

USING ENZYMES TO IMPROVE FROZEN-DOUGH BREAD QUALITY

by

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Abstract

Potassium bromate is a well-known strong chemical oxidant. It was once widely used by the baking industry all over the world, especially for making frozen doughs. Since potassium bromate has been banned in many countries, many researchers have studied in this area to find a replacement. Ascorbic acid was often combined with potassium bromate in frozen dough making as an oxidant dough additive. In addition, ascorbic acid has different chemical oxidant activity, and its function in yeast leavened dough is not as strong as is potassium bromate. More dough additives have been found, such as enzymes. Enzymes play key roles in bread making. In recent years, enzyme usage in bread making has been increasing, especially for shelf-life extension.

Based on the results from this research, potassium bromate use can be replaced by a combination of ascorbic acid and hemicellulase/endoxylanase. However, using hemicellulase/endoxylanase alone cannot benefit frozen dough quality such as finer crumb cell or increasing final bread volume.

These experimental results also show that using a combination of ascorbic acid and hemicellulase/endoxylanase can delay the development of bread firmness (staling) after baking. As frozen storage time increased, the firmness of frozen dough bread increased, and the bread tended to have a coarser texture. Hence, larger and uneven grain cells reflect a gray or dark crumb color.

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CHAPTER 1 - Introduction

Bread is an important staple for human consumption in many countries of the world. Baking is also one of the oldest crafts in the world. However, most breads have a short shelf-life as a result of staling. Hence, frozen dough technology has been developed since the early twentieth century (Sluimer 2005). This technique provides customer benefits by permitting the baker or retailer to provide fresh bread at almost any time. As a result, this technology has become one of the most important technologies practiced by today's baking industry. Many studies related to frozen dough have been published, but much of the information necessary to produce high-quality frozen doughs has been kept as proprietary technology and is not publicly available. This has hindered the development of better and improved processes. Currently, frozen dough is being used all-over the world, resulting in economic advantages to the producer and increasing convenience for the user. Although there are many advantages, frozen dough also exhibits some problems in the quality of the final products.

A major factor affecting frozen dough is the yeast's stability after freezing. Hence, yeast problems have been widely studied (Gélinas et al 1993 and 1994; Ribotta et al 2003; Hsu et al 1979b; Wolt and D'appolonia 1984a & b; Hino et al 1987; Bruinsma and Giesenschlag 1984). During dough processing, when the temperature approaches the freezing point, ice crystals damage the yeast cell walls. Consequently, yeasts release glutathione, a protein reducing agent, which speeds up the weakening of the dough. To prevent these effects, oxidants such as ascorbic acid and potassium bromate (KBrO_3) are added into yeast-leavened doughs. Ascorbic acid is now commonly used in baked products, especially bread. Potassium bromate is a physically powerful oxidant that was once used also, although its use has declined in recent years. During dough mixing, and for potassium bromate in the oven, an oxidant functions to strengthen the gluten network. During the baking process proper, it also contributes to greater oven spring and improves the volume and internal texture of the final product.

However, after baking, the residue of potassium bromate could conceivably be injurious if consumed (Silverglade and Sperling 2005). Consequently, its application as an additive to food products has been banned in many countries including The United Kingdom, Canada, and

China. According to the United States FDA (Food and Drug Administration), potassium bromate may still be legally used (up to 75 ppm based on flour weight) for baked foods in the U.S. However, in California, strict labeling is required if used. But it is also recommended that its use be discontinued in the U.S., and in fact very little is now used.

Many food additives such as amino acids and enzymes have been used in breadmaking as bromate replacements (Morita et al 1997). After usage of potassium bromate was banned in many countries, interest increased in finding an enzyme to replace this chemical oxidant (Mathewson 1998). The advantages of enzyme usage has been demonstrated in breadmaking. Numerous studies (Gil et al 1999; Ribotta and Le Bail 2007; Hille and Schooneveld-Bergmans 2004; Guy 2001) report that enzymes play key roles in bread making such as increasing loaf volume, producing finer crumb cells, and extending shelf-life. Furthermore, Japanese Patent specification No. 5701/1968 discloses a method for bread quality improvement by adding to the dough a component which contained hemicellulase (U.S. Patent No. 4,990,343., 1991). The Patent claims “the combination of the enzyme preparation of the invention and lecithin can advantageously replace bromate conventionally used as a baking additive.” (U.S. Patent No. 4,990,343., 1991). In another, similar, study the combination of ascorbic acid and hemicellulase was used to study the thermo-mechanical behavior of dough systems during research (Ribotta and Le Bail 2007).

