale [1)]

Bread making treatments	Water absorption (%)	GRD (ml)	GP (ml)		
			1h	2h	3h
Control	68	105.00 ± 0.00 [b]	32.77 ± 0.17 [a]	65.57 ± 0.53 [a]	91.46 ± 0.73 [b]
WWF	69	97.78 ± 1.92 [c]	31.16 ± 0.64 [b]	66.93 ± 1.31 [a]	101.21 ± 1.40 [a]
WWF+E	69	121.11 ± 4.19 [a]	31.69 ± 0.64 [b]	67.51 ± 2.25 [a]	103.60 ± 2.68 [a]

[1)] GRD: gas retention of dough, GP: gassing power of dough,WWF: whole wheat flour, E: enzymes. Optimal amounts of enzymes, α-amylase and hemicellulase, were added in dough of WWF+E. Each value is the mean ± SD (GRD: n=3, GP: n=4,). The values followed by different letters within column are significantly different ($p<0.05$).

Table 4.2 Color of bread crusts and crumbs made from doughs supplemented with WWF and treated with optimal concentration of enzymes at Pullman-scale [1)]

Bread making treatments	Bread crust color (-)			Bread crumb color (-)		
	L*	a*	b*	L*	a*	b*
Control	46.00 ± 0.52 [a]	15.50 ± 0.10 [a]	29.00 ± 0.36 [a]	75.21 ± 0.72 [a]	-2.19 ± 0.09 [b]	9.67 ± 0.60 [b]
WWF	43.79 ± 0.64 [b]	13.97 ± 0.21 [b]	25.21 ± 0.91 [b]	69.82 ± 1.62 [b]	-0.37 ± 0.19 [a]	10.94 ± 0.55 [a]
WWF+E	39.80 ± 1.08 [c]	13.68 ± 0.26 [b]	21.23 ± 1.13 [c]	69.28 ± 0.89 [b]	-0.38 ± 0.30 [a]	10.35 ± 0.58 [ab]

[1)] WWF: whole wheat flour, E: enzymes. Optimal amounts of enzymes, α-amylase and hemicellulase, were added in dough of WWF+E. Each value is the mean ± SD (Bread crust color: n=5, Bread crumb color: n=8). The values followed by different letters within column are significantly different ($p<0.05$).

Table 4.3 Changes in rupture properties of bread crumb made from doughs supplemented with WWF and treated with optimal concentration of enzymes at Pullman-scale during storage [1)]

Bread making treatments	Rupture force (N)		Rupture deformation (mm)		Rupture energy ($J \times 10^{-3}$)	
	Storage time (d)		Storage time (d)		Storage time (d)	
	1	3	1	3	1	3
Control	6.83 ± 1.42 [a]	5.68 ± 1.18 [a]	24.50 ± 0.97 [ab]	22.50 ± 1.05 [b]	4.24 ± 0.81 [a]	3.87 ± 0.43 [a]
WWF	5.89 ± 1.57 [ab]	5.48 ± 1.78 [ab]	24.05 ± 0.76 [b]	22.75 ± 1.42 [ab]	3.79 ± 1.14 [ab]	4.01 ± 1.25 [a]
WWF+E	4.87 ± 2.19 [b]	4.19 ± 0.91 [b]	26.55 ± 3.35 [a]	24.10 ± 1.22 [a]	2.94 ± 1.13 [b]	3.10 ± 0.84 [a]

[1)] WWF: whole wheat flour, E: enzymes. Optimal amounts of enzymes, α-amylase and hemicellulase, were added in dough of WWF+E. Each value is the mean ± SD (n=10). The values followed by different letters within column are significantly different ($p<0.05$).

Table 4.4 Changes in viscoelastic parameters of various bread crumb from doughs supplemented with WWF and treated with optimal concentration of enzymes at Pullman-scale during storage [1)]

Bread making treatments	E_0 (Pa$\times 10^3$)		E_1 (Pa$\times 10^4$)		η_1 (Pa·s$\times 10^4$)		η_N (Pa·s$\times 10^6$)	
	Storage time (d)		Storage time (d)		Storage time (d)		Storage time (d)	
	1	3	1	3	1	3	1	3
Control	8.24 ± 1.38 [a]	12.50 ± 2.15 [a]	1.15 ± 0.27 [a]	1.98 ± 0.64 [a]	2.39 ± 0.41 [a]	3.76 ± 1.52 [a]	1.27 ± 0.32 [a]	2.17 ± 0.51 [a]
WWF	7.87 ± 0.97 [a]	13.13 ± 1.34 [a]	0.84 ± 0.12 [b]	1.95 ± 0.49 [a]	1.73 ± 0.28 [b]	3.60 ± 1.32 [ab]	0.97 ± 0.15 [b]	2.16 ± 0.47 [a]
WWF+E	7.84 ± 1.44 [a]	10.29 ± 2.36 [b]	0.72 ± 0.28 [b]	1.16 ± 0.29 [b]	1.89 ± 0.50 [b]	2.50 ± 0.47 [b]	0.72 ± 0.31 [b]	1.26 ± 0.36 [b]

[1)] E_0: instantaneous elasticity, E_1: retardation elasticity, η_1: retardation coefficient of viscosity, η_N: regularity coefficient of viscosity, WWF: whole wheat flour, E: enzymes. Optimal amounts of enzymes, α-amylase and hemicellulase, were added in dough of WWF+E. Each value is the mean ± SD (n=10). The values followed by different letters within column are significantly different ($p<0.05$).

Control WWF WWF+E

Figure 4.1 Photographs of appearance and scanned crumb images of bread made from doughs supplemented with WWF and treated with optimal concentration of enzymes at Pullman-scale [1)]

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

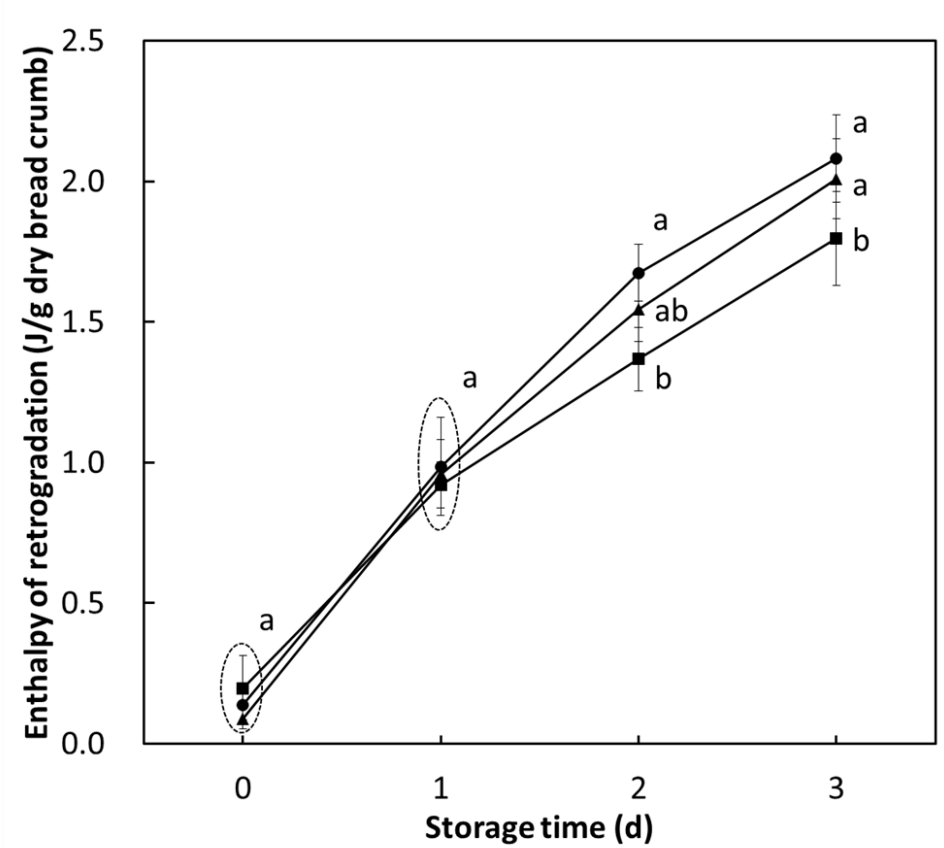


Figure 4.2 Changes in enthalpy of amylopectin retrogradation of various bread crumb made from doughs supplemented with WWF and treated with optimal concentration of enzymes at Pullman-scale during storage [1)]

[1)] WWF: whole wheat flour, E: enzymes. Optimal amounts of enzymes, α-amylase and hemicellulase, were added in dough of WWF+E. The vertical bar is the standard deviation of each value (n=10). The data points followed by different letters are significantly different ($p < 0.05$). ●: Control, ▲: WWF, ■: WWF+E.

