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Effect of a Maltogenic Amylase and High-Performing Maltogenic Amylase on Freshness and Other Quality Attributes of White Sandwich Bread

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Abstract

The effects of a maltogenic amylase and a high-performing maltogenic amylase were compared to a control (no enzyme added), and were evaluated for their effect on the quality and shelf life of white sandwich bread. The maltogenic amylase and high-performing maltogenic amylase were tested at 0.0025%, 0.0050%, and 0.0100%. Breads were evaluated by bread moisture, loaf volume, crust and crumb color, firmness, springiness by texture analyzer, foldability, ball kneading test (for crumbliness/cohesiveness), and sensory. Both maltogenic amylases extended the shelf life of white sandwich bread over 21 days of shelf life at room temperature. Breads with both enzymes were significantly softer in slice firmness than the control on day 21. Bread with 0.0050% and 0.0100% high-performing maltogenic amylase rated significantly moister and better in foldability than control, and prolonged softness in slice and loaf firmness by 20 days. Overall, the high-performing maltogenic amylase was found to be more effective than the maltogenic amylase in extending freshness of bread, in terms of prolonging softness and resilience.

Keywords: Extended shelf life, softness, resilience, foldability, springiness, enzymes.

Study Area: Food Microbiology.

1. INTRODUCTION

Staling of baked goods causes significant food waste all over the world [1-2]. Staling of bakery products can result in moisture migration from the crumb to the crust, decreased resilience, increased firmness of the crumb, reduced crispness of the crust, and a decrease in the intensity of a pleasant taste and smell [3-4], which can negatively affect the shelf life of a product. Consumers look for baked goods that do not stale quickly and have a similar taste and texture to fresh baked goods [4]. Important qualities for bread shelf life include softness, springiness, and foldability, where soft and springy bread that folds without breaking or cracking is desirable. During staling, bread becomes harder and tends to break when folded, which is unacceptable for shelf life [5]. During the staling process, there is a gradual retrogradation (recrystallization) of branched starch chains (amylopectin) [6]. The baked goods industry has been evolving towards products with cleaner labels, prolonged softness, and fresher taste. Enzymes can provide clean label solutions for extending the shelf life of baked goods.

Enzymes are proteins with catalytic properties, and can be found in plants, animals, and microorganisms. Enzymes are specific to their substrate and the bonds that they cleave, can be used in very low doses to catalyze reactions, and are used as natural solutions for brewing, juice, dairy, and baking applications. In baking, enzymes can be used to improve dough handling, reducing fat, reducing eggs, and increasing volume, softness, and overall shelf life in baked goods. Enzyme activity can be affected by several factors including pH, temperature, salt, sugar, water activity, and ionic strength [7-8].

α-amylases make up 25% of the industrial enzyme market [9]. α-amylases (EC 3.2.1.1) are endoenzymes that speed up the cleavage of α-1,4-glycosidic bonds in the interior part of the amylose or amylopectin chain. Different α-amylases can have diverse thermal stability profiles and can vary in the resulting products produced due to differences in the number of binding sites and location of the catalytic regions [10].

Maltogenic α-amylases (EC 3.2.1.133) are exoenzymes that mainly release maltose from starch, by hydrolyzing α-1,4 glucosidic linkages to remove successive maltose units from non-reducing ends of the starch chains [10]. Maltogenic amylases are commonly used in the food industry for extending shelf life by delaying staling in baked goods [11], such as prolonging freshness in tortillas without gumminess [12]. Maltogenic amylases delay staling by shortening the amylopectin chains, which slows the retrogradation or reassociation of amylopectin chains [5]. Maltogenic amylases prefer cyclodextrins to starch or pullulan as substrates [13].

Different maltogenic amylases have been evaluated in the literature for their effects on bread and tortillas [14-16]. Little research has been published on the effects of maltogenic amylases on bread loaf firmness (as measured with an inverted V-shaped probe to simulate a consumer squeezing a loaf of bread) and ball kneading test. Slice firmness (also called crumb firmness) is more commonly measured.

This study evaluated the effects of a high-performing maltogenic amylase and a maltogenic amylase compared to a control (no enzyme added) in extending the freshness and other quality attributes of white sandwich bread. The effects of these two enzymes were evaluated on bread quality including slice firmness, loaf firmness, crumbliness/cohesiveness by a ball kneading test, and changes in the slice and loaf firmness over time.

2. MATERIALS AND METHODS

2.1 Bread

2.1.1 Materials

Wheat flour of 11.5-12.1% protein and 0.49-0.55% ash, salt, sugar, calcium propionate, yeast, shortening, and filtered tap water was used for the testing. The maltogenic amylase (SEBake Fresh 10P) and high-performing maltogenic amylase (SEBake Fresh Ultra) were supplied by Enzyme Innovation (a division of Specialty Enzymes & Probiotics). Both maltogenic amylases had a declared activity of 10,000 MANU/g.

2.1.2 Experimental procedures

2.1.2.1 Wheat flour quality

Wet gluten (moist gluten) was measured using AACC Method 38-10 [17]. Moisture of flour was tested using a loss on drying method at 140^{o}C using the auto mode. Diastatic power was tested using the Diastase (DP^o) method from the Food Chemicals Codex [18].

2.1.2.2 Baking conditions

Bread was made using a no-time dough process, which was adapted from Pyler and Gorton [19] and AACC Method 10-10B [20] with modifications. See Table 1 for the formulas tested. Dough was mixed for 3 minutes on speed 1 (60 rpm) and 6 minutes on speed 2 (90 rpm) to a dough temperature of 28 to 30^{o}C. The dough was then bulk fermented for 20 minutes of floor time at room temperature, proofed at 38^{o}C and 85%RH for 60 minutes, and baked at 218^{o}C for 19 minutes in a rotating rack oven. Baked bread was cooled for one hour on a cooling rack before storing in low density polyethylene bags sealed with a twist tie. Breads were stored at room temperature (23-26°C).

Table 1. Formulas used for breads tested (on a flour weight basis).

Ingredients	B1	B2	B3	B4	B5	B6	B7
Wheat flour	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
Sugar	4.0%	4.0%	4.0%	4.0%	4.0%	4.0%	4.0%
Instant yeast	2.0%	2.0%	2.0%	2.0%	2.0%	2.0%	2.0%
Shortening	2.0%	2.0%	2.0%	2.0%	2.0%	2.0%	2.0%
Salt	1.0%	1.0%	1.0%	1.0%	1.0%	1.0%	1.0%
Calcium propionate	0.5%	0.5%	0.5%	0.5%	0.5%	0.5%	0.5%
Maltogenic amylase	---	0.0025%	0.0050%	0.0100%	---	---	---
High-performing maltogenic amylase	---	---	---	---	0.0025%	0.0050%	0.0100%
Water	60.0%	60.0%	60.0%	60.0%	60.0%	60.0%	60.0%

2.1.2.3 Dough and Bread Quality

Bread moisture (by loss on drying) was measured using a halogen moisture analyzer (DSH-10A) at 135°C with the auto mode. Bread was ground using a coffee grinder for 5-10 seconds (until finely ground). The ground bread was used for testing bread moisture, and samples were run in duplicate. Dough pH was measured using a HALO wireless pH meter.

Loaf volume was measured in mL using a volume analyzer. Specific volume was calculated as $Specific\ Volume = \frac{(Loaf\ Volume\ in\ mL)}{(Bread\ Weight\ in\ g)}$. Breads were tested in duplicate.

Crust and crumb color were measured using a chroma meter with a 2° observer and C illuminant. Color values were reported as CIELAB L*a*b* values. For each test, crust color readings were run in triplicate, and crumb color readings were run in quadruplicate.

2.1.2.4 Bread Texture Analysis

Breads were sliced with a bread slicer to a slice thickness of 16mm for texture analysis. Breads slices were evaluated using a texture analyzer for slice firmness, springiness, and loaf firmness. Slice firmness was measured using a method modified from AACC Method 74-09 [21]. Two bread slices were stacked together for each test sample, and a 75 mm compression plate was used to compress the bread by 6.2 mm at a test speed of 250mm/min. Samples were run in triplicate for slice firmness. Lower firmness values represent softer breads. For springiness, the same conditions in the firmness test were used in a second compression cycle after a 30 second pause to give the bread time to spring back after compression. Springiness was calculated as a $\frac{F_2}{F_1} \times 100\%$, where F_1 is the force to compress the bread to 6.2 mm in the first compression cycle and F_2 is the force to compress the bread to the same distance in the second compression cycle. Higher springiness values represent springier breads. Bread loaves were tested for loaf firmness using an inverted V-shaped probe, where each bread loaf was compressed by 25 mm at a test speed of 250mm/min. Samples were run in duplicate for the loaf firmness test. Texture analysis was run after 1, 7, 14, and 21 days of room temperature storage.

2.1.2.5 Ball Kneading Test

A ball kneading test was adapted from Stöllman and Lundgren [22] with modifications. A 45 mm circular cookie cutter was used to cut a consistent sample of bread crumb from the center of each test bread. The cut portion of bread crumb was kneaded into a ball in 7 revolutions, and the ability to form a ball was rated on a scale of 1 to 5, where 1=all crumbled, 3=half-crumbled ball, and 5=round ball. Tests were run in duplicate.

2.1.2.6 Sensory

Breads were evaluated by a sensory panel for crumb grain, aroma, springback, foldability, softness, mouthfeel, and taste. Tests for springback and softness were adapted from Stöllman and Lundgren [22] with modifications. Samples were presented with randomized 3-digit codes and were rated on a subjective scale of 1 to 9. See Table 2 for more information on the sensory attributes tested.

Table 2. Bread sensory attributes were tested using the following procedures and scoring.