The objective of this study was to evaluate the effects of a combination of hemicellulase, endoxylanase, lipase, and ascorbic acid as a replacement for the potassium bromate – ascorbic acid combination in frozen dough making, particularly, on the final loaf volume and staling rate over various frozen dough storage times.

CHAPTER 2 - Literature Review

Dough freezing and storage processes create large challenges to yeast survival in the frozen dough process. Many researchers have widely investigated and experimented in this area. The quality of final frozen dough bread is known to be affected by dough formulation, quality, quantity, and type of yeasts, dough additives, mixing methods, mixing time, dough process, freezing rate, and storage time and conditions, among others (El-Hady et al 1996; Hino et al 1987; Selomulyo and Zhou 2006; Wolt and D'appolonia 1984a & b).

Effects of dough formulation

For satisfactory dough, flour for frozen dough contains a higher protein level than is used for equivalent non frozen dough products. Normally, a protein content of 11 to 13 percent is preferred, with a low level of damage starch (Marston 1978). If needed, vital wheat gluten (VWG) can be added to enhance the gluten level. An increase in one percent of VWG increases the total effective protein by about 0.6 percent and absorption by about 1.5 percent (Rogers 2004).

When water molecules are at their freezing point, ice crystals form and damage the yeast cell walls. To minimize such damage, frozen dough moisture is usually maintained at a slightly lower level than in a commercial fresh dough formula (Brümmer, 1993). The yeast content of frozen dough should be higher than normal to compensate for inevitable losses of activity during freezing and storage (Wolt and D'appolonia 1984a, Marston 1978). Due to its osmotic pressure effect on yeast, the suitable amount of salt in frozen formulation is no more than 2 percent (based on flour weight).

Dough additives

Sodium stearoyl-2-lactylate (SSL)

The surfactant sodium stearoyl-2-lactylate (SSL) is a reaction product of stearic and lactic acids neutralized to sodium salts. It is insoluble in water but soluble in oil. In baking, it has been shown to be effective in maintaining bread volume and crumb softness during storage. It also can decrease the effects of frozen storage on rheological properties, without reducing the proofing time (Wolt and D'appolonia 1984b). The same authors concluded that frozen dough containing SSL had a greater loaf volume after baking than did the dough with no SSL, and that this was because of its greater oven spring.

Ascorbic Acid (AA)

Oxidizing agents are required to be added to frozen dough formulas to strengthen their gluten network and to improve the final product's volume as well. The combination of ascorbic acid and potassium bromate are often added into a frozen dough formula (Marston 1978).

Ascorbic acid is well-known as vitamin C and dietary quantities are sourced from vegetables and fruits. It has also been widely used in the baking industry as a dough conditioner. As an oxidant, L-ascorbic acid “exhibits an intermediate reaction rate and is, therefore, capable of sustained action through most of the dough phase.” (Pylar 1988). It is a reducing agent (or sometimes called an anti-oxidant), “it must first be oxidized to dehydro-L-ascorbic acid (DHA) (Fig. 2.1) in order to act as an oxidant.” (Pylar 1988; Cauvain et al 2001). It may create - S - S - (disulphide) bonds reinforcing the gluten network, thus improving dough gas retention (Selomulyo and Zhou 2006, Cauvain et al 2001). Furthermore, ascorbic acid provides other benefits to breadmaking, such as providing resistance to dough deformation during mixing, increases oven spring, and finer crumb grain. Ascorbic acid cannot over-oxidize the dough. Consequently, ascorbic acid is best suited to no-time doughmaking systems. (Cauvain et al 2001). However, adding ascorbic acid in bread cannot be a gateway for dietary enrichment since most of it is decomposed during bread baking (Pylar 1988).