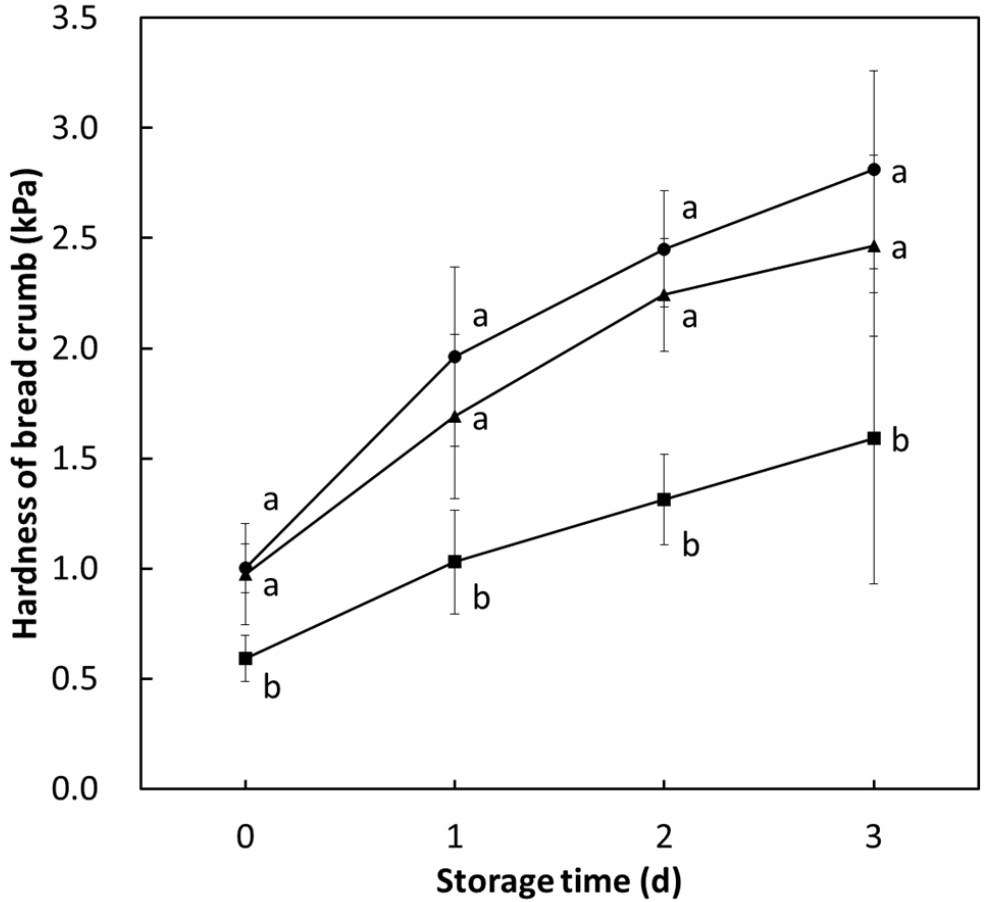


Figure 4.3 Changes in hardness of various bread crumbs made from doughs supplemented with WWF and treated with optimal concentration of enzymes at Pullman-scale during storage [1)]

[1)] WWF: whole wheat flour, E: enzymes. Optimal amounts of enzymes, α-amylase and hemicellulase, were added in dough of WWF+E. The vertical bar is the standard deviation of each value (n=10). The data points followed by different letters are significantly different ($p < 0.05$). ●: Control, ▲: WWF, ■: WWF+E.

Chapter 5. Bread Making Improvement of Mashed Potato-Supplemented Dough by Treating with Optimal Bakery Enzymes

5.1. Introduction

Potato is major crops globally and being produced widely in many countries of the world. In Japan, potato is also major agricultural crop and about 2.5 million tons are produced per year. Those are used for much utilization such as table food, processing and starch extraction, etc. However, potato is not used much in bread making in Japan and mainly used for other purposes, such as potato salad for sandwiches, than bread dough production. The main reason is that when mashed potato is added to the dough, the gluten network in the dough deteriorates due to DS and DFs, especially insoluble DFs, and the bread making properties remarkably decrease. On the other hand, using raw materials containing a large amount of gelatinized or swollen starch such as MP for bread making results in a positive effect that the bread has slightly sweet taste, low staling, and sticky texture as reported by Murayama et al. (2015) and Yamauchi et al. (2014). In addition, in particular, potato starch in various starch has been found to have very high swelling power and viscosity when heated in water, and its DS retains a large amount of water (Hossen et al. 2011; Li and Yeh 2001). Therefore, by adding MP to dough, it is expected to improve the water absorption of dough and bread qualities. Recently, it is also reported that although the BMQ of the dough containing a large amount of DS or DFs greatly degradate, it can be remarkably improved 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 the BMQ, it is so important to reasonably derive the optimal amounts without performing many experiments. Because, this makes it possible to efficiently develop high quality bread.

Therefore, in this Chapter, we adopted a CCF as a reasonable and effective method for acquiring the evaluation data to determine reasonable optimum amounts of multiple enzymes for maximum improvement in the BMQ of MP-supplemented dough (Flander et al. 2007). A 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, in order to validate the effectiveness of these methods, the bread making experiment with the optimal amounts of multiple enzymes was conducted, and the effectiveness was verified from the BMQ of the dough and the various evaluation of the bread.

5.2. Materials and Method

5.2.1. Flour, Enzymes, and MP used

Strong wheat flour and two commercial enzymes were same with those used in the previous Chapters. A commercial table potato variety, *Solanum tuberosum L.* cv. May Queen, (potato) 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 -30ºC for 30 min and then stored at -20ºC until use. The moisture content of this MP

was 81.6 %.

5.2.2. Optimization of Concentrations of added Enzymes

CCF as reported by Flander et al. (2007) was used with two variables to determine optimal concentrations of enzymes. This CCF is composed by twelve runs with four replicates at the center point (Table 5.1). 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 were ranged from 0.00 to 0.100 (g/100 g flour). These minimum and maximum concentrations of enzymes were determined by using the data of bread making tests to add the various amount of these enzymes and the bread making tests determined by using CCF were done randomly. In this study, SLV and amounts of added enzymes (AM and HC) were respectively adopted as a response and factors on analysis of RSMd. The reason for choosing SLV as a response is that it is a representative index of BMQ. From the results of twelve runs based on CCF, a RSMd between a response and factors was derived by using multiple regression analysis. Selection of the explanatory variables of the RSMd was determined by stepwise back selection method for the variable with 2.0 of F value 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 Excel add-in software Solver. After CCF experiments, bread making tests of dough to add MP and the optimal concentrations of enzymes (MP+E dough) were done with the Control and MP supplemented dough (MP dough) and the improving effects were evaluated in detail.

5.2.3. Dough Preparation and Bread Making

Bread making tests were done 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 was the maximum percentage instead of wheat flour at which the BMQ could be improved with optimal enzymes.

