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

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

Keywords Whole wheat flour · Bread making quality · Enzymes · Response surface methodology

Introduction

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

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

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gluten network, thus improving BMQ (Santiago et al. 2015a).

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

Therefore, in this study, we adopted a central composite face-centered design (CCF) (Flander et al. 2007) as a reasonable and effective method to acquire the evaluation data to determine the optimum amounts of multiple enzymes that would maximum the BMQ of dough with whole wheat flour. A response surface model (RSMd) was created using the data acquired, based on the CCF, and then the optimal amounts of multiple enzymes were determined by using an optimization technique (OT) with Solver (Excel add-in software). Finally, in order to validate the effectiveness of these methods, bread making experiments with the optimal amounts of multiple enzymes were conducted, and the effectiveness of each combination was verified from the BMQ of the dough and various evaluations of the bread.

Materials and methods

Flour and enzymes used

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

Optimal concentrations of added enzymes

A 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 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.

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

Run	Scaled value ^a		Actual concentration	
	X ₁	X ₂	AM (g/100 g flour)	HC (g/100 g flour)
1	0.0	0.0	0.1	0.1
2	0.0	− 1.0	0.1	0.0
3	− 1.0	− 1.0	0.0	0.0
4	0.0	+ 1.0	0.1	0.2
5	0.0	0.0	0.1	0.1
6	− 1.0	0.0	0.0	0.1
7	+ 1.0	+ 1.0	0.2	0.2
8	0.0	0.0	0.1	0.1
9	− 1.0	+ 1.0	0.0	0.2
10	0.0	0.0	0.1	0.1
11	+ 1.0	0.0	0.2	0.1
12	+ 1.0	− 1.0	0.2	0.0

Scaled values and actual concentrations of AM and HC are shown

AM α -amylase, HC hemicellulase

^aX₁ = (AM − 0.1)/0.1, where the actual concentration of AM ranged from 0.0 to 0.2/100 g flour, X₂ = (HC − 0.1)/0.1, where the actual concentration of HC ranged from 0.0 to 0.2/100 g flour

In this study, specific loaf volume (SLV) was adopted as the response and the amounts of added enzymes (AM and HC) were the factors in analysis of response surface methodology. 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.

Dough preparation and bread making

The Control and whole wheat flour doughs were prepared according to the formula described by Matsushita et al. (2017). The optimal amount of water was determined using a Farinograph at 500 BU according to the method used by the AACC (1991). Forty percent of the standard white wheat flour formulation used for the Control was replaced with whole wheat flour because it is the maximum percentage at which the BMQ can be improved with enzymes

(Matsushita et al. 2017). The no-time method and the standard wheat bread formulation were employed (Yamauchi et al. 2001).

Dough properties and bread evaluation

The gas retention of dough (GRD) was evaluated by measuring the maximum expansion volume of 20 g of dough proofed at 38 °C and 85% relative humidity (RH) in a cylinder subjected to low pressure from 0 to 75 cm Hg (Yamauchi et al. 2000). The gassing power (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.

DS and DF analysis

Sample preparation, before DS and DF analysis, was done according to the method reported by Santiago et al. (2015a). The DS content in dough was measured with a Megazyme assay kit (Megazyme International Ireland Ltd., Wicklow, Ireland) based on the method of Gibson et al. (1991). Neutral detergent fiber (NDF), which are cellulose, hemicellulose and lignin content and acid detergent fiber (ADF), which are cellulose and lignin content, were measured using the official AOAC (2000). The difference between NDF and ADF was calculated and used as a rough number for hemicellulose content. The DS and DF of doughs, after final proofing, were measured using eight and four samples, respectively, of the same replicate.

Bread staling evaluation

The temporal changes of crumb hardness were measured at 1, 2, and 3 days of storage (Yamauchi et al. 2001). The loaves were cut into 2 cm thick slices and a 3 × 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 × 6 cm width × 2 cm height).

Statistical analysis

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

Results and discussion

Optimal concentrations of added enzymes

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

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

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 showed minus values. Therefore, especially, when both enzymes were added to the dough in a large excess, these explanatory variables had the effect of largely lowering 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 shows 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, an Excel add-in software, were 0.128 and 0.1 g/100 g flour, respectively. In whole wheat flour dough, SLV increased with the amount of HC added, but the dough became very sticky and extremely difficult to handle, and the improving effect plateaued when added HC exceeded 0.1 g/100 g flour. Therefore, the maximum concentration of HC was limited 0.1 g/100 g flour.

BMQ evaluation

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

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

The WWF bread had significantly lower SLV than the others. On the other hand, the SLV of the WWF + E bread was significantly the highest among all breads. The 5.66 value of SLV of the WWF + E bread was very close to the 5.54 value calculated using the RSMd. The experiments verified the effectiveness of this model.

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

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

In terms of GRD and SLV, the WWF + E dough and bread had significantly the highest values among all the samples. This might be attributed to the combined catalytic activities of AM and HC that decreases DS and insoluble hemicellulose (equivalent to NDF–ADF) in the dough (Table 4). The Control dough and bread had higher GRD and SLV values despite having high DS content (4.16%),

which might be attributed to the lower values of total DF (equivalent to NDF), especially hemicellulose (equivalent to NDF–ADF) compared to the WWF dough. The GP of WWF and WWF + E doughs were significantly higher than the Control at 3 h fermentation. This may be related to that high concentrations of various nutrients in whole wheat flour promote fermentation of yeast.