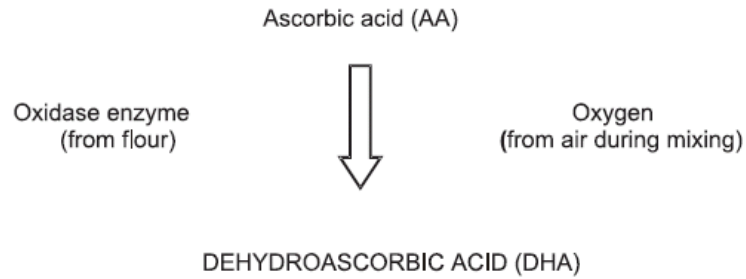


Figure 2.1 Ascorbic acid reaction in dough
(adapted from Cauvain and Young 2001)

Potassium Bromate (KBrO₃)

The introduction listed some reasons for using potassium bromate (KBrO₃) in breadmaking. More studies have shown advantages and disadvantages to its incorporation. It is one of the oxidants approved by the Food and Drug Administration (Pylar 1988). It is typically a very powerful oxidant and mainly functions to strengthen bread dough. It is often used during frozen dough production in combination with ascorbic acid. According to Inoue and Bushuk's study (1991), the function of this combination in dough formulation is superior to using potassium bromate alone. The United States FDA has ruled that potassium bromate can be added into the dough at up to 75 ppm in the U.S. However, in California, strict labeling is required if used. "The oxidants differ in their critical levels of application. For instance, bromates are less critical at higher use levels, but are more prone to create problems associated with under-oxidation when the treatment level is inadequate" (Pylar 1988). Because potassium bromate is a slow acting oxidant, "it does not exert its full effect until the dough reaches the late stages of proving and the early stages of baking" (Pylar 1988, Cauvain and Young 2001). Conversely, if bread is not baked long enough or baking temperature is not high enough, then residual bromate may cause health problems; for example, it may be a carcinogen if consumed (Silverglade and Sperling 2005).

Yeasts

The primary function of yeast in the yeast-raised dough is leavening. During dough fermentation, yeast converts sugars to carbon dioxide (CO₂) and alcohol. Also, dough temperature is increased by fermenting. Meantime, fermentation flavors are developed (Rogers 2004).

Baker's yeast has been classified into two general types based on its stability and how it is processed. The first, fresh yeasts, can be compressed, crumbled, or cream types. The other type, dry yeasts, can be active dry or instant dry yeast. The type of yeast that is better for frozen dough making has been somewhat controverted over time. For example, Wolt and D'appolonia (1984a) mentioned that fresh compressed yeast usually performed better than did active dry yeast in proof-time stability over a storage period. On the other hand, El-Hady and his coworkers (1996) pointed out that active dry yeast is superior to compressed yeast in maintaining shelf life in frozen dough. (Fig. 2.2) Currently, many frozen dough industries use new freeze-tolerant yeasts for frozen dough making. This yeast was isolated from banana peel and identified as *Kluyveromyces thermotolerans* and *Saccharomyces cerevisiae*. The new freeze-tolerant yeasts are claimed to provide good quality bread similar to that made from unfrozen dough (Hino et al 1987).

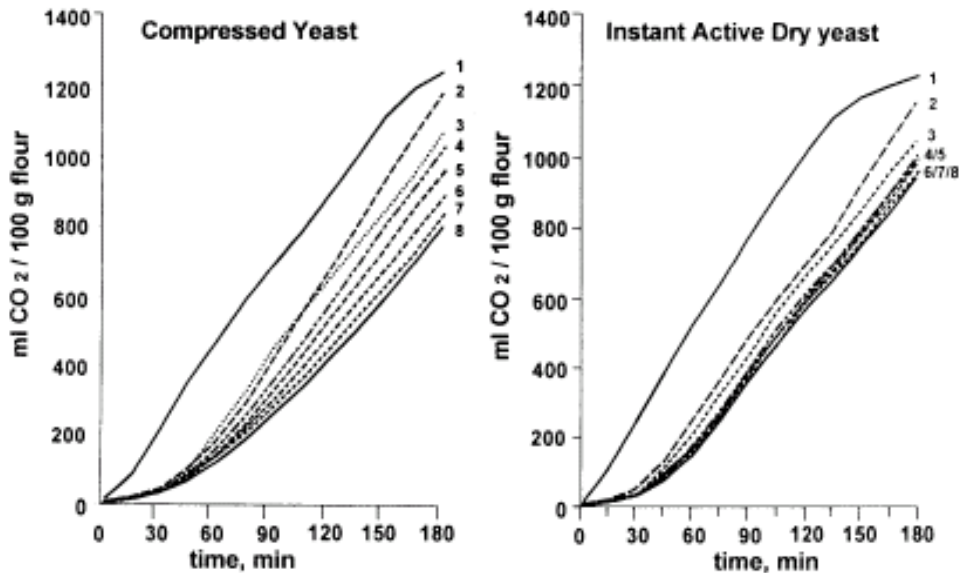


Figure 2.2 Gas production (risograph) and yeast type (1 = unfrozen; 2 = deep freezing and -20°C for 1 day; 3 = 2 weeks; 4 = 4 weeks; 5 = 6 weeks; 6 = 8 weeks; 7 = 10 weeks; 8 = 12 weeks). (adapted from El-Hady 1996)

The introduction described the negative influence of freezing and storage on yeast viability. Hence the frozen dough making process is different from non-frozen dough. In general, the more fermentation a dough is given, the higher the final product quality. However, this relationship does not apply to frozen dough. Figure 2.3 shows this (Inoue and Bushuk 1991, Sluimer 2005). Therefore, no-time and short-time dough methods are often applied to frozen dough making (Marston 1978). Yeasts, after activation, are more susceptible to freeze damage than are nonactivated yeasts (Hsu 1979a). Freeze damage to the dough results in longer proofing times and lower bread volumes. As dough loses strength, oven spring decreases and causes lower final product loaf volume and inferior texture, not because of a lower volume at the start of baking (Sluimer 2005).

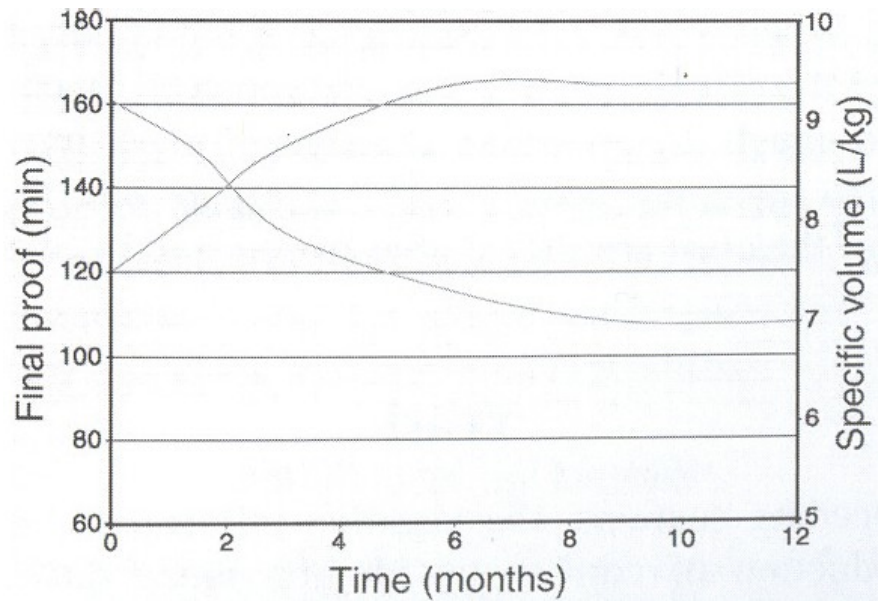


Figure 2.3 Final proof time (upper curve) and specific volume (lower curve) of croissants as a function of storage time. As storage time increases from 0 to 12 months, final proof time increases from 120 to more than 160 min, and specific volume decreases from 9 to about 7.4 (L/kg).

(adapted from Sluimer 2005)

Study on Hemicellulases

Definition

Hemicellulase is a hydrolytic enzyme which can hydrolyze the hemicellulose present in plant cell walls. Hemicellulose is a minor gum-like fraction (~3%) in white flour. In whole wheat flour, the total content ranges from 4 to 7% (Hille and Schooneveld-Bergmans 2004). “It categorizes a variety of polysaccharides that are more complex than sugars and less complex than cellulose. The hemicellulase breaks down the hemicellulose fiber to disengage smaller fragments of cellulose which is then further attacked by exo-cellulase to liberate glucose.” (<http://www.enzymeindia.com/enzymes/hemicellulase.asp>). Hemicellulase is classified as a carbohydrase. (Fig. 2.4)

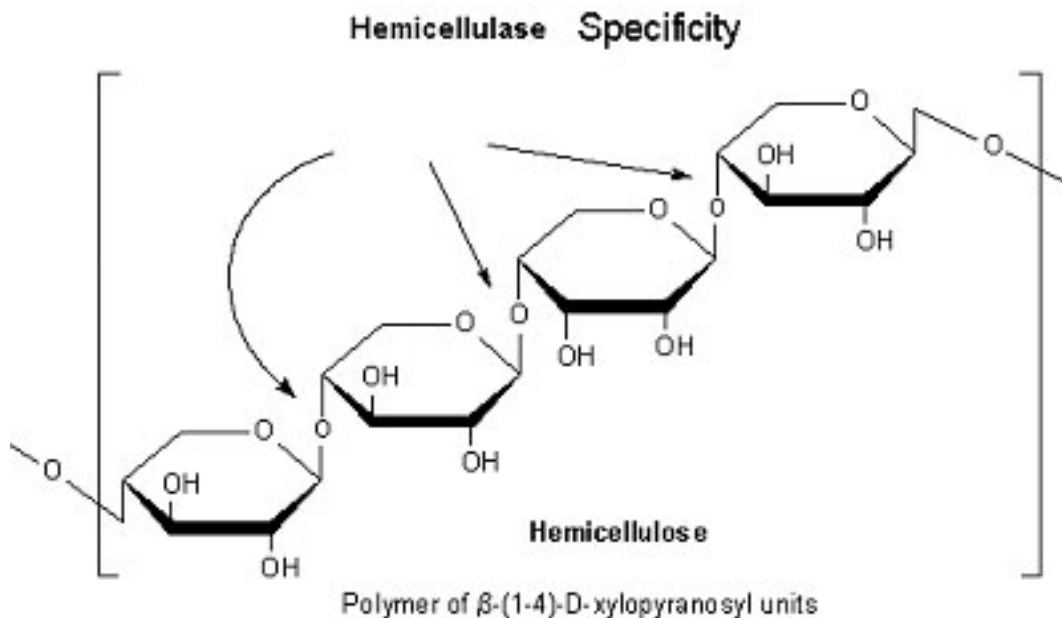


Figure 2.4 Structure of hemicellulose.

(<http://www.enzymeindia.com/enzymes/images2/hemicellulase-image.jpg>)

Application of hemicellulase to baked products

The book “Enzymes” (Mathewson 1998) contains a description of the dough improvement/oxidizing functions of enzyme usage in breadmaking. It concludes that, increasingly, researchers have tried to find a single enzyme to act as a bromate replacement but that “no single enzyme has been identified that can replace the addition of oxidizing agents. A number of companies have introduced enzyme preparations containing multiple forms of enzyme activity, including amylase, proteases, and several hemicellulases in combination with ascorbic acid.”

Hille and Schooneveld-Bergmans (2004) concluded that both fungal and bacterial hemicellulases are able to improve fresh bread quality as measured by loaf size and shape, and crumb texture and softness. Hammond (1994) showed that hemicellulase increased loaf volume of pan bread, coupled with an enhancement of shape and symmetry, as well as finer cell structure and a resilient loaf. Hemicellulases also contributed finer cell structure, whiter crumb, and significantly increased softness in French baguettes. When a combination of hemicellulase and fungal alpha-amylase was added into white and wholemeal loaf formulae, the results showed significantly greater volume than when using fungal alpha-amylase alone (Hammond 1994). Guy (2001) pointed out that “hemicellulases have been claimed to improve on the effects achieved with fungal amylase and to also improve the fineness of the cellular structure of the crumb.” Figure 2.5 shows one model to explain what happens in the dough and why hemicellulase improves bread quality. Hemicellulose particles may disturb the gluten network. When endoxylanase degrades the WU-AX (water-unextractable arabinoxylan) polymer into a WE-AX (water-extractable arabinoxylan), the gluten net can have better gas retention, resulting in extra volume (DSM 2005).

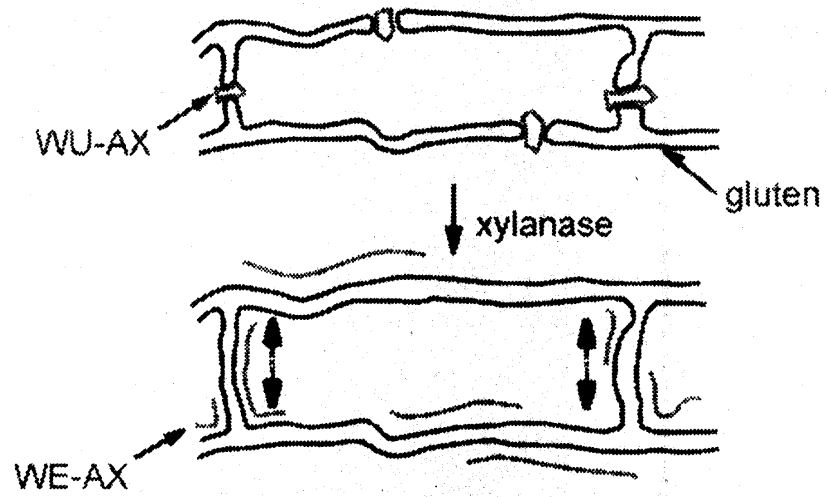


Figure 2.5 Interactions of arabinoxylan and gluten in dough. WU-AX is water-unextractable arabinoxylans. WE-AX is water-extractable arabinoxylans.

(adapted from Bakezyme BXP 5001 BG application data sheet, DSM)

Lipase(s)

Lipases are widely distributed in nature, in animals, plants, and microorganisms. Lipases have been defined as enzymes which hydrolyze insoluble fats and fatty acid esters occurring in separate, non-aqueous phases (Underkofler 1972). According to Hosney (1998), “all cereals have lipase activity, but the activity varies widely between cereals, with oats and pearl millet having relatively high activity compared to that of wheat or barley.” When different techniques or different substrates are used, it is very difficult to compare the lipases from different cereals.

Lipase hydrolyzes a triglyceride into mono- and diglycerides. The reaction products function as dough emulsifiers and then improving action is based on this mechanism (Sluimer 2005). Even though lipase is not commonly used in baked foods, it has been widely known to benefit bread quality (Sluimer 2005, Sahi and Guy 2004). For example, Gélinas et al (1998) concluded that lipase has dough bleaching activity if combined with peroxidase and linoleic acid. Also, the combinations of lipase, oxidized oil, and linoleic acid significantly degraded flour pigments (Mercier and Gélinas 2001). However, Underkofler (1972) pointed out that “lipase activity in flour for baking is undesirable because free fatty acids have a detrimental effect in doughs.”

Dough Rheological Tests

Mixograph

The Mixograph was developed at Kansas State University by Dr. Swanson and Dr. Working in 1926. The purpose of the Mixograph is very similar to the Farinograph. Both are recording mixers and are well-known as dough rheological tests, although they have different mixing actions. The Mixograph is a rapid tool for measuring the mixing behavior of dough because of the reduced small sample size (Chung et al 2001). The Mixograph parameters most commonly used in dough quality evaluation include mixing time, water absorption, and mixing tolerance (Finney 1985). The predicted optimum water absorption is calculated using the flour's protein content. That calculation does not reflect damaged starch content and protein quality, so different flours which have the same protein content might show differences in their resulting mixing curves and require different amounts of water.

A sample curve with its measurements is shown in Figure 2.6.

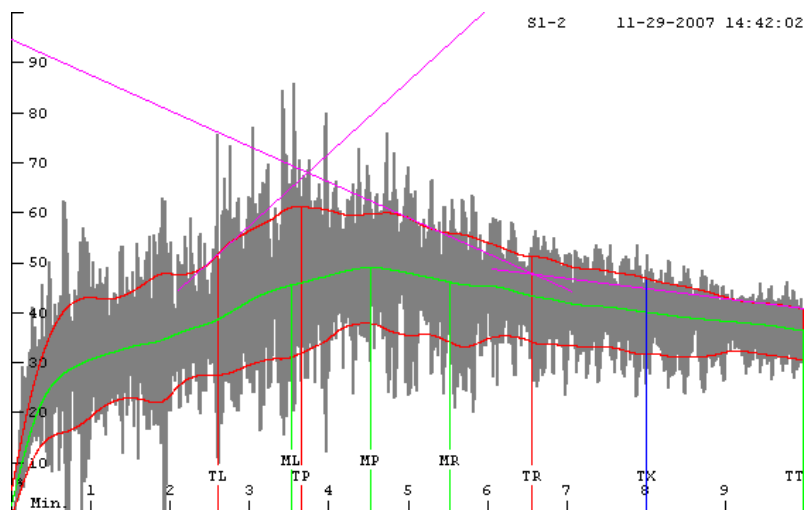


Figure 2.6 A sample computer-analyzed Mixograph curve.

Alveograph

The Alveograph, developed by Marcel Chopin in France in the 1920s, has been widely used in Europe. Its use is increasing in the U.S. This instrument provides an empirical physical test to assess the breadmaking quality of European and for soft wheat flours, but does not work quite as well for stronger North American hard wheat flours. It is one of the common instruments used to measure gluten quality and dough elasticity (Gaines et al 2006). It imitates the inflation of bubbles in dough by CO₂ produced by yeast fermentation. However, the rate of inflation and consequently shear rate during the test is much higher than that experienced during fermentation. Also, it makes only one large dough bubble rather than many small ones.

This curve resulting from the test provides a measure of dough tenacity (P or pressure), extensibility (L or length), and energy to stretch a dough (W). The P value, which is the peak height of the curve, relates to the resistance or strength of a dough. The L value reflects dough extensibility. The W value, which is the surface area under the curve, or work performed, is related to the baking strength. The value of P/L indicates the configuration ratio of the curve, and is useful for comparing different flours.

A sample curve is shown in Figure 2.7.

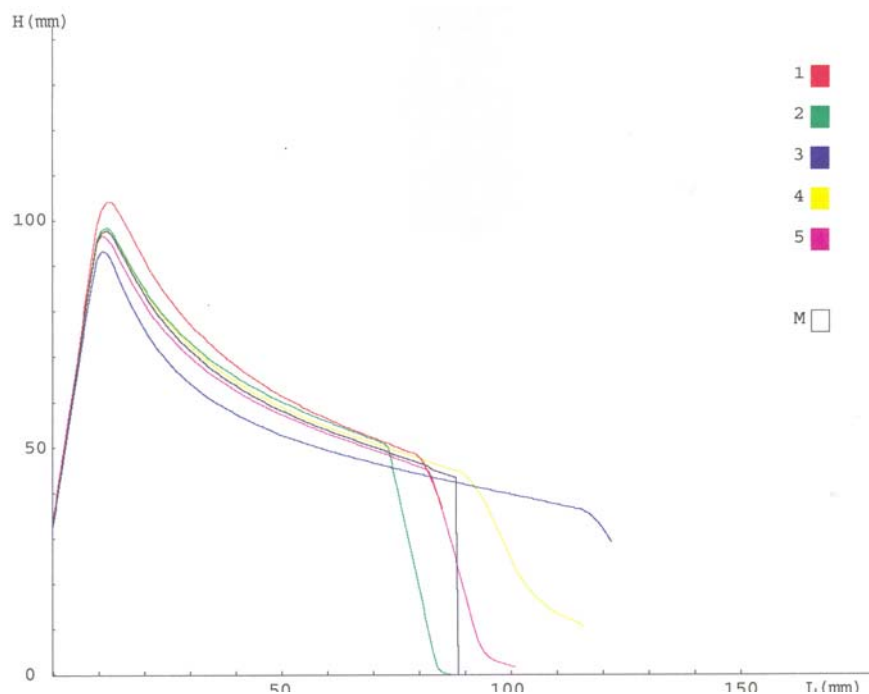


Figure 2.7 A sample Alveogram curve. The black curve shows the average of the five dough pieces.

Bread Storage & Staling

Bread has a relatively short shelf life. After baking, it rapidly loses its freshness and a number of physical and chemical changes occur. Changes in flavor and texture during storage are commonly called staling. The loss of freshness is shown by an increase in crumb firmness and a decrease in flavor and aroma. This phenomenon is frequently attributed to starch retrogradation, a term used to denote partial recrystallization. