The effects of certain enzymes on the rheology of dough and the quality characteristics of bread prepared from wheat meal

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Abstract This study was carried out to evaluate the effects of amyloglucosidase, glucose oxidase, hemicellulase (mainly consist of endo-1,4- β -xylanase), cellulase, lipase, and the combination of phospholipase and hemicellulase (phospholipase + hemicellulase) on the extensographic properties of dough and the quality characteristics of bread prepared from wheat meal. The enzymes were added separately in two different amounts. The addition of glucose oxidase (at 0.0003–0.001%) caused a significant decrease in the resistance to extension, ratio of resistance to extensibility and energy values of the wheat meal dough compared with the control dough. The addition of hemicellulase (at 0.001–0.005%) and phospholipase + hemicellulase (at 0.0006–0.0009%) also improved the wheat meal dough rheology by reducing the resistance to extension and the ratio of resistance to extensibility. Glucose oxidase (at 0.0003–0.001%), hemicellulase (at 0.001–0.005%) and phospholipase + hemicellulase (at 0.0006–0.0009%) addition improved the specific volume of wheat meal bread compared with the control bread. Increasing the dosage of glucose oxidase from 0.0003 to 0.001% caused a further increase in the specific volume of wheat meal bread. The addition of hemicellulase (at 0.001–0.005%) caused a significant decrease in the baking loss and an increase in the moisture content of wheat meal bread compared with the control bread. The addition of amyloglucosidase (at 0.000875–0.001%), lipase (at 0.0002–0.001%) and cellulase (at 0.0003–0.0005%) did not

considerably affected the dough rheological and the quality characteristics of wheat meal bread.

Keywords Bakery enzymes · Wheat meal dough · Wheat meal bread · Extensograph · Specific volume

Introduction

Bread is one of the oldest forms of prepared foods and it has been a staple source of nutrients for humans for several thousands years. As bread is one of the major constituents of the human diet, wheat is by far the most important cereal in breadmaking (Geng et al. 2016). The most of cereal products are traditionally prepared from wheat flour (WF). However, the endosperm, germ and bran parts of the wheat kernel are separated during the milling processes which causes the loss of many health beneficial micronutrients, minerals, and fibre. Whole wheat flour is one of the most common and important whole grain products. It is a rich source of dietary fibre, vitamins, minerals, and antioxidants (Hirawan et al. 2010). Epidemiological studies have reported that the long-term intake of whole grain products can reduce the risk of several chronic diseases, such as diabetes, cardiovascular diseases and cancer (Vitaglione et al. 2008). Therefore, as public awareness of eating healthy foods grows, whole grains and whole grain products, such as wheat meal (WM), are increasingly used in the production of many kinds of cereal based foods. Despite the fact that foods containing whole grain products have health beneficial effects, the production of these foods in good quality is very challenging from the standpoint of consumers' acceptance. Gan et al. (1992) reported that the public's acceptance of whole wheat food products is limited due to their poor taste and texture. As consumers'

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preferences are shifting towards the consumption of healthier foods, the bakery industry is focused on the production of food products containing whole grain products in good quality.

Various studies have been conducted to modify dough rheology and bread quality. Grosch and Wieser (1999) indicated that ascorbic acid is used as a bread improver to improve bread volume and crumb structure. Miyazaki et al. (2004) have examined the effects of dextrins on bread quality and dough properties. Gómez et al. (2004) reported that some emulsifiers, like sodium stearoyl lactylate, diacetyl tartaric acid esters of monoglycerides and polysorbate exhibit their positive effects during the mechanical handling and proofing of dough. Emulsifiers also improve bread volume and crumb structure. However, today, one of the most common alternatives in breadmaking to improve dough properties and bread quality, is the use of enzymes.

Starch and proteins are the major constituents of wheat flour and they affect the rheological properties of dough and consequently the characteristics of the final baked product (Kaur et al. 2016). Siddhartha Kumar et al. (2012) defined that glucoamylase (amyloglucosidase) hydrolyzes both 1,4- α - and 1,6- α -glucosidic linkages and releases β -D-glucose from the non-reducing ends of starch and related oligo- and poly-saccharides. Glucose oxidase is an oxidative enzyme that catalyzes the oxidation of β -D-glucose to gluconic acid and hydrogen peroxide. In breadmaking, glucose oxidase induces the formation of disulfide bonds in gluten proteins. The use of the proper dosage of glucose oxidase improves dough properties and bread quality (Bonet et al. 2006). The minor wheat flour constituents, e.g. non-starch polysaccharides, such as arabinoxylans (AX) and β -glucans, also play a significant role in the bread-making process, affecting the properties of the dough and the final baked product. AX are classified as water extractable AX (WE-AX) and water unextractable AX (WU-AX) (Courtin and Delcour 2002). Courtin et al. (2001) indicated that WU-AX are detrimental to breadmaking while WE-AX with medium to high molecular weight have a positive impact on bread volume (Shah et al. 2006). Hemicellulases, which hydrolyze pentosans, and cellulases, which hydrolyze complex cell wall carbohydrates, improve the handling properties of dough and bread quality (Harada et al. 2000). Among hemicellulase enzymes endo-xylanases and β -xylosidases are the two key enzymes responsible for the hydrolysis of xylan, the major component of hemicellulose (Juturu and Wu 2012). Xylanases are able to hydrolyze the xylan backbones of WU-AX releasing WE-AX, which have positive effects on dough and bread characteristics (Courtin and Delcour 2002). Lipids and phospholipids are also minor components of wheat kernel. Lipases hydrolyze triglycerides into monoglycerides, diglycerides, fatty acids and glycerol.