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 with polished wheat flour, but SLV was increased by the addition of AM. Patel et al. (2012) also had a similar observation that the addition of fungal AM increased SLV in chemically leavened bread. Likewise, Jiang et al. (2005) reported that HC catalyzes the degradation of polysaccharides into mono-sugars and short chain saccharides, resulting in superior gluten network formation. The catalytic activity of HC may have led to higher GRD and SLV in WWF + E dough and bread compared to those with WWF. The addition of xylanase, a kind of HC enzyme, improved SLV of whole wheat flour bread (Shah et al. 2006) and a millet/wheat composite bread (Schoenlechner et al. 2013).

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

Bread color and appearance

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

In terms of crumb color, the addition of whole wheat flour significantly decreased the value of L*, while it

Table 2 Bread making qualities of doughs: the Control, WWF, and WWF + E

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

Optimal amounts of enzymes, α -amylase and hemicellulase, were added to WWF + E dough. Each value, except for water absorption, is the mean ± SD (the others: n = 6, moisture content of crumb: n = 10). The values followed by different letters within a column are significantly different ($p < 0.05$)

GRD gas retention of dough, GP gasing power of dough, SLV specific loaf volume, WWF whole wheat flour, E enzymes

^aMoisture content of crumb was measured with the samples stored 1 day into polyethylene bags after baking

Table 3 Color of the breads: the Control, WWF, and WWF + E

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

Optimal amounts of enzymes, α -amylase and hemicellulase, were added to WWF + E dough. Each value is the mean $\pm$ SD ($n = 8$). The values followed by different letters within a column are significantly different ($p < 0.05$)

WWF whole wheat flour, E enzymes, L* level of lightness, a* level of redness, b* level of yellowness

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

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

Fig. 1 The appearance and scanned crumb images of breads: the Control, WWF, and WWF + E. WWF whole wheat flour, E enzymes. Optimal amounts of enzymes, α -amylase and hemicellulase, were added to WWF + E dough

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

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

DS and DF contents of dough

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

Table 4 also shows the DF content of the various doughs. The WWF and WWF + E doughs had significantly higher values than the Control dough except for the NDF–ADF of WWF + E dough. Furthermore, the values of WWF + E dough were lower than that of WWF dough except for ADF. In addition, NDF–ADF of WWF + E dough was significantly lower compared to that of WWF dough.

Table 4 DS and DF contents of doughs: the Control, WWF, and WWF + E

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

Optimal amounts of enzymes, α -amylase and hemicellulase, were added to WWF + E dough. Each value is the mean $\pm$ SD (DS: $n = 8$, the others: $n = 4$). The values followed by different letters within a column are significantly different ($p < 0.05$)

WWF whole wheat flour, E enzymes, DS damaged starch, NDF neutral detergent fiber, ADF acid detergent fiber, NDF-ADF crude hemicellulose content

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

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

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

Hardness of bread

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

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

AM mainly breaks down DS and GSG in dough into low molecular weight dextrans and oligo-saccharides during

Fig. 2 Temporal hardness changes of bread crumbs: the Control, WWF, and WWF + E during storage. WWF whole wheat flour, E enzymes. Optimal amounts of enzymes, α -amylase and hemicellulase, were added to WWF + E dough. The vertical bar is the standard deviation of each value ($n = 8$). The symbols followed by different letters are significantly different ($p < 0.05$). Circle: Control, triangle: WWF, square: WWF + E

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 increased the content of low molecular weight saccharides (LMWSs) in bread. It was reported that these LMWSs retard the retrogradation of GSG and reduce the amount of available starch for the retrogradation in bread (Duran et al. 2001; Palacios et al. 2004; Goesaert et al. 2009). Caballero et al. (2007) and Palacios et al. (2004) also reported that the AM has an anti-staling effect on bread during the storage. Martin and Hoskeney (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 Hoskeney 1991; Martin et al. 1991). The SLV of WWF + E

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

The insoluble pentosan in dough interferes with the formation of a desirable gluten network, and HC attacks the insoluble pentosan, resulting in the improvement of BMQ. It was reported that the addition of HC improved SLV and increased LMWSs in dough (Caballero et al. 2007; Matsushita et al. 2017; Ghoshal et al. 2013). The WWF + E dough had significantly lower amounts of crude hemicellulose (NDF–ADF) than WWF dough (Table 4).

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

Overall BMQ

This study established that a treatment with an optimal amount of AM and HC drastically improves BMQ of whole wheat flour dough and bread. The most improved properties of BMQ were GRD and SLV, which increased, and the suppression of bread staling (Table 2 and Fig. 2). These WWF + E dough and bread properties were significantly improved compared to those of WWF dough and bread, which were also significantly better than the Control. On the other hand, a negative effect of the treatment was a reduction in the bread color evaluation, especially a decrease in L^* value of crust, (Table 3). In the WWF bread, the decrease in L^* value of the bread was comparable to the Control and was considered to be an acceptable characteristic. However, the addition of enzymes resulted in increased browning of the bread crust, an effect of promoting the Maillard reaction during the baking process, and lowering the bread color evaluation. This seems to be a negative effect of adding enzymes. In this study, the optimal amounts of enzymes (AM and HC) were derived using SLV as a response in an RSM and OT. The optimal value calculated for SLV using the RSMd was 5.54, which 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.

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.

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