5.2.4. Evaluation of BMQ

The GRD was evaluated by measuring the maximum expansion volume of 20 g dough proofed for 70 min at 38ºC and 85 % RH in a cylinder subjected to 0 to 75 cmHg, following the report of Yamauchi et al. (2000). The GP of 20 g dough after bench time was measured at 30ºC 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 rapeseed-displacement method according to the AACCI (2000). Photographs and images of the breads were also recorded using the method as reported by Santiago et al. (2015a). Color of the bread top crust and crumb was measured with a colorimeter by as reported by Matsushita at al. (2017).

5.2.5. Moisture Contents of MP and Bread Crumb

The moisture contents of MP just after preparation and bread stored for 3 days

in polyethylene bags at 20ºC and 70 % RH were measured with homogenized MP and bread crumbs by using the method of Santiago et al. (2015a).

5.2.6. Soluble Sugar Contents of Bread Crumb

Soluble sugars contents, such as total, reducing, mono- and di-saccharides, were also analyzed using the method reported by Santiago et al. (2015b).

5.2.7. DFs and DS Analysis

The sample preparation and storage before DFs and DS analysis was carried out according to the method reported by Santiago et al. (2015a). NDF, the amounts of the hemicellulose, cellulose, and lignin, and ADF, the amounts of the 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 DS contents in doughs were measured with the Megazyme assay kit (Megazyme International Ireland Ltd., Wicklow, Ireland) based on the method of Gibson et al. (1991).

5.2.8. Hardness of Bread Crumb

The temporal changes of crumb hardness (bread staling) was 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 and a square of crumb (3 x 3 cm) was cut from the crumb 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.

5.2.9. Sensory Evaluation of Breads

Sensory evaluation of breads was done by using the samples stored into polyethylene bags for 1 day at 20ºC and 70 % RH. Quantitative descriptive analysis of MP supplemented and MP+E supplemented breads (MP and MP+E breads) were evaluated and compared with the Control by 12 panelists; undergraduate and graduate students of Obihiro university of agriculture and veterinary medicine. The appearance evaluation is the three items of volume, shape, and color. The crumb evaluation is also crumb grain, color, texture, flavor, and taste. The full points of volume are 30. Those of other items points 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 evaluation of the above two kinds of breads was carried out by two samples comparison method compared with the Control.

5.2.10. Statistical Analysis

Significant differences were evaluated using same method with that used in the previous Chapters.

5.3. Results and Discussion

5.3.1. Optimization of Concentrations of added Enzymes

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

The optimal concentrations of AM and HC calculated with the optimization method using Excel add-in software Solver were 0.059 and 0.05 g/100 g flour, respectively. In dough with MP, the SLV increased with amount of added HC, but the improving effect became a plateau. When excessive HC was added, the dough became very sticky and its 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 an upper concentration.

5.3.2. Evaluation of BMQ

BMQ of Control, MP, and MP+E doughs were presented in Table 5.2. The doughs with MP and MP+E showed higher water absorption than that of Control. This main reason is speculated to be due to the high-water absorption of starch and DFs in MP. The dough with MP showed the significantly lowered GRD compared to the others, while the dough with MP+E showed the significantly highest GRD among all samples.

The doughs with MP showed significant decrease in GP compared to the

Control at 1 and 2 h fermentation. On the other hand, GP of these doughs were significantly higher than the Control at 3 h fermentation.

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

Lower GRD and SLV of dough and bread with MP can be due to the highest amounts of DS and DFs among the all samples as shown in Table 5.6. It was suggested that the excessive DS and DFs of MP dough disrupt the gluten network formation in dough, resulting in a weaker gluten network (Lai et al. 1989; Ozboy and Koksel 1997; Wang et al. 2002).

In terms of GRD and SLV, the dough and bread 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 bread, which might be attributed to the optimal combined catalytic activities of AM and HC. Those enzymes significantly decreased the contents of DS and DFs, especially approximate hemicellulose (equivalent to NDF-ADF) in the dough, as shown in Table 5.6. The Control dough and bread also had higher values in GRD and SLV despite high DS and NDF-ADF contents, 3.63 % and 0.45 %, which might be attributed to the rather low values of total DFs (equivalent to NDF), especially ADF, compared to the dough with MP. 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 the GP during the fermentation. However, the GP of

two doughs with MP before 3 h fermentation was significantly lower compared with the Control as shown in Table 5.2. While, the GP of the doughs with MP at 3 h fermentation was significantly high value as well as above reference compared to the Control. High concentrations of various components like mono- and di-saccharides in MP seemed to promote the fermentation of yeast at final stage of fermentation. The GP of the dough with MP+E is slightly suppressed compared to those of MP dough after 2 h fermentation. It seems that this is related to the slight suppression of fermentation by low molecular saccharides, etc. produced by the added enzyme.

Regarding the addition effect of each enzyme, the endogenous AM and β-amylase of flour firstly hydrolyzes damaged and gelatinized starch to maltose, 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) also suggested a similar observation that the addition of fungal AM increased the SLV of chemically leavened bread. Likewise, Jiang et al. (2005) and Rouau et al. (1994) reported that HC catalyzes the degradation of polysaccharides (mainly hemicellulose) into mono sugars and short chain saccharides, resulting in the 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 including high DFs and millet/wheat composite bread as reported by Shah et al. (2006) and Schoenlechner et al. (2013), respectively.

From above findings, it is considered that the drastic improvements of GRD

and SLV in dough and bread with MP and optimal enzymes (AM and HC) is reasonable.

5.3.3. Bread Color and Appearance

Table 5.3 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. The addition of MP also decreased the values of L*, a*, and b*. In addition, the all values of 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 values of b*. L* and a* of crumb of MP+E bread was significantly lower, while b* value was significantly higher compared to the Control. These results seem to be related to the high reducing sugar content of doughs with MP and MP+E and the yellow color of MP.

Figure 5.1 shows the bread appearances and crumb images. The addition of MP made the external bread color darker, especially the color of MP+E bread, and the crust of breads with MP and MP+E was dark compared to the Control. The crumbs of breads with MP and MP+E were somewhat yellow compared with the Control crumb. These results were consistent with the color data measured with a colorimeter in Table 5.3. Appearance of MP bread was significantly smaller than the Control, while the bread with MP+E was nearly same compared with the Control. These results were congruent with their SLV presented in Table 5.2. The crust color of bread added MP became darker than the Control. In addition, the bread with MP+E showed remarkable darker color compared with the Control and MP breads, which corresponded with their greatly lower values of L* and b*, and the photographs shown in Table 5.3 and Figure

5.1, respectively. MP addition also resulted in the decrease of all crust color values compared to the Control. Especially, these values in crust were significantly decreased by the addition of optimum amounts of enzymes compared to the MP bread, which is evidenced by the significantly lower L*, a*, and b* values of crust in Table 5.3. These results corresponded with that the bread with MP+E has an inferior color in appearance evaluation in Table 5.7, which result in the excess darkness of crust color. It seems that, as one factor, the high reducing sugar content of the dough with MP+E in Table 5.5 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, which result in the enhancement of the Maillard reaction. From these previous findings, it is considered that the remarkable darkness of the crust color of MP+E bread is greatly affected by the increases of reducing sugars by enzymes reaction and free amino acids with addition of MP.

The L* and a* of crumbs of breads with MP and MP+E, especially the latter, were lower than the Control and conversely, the b* of crumbs of MP and MP+E breads significantly was high value compared with the Control as shown in Table 5.3. In particular, the significantly higher b * values of MP and MP+E breads crumbs of the latter are good consistent with that the crumbs color of breads with MP (MP and MP+E breads) showed more yellow color compared with the Control as shown in Figure 5.1. It also seems that these related to the fact that MP shows a more yellow color as compared with wheat flour. The greatly dark color of crust and somewhat dark and yellow color of crumb in MP+E bread seemed to result in the high reducing sugars in the dough that resulted in browning and a little yellow color of MP.