Lipases improve volume, texture and shelf-life of bread (Hasan et al. 2006). Phospholipases convert phospholipids into fatty acids and other lipophilic substances (Salehifar et al. 2012). Phospholipases provide the dough with a suitable degree of elasticity and extensibility, and also improve bread volume (Néron et al. 2004).

The main objective of our study was to evaluate the effects of the addition of commercial amyloglucosidase, glucose oxidase, hemicellulase (mainly consist of endo-1,4- β -xylanase), cellulase, lipase and the combination of phospholipase and hemicellulase (phospholipase + hemicellulase) on the extensographic properties of dough and the quality characteristics of bread prepared from WM.

Materials and methods

Materials

Commercial type WM and WF were supplied from a commercial miller in Turkey. The following enzymes used in this study were provided from an enzyme supplier in Turkey, which imported the enzymes from an enzyme manufacturer in Europe: amyloglucosidase (AGL; standardized activity: 65,000 AGI/g), glucose oxidase (GOX; standardized activity: 1500 SRU/g), hemicellulase (HML; mainly consist of endo-1,4- β -xylanase; standardized activity: 6000 EDX/g), cellulase (CLL; standardized activity: 25,000 CXU/g), lipase (LPS; standardized activity: 80,000 PLI/g), and the combination of phospholipase and hemicellulase (PHM; standardized activity: 1650 DLU/g). AGL, GOX, HML, CLL, LPS and PHM were added to WM separately (not in combinations) at two different dosages, according to the enzyme supplier's recommendations. The enzyme dosages added to WM were: AGL = 0.000875 and 0.001%; GOX = 0.0003 and 0.001%; HML = 0.001 and 0.005%; CLL = 0.0003 and 0.0005%; LPS = 0.0002 and 0.001%; PHM = 0.0006 and 0.0009% (w/w, flour weight basis). WF, WM and enzyme added WM were mixed for 1 h using a mixing machine (Type LDK M73, Apparatebau JEL J. Engelsmann AG, Ludwigshafen/Rhein, Germany) before analyses.

Characterization of wheat meal and flour

Moisture content was determined according to ICC Standard Method Nr. 110/1 (2004) using a laboratory scale oven (Köttermann Typ 2702, Germany). Ash content was determined according to ICC Standard Method Nr. 104/1 (2004) using an ash oven (Carbolite, Type: ELF 11/68, England). Protein content was determined according to AACC International Method 46-12 (2000) using an automatic distillation system (Gerhardt Vapodest 20, Type

VAP 20, Germany). The total and soluble pentosan contents were determined according to the method stated in Hashimoto et al. (1987) using an UV–visible spectrophotometer at 670 nm (Varian Cary 50 Bio UV–visible Spectrophotometer, USA). Damaged starch content was determined according to McDermott (1980) using the UV–visible spectrophotometer at 600 nm. Wet gluten quantity was determined according to ICC Standard Method Nr. 155 (2004) using a gluten washer apparatus (Yücebaş Makine, Type 111, Turkey). α -amylase activity was determined according to ICC Standard Method Nr. 107/1 (2004) using a Falling Number apparatus (Falling Number AB S-12611, Type 1402, Sweden). Chemical composition analyses of WM and WF were performed in triplicate. All chemicals and laboratory reagents used in this study were analytical grade.

Farinograph and extensograph tests

Farinograph tests were carried out according to ICC Standard Method Nr. 115/1 (2004) using the farinograph equipped with a 300 g stainless steel bowl (Mod. PL Nr. 952065, Brabender, Duisburg, Germany). Water absorption (WA, %), development time (DT, min), stability (ST, min) and degree of softening (DS, BU) were determined from the farinograph curves.

Extensograph tests were carried out according to ICC Standard Method Nr. 114/1 (2004) using the extensograph (Mod. FXEK/6 Nr. 466, Brabender, Duisburg, Germany). The parameters obtained from the extensograph curves were resistance to extension up to 50 mm (R_{50} , BU), extensibility (E_x , mm), ratio of resistance to extensibility (R_{50}/E_x) and energy (A_e , cm²). The farinograph and extensograph tests were performed in duplicate.

Breadmaking procedure

The breadmaking procedure was carried out according to the procedure described in the literature (Standard-Methoden für Getreide Mehl und Brot. 6, 1978, Verlag Moritz Schafer, Detmold, Germany) with slight modifications. In breadmaking trials, 100% flour (WM and WF), enzyme (when added), 1.2% salt, 3% compressed yeast (based on flour weight at 14% moisture), water at 30 ± 1 °C (according to the WA determined by the farinograph) were kneaded using a laboratory scale L-shaped mixer (Diosna Dierks & Söhne GmbH, Germany) at 100 rpm for 7 min (we determined the optimal kneading time by pre-studies). At the end of kneading, the leavening step was performed twice using a proofing cabinet (at 32 ± 2 °C and 75–80% relative humidity) each for 30 min. The dough was aerated in the mixer for 1 min at the end of each leavening step. After the second (last) aeration step, the dough was divided

into 500 g pieces, hand rounded, molded and proofed for 45 min at 32 ± 2 °C and 75–80% relative humidity. Baking was carried out for 35 min at 220 °C in a ventilated oven (Özköseoğlu Rotatherm Oven, Turkey) following a steam injection at the beginning.