Sensory Attribute	Method	Scoring
Crumb grain	Evaluate the overall size of the air cells in the bread crumb.	9= Fine, compact 5= Medium 1= Coarse, open-grain crumb
Aroma	Rate the smell of the bread.	9= Pleasant 5= Neutral 1= Strong off-flavor
Springback	Press down on the bread crumb in the center of the bread slice halfway with finger, and hold for 5 seconds. Evaluate the speed of springback.	9= Fast (≤ 1 second) 5= Medium (5 seconds) 1= Slow (≥ 10 seconds)
Foldability	Fold the bread sample in the middle, and rate how well the bread folds without breaking.	9= Slice stays completely intact 5= Half of slice stays intact 1= Breaks completely
Softness	Bite through the crumb with your front teeth from the center of the slice, and evaluate its softness.	9= Soft 1= Hard
Mouthfeel	Evaluate the mouthfeel by the moistness/dryness of the bread.	9= Soft and moist 5= Neither dry nor moist 1= Dry and firm
Taste	Rate the flavor of the bread.	9= Pleasant 5= Neutral 1= Strong off-flavor

2.2 Data Analysis

A one-way ANOVA was used to analyze the data, and a Tukey's t-test was used for comparing the values using a program from Assaad et al. [23]. Differences were considered statistically significant at a p value < 0.05.

3. RESULTS AND DISCUSSION

3.1 Bread

3.1.1 Flour Quality

The wheat flour used had 31.7% ± 0.4% wet gluten (SEM, n=2), and 10.46% ± 0.06% moisture (SEM, n=2). Diastase activity was 1,170 ± 67 DP°/g (SEM, n=2).

3.1.2 Dough and Bread Quality

Table 3 shows the dough pH, bread moisture, volume, and color data for breads made with the maltogenic amylase (B2-B4) and high-performing maltogenic amylase (B5-B7), and the control (B1, bread made without maltogenic amylase). As shown, the maltogenic amylase and high-performing maltogenic amylase did not have a significant effect on dough pH, bread moisture, crust color, and crumb color. Overall, volume and specific volume were similar for most of the breads (B1-B2, and B4-B7). 0.0050% maltogenic amylase (B3) was 6.0% higher in loaf volume and 6.5% higher in specific volume than the control (B1), and this difference was statistically significant.

Table 3. Dough pH, bread moisture, volume, and color data.

	B1	B2	B3	B4	B5	B6	B7
Dough pH	5.80 ± 0.01[a]	5.82 ± 0.05[a]	5.82 ± 0.04[a]	5.82 ± 0.03[a]	5.80 ± 0.00[a]	5.83 ± 0.03[a]	5.81 ± 0.01[a]
Moisture %	34.0% ± 1.0% [a]	35.0% ± 0.5% [a]	34.9% ± 0.0% [a]	34.7% ± 0.3% [a]	35.8% ± 0.1% [a]	34.4% ± 0.4% [a]	35.5% ± 0.0% [a]
Loaf Volume (mL)	1840 ± 18[b]	1860 ± 17[ab]	1950 ± 31[a]	1930 ± 4[ab]	1840 ± 2[b]	1890 ± 20[ab]	1910 ± 15[ab]
Specific Volume (mL/g)	4.10 ± 0.04[b]	4.15 ± 0.04[ab]	4.37 ± 0.07[a]	4.32 ± 0.01[ab]	4.11 ± 0.01[b]	4.23 ± 0.05[ab]	4.28 ± 0.04[ab]
Crust Color (L*)	40.06 ± 0.37[a]	40.65 ± 0.13[a]	41.05 ± 0.41[a]	40.89 ± 0.05[a]	40.71 ± 0.04[a]	40.56 ± 0.14[a]	40.89 ± 0.24[a]
Crust Color (a*)	15.24 ± 0.07[a]	15.55 ± 0.01[a]	15.36 ± 0.08[a]	15.37 ± 0.04[a]	15.21 ± 0.16[a]	15.36 ± 0.05[a]	15.28 ± 0.08[a]
Crust Color (b*)	21.70 ± 0.61[a]	22.59 ± 0.03[a]	22.55 ± 0.30[a]	22.55 ± 0.09[a]	22.09 ± 0.04[a]	22.35 ± 0.16[a]	22.52 ± 0.30[a]
Crumb Color (L*)	78.73 ± 0.23[a]	78.69 ± 0.09[a]	78.27 ± 0.02[a]	78.06 ± 0.35[a]	78.38 ± 0.23[a]	78.14 ± 0.06[a]	78.13 ± 0.02[a]
Crumb Color (a*)	-1.37 ± 0.04[a]	-1.34 ± 0.04[a]	-1.54 ± 0.03[a]	-1.47 ± 0.02[a]	-1.43 ± 0.06[a]	-1.41 ± 0.04[a]	-1.38 ± 0.01[a]
Crumb Color (b*)	20.40 ± 0.07[a]	20.66 ± 0.47[a]	20.50 ± 0.23[a]	20.54 ± 0.09[a]	20.36 ± 0.02[a]	20.62 ± 0.16[a]	20.41 ± 0.04[a]

Values are means ± SEM, n = 2 per treatment group.
Means in a row without a common superscript letter differ (P<0.05) as analyzed by one-way ANOVA and the TUKEY test.

Fig. 1 shows a side view of the bread loaves from each test. Fig. 2 shows a cross-section of cut bread loaves from each test. The visual crust and crumb color were overall similar between tests.

Figure 1. Side view of bread loaves (B1-B7 from left to right).

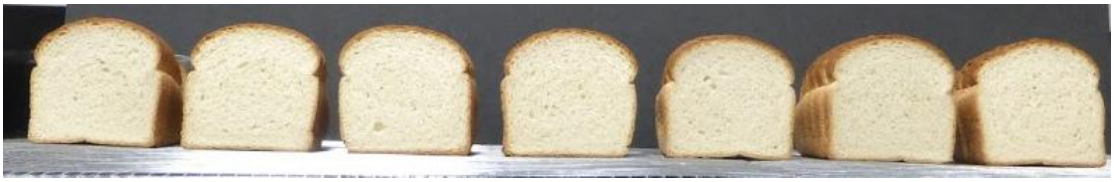

Figure 2. Side view of bread loaves (B1-B7 from left to right).

3.1.3 Bread Texture Analysis

Fig. 3 and Table 4 show the effect of the maltogenic amylase and high-performing maltogenic amylase on bread slice firmness over 21 days of room temperature shelf life. On day 1, breads with added enzyme (B2-B7) were 22-38% softer in slice firmness than the control (B1), and this difference was statistically significant (see Table 4). On day 21, breads with added enzyme (B2-B7) were significantly softer in slice firmness than the control (B1), and bread with 0.0100% high-performing maltogenic amylase was the softest bread (lowest slice firmness value). The difference in slice firmness between breads with maltogenic amylase (B2-B4) and high-performing maltogenic amylase (B5-B7) on day 21 was not statistically significant, when compared at the same dosage. Breads with high-performing maltogenic amylase were 55-73% softer in slice firmness than the control. 0.0050% and 0.0100% high-performing maltogenic amylase prolonged softness in slice firmness by 20 days, in that these tests had slice firmness values on day 21 that were similar to the slice firmness of the control on day 1.

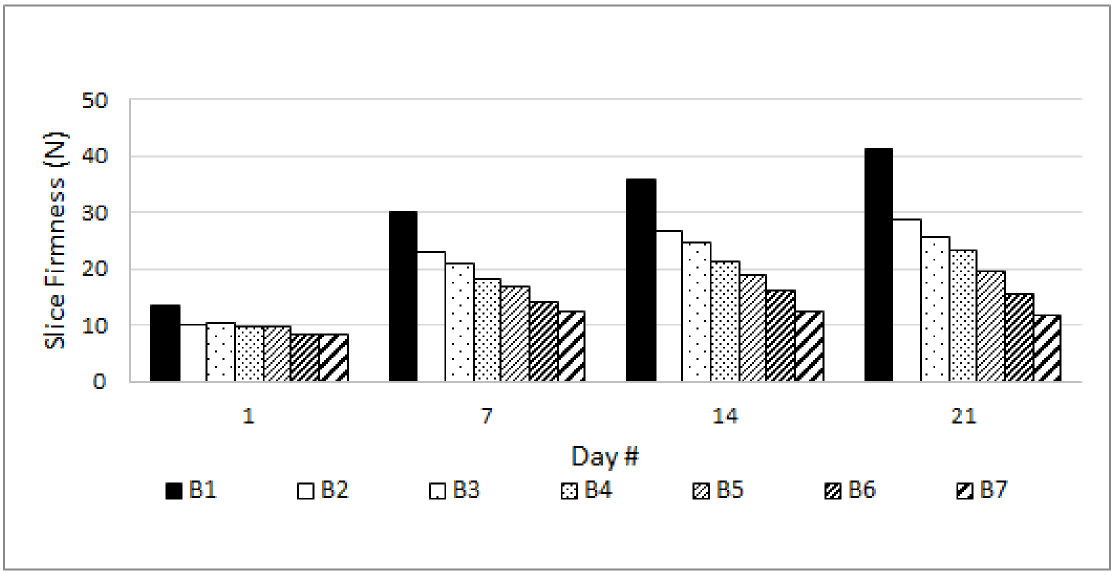

Figure 3. Bread slice firmness over 21 days of room temperature storage.

In addition, the breads with added enzyme (B2-B7) staled at a slower rate in slice firmness, in that their increase in hardness by slice firmness was lower than the control (B1). Interestingly, the breads with high-performing maltogenic amylase (B5-B7) staled more slowly (had a smaller increase in slice firmness after 21 days of shelf life) than the breads with maltogenic amylase (B2-B4), when compared at the same dosage. Specifically, breads with high-performing maltogenic amylase were 1.4 – 2.0 times harder in slice firmness on day 21 than day 1, while breads with maltogenic amylase were 2.5 – 2.8 times harder in slice firmness on day 21 than day 1 (see Table 4). Thus, the high-performing maltogenic amylase was more effective in delaying staling in slice firmness than the maltogenic amylase.

Table 4. Bread slice firmness (N) over 21 days of room temperature storage.

Day	B1	B2	B3	B4	B5	B6	B7
1	13.30 ± 0.81^{a}	10.20 ± 0.24^{b}	10.30 ± 0.43^{b}	9.84 ± 0.97^{b}	9.61 ± 0.30^{b}	8.42 ± 0.38^{b}	8.28 ± 0.51^{b}
7	30.10 ± 0.73^{a}	22.90 ± 1.01^{ab}	20.90 ± 1.53^{bc}	18.30 ± 2.81^{bd}	16.80 ± 1.54^{bd}	14.20 ± 1.59^{cd}	12.40 ± 0.63^{d}
14	36.00 ± 3.61^{a}	26.70 ± 1.57^{ab}	24.60 ± 1.20^{b}	21.40 ± 3.26^{bc}	19.00 ± 1.46^{bc}	16.10 ± 2.04^{bc}	12.60 ± 1.10^{c}
21	41.20 ± 4.36^{a}	28.60 ± 1.48^{b}	25.60 ± 2.13^{bc}	23.40 ± 3.34^{bd}	19.60 ± 1.54^{bd}	15.40 ± 2.74^{cd}	11.80 ± 1.11^{d}

Values are means ± SEM, n = 3 per treatment.
Means in a row without a common superscript letter differ (P<0.05) as analyzed by one-way ANOVA and the TUKEY test.

Fig. 4 and Table 5 show the effect of the maltogenic amylase and high-performing maltogenic amylase on bread springiness over 21 days of room temperature shelf life. Bread with 0.0100% maltogenic amylase, and 0.0025-0.0100% high-performing maltogenic amylase (B4-B7) were significantly springier than the control (B1) on day 7. Bread with highest springiness values was bread with 0.0100% high-performing maltogenic amylase (B7), which was significantly springier than the control (B1) on both days 7 and 14. At day 21, breads with maltogenic amylase and high-performing maltogenic amylase (B2-B7) had higher springiness values than the control (B1), but the differences in springiness from the control were not statistically significant. Overall, the high-performing maltogenic amylase was more effective at maintaining springiness than the maltogenic amylase.

Table 5. Springiness of bread over 21 days of room temperature shelf life.

Day	B1	B2	B3	B4	B5	B6	B7
1	93.1% ± 0.8%^{b}	94.4% ± 0.2%^{ab}	94.7% ± 0.6%^{ab}	95.3% ± 0.6%^{ab}	95.5% ± 0.1%^{ab}	95.8% ± 0.3%^{a}	95.2% ± 0.5%^{ab}
7	90.9% ± 0.5%^{b}	91.7% ± 0.8%^{b}	93.1% ± 0.2%^{ab}	94.0% ± 0.4%^{a}	94.1% ± 0.1%^{a}	94.7% ± 0.7%^{a}	95.0% ± 0.1%^{a}
14	92.5% ± 0.4%^{c}	93.0% ± 0.3%^{bc}	92.7% ± 0.8%^{bc}	94.7% ± 0.2%^{ac}	94.4% ± 0.7%^{ac}	95.3% ± 0.7%^{ab}	95.9% ± 0.5%^{a}
21	92.5% ± 0.9%^{a}	93.3% ± 0.2%^{a}	94.3% ± 0.2%^{a}	95.4% ± 0.3%^{a}	94.5% ± 0.4%^{a}	94.8% ± 1.3%^{a}	95.7% ± 0.8%^{a}

Values are means ± SEM, n = 3 per treatment group.
Means in a row without a common superscript letter differ (P<0.05) as analyzed by one-way ANOVA and the TUKEY test.

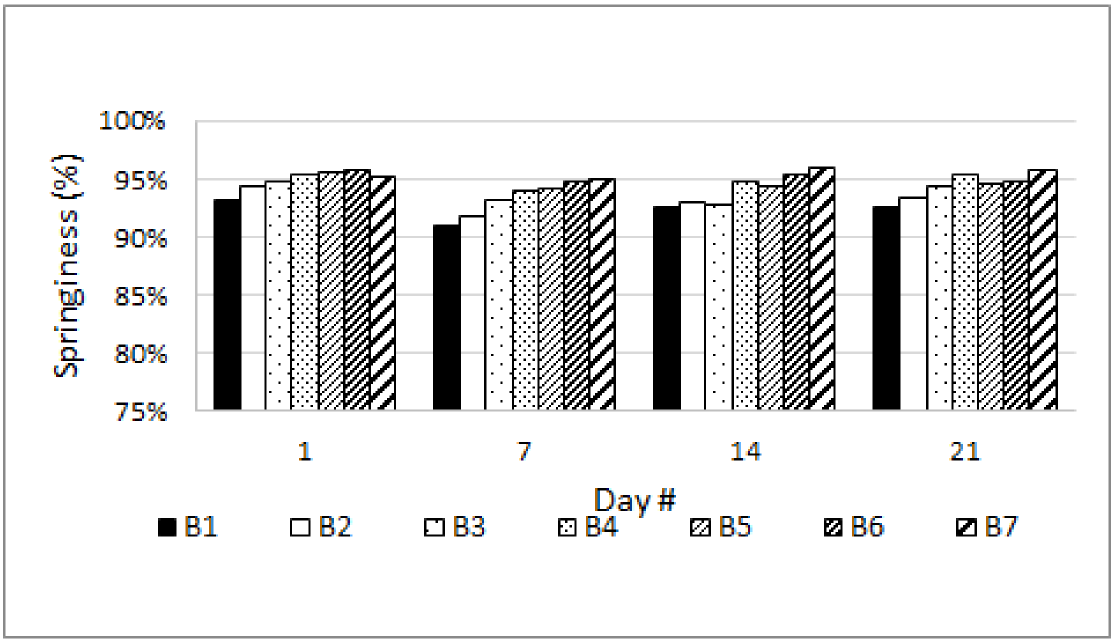

Figure 4. Springiness of bread over 21 days of room temperature shelf life.

Fig. 5 and Table 6 show the effect of the maltogenic amylase and high-performing maltogenic amylase on loaf firmness. On day 21, 0.0100% maltogenic amylase (B4) and 0.0050%-0.0100% high-performing maltogenic amylase (B6-B7) were significantly softer in loaf firmness than the control (B1). 0.0100% maltogenic amylase and 0.0025%-0.0100% high-performing maltogenic amylase (B4-B7) prolonged softness by 20 days, in that these tests had loaf firmness values on day 21 that were similar in loaf firmness to the control on day 1. This suggests that the high-performing maltogenic amylase was more effective at prolonging softness in loaf firmness than the maltogenic amylase since a lower dosage of high-performing maltogenic amylase (0.0025%) can be used to prolong softness in loaf firmness by 20 days than the maltogenic amylase (0.0100%).

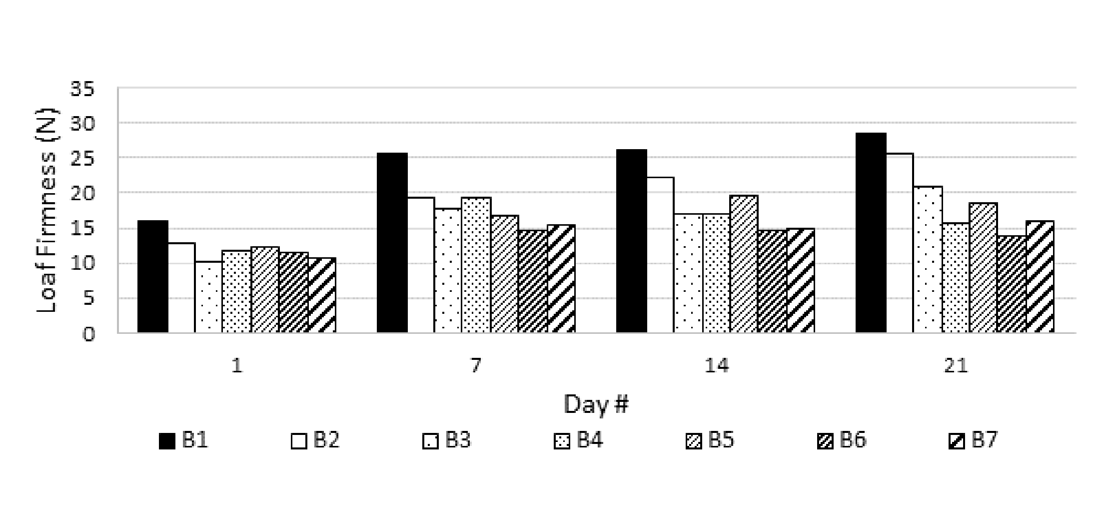

Figure 5. Loaf firmness of bread over 21 days of room temperature shelf life.

Table 6. Loaf firmness of bread over 21 days of room temperature shelf life.

Day	B1	B2	B3	B4	B5	B6	B7
1	16.02 ± 0.24^{a}	12.8 ± 0.19^{b}	10.29 ± 0.15^{c}	11.72 ± 0.94^{bc}	12.22 ± 0.28^{bc}	11.42 ± 0.04^{bc}	10.61 ± 0.02^{c}
7	25.48 ± 0.11^{a}	19.42 ± 0.06^{b}	17.83 ± 1.64^{b}	19.32 ± 0.86^{b}	16.73 ± 1.47^{b}	14.72 ± 0.015^{b}	15.33 ± 0.00^{b}
14	26.06 ± 2.14^{a}	22.31 ± 0.75^{ab}	17.03 ± 0.84^{bc}	16.90 ± 0.64^{bc}	19.55 ± 0.79^{bc}	14.53 ± 0.80^{c}	14.75 ± 0.13^{c}
21	28.61 ± 0.15^{a}	25.61 ± 2.14^{ab}	21.02 ± 2.67^{ac}	15.62 ± 3.05^{bc}	18.54 ± 2.42^{ac}	13.95 ± 1.57^{c}	15.84 ± 0.36^{bc}

Values are means ± SEM, n = 2 per treatment group.
Means in a row without a common superscript letter differ (P<0.05) as analyzed by one-way ANOVA and the TUKEY test.

Breads with 0.0025% and 0.0050% maltogenic amylase (B2-B3) increased in loaf firmness from day 1 to day 21 slightly more than the control (B1) (see Table 6). However, since the initial loaf firmness of the breads with 0.0025% and 0.0050% maltogenic amylase was significantly softer than the control on day 1, the breads with 0.0025% and 0.0050% maltogenic amylase had lower final loaf firmness values than the control on day 21. This difference was not statistically significant. On the other hand, the breads with high-performing maltogenic amylase (B5-B7) were significantly softer in initial loaf firmness (day 1) than the control (B1), and also staled more slowly in loaf firmness than the control, in that the bread had a smaller increase in loaf firmness after 21 days of shelf life than the control. For example, breads with high-performing maltogenic amylase (B5-B7) were 1.2-1.5 times harder on day 21 than day 1, while the control bread (B1) was 1.8 times harder on day 21 than day 1. This suggests that the high-performing maltogenic amylase was more effective in delaying staling in loaf firmness compared to the maltogenic amylase.

Interestingly, the control bread (B1) staled more quickly in slice firmness (which is a measure of crumb firmness) than in loaf firmness, in that the control bread was 3.1 times harder in slice firmness but only 1.8 times harder in loaf firmness after 21 days of room temperature shelf life (compared to its respective day 1 firmness value). The slice firmness is a measure of the crumb firmness, since it compresses only the crumb portion of the bread slice, while the loaf firmness test compresses both the crust and crumb of a bread loaf. Since the loaf firmness test also compresses the crust, it is also a relative measure of the crust firmness. The observed results suggest that the crumb of the control bread may have staled more quickly than the crust.

Overall, the high-performing maltogenic amylase was more effective in delaying staling (by texture analyzer results) than the maltogenic amylase. For example, all levels tested of the high-performing maltogenic amylase (B5-B7) prolonged softness in both slice firmness and loaf firmness by 20 days, while only the highest level of maltogenic amylase tested (B4) prolonged softness in loaf firmness and was significantly harder in slice firmness on day 21 than the control on day 1. Also, bread with the high-performing maltogenic amylase increased in slice firmness more slowly over 21 days of shelf life than bread with the maltogenic amylase, when compared at the same dosage.

Table 7 shows average texture values for slice firmness, springiness, and loaf firmness, which were calculated for an overall comparison of texture between the different test breads. Breads with added enzyme (B2-B7) were all on average softer (in loaf firmness and slice firmness) and springier than the control (B1). Breads with high-performing maltogenic amylase (B5-B7) were on average softer (lower slice firmness and loaf firmness values) and springier (higher springiness values) than breads with maltogenic amylase (B2-B4), when compared at the same dosage.

Table 7. Average bread texture values.

Day	B1	B2	B3	B4	B5	B6	B7
Slice Firmness (N)	34.38	24.00	21.94	20.41	17.58	14.22	11.80
Springiness (%)	91.8%	93.0%	93.3%	94.7%	93.5%	95.1%	95.7%
Loaf Firmness (N)	24.04	20.04	16.54	15.89	16.76	13.66	14.13

Texture values were averaged over all evaluation days for each test.

3.1.4 Ball Kneading Test

Table 8 and Fig. 6 show the effect of the maltogenic amylase and high-performing maltogenic amylase on the ball kneading test. Breads with maltogenic amylase and high-performing maltogenic amylase had significantly higher scores in the ball kneading test than the control on day 21, indicating that the breads with added enzyme were more cohesive (stayed intact better) and crumbled less in the ball kneading test. 0.0025% high-performing maltogenic amylase (B5) performed significantly better in the ball kneading test than 0.0025% maltogenic amylase (B2). Visually (see Fig. 6), the high-performing maltogenic amylase (B5-B7) formed a more cohesive ball than the maltogenic amylase (B2-B4). Overall, breads with either enzyme (B2-B7) had 40%-94% higher total ball kneading scores than the control (where the ball kneading scores of each evaluation day were totaled for each test).

Figure 6. Listed from top left to top right and then from bottom left to bottom right are pictures of the ball kneading test for tests B1-B7 on day 21.

Table 8. Ball kneading ratings.

Day	B1	B2	B3	B4	B5	B6	B7
1	4.0 ± 0.0^{b}	4.5 ± 0.0^{a}	4.5 ± 0.0^{a}	4.5 ± 0.0^{a}	4.5 ± 0.0^{a}	4.5 ± 0.0^{a}	4.5 ± 0.0^{a}
7	1.8 ± 0.2^{c}	2.5 ± 0.5^{bc}	3.5 ± 0.5^{ab}	4 ± 0.0^{ab}	4 ± 0.0^{ab}	4.5 ± 0.0^{a}	4.5 ± 0.0^{a}
14	2.0 ± 0.0^{c}	3.0 ± 0.0^{b}	4.0 ± 0.0^{a}	4.5 ± 0.0^{a}	4.3 ± 0.2^{a}	4.5 ± 0.0^{a}	4.5 ± 0.0^{a}
21	1.5 ± 0.0^{c}	3.0 ± 0.0^{b}	4.0 ± 0.0^{a}	4.5 ± 0.0^{a}	4.3 ± 0.2^{a}	4.5 ± 0.0^{a}	4.5 ± 0.0^{a}
Total	**9.3**	**13.0**	**16.0**	**17.5**	**17.1**	**18.0**	**18.0**

Values are means ± SEM, n = 2 per treatment group.
Means in a row without a common superscript letter differ (P<0.05) as analyzed by one-way ANOVA and the TUKEY test.

3.1.5 Sensory

Table 9 shows the sensory ratings for crumb grain, aroma, springback, foldability, softness, mouthfeel, and taste for bread rated on day 14. Crumb grain, aroma, and taste were rated similar.

Table 9. Sensory data for bread evaluated on day 14.

Attribute	B1	B2	B3	B4	B5	B6	B7
Crumb Grain	5.50 ± 0.29[a]	6.00 ± 0.58[a]	5.25 ± 0.25[a]	4.75 ± 0.48[a]	4.75 ± 0.25[a]	5.00 ± 0.00[a]	5.25 ± 0.63[a]
Aroma	7.75 ± 0.48[a]	7.50 ± 1.19[a]	7.25 ± 1.18[a]	7.00 ± 1.23[a]	7.00 ± 0.41[a]	7.00 ± 1.23[a]	7.50 ± 0.50[a]
Springback	3.25 ± 1.03[b]	5.50 ± 0.65[ab]	5.00 ± 1.23[ab]	6.50 ± 0.65[ab]	6.50 ± 0.50[ab]	6.00 ± 0.815[ab]	7.50 ± 0.65[a]
Foldability	2.25 ± 0.95[c]	3.00 ± 1.08[bc]	4.50 ± 1.04[ac]	5.25 ± 0.63[ac]	6.00 ± 0.41[ab]	7.00 ± 0.41[a]	7.50 ± 0.50[a]
Softness	4.50 ± 1.19[a]	4.75 ± 0.95[a]	5.75 ± 0.25[a]	6.25 ± 0.25[a]	6.50 ± 0.50[a]	7.25 ± 0.48[a]	7.25 ± 0.25[a]
Mouthfeel	1.75 ± 0.48[b]	3.00 ± 1.08[ab]	5.00 ± 1.08[ab]	4.75 ± 0.95[ab]	5.50 ± 0.50[ab]	6.00 ± 0.58[a]	6.75 ± 0.86[a]
Taste	5.25 ± 1.32[a]	5.00 ± 1.58[a]	6.50 ± 1.04[a]	6.00 ± 1.73[a]	6.00 ± 1.29[a]	7.25 ± 0.86[a]	7.25 ± 1.18[a]
Overall Score	30.3	34.8	39.3	40.5	42.3	45.5	49.0

Values are means ± SEM, n = 4 per treatment group.
Means in a row without a common superscript letter differ (P<0.05) as analyzed by one-way ANOVA and the TUKEY test.

For foldability, breads with maltogenic amylase (B2-B4) had higher foldability values than the control (B1), but the difference was not statistically significant. Breads with high-performing maltogenic amylase (B5-B7) were more resilient in that they had significantly better foldability than the control (B1). Overall, breads with the high-performing maltogenic amylase (B5-B7) had the highest foldability values, but their difference in foldability between breads with maltogenic amylase was not statistically significant.

For softness, breads with 0.0050-0.0100% maltogenic amylase and 0.0025-0.0100% high-performing maltogenic amylase (B3-B7) had higher softness ratings by sensory than the control, but the difference in softness ratings by sensory was not found to be statistically significant. This may be due to the nature of having more variation in scoring between sensory panelists, since the ratings were subjective. It is noted that the slice firmness test by texture analyzer is a more precise test for texture since it is an objective test. Table 4 shows that the 0.0050%-0.0100% maltogenic amylase and 0.0025%-0.0100% high-performing maltogenic amylase (B3-B7) were significantly softer in slice firmness than the control (B1) on the same day that the sensory was conducted (day 14).

For mouthfeel, breads with 0.0050-0.0100% high-performing maltogenic amylase (B6-B7) were rated the moistest in mouthfeel (highest mouthfeel scores), and this difference from the control (B1) was statistically significant. The difference in mouthfeel scores between breads with maltogenic amylase (B2-B4) and high-performing maltogenic amylase (B5-B7) was not statistically significant.

For springback, bread with 0.0100% high-performing maltogenic amylase (B7) had the best score for springback. The difference in springback scores between this bread (B7) and the control (B1) was statistically significant, indicating that this bread was significantly springier than the control. Breads with high-performing maltogenic amylase had higher springback scores than breads with maltogenic amylase at the same dosage, but the difference was not found to be statistically significant. Overall score was calculated as a sum of the scores of all sensory attributes for each test. Both the maltogenic amylase and high-performing maltogenic amylase (B2-B7) had a higher overall sensory score than the control (B1). The high-performing maltogenic amylase (B5-B7) had higher overall scores than the maltogenic amylase (B2-B4) for the same dosage level, suggesting that bread with the high-performing maltogenic amylase performed better in overall sensory than bread with the maltogenic amylase for the same dosage level.

4. CONCLUSION

Breads with both maltogenic amylases were significantly softer in slice firmness than the control bread after 21 days of room temperature storage. The high-performing maltogenic amylase was more effective at delaying staling (as measured by the increase in slice and loaf firmness after 21 days of shelf life) than the maltogenic amylase. Breads with high-performing maltogenic amylase rated better in overall sensory score than breads with the maltogenic amylase at the same dosage. Bread with 0.0100% high performing maltogenic amylase was softer and springier than the control bread by texture analyzer results, and was softer, moister in mouthfeel, springier, and had better foldability in sensory than the control bread. The high performing maltogenic amylase offers a promising solution to further extend shelf life of bread, such as by prolonging softness, springiness, and moistness.

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