5.3.4. Moisture Contents of Bread Crumb

Table 5.4 shows the temporal moisture contents of bread crumbs during storage. The moisture content of MP and MP+E breads showed higher values than the Control during the storage period. This is considered to be mainly related to high water absorption of the doughs with MP compared to the Control as shown in Table 5.2. In difference (1day-3days) of moisture contents of bread crumbs, MP bread also showed low value compared to the others. This main reason 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 becomes small and the moisture evaporation of this bread during storage is suppressed. These results also correspond to the report that the breads with the smaller SLV or with gelatinized starch has less moisture evaporation during baking and storage (Santiago et al. 2015b; Tsai et al. 2012; Yamauchi et al. 2014).

5.3.5. Soluble Sugar Contents of Bread

The saccharide contents in water soluble fraction of bread crumbs are shown in Table 5.5. As the sucrose contents of all samples were nearly zero, the data was omitted. All saccharides contents in MP+E bread except of glucose and fructose were significantly higher than those of the others. The reducing sugar, glucose, fructose and maltose, especially maltose, of MP and MP+E breads were also significantly high values compared with the Control. These results agreed with those of previous papers reported concerning the Yudane bread produced with gelatinized and swollen flour paste and the bread made by adding 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, apparently more total and reducing saccharides, and maltose in the dough are produced compared to the Control without these materials. It is reported that the gelatinized and swollen starch added to the dough are decomposed by endogenous various amylases in wheat flour and added AM and HC, and the latter effect is greater (Santiago et al. 2015b).

5.3.6. DFs and DS Analysis

Table 5.6 shows the DFs and DS contents of the final proofing doughs from different treatments. MP dough had higher DFs contents (NDF, ADF, and NDF-ADF), which is inherent with the MP added. Generally, excess fibers cause a negative effect on the formation of optimal gluten network, resulting in the reduction of GRD and SLV (Lai et al. 1989; Matsushita et al. 2017). Conversely, the dough with MP+E showed lower DFs contents, especially NDF-ADF, except for the ADF, which was attributable to xylanase activity of HC, compared to the MP dough. The HC hydrolyzes the DFs such as xylan and arabinoxylan, etc. resulting in low contents of NDF and approximate hemicellulose (NDF-ADF) in the dough with MP+E (Jiang et al. 2005; Stojceska and Ainsworth 2008). The Control and MP doughs had higher value in the DS content than the MP+E dough. The value of dough with MP+E had the lower value than the Control and significantly lower than the dough with MP. The addition of optimal enzymes decomposes the much amounts of DS in dough, therefore the dough with MP+E had the lowest DS content among all samples. Table 5.6 also showed the DFs contents of doughs. The ADF of dough with MP or MP+E had significantly higher value than the Control dough and the NDF of dough with MP also had significantly

high 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 amounts of DS generated by the physical damages during the milling process and gelatinized starch in MP. Excess amounts of DS cause unsuitable effects on BMQ (Murayama et al. 2015; Santiago et al. 2015a; Yamauchi et al. 2014). The dough with MP+E had lower or significantly lower DS than the others, which can be mainly related to the enzymatic decomposition of DS by added AM. Ultimately, the improvement of GRD and SLV of MP+E dough and bread can be associated with the reduction of the amounts of DS and DFs (mainly insoluble hemicellulose (pentosan)).

5.3.7. Hardness of Bread Crumb

Figure 5.2 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 higher or significantly higher than that of MP+E bread at 3 days storage. MP bread had the significantly highest value of hardness at 3 days among all samples, while the bread with MP+E had lower or significantly lower than the others at 3 days storage. There are various factors which relate to the temporal changes in bread crumb hardness during the storage: such as retrogradation rate of GSG in bread, SLV, low molecular saccharides content, and bread moisture, etc. The AM mainly breaks down DS in dough into low molecular weight dextrins, oligo-saccharides, etc. during the bread making. In addition, the endogenous β-amylase in wheat flour converts above saccharides into maltose. These

complementary functions during bread making process bring about partial decompositions of DS. In addition, 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 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 AM has the 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 starch-protein interactions are interfered by these LMWSs produced by AM hydrolysis in dough, resulting in the few and weak crosslinks between the starch and protein, and the reduction of hardening rate of the bread (Martin and Hoseney 1991; Martin et al.1991). From Table 5.2 and Figure 5.2, the SLV of MP+E bread with optimum enzymes including AM was significantly larger than the MP bread and nearly same compared to the Control. Maleki et al. (1980) also reported that the staling rate of bread is clearly decreased with the large SLV. The staling suppression of the bread accompanying the increase of SLV is considered to be mainly due to the increase of the porosity of crumb.

On the other hand, the insoluble hemicellulose (mainly insoluble pentosan) interferes with the desirable formation of the gluten network, while HC mainly attacks the insoluble pentosan and changes to LMWs, 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; Ghoshal et al. 2013; Matsushita et al. 2017). In this study, the dough with MP+E actually had significantly lower amounts of approximate hemicellulose (NDF-ADF) than the Control and MP doughs in Table 5.6. Roger et al. (1980) and Zeleznak et al. (1986) also reported that high moisture content bread shows

low staling. As shown in Table 5.4, the breads with MP showed higher moisture contents than the Control during storage. Therefore, in the breads with MP, it is considered that the high moisture contents of these breads positively influence the suppression of these bread staling.

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

Figure 5.2 also showed that the addition of optimum enzymes obviously suppressed the staling rate of bread compared to the Control and MP breads. It was caused by the increase of some saccharides with decompositions of DS and DFs in bread by the optimal addition of AM and HC, the high SLV accompanying decompositions of DS and insoluble DFs, and high moisture content compared with the Control, resulting in the anti-bread staling effects. These results agree with previous data reported by Caballero et al. (2007), Matsushita et al. (2017), Ghoshal et al. (2013), and Goesaert et al. (2009).

5.3.8. Sensory Evaluation of Breads

The sensory evaluation results of breads are shown in Table 5.7. From the results, as the volume and color of appearance evaluation in MP bread was significantly lower than those of the Control, the total appearance evaluation of MP bread showed significantly lower value compared with the Control. On the other hand, in the bread of MP+E, the evaluation of color was significantly lower than the Control,

but the evaluation of volume was significantly high. Therefore, the total appearance evaluation was similar with the Control. In 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. As such, the total crumb evaluation of these breads showed higher values than the Control and especially that of the MP+E bread showed significantly higher value. The overall total evaluation of MP bread had almost equivalent value compared with the Control and that of MP+E bread showed significantly higher value, which reflect the above total appearance and crumb evaluation results.

These results are approximately consistent with the above-mentioned bread making evaluation of these bread dough (Table 5.2) and various evaluations of these breads (Table 5.3, Figure 5.1 and 5.2). Namely, although the BMQ of the dough greatly decreases due to the addition of MP, the texture, flavor, and taste in crumb evaluation were greatly improved by the effect of MP. In addition, the volume in appearance evaluation of MP+E bread was greatly improved by the effect of optimum enzyme addition 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 MP+E bread in appearance evaluation was extremely lower than the MP bread, which was the main factor that the total appearance evaluation of MP+E bread was similar with the Control.

Although described in the following sections of the Overall BMQ and bread evaluation, the inferior crust color of MP+E bread may have seemed to be caused by excessive production of reducing saccharides by the added enzymes, as shown in Table 5.5. Therefore, to further improve the overall qualities of the MP+E bread, it seems

that to include the crust color in optimization evaluation index or to check if baking MP+E dough at low temperature is suitable is effective. Consequently, overall total evaluation result of MP+E bread could further be improved.

5.3.9. Overall BMQ and Bread Evaluation

Overall, this study established that the treatment with optimal AM and HC drastically improved the BMQ of dough supplemented with 5 % MP. The most improved BMQ 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 5.2, Figure 5.2, and Table 5.7. These properties of dough and bread with optimal enzymes were dramatically and greatly improved compared to those of MP dough and bread, which were totally better than the Control. On the other hand, as a negative effect with the treatment of optimal enzymes addition, the reduction in bread color evaluation, especially large decrease in L* and b* values of crust, is presented as shown in Table 5.3. In MP bread, the L*, a* and b* values of the bread also decrease compared with the Control, but the color is considered to be an acceptable characteristic. However, the crust browning of MP+E bread accompanying promotion of the Maillard reaction during baking process proceeded more than necessary by addition optimum enzymes and the crust color evaluation greatly decreased. As such, the crust color of MP+E bread seemed to be unacceptable. Therefore, it seemed that this is a negative point of the effect with adding the optimum amounts of enzymes. In this study, the amounts of optimum enzymes were decided to maximize the SLV as a response by using RSMd and OT. As

the calculated value (5.20) of optimal SLV with the RSMd considerably corresponded to the actual experimental value (5.08), the validity of this model was verified. While, this model has a limit to optimize the bread making condition by using SLV as an index of optimum bread quality and, as such, the degradation on crust color of bread obtained in the optimum enzymes addition was observed in this study.

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

5.4. Conclusion

Although the high amounts of DS and DFs in MP had a beneficial effect on bread qualities such as texture, flavor, and taste, these made to decrease general bread making properties. The DS and hemicellulose (mainly insoluble pentosan) among DFs especially interfere with fine gluten network formation, resulting in the reduction of GRD and SLV, and the acceleration of staling rate during the storage. The addition of optimum enzymes (AM and HC) to solve these problems were reasonably determined using RSM and OT, which resulted in the formation of the desirable gluten network and the remarkable improvement of dough and bread properties such as GRD and SLV. It is attributed to the degradations of DS and hemicellulose (mainly insoluble types) into soluble low molecular weight saccharides, which do not give the negative influence on the gluten network formation. As such, the addition of optimum enzymes

in MP-supplemented dough enable the production of satisfactory bread supplemented with 5 % MP except of bread crust color, which has several suitable properties such as high GRD and SLV, retarded staling rate, and good crumb evaluation. The above findings can be suggested that RSM and OT (Solver) are effective methods for establishment of optimum conditions in bread making. By using these methods, the optimal conditions can be reasonably and easily determined.

Table 5.1 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.

Table 5.2 BMQ of doughs supplemented with MP and treated with optimal concentration of enzymes [1)]

Bread making treatments	Water absorption (%)	GRD (ml)	GP (ml)			SLV (ml/g)
			1h	2h	3h	
Control	66.5	100.56 ± 1.72 [b]	26.80 ± 0.30 [a]	61.45 ± 0.38 [a]	92.76 ± 0.26 [b]	5.32 ± 0.05 [a]
MP	70.7	82.78 ± 5.10 [c]	25.39 ± 0.32 [b]	59.55 ± 0.48 [b]	100.16 ± 0.71 [a]	4.62 ± 0.06 [b]
MP+E	70.7	117.78 ± 2.60 [a]	25.63 ±0.31 [b]	59.31 ± 0.51 [b]	99.68 ± 0.79 [a]	5.20 ± 0.28 [a]

[1)] BMQ: bread making qualities, Water absorption (%): Optimal amount of water for bread (%), MP: mashed potato, E: enzymes, GRD: gas retention of dough, GP: gassing power of dough, SLV: specific loaf volume. 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 ($p<0.05$).

Table 5.3 Color of bread crusts and crumbs made from doughs supplemented with MP and treated with optimal concentration of enzymes [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, b*: level of yellowness. 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 5.4 Temporal moisture contents of bread crumbs made from doughs supplemented with MP and treated with optomal concentration of enzymes [1)]

Bread making treatments	Moisture contents of bread crumbs (%)			Difference (1day - 3days)of moisture contents of bread crumbs during storage (%)
	Storage time (d)			
	1	2	3	
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 ($p<0.05$).

Table 5.5 Soluble sugar contents of bread crumbs made from doughs supplemented with MP and treated with optimal concentration of enzymes [1)]

Bread making treatments	Saccharide contents in water soluble fraction of bread crumbs (mg/g bread crumb)				
	Glucose	Fructose	Maltose	Reducing saccharide	Total saccharide
Control	10.14 ± 0.41 [b]	19.18 ± 0.63 [b]	26.46 ± 0.79 [c]	66.09 ± 0.96 [c]	73.24 ± 2.72 [b]
MP	12.99 ± 0.90 [a]	22.20 ± 1.03 [a]	33.76 ± 1.68 [b]	69.45 ± 0.62 [b]	81.09 ± 0.92 [b]
MP+E	14.55 ± 1.76 [a]	21.10 ± 0.57 [a]	49.75 ± 1.33 [a]	100.84 ± 1.29 [a]	135.35 ± 5.30 [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 5.6 DFs and DS contents of doughs supplemented with MP and treated with optimal concentration of enzymes [1)]

Bread making treatments	NDF (%)	ADF (%)	NDF-ADF (%) [2)]	DS (%)
Control	0.86 ± 0.09 [b]	0.42 ± 0.03 [b]	0.45 ± 0.13 [a]	3.63 ± 0.32 [ab]
MP	1.28 ± 0.21 [a]	0.74 ± 0.08 [a]	0.54 ± 0.14 [a]	4.76 ± 0.42 [a]
MP+E	0.80 ± 0.03 [b]	0.69 ± 0.08 [a]	0.11 ± 0.04 [b]	2.65 ± 0.08 [b]

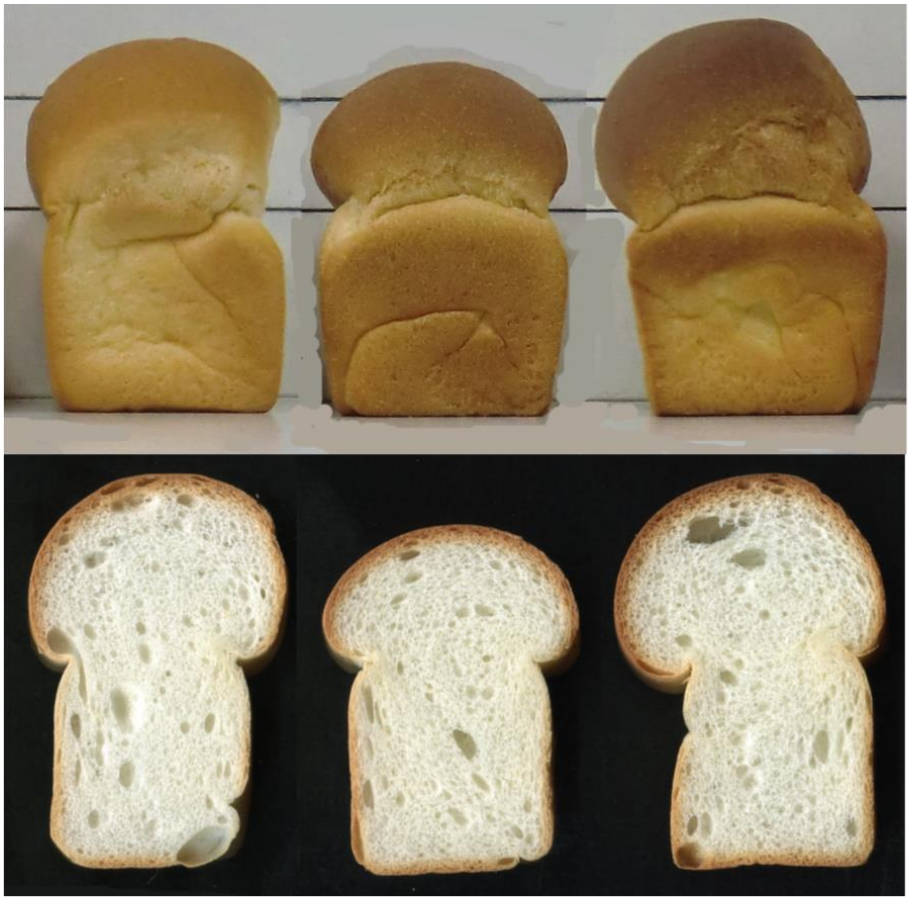
[1)] DFs: dietary fibers, DS: damaged starch, MP: mashed potato, E: enzymes, NDF: neutral detergent fiber, ADF: acid detergent fiber. The DS and DFs contents are percentage based on the dry base weight of samples. Optimal amounts of enzymes, α-amylase and hemicellulase, were added in dough of MP+E. Each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

[2)] NDF-ADF: crude hemicellulose content.

Table 5.7 Sensory evaluation of breads made from doughs supplemented with MP and treated with optimal concentration of enzymes[1)]

Bread making treatmens	Apearance evaluation (-)				Crumb evaluation (-)						Overall total evaluation (-)
	Volume	Shape	Color	Total	Crumb grain	Color	Texture	Flavor	Taste	Total	
Control	15.00 ± 0.00 [b]	5.00 ± 0.00 [a]	5.00 ± 0.00 [a]	25.00 ± 0.00 [a]	5.00 ± 0.00 [a]	5.00 ± 0.00 [a]	5.00 ± 0.00 [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 ($p<0.05$).



Control MP MP+E

Figure 5.1 Photographs of appearance and scanned crumb images of breads made from doughs supplemented with MP and treated with optimal concentration of enzymes [1)]

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

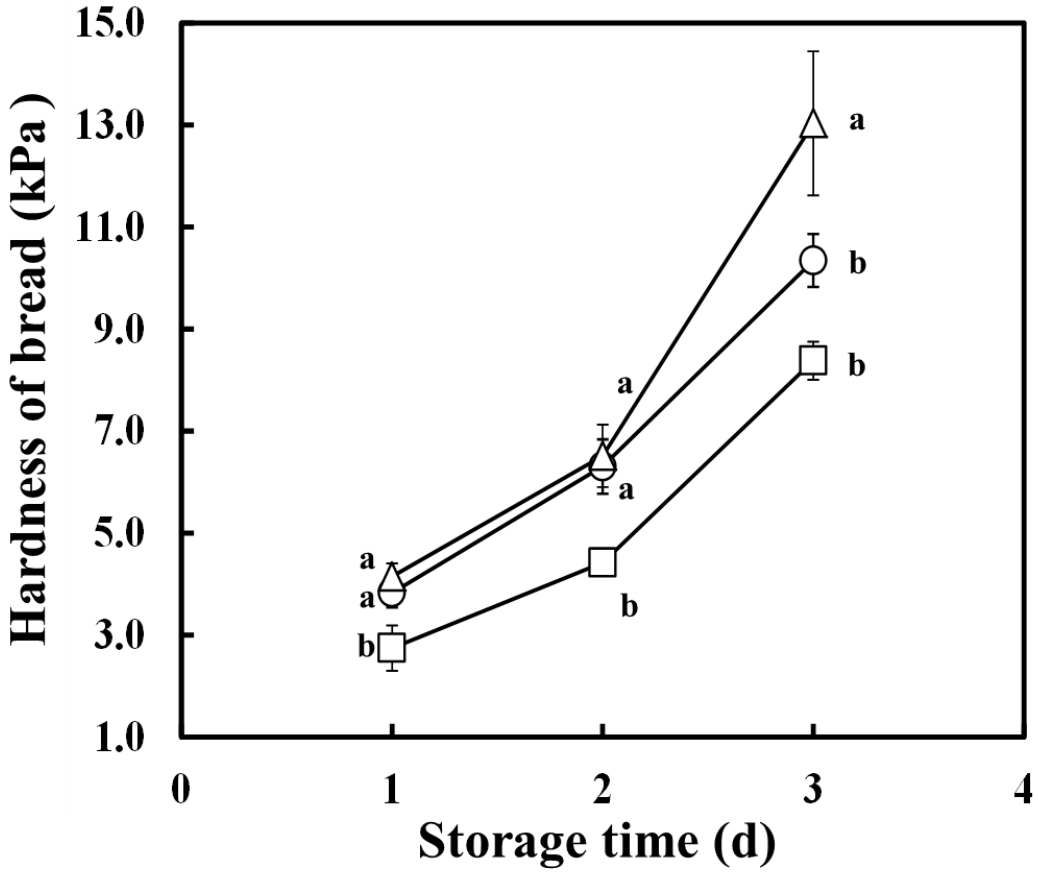


Figure 5.2 Temporal hardness changes of bread crumbs made from doughs supplemented with MP and treated with optimal concentration of enzymes during storage [1)]

[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.

Chapter 6. Effect of Combining Additional Bakery Enzymes and High-Pressure Treatment on Bread Making Qualities

6.1. Introduction

Generally, DS and insoluble DFs (especially insoluble pentosan) in wheat flour inhibit the formation of a suitable gluten network in dough, and they are considered as factors reducing BMQ (Wang et al. 2002; Hung et al. 2007; Santiago et al. 2015a). Various enzymes for bread making are used to improve BMQ; especially AM and HC, 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 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 Chapter, we conducted bread making tests according to the central composite plan and determined the regression coefficients from the subsequent data to develop a 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.

6.2. Materials and Methods

6.2.1. 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.

6.2.2. Optimal Concentrations of Bakery Enzyme and High-Pressure Level

A 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 (Table 6.1). 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.

6.2.3. 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% RH 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).

6.2.4. DFs and DS Analysis

Samples for DFs and DS analysis were prepared according to the methods reported by Santiago et al. (2015a) using dough after proofing. NDF, which are cellulose, hemicellulose and lignin content, and 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. 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).

6.2.5. Soluble Sugar Contents of Bread Crumb

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).

6.2.6. Evaluation of BMQ

The GRD and the GP 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 GRD, GP 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 methods described by Santiago et al. (2015a).

6.2.7. 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.

6.2.8. Hardness of Bread Crumb

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).

6.2.9. Statistical Analysis

Significant differences were evaluated using same method with that used in the previous Chapters.

6.3. Results and Discussion

6.3.1. 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 0.200 % flour and 43 MPa, respectively. In other words, BE were supplemented with bakery enzyme at 0.200 %, HP were treated with high-pressure at 43 MPa, and BE/HP were supplemented and treated with bakery enzyme and high-pressure at 0.200 % and 43 MPa, respectively.

6.3.2. DFs and DS Analysis

Table 6.2 shows the contents of DFs and DS 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. In terms of DS content, 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.

Generally, DFs negatively affect the formation of an optimal gluten network, resulting in the reduction of GRD 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 HC 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 HC 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). 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 AM, on the dough. Ultimately, the improvement of GRD 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 6.4.

6.3.3. Soluble Sugar Contents of Bread Crumb

Table 6.3 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 bread, 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 AM 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 HC 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 6.3. Santiago et al. (2015b) also reported that the addition of AM and HC increases the soluble sugar contents in bread.

6.3.4. Evaluation of BMQ

Table 6.4 shows the BMQ of various doughs. The BE, HP and BE/HP doughs had decreased GP compared with the Control dough. The GP 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 GRD, the bakery enzyme heightened the GRD, 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 GRD 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 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 the fermentation. However, in this study, the GP of two doughs with BE during fermentation was significantly lower or lower compared with the Control (Table 6.4). 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 GP of BE and BE/HP doughs were slightly suppressed compared to the Control. While it seems that the increased GRD 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 HC catalyzes the degradation of polysaccharides (mainly hemicellulose) into mono sugars and short chain saccharides, resulting in

superior gluten network formation. The catalytic activity of AM and HC may have led to the dough and bread of BE and BE/HP having higher GRD 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 OT 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).

6.3.5. Bread Color and Appearance

Table 6.4 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 6.4 and Figure 6.1, respectively. Figure 6.1 shows the bread appearances and crumb images. The crust redness of BE/HP bread in Figure 6.1 was also significantly stronger compared to the Control, which is evidenced by the significantly higher a* values of crust (Table 6.4). 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 6.4). 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 AM increased concentrations of reducing sugars such as glucose, fructose, resulting in the enhancement of the Maillard reaction.

6.3.6. Scanning Electron Microscopy Observation

Figure 6.2 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 (Figure 6.2a and c) had the crosslink between GSG 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 (Figure 6.2b and d). The Control and HP bread crumbs (Figure 6.2a 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 (Figure 6.2b 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

GRD and SLV (Table 6.4). Santiago et al. (2015b) also reported that the addition of AM and HC improved the crumb structure (gluten network and crosslink starch gel and gluten) of bread made from the dough supplemented with sweet potato powder.

6.3.7. Hardness of Bread Crumb

Figure 6.3 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 GSG in bread, SLV, low molecular saccharides content, and bread moisture. It was reported that AM decomposes DS and gelatinized starch to low molecular weight saccharides which retards the retrogradation of GSG 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 AM 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 AM hydrolysis in dough, resulting in a few and weak crosslinks between the starch gel and protein (Figure 6.2), and reduces the hardening rate of the bread (Martin and Hoseney 1991; Martin et al. 1991). The SLV of BE/HP bread (Table 6.4 and Figure 6.1) 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 HC 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 HC 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 6.2).

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 6.3) support the conclusions reported by Caballero et al. (2007), Matsushita et al. (2017), Ghoshal et al. (2013), and Goesaert et al. (2009).

6.4. 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 (0.200 % and 43 MPa) were established using RSMd and the OT by Solver. The high-pressure treatment enhances enzymatic activity (degradation of DS 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 GRD 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 OT 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.

Table 6.1 Central composite face-centered design on scaled values and actual concentrations of bakery enzyme and high-pressure levels [1)]

Run	Scaled value (-) [2)]		Actual value [3)]	
	X_1	X_2	Bakery enzyme (%)	High-pressure level (MPa)
1	-1.0	-1.0	0.000	0
2	-1.0	0.0	0.000	50
3	-1.0	1.0	0.000	100
4	0.0	-1.0	0.125	0
5	0.0	0.0	0.125	50
6	0.0	0.0	0.125	50
7	0.0	0.0	0.125	50
8	0.0	0.0	0.125	50
9	0.0	1.0	0.125	100
10	1.0	-1.0	0.250	0
11	1.0	0.0	0.250	50
12	1.0	1.0	0.250	100

[1)] Sacaled and actual values of bakery enzyme and high-pressure level are shown in above Table.

[2)] X_1=(Bakery enzyme-0.125)/0.125, where the bakery enzyme* ranged from 0.00 to 0.250 (%) based on flour.

X_2=(High-pressure level-50)/50, where the high-pressure* ranged from 0 to 100 (MPa).

[3)] Bakery enzyme: actual value of bakery enzyme (%) based on flour, High-pressure level: actual value of high-pressure level (MPa).

Table 6.2 DFs and DS content of doughs with bakery enzyme and treated with high-pressure [1)]

Bread making treatments	NDF (%)	ADF (%)	NDF-ADF (%) [2)]	DS (%)
Control	1.15 ± 0.08 [a]	0.29 ± 0.04 [a]	0.86 ± 0.05 [a]	3.95 ± 0.49 [a]
BE	0.93 ± 0.12 [ab]	0.23 ± 0.14 [a]	0.69 ± 0.06 [b]	3.33 ± 0.25 [bc]
HP	1.13 ± 0.18 [ab]	0.28 ± 0.06 [a]	0.85 ± 0.12 [ab]	3.67 ± 0.24 [ab]
BE/HP	0.84 ± 0.09 [b]	0.29 ± 0.02 [a]	0.55 ± 0.07 [b]	3.05 ± 0.13 [c]

[1)] DFs: dietary fibers, DS: damaged starch, NDF: natural dertergent fiber, ADF: acid detergent fiber, BE: bakery enzymes-supplemented dough, HP: high pressure-treated dough, BE/HP: optimal bakery enzymes-supplemented and high pressure-treated dough. Each value is the mean ± SD (NDF and ADF: n=4, DS: n=8). The analysis of variance between the data was evaluated by using Tukey's multiple range test of Excel statistical software 2012. The values followed by the same letter within the column are not significantly different ($p<0.05$).

[2)] NDF-ADF: crude hemicellulose content.

Table 6.3 Soluble sugar contents of bread crumbs made from doughs with bakery enzyme and treated with high-pressure [1)]

Bread making treatments	Saccharide contents in water soluble fraction of bread crumbs (mg/g bread crumb)					
	Glucose	Fructose	Sucrose	Maltose	Reducing Sugar	Total Sugar
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]

[1)] BE: bakery enzymes-supplemented bread, HP: high pressure-treated bread, BE/HP: optimal bakery enzymes-supplemented and high pressure-treated bread. Each value is the mean ± SD (n=4). The analysis of variance between the data was evaluated by using Tukey's multiple range test of Excel statistical software 2012. The values followed by the same letter within the column are not significantly different ($p<0.05$).

Table 6.4 BMQ of doughs and bread with bakery enzyme and treated with high-pressure [1)]

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

[1)] BMQ: bread making qualities, GRD: gas retention of dough, GP: gassing power of dough, SLV: specific loaf volume, BE: bakery enzymes-supplemented dough and bread, HP: high pressure-treated dough and bread, BE/HP: optimal bakery enzymes-supplemented and high pressure-treated dough and bread. Each value is the mean ±SD (GRD: n=4, GP: n=3, SLV: n=5, Color of bread crust: n=15). The analysis of variance between the data was evaluated by using Tukey's multiple range test of Excel statistical software 2012. The values followed by the same letter within the column are not significantly different ($p<0.05$).

Figure 6.1 Photographs of appearance and scanned crumb images of breads made from doughs with bakery enzyme and treated with high-pressure [1)]

[1)] BE: bakery enzymes-supplemented bread, HP: high pressure-treated bread, BE/HP: optimal bakery enzymes-supplemented and high pressure-treated bread.

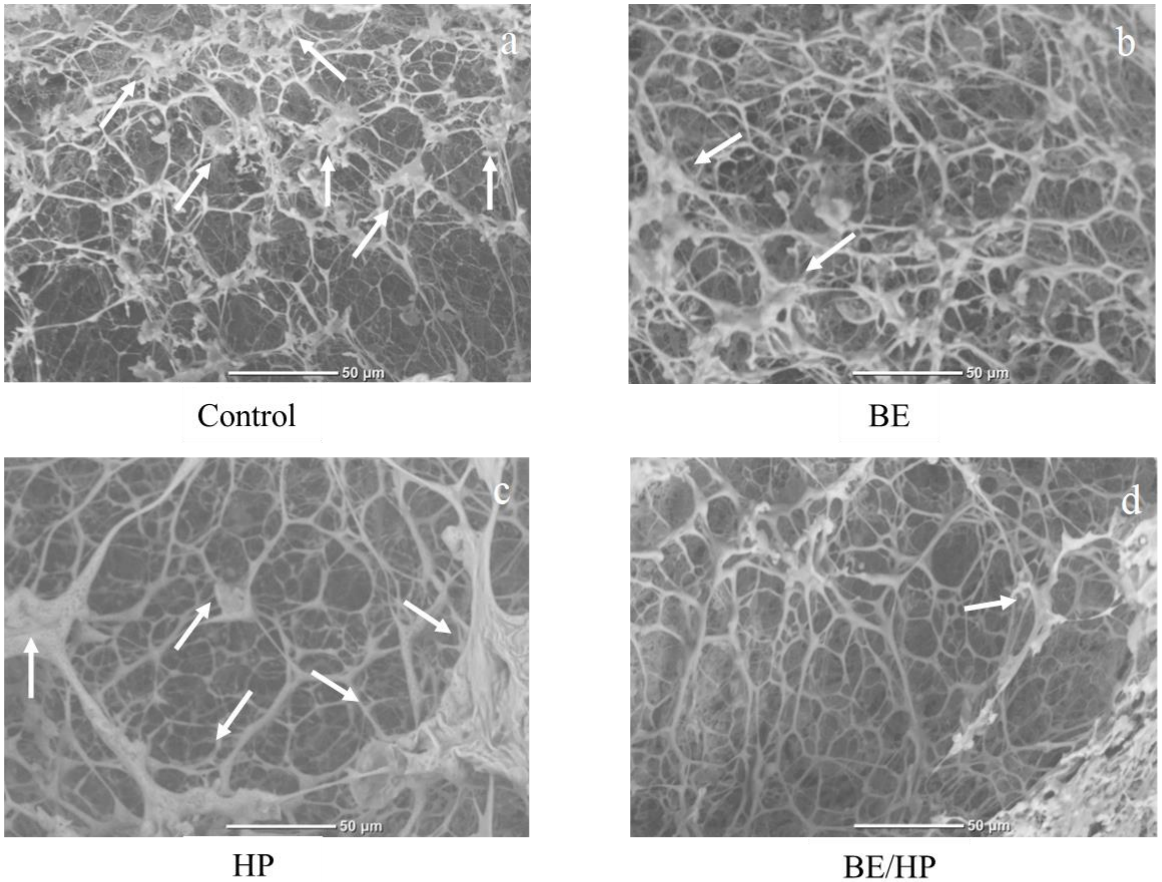


Figure 6.2 Electron microscope photographs of bread crumbs made from doughs with bakery enzyme and treated with high-pressure [1)]

[1)] 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.

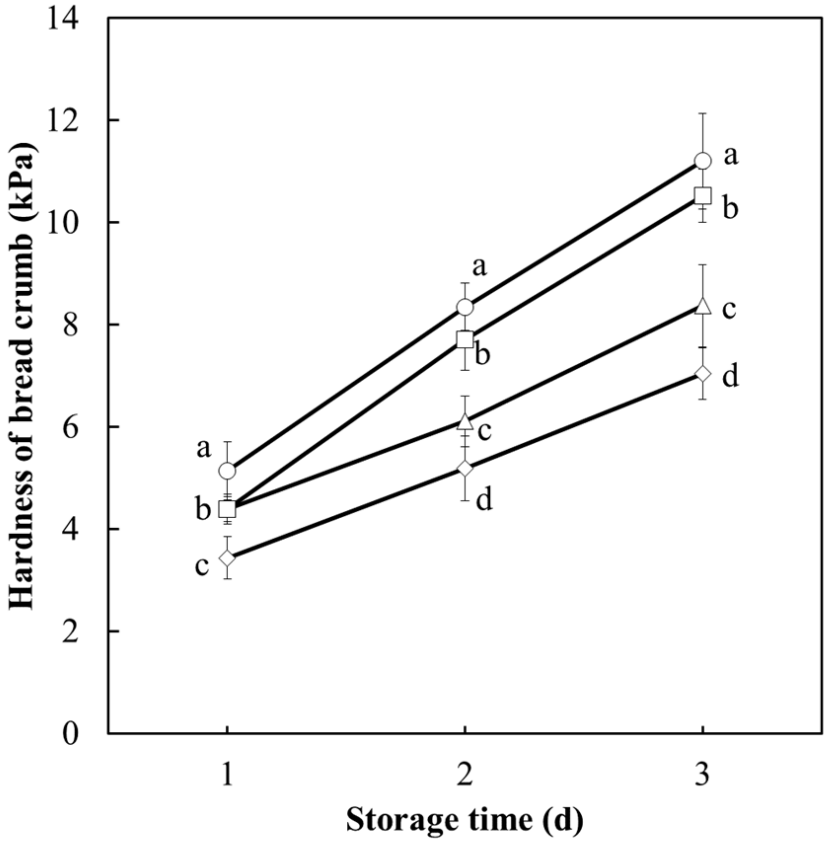


Figure 6.3 Temporal hardness changes of bread crumbs made from doughs with bakery enzyme and treated with high-pressure [1)]

[1)] 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, △: E, □: HP, ◇: E/HP. BE: bakery enzymes-supplemented bread, HP: high pressure-treated bread, BE/HP: optimal bakery enzymes-supplemented and high pressure-treated bread.

Chapter 7. Summary

The utilization of bakery enzymes and high-pressure treatment could improve BMQ such as GRD, SLV, and staling rate of bread crumb. In addition, it is expected to improve the water absorption of dough and bread qualities by adding mashed potato MP to dough. On the other hand, it is necessary to experiment with a large number of combinations in order to determine the optimum conditions for the utilization of enzymes, high-pressure treatment, and MP. Thus, in this study, response surface model RSMd was created using the data acquired, based on the CCF, and then the optimal conditions were determined by using an OT with Solver (Excel add-in software). Finally, in order to validate the effectiveness of these methods, bread making experiments with determined condition were conducted, and the effectiveness of each combination was verified from the BMQ of the dough and various evaluations of the bread.

The objective of the study in Chapter 2 was to investigate the effects of whole wheat flour substitution and enzyme treatments using AM and HC on BMQ. Results showed that the addition of whole wheat flour produced dough with low GRD and SLV. However, AM and HC drastically improved both GRD and SLV of whole wheat flour substituted dough and bread by degrading DS and hemicellulose. Thus, these results indicated that the treatments with suitable enzymes could drastically improve the BMQ of dough made with whole wheat flour.

In Chapter 3, RSM created a RSMd and Solver (Excel add-in software) calculated the optimal amounts of the enzymes. Adding optimum concentrations of AM and HC drastically improved BMQ (GRD, SLV, 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 that determines optimal concentrations of enzymes to obtain the highest quality bread when using whole wheat flour.

A method for investigating the mechanical properties of bread crumb has been established in previous studies. However, there are few reports describing the effects of using whole wheat flour and the addition of enzymes (AM and HC) on the mechanical properties of bread crumb during storage. Therefore, in Chapter 4, we investigated the effects of storage on the properties of bread crumbs by using pullman scale bread making. Rupture force, rupture deformation and rupture energy were decreased using whole wheat flour, as the higher amount of insoluble DF in whole wheat flour disturbs the fine gluten network formation in dough, resulting in a weakened bread crumb structure. In comparison, the bread crumb made from the dough with whole wheat flour treated with enzymes had a lower rupture force, a higher rupture deformation and lower viscoelastic values compared with both the Control and bread crumb made from dough with whole wheat flour since AM and HC digest DS, insoluble DFs and gelatinized starch, which decrease BMQ, resulting in improved bread crumb texture. This study elucidates the effects of using whole wheat flour and the addition of enzymes on the mechanical properties of bread crumb during storage. The addition of enzymes made it possible to obtain high quality pullman bread using whole wheat flour.

The bread substituted with MP instead of wheat flour have been attracting attention because of their added compositions such as DS, DFs, vitamins, minerals, etc. that has a beneficial effect on bread's nutritional value, texture, flavor and taste. On the

other hand, excess amount of DS and DFs in MP inhibits the gluten network formation in dough and greatly deteriorates the BMQ. In Chapter 5, we investigated the optimal addition of two types of bakery enzymes, AM and HC, to improve the BMQ of MP-added dough. The reasonable optimum addition amount of the enzymes was determined using the RSM and OT. As the results, the BMQ such as SLV and GRD, and the 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, which resulted in obtaining the good quality MP-supplemented bread with high SLV, desirable texture, flavor and taste except of crust color.

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. In Chapter 6, we 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 RSM and OT. Bread dough was prepared by the optimal condition, 0.200% 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 BMQ such as increased SLV, higher concentrations of reducing sugar, and lower concentrations of DS and insoluble DF 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 during storage.

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