Evaluation of bread quality

Breadmaking trials were performed in duplicate. Four bread samples were obtained at each trial and the best two of four bread samples in terms of shape uniformity from each trial were chosen for bread quality assessment. Chosen breads were cooled for 2 h at 25 ± 2 °C and then weighed before quality analyses. Quality characteristics of breads were assessed in terms of specific volume (SV, cm³/g), baking loss percentage (BL, %) and moisture content (MC, %). Loaf volume was measured according to the rapeseed displacement method using a mechanical volumeter. SV was calculated as the ratio between the volume and the mass of the bread. BL was calculated as a percentage ratio of the mass of water removed from the dough during baking and the mass of dough before baking. MC was determined according to AACC International Method 44-15A (2000). Bread quality analyses—comprised of SV, BL and MC analyses—were conducted in four replicates for each chosen bread sample. The mean values of the results of the analyses were used in statistical analyses.

Statistical analyses

The data obtained in the present study were subjected to analysis of variance (ANOVA) using the software of Statistical Package for the Social Sciences—SPSS v.15.0 (2006, SPSS Inc., USA). The mean values were compared using Tukey HSD Post Hoc Test ($P < 0.05$).

Results and discussion

Chemical composition of wheat meal and flour

Chemical composition data of WM and WF are presented in Table 1.

The ash content of WM was found to be higher than WF because of the higher bran content of WM. This was in agreement with the findings of Bruckner et al. (2001) who found that whole meal has higher ash content compared with WF. In the present study, the ash content of WF was found to be 0.62%, which is in agreement with the findings of Sakhare et al. (2014) who found 0.54–0.95% ash contents for straight run flours and bran duster flours. McCleary (1986) reported that whole meal contains 5% and WF 2.5–3% of hemicellulose. In our

Table 1 Chemical composition of wheat meal and flour

	WM	WF
Mositure (%)	11.61 ± 0.77	12.44 ± 0.21
Ash ^a (%)	1.69 ± 0.02	0.62 ± 0.02
Protein ^{a,b} (%)	10.24 ± 0.18	11.68 ± 0.18
Total pentosan ^a (%)	6.37 ± 0.11	1.35 ± 0.03
Soluble pentosan ^a (%)	1.07 ± 0.05	0.76 ± 0.07
Insoluble pentosan ^{a,c} (%)	5.30 ± 0.06	0.59 ± 0.04
Damaged starch ^a (%)	5.27 ± 0.10	5.56 ± 0.13
Gluten (%)	22.7 ± 0.40	28.1 ± 0.50
Falling number (s)	211.3 ± 4.16	343.7 ± 9.07

WM, wheat meal; WF, wheat flour

Data are expressed as the mean values ± standard deviations (n = 3)

^a As dry matter^b N × 5.7^c Calculated using the data of total and soluble pentosan contents of wheat meal and flour

study, total pentosan contents of WM and WF were found to be 6.37 and 1.35%, respectively. The total, soluble and insoluble pentosan contents of WM were found to be higher than WF, which was related with the high amount of bran content of WM. Damaged starch contents of WM and WF were found to be between 5 and 6% with no remarkable differences ($P < 0.05$). The α -amylase activity was inversely correlated to the falling number thus a low value of this parameter corresponded to a high α -amylase activity. In the present study, the falling number of WM (211.3 s) was found to be lower than WF (343.7 s). The falling number of WF was in agreement with the findings of Sakhare et al. (2014) who found 301.3–396.3 s falling numbers for straight run flours and bran duster flours.

Farinographic properties of wheat meal and flour

The farinographic properties of non-enzyme added WM and WF are presented in Table 2. The addition of AGL, GOX, HML, CLL, LPS and PHM to the WM presented slight and negligible changes in the farinographic

properties. Therefore, the farinographic properties of AGL, GOX, HML, CLL, LPS and PHM added WM are not presented.

As seen in Table 2, WA of WM was found to be higher than WF as expected. This was in agreement with the findings of Bruckner et al. (2001) who found that whole meal has higher WA compared with WF. Finney et al. (1987) reported that WA of flour was affected by the protein content, damaged starch content and the non-starch carbohydrates content of flour. In the present study, the increase in WA of WM compared with WF was most probably related with the higher bran content consequently the higher total pentosan content of WM. Schmieles et al. (2012) pointed out that the addition of whole wheat flour or wheat bran to WF causes an increase in the bran content of flour blend leading to an increase in WA (Bucella et al. 2016).

DT and ST of WM dough (WMD) were found to be higher than WF dough (WFD). This was in agreement with the findings of Sakhare et al. (2014) who found that DT and ST of bran duster flours (high extraction rate flours) were higher than straight run flours. Kaur et al. (2016) reported that dough strength (in particular DT and ST) is not positively related to the protein content of flours, from which it can be inferred that flours with higher protein content may not necessarily have higher dough strength. In a study conducted by Chaudhary et al. (2016), it was noticed that the DT and ST increased in wheat varieties as per the quantity of glutenin in their composition. This was attributed to the intermolecular disulfide bonding between the glutenin polypeptides and formation of long chain polymers exhibiting a stronger network in dough. In the present study, the increase in DT of WMD compared with WFD may be attributed to the effect of the interaction between fibre and gluten (Rosell et al. 2006). Since bran particles were able to absorb high amount of water during mixing, protein hydration was prevented; consequently, the DT of dough increased. Mudgil et al. (2016) found that DT of dough increased significantly with the addition of partially hydrolyzed guar gum to wheat flour. The increase in levels of partially hydrolyzed guar gum fortification caused an increase in DT of dough may be due to higher WA of partially hydrolyzed guar gum. They also found that a remarkable increase in DS of dough with the addition of partially hydrolyzed guar gum at 4 and 5% levels. In our study, DS of WMD was found to be lower than WFD which was similar to the findings of Rosell et al. (2006) found that ST showed negative correlation with DS for fibre enriched flour.

Table 2 Farinographic properties of wheat meal and flour

	WA (%)	DT (min)	ST (min)	DS (BU)
WM	67.3 ± 0.49	6.07 ± 0.11	7.04 ± 0.09	82.5 ± 4.95
WF	56.8 ± 0.42	1.48 ± 0.05	5.11 ± 0.11	100.0 ± 2.83

WM, wheat meal; WF, wheat flour; WA, water absorption; DT, development time; ST, stability; DS, degree of softening

Data are expressed as mean values ± standard deviations (n = 2)

Extensographic properties of dough

The extensographic properties of enzyme added WMD, non-enzyme added WMD (WMD^C) and WFD at 45, 90 and 135 min resting time periods are presented in Table 3.

R_{50} and R_{50}/E_x of WMD^C were found to be higher than WFD, and E_x and A_e of WMD^C were found to be lower than WFD, throughout 135 min resting time period (Table 3). The high amount of bran present in WM, in particular the high amount of non-starch polysaccharides content in WM could most probably cause an increase in R_{50} and R_{50}/E_x , and a decrease in E_x and A_e of dough. This was similar to the findings of Miś et al. (2012) who reported that the interactions between gluten matrix of the dough and carob fibre were strong enough to counteract the weakening effect of hydration on dough structure which resulted in an increase in resistance to extension of dough containing carob fibre. Courtin et al. (1999) pointed out that resistance to extension of dough increased and extensibility of dough decreased with increase in WU-AX content of flour (Courtin and Delcour 2002). Water unextracted solids, mainly containing WU-AX negatively affect the dough properties because they have a higher water binding capacity, which was reflected in a higher maximum resistance to extension and a smaller extensibility at maximum resistance to extension of dough (Wang et al. 2003).

R_{50} of WMD^C, WFD and enzyme added WMD increased with the increase in resting time from 45 to 135 min (regardless 90 min). At 135 min resting time, the highest R_{50} was found in WMD + AGL^H (691 BU). Diler et al. (2015) indicated that amyloglucosidase uses a part of the water during mixing, slowing down dough hydration. At 135 min resting time 0.0003 and 0.001% GOX added WMD had the lowest R_{50} among all WMD samples tested in our study. Also, no significant difference was observed between R_{50} of GOX added WMD and WFD ($P < 0.05$). In Table 3, it is apparent that R_{50} of 0.0003 and 0.001% GOX added WMD (447 and 471 BU, respectively) is very close to R_{50} of WFD (458 BU). These findings led to the conclusion that the addition of GOX causes a considerable decrease in R_{50} of WMD. This was most probably be related with the hydrogen peroxide produced during glucose oxidase reaction. The hydrogen peroxide promotes a weakening effect on the glutenin network structures, and in consequence the modification of dough viscoelastic properties (Bonet et al. 2006). However, increasing dosage of GOX from 0.0003 to 0.001% did not cause a further decrease in R_{50} of WMD. The addition of AGL at 0.000875%, HML at 0.001–0.005%, CLL at 0.0003%, LPS at 0.001% and PHM at 0.0006–0.0009% also caused a significant decrease in R_{50} of WMD compared with WMD^C at

135 min resting time ($P < 0.05$). Primo-Martín et al. (2003) indicated that xylanases soften the dough structure, which is similar to our findings. The addition of CLL at 0.0005% and LPS at 0.0002% did not show any significant effects on R_{50} of WMD compared with WMD^C at 135 min resting time ($P < 0.05$).

As expected, WFD had the highest E_x values throughout 135 min resting time among all the dough samples tested. E_x of WMD^C and enzyme added WMD samples decreased with the increasing resting time from 45 to 135 min (regardless 90 min). However, no significant difference ($P < 0.05$) was observed between E_x of WMD^C and enzyme added WMD samples at 135 min resting time, respectively (Table 3). At 135 min resting time, although not statistically significant ($P < 0.05$), E_x of 0.005% HML and 0.0006% PHM added WMD was found to be higher than all WMD samples tested in the study. Rouau et al. (1994) reported that xylanases led to moderate release of water initially absorbed by WU-AX, which caused a redistribution of water that became available for gluten leading to optimal development. Solubilisation of AX plays a positive role in dough rheology, improving the extensibility and gas retention of dough (Jiménez and Martínez-Anaya 2001). Phospholipases also provide the dough with a suitable degree of elasticity and extensibility (Néron et al. 2004). However, although not statistically significant ($P < 0.05$), E_x of 0.0009% PHM added WMD was found to be lower than E_x of 0.0006% PHM added WMD in the present study.

The highest R_{50}/E_x was found in WMD^C and 0.001% AGL added WMD, and the lowest R_{50}/E_x was found in WFD at 135 min resting time among all the doughs tested in the study (Table 3). The addition of GOX (at 0.0003 and 0.001%), HML (at 0.005%) and PHM (at 0.0006 and 0.0009%) caused a significant decrease in R_{50}/E_x of WMD compared with WMD^C at 135 min resting time ($P < 0.05$). The highest A_e was found in WFD throughout 135 min resting time period. At 135 min resting time, A_e of 0.0003 and 0.001% GOX added WMD was found to be lowest among all WMD doughs tested in our study. This finding led to the conclusion that the energy required for handling of WMD reduces with the use of GOX.

Lipases are used in breadmaking to improve dough and bread characteristics (Moayedallaie et al. 2010). However, the addition of LPS at 0.0002 and 0.001% did not considerably affect the extensographic properties of WMD, in our study. Underkofler (1972) pointed out that lipase activity in flour for baking could be undesirable because free fatty acids have a detrimental effect on dough (Lin 2008). In the present study, the addition of CLL at 0.0003 and 0.0005% did not considerably affect the extensographic properties of WMD.

Table 3 Extensographic properties of doughs at 45, 90 and 135 min resting time periods

Extensograph parameters										
R ₅₀ (BU)			E _x (mm)			R ₅₀ /E _x			A _e (cm ²)	
45 min	90 min	135 min	45 min	90 min	135 min	45 min	90 min	135 min	45 min	135 min
WMD ^C	519 ± 5.66 ^a	581 ± 8.49 ^{bc}	653 ± 7.07 ^b	87 ± 1.41 ^{bc}	77 ± 2.83 ^c	70 ± 1.41 ^b	5.97 ± 0.04 ^{ab}	7.55 ± 0.17 ^a	74 ± 4.24 ^{ab}	66 ± 0.71 ^{de}
<i>WMD</i>										
+AGL ^L	485 ± 15.56 ^{abc}	564 ± 7.07 ^c	581 ± 7.07 ^d	87 ± 4.24 ^{bc}	77 ± 4.24 ^c	70 ± 2.83 ^b	5.58 ± 0.09 ^{abc}	7.33 ± 0.31 ^a	70 ± 5.66 ^{bc}	67 ± 4.24 ^{cde}
+AGL ^H	481 ± 11.31 ^{abc}	608 ± 11.31 ^{ab}	691 ± 7.07 ^a	87 ± 0.71 ^{bc}	77 ± 1.41 ^c	77 ± 2.83 ^b	5.56 ± 0.08 ^{abc}	7.90 ± 0.01 ^a	68 ± 4.24 ^{bc}	69 ± 2.83 ^{bcde}
+GOX ^L	424 ± 4.95 ^{ef}	451 ± 15.56 ^d	447 ± 7.07 ^f	94 ± 0.71 ^b	87 ± 0.71 ^{bc}	74 ± 1.41 ^b	4.53 ± 0.08 ^d	5.22 ± 0.13 ^b	65 ± 0.71 ^{bc}	63 ± 4.24 ^e
+GOX ^H	444 ± 8.49 ^{cdef}	458 ± 11.31 ^d	471 ± 9.90 ^f	93 ± 2.83 ^b	93 ± 2.83 ^b	74 ± 0.71 ^b	4.78 ± 0.05 ^{cd}	4.93 ± 0.03 ^b	67 ± 4.24 ^{bc}	70 ± 4.24 ^{bcde}
+HML ^L	495 ± 9.90 ^{ab}	574 ± 8.49 ^{bc}	578 ± 8.49 ^d	80 ± 0.71 ^c	77 ± 4.24 ^c	74 ± 2.12 ^b	6.23 ± 0.18 ^a	7.47 ± 0.30 ^a	65 ± 2.83 ^{bc}	65 ± 2.83 ^{bcde}
+HML ^H	406 ± 7.07 ^f	564 ± 9.90 ^c	612 ± 4.24 ^{cd}	90 ± 4.24 ^{bc}	80 ± 5.66 ^{bc}	83 ± 2.83 ^b	4.52 ± 0.13 ^d	7.07 ± 0.37 ^a	60 ± 1.41 ^c	67 ± 2.83 ^{cde}
+CLL ^L	451 ± 15.56 ^{cde}	591 ± 7.07 ^{bc}	612 ± 15.56 ^{cd}	90 ± 4.24 ^{bc}	83 ± 5.66 ^{bc}	70 ± 5.66 ^b	5.02 ± 0.41 ^{cd}	7.14 ± 0.57 ^a	68 ± 2.83 ^{bc}	76 ± 2.83 ^{bc}
+CLL ^H	468 ± 5.66 ^{bcd}	639 ± 15.56 ^a	642 ± 8.49 ^{bc}	90 ± 5.66 ^{bc}	80 ± 2.83 ^{bc}	74 ± 2.83 ^b	5.21 ± 0.27 ^{bcd}	7.99 ± 0.47 ^a	70 ± 3.54 ^{bc}	76 ± 1.41 ^{bc}
+LPS ^L	475 ± 9.90 ^{bcd}	598 ± 4.24 ^{abc}	673 ± 7.07 ^{ab}	87 ± 1.41 ^{bc}	87 ± 2.83 ^{bc}	77 ± 4.24 ^b	5.46 ± 0.20 ^{abc}	6.88 ± 0.27 ^a	67 ± 3.54 ^{bc}	79 ± 2.83 ^b
+LPS ^H	471 ± 11.31 ^{bcd}	601 ± 15.56 ^{abc}	602 ± 12.02 ^d	90 ± 4.24 ^{bc}	80 ± 4.24 ^{bc}	77 ± 4.24 ^b	5.25 ± 0.37 ^{bcd}	7.53 ± 0.59 ^a	71 ± 4.24 ^{abc}	74 ± 0.71 ^{bcd}
+PHM ^L	444 ± 7.07 ^{cdef}	571 ± 11.31 ^{bc}	591 ± 8.49 ^d	94 ± 1.41 ^b	80 ± 1.41 ^{bc}	83 ± 5.66 ^b	4.73 ± 0.15 ^{cd}	7.14 ± 0.27 ^a	69 ± 2.83 ^{bc}	74 ± 3.54 ^{bc}
+PHM ^H	434 ± 15.56 ^{def}	567 ± 7.07 ^{bc}	533 ± 9.90 ^e	87 ± 2.83 ^{bc}	80 ± 2.83 ^{bc}	74 ± 1.41 ^b	4.99 ± 0.35 ^{cd}	7.09 ± 0.34 ^a	62 ± 1.41 ^{bc}	60 ± 2.83 ^{cde}
WFD	351 ± 14.85 ^g	437 ± 12.73 ^d	458 ± 7.07 ^f	137 ± 4.24 ^a	134 ± 0.71 ^a	140 ± 5.66 ^a	2.56 ± 0.03 ^c	3.27 ± 0.11 ^c	84 ± 2.83 ^a	112 ± 5.66 ^a

WMD, wheat meal dough; WMD^C, non-enzyme added wheat meal dough (control dough); WFD, wheat flour dough; +AGL^L, 0.000875% amyloglucosidase; +AGL^H, 0.001% amyloglucosidase; +GOX^L, 0.0003% glucose oxidase; +GOX^H, 0.001% glucose oxidase; +HML^L, 0.001% hemicellulase; +HML^H, 0.005% hemicellulase; +CLL^L, 0.0003% cellulase; +CLL^H, 0.0005% cellulase; +LPS^L, 0.0002% lipase; +LPS^H, 0.001% lipase; +PHM^L, 0.0006% phospholipase + hemicellulase; +PHM^H, 0.0009% phospholipase + hemicellulase addition (w/w); R_{50} , resistance to extension up to 50 mm; E_x , extensibility; R_{50}/E_x , ratio of resistance to extensibility; A_e , energy

Data are expressed as mean values ± standard deviations (n = 2); mean values within the same column with different superscripts are significantly different ($P < 0.05$); the letter “a” denotes the highest value

Fig. 1 Specific volume values of breads. WMB, wheat meal bread; WFB, wheat flour bread; (C), non-enzyme added wheat meal bread (control bread); +AGL (L), 0.000875% amyloglucosidase; +AGL (H), 0.001% amyloglucosidase; +GOX (L), 0.0003% glucose oxidase; +GOX (H), 0.001% glucose oxidase; +HML (L), 0.001% hemicellulase; +HML (H), 0.005% hemicellulase; +CLL (L), 0.0003% cellulase; +CLL (H), 0.0005% cellulase; +LPS (L), 0.0002% lipase; +LPS (H), 0.001% lipase; +PHM (L), 0.0006% phospholipase + hemicellulase; +PHM (H), 0.0009% phospholipase + hemicellulase addition (w/w). Data are expressed as mean values $\pm$ standard deviations ($n = 4$); mean values over the columns with different superscripts are significantly different ($P < 0.05$); the letter “a” denotes the highest value

Bread properties

SV values of breads are presented in Fig. 1, BL and MC values of breads are presented in Table 4. WMB represents WM bread, WMB^C represents non-enzyme added WM bread, WFB represents WF bread.

Loaf volume is one of the most important measures of bread quality (Bruckner et al. 2001). It is well known that the volume of bread produced from WM is lower than the volume of bread produced from WF. In the present study, volume values of all WM breads were found to be significantly lower than WF bread ($P < 0.05$). This was in agreement with the findings of Bruckner et al. (2001) who found that whole meal bread has lower loaf volume compared with the volume of bread produced from WF. Noort et al. (2010) pointed out that the bread produced from whole grain flour showed reduced loaf volume (Bucella et al. 2016). This was explained by the mechanical disruption of the gluten network by hard bran particles in

Table 4 Baking loss values and moisture contents of breads

	BL (%)	MC (%)
WMB ^C	13.48 $\pm$ 0.52 ^{bc}	38.85 $\pm$ 0.76 ^{bcd}
WMB		
+AGL ^L	12.04 $\pm$ 0.53 ^{def}	39.64 $\pm$ 0.37 ^{abcd}
+AGL ^H	11.78 $\pm$ 0.25 ^{efg}	39.89 $\pm$ 0.79 ^{abc}
+GOX ^L	12.78 $\pm$ 0.42 ^{cd}	39.23 $\pm$ 0.57 ^{abcd}
+GOX ^H	13.34 $\pm$ 0.31 ^{bc}	38.76 $\pm$ 0.36 ^{bcd}
+HML ^L	10.91 $\pm$ 0.26 ^g	40.53 $\pm$ 0.67 ^a
+HML ^H	11.26 $\pm$ 0.56 ^{fg}	40.21 $\pm$ 0.40 ^{ab}
+CLL ^L	13.83 $\pm$ 0.22 ^{ab}	38.21 $\pm$ 0.80 ^{de}
+CLL ^H	14.51 $\pm$ 0.42 ^a	38.43 $\pm$ 0.81 ^{cde}
+LPS ^L	12.61 $\pm$ 0.22 ^{cde}	39.58 $\pm$ 0.63 ^{abcd}
+LPS ^H	13.22 $\pm$ 0.33 ^{bc}	38.15 $\pm$ 0.77 ^{de}
+PHM ^L	12.60 $\pm$ 0.14 ^{cde}	39.42 $\pm$ 0.63 ^{abcd}
+PHM ^H	14.51 $\pm$ 0.31 ^a	38.06 $\pm$ 0.93 ^{de}
WFB	8.42 $\pm$ 0.46 ^h	37.08 $\pm$ 0.27 ^e

WMB, wheat meal bread; WMB^C, non-enzyme added wheat meal bread (control bread); WFB, wheat bread; +AGL^L, 0.000875% amyloglucosidase; +AGL^H, 0.001% amyloglucosidase; +GOX^L, 0.0003% glucose oxidase; +GOX^H, 0.001% glucose oxidase; +HML^L, 0.001% hemicellulase; +HML^H, 0.005% hemicellulase; +CLL^L, 0.0003% cellulase; +CLL^H, 0.0005% cellulase; +LPS^L, 0.0002% lipase; +LPS^H, 0.001% lipase; +PHM^L, 0.0006% phospholipase + hemicellulase; +PHM^H, 0.0009% phospholipase + hemicellulase addition (w/w); BL, baking loss; MC, moisture content

Data are expressed as mean values $\pm$ standard deviations ($n = 4$); mean values within the same column with different superscripts are significantly different ($P < 0.05$); the letter “a” denotes the highest value

dough containing high amount of bran (Gan et al. 1992). Lai et al. (1989) reported that bran added white flour binds large volume of water and so gluten is not properly hydrated. Poorly hydrated gluten causes lower loaf volume (Bruckner et al. 2001). Mudgil et al. (2016) found that the loaf volume decreased with the addition of increasing levels of partially hydrolyzed guar gum. This was attributed to the diluting effect of gluten caused by partially hydrolyzed guar gum addition. Ognean et al. (2011) indicated that the WU-AX present in bran reduce the gas retention capacity of dough and volume of bread. Protein content and amylase activity of flour also played an important role on the loaf volume. Chaudhary et al. (2016) found that loaf volume was positively influenced by protein content. Cauvain and Young (2001) pointed out that flour with low falling number produced weaker dough and lower loaf volume (Chaudhary et al. 2016).

Specific volume which comprises loaf volume and loaf weight is an important parameter to analyze the quality of bread (Mudgil et al. 2016). Since a remarkable decrease or

increase in specific volume is due to the strength of the gluten network, specific volume can be elucidated as the potential of gluten network to entrap CO₂ produced during proofing in breadmaking (Chaudhary et al. 2016).

As seen in Fig. 1, SV of WFB (2.51 cm³/g) was found to be significantly higher than WMB^C (1.66 cm³/g) and enzyme added WMB samples (varied between 1.57 and 1.83 cm³/g), in the present study ($P < 0.05$). This result was an expected result because the total pentosan content of WM was found to be higher than WF, and the protein content and falling number values of WM were found to be lower than WF. This was also in agreement with the findings of Miš et al. (2012) who reported that fibre-rich breads were characterized by reduced volume.

In the present study, the addition of GOX at 0.001% significantly improved SV of WMB compared with WMB^C ($P < 0.05$). Although not statistically significant, SV of 0.0003% GOX added WMB was found to be higher than SV of WMB^C ($P < 0.05$). Additionally, the increasing dosage of GOX from 0.0003 to 0.001% caused a further increase in SV of WMB. These findings indicate that the use of proper dosage of GOX improves the volume of WMB. This effect is most probably related with the positive effects of GOX on WMD rheology (Table 3).

Courtin and Delcour (2002), Martínez-Anaya and Jiménez (1997, 1998) and Rouau et al. (1994) indicated that the utilization of xylanases exhibits positive effects in baking. Shah et al. (2006) reported that the addition of xylanases increased SV of WMB, significantly. In our study, the addition of HML at 0.001 and 0.005% significantly improved SV of WMB compared with WMB^C ($P < 0.05$). Ognean et al. (2011) indicated that the conversion of WU-AX in WE-AX by xylanases has positive effects on bread characteristics. Gan et al. (1995) reported that WE-AX form highly viscous solutions in dough aqueous phase which they increase the stability of the liquid films surrounding gas cells. This phenomenon prevents the gas diffusion from dough and increases the gas retention capacity of dough (Courtin and Delcour 2002). Therefore, volume of bread increases. Although not statistically significant ($P < 0.05$), SV of 0.005% HML added WMB was found to be lower than SV of 0.001% HML added WMB, in the present study.

Néron et al. (2004) reported that phospholipases provide the dough with a suitable degree of elasticity and extensibility resulting in an increase in bread volume. In our study, the addition of PHM (at 0.0006 and 0.0009%) significantly improved SV of WMB compared with WMB^C ($P < 0.05$). Although not statistically significant ($P < 0.05$), SV of 0.0009% PHM added WMB was found to be lower than SV of 0.0006% PHM added WMB.

In the present study, WMB samples containing 0.000875% AGL and 0.0002% LPS exhibited lower SV values than WMB^C, significantly ($P < 0.005$). Free fatty acids and other lipid degradation products produced during lipase reaction in dough can act as foam destabiliser, which causes reduced bread volume (Gan et al. 1995). The addition of 0.001% AGL and 0.001% LPS did not cause any significant differences in SV of WMB samples compared with WMB^C. Although not statistically significant ($P < 0.05$), SV of 0.001% AGL added WMB was found to be higher than SV of 0.000875% AGL added WMB. Similarly, SV of 0.001% LPS added WMB was found to be higher than SV of 0.0002% LPS added WMB. The addition of 0.0003 and 0.0005% of CLL did not significantly improve SV of WMB compared with WMB^C ($P < 0.05$). However, although not statistically significant ($P < 0.05$), SV of 0.0003% CLL added WMB was found to be higher than SV of WMB^C.

During baking, when dough is transformed into bread, one of the most significant phenomena is moisture loss. If too much moisture is lost during the baking, the product become underweight. In addition, water loss during the baking process has disadvantageous effects on the freshness of baked get staled (Kotoki and Deka 2010). In our study, all WMB samples exhibited higher BL than WFB (Table 4). This was most probably related to the higher WA of WM than WF (Table 2). The BL of WFB and WMB^C was found to be 8.42 and 13.48%, respectively. The BL of enzyme added WMB samples varied between 10.91 and 14.51%. The BL of 0.001 and 0.005% HML, and BL of 0.000875 and 0.001% AGL added WMB samples were found to be significantly lower than WMB^C ($P < 0.05$). Among all WMB samples, the lower BL values were observed in WMB samples containing 0.001 and 0.005% HML. Our findings on BL of HML added WMB samples are most probably related to highly viscous solutions in the dough aqueous phase formed by WE-AX with the addition of HML. We assumed that highly viscous solutions in the dough aqueous phase prevented the release of water during baking. The BL of WMB samples containing 0.0005% CLL and 0.0009% PHM was found to be significantly higher than WMB^C ($P < 0.05$). Among all WMB samples, the highest BL values were observed in WMB samples containing 0.0005% CLL and 0.0009% PHM. It should be noticed that the second highest BL was observed in WMB containing 0.0003% CLL among all WMB samples. We assumed that the water absorbed by bran particles in WMD could be released by the hydrolyzation of complex cell wall carbohydrates with the addition of CLL, from which more water was removed from dough during baking. Therefore, the BL values of CLL added WMB samples were found to be higher than WMB^C, in our study.

MC of WFB and WMB^C was found to be 37.08 and 38.85%, respectively. MC of enzyme added WMB samples varied between 38.06 and 40.53%. The peak values of MC were observed in WMB samples containing 0.001 and 0.005% HML. This finding is most probably related with the formation of highly viscous solutions in the dough aqueous phase with the addition HML. The formation of highly viscous solutions in the dough aqueous phase prevents the release of water during baking.

Conclusion

The results obtained in our study clearly indicated that the use of glucose oxidase improved the extensographic properties of dough prepared from wheat meal. The addition of glucose oxidase, hemicellulase and phospholipase + hemicellulase to wheat meal increased the loaf volume, considerably. These findings led to the conclusion that glucose oxidase, hemicellulase and phospholipase + hemicellulase can be used to improve the characteristics of dough and bread prepared from wheat meal. However, it should be noticed that enzymes can exhibit unexpected or unpredictable effects on the characteristics of dough and bread, depending on many factors such as substrate specificity and action pattern of enzymes, activity and dosage of enzymes, and the type of flour and flour quality, in breadmaking. Therefore, further research is needed to confirm the results that we obtained in the present study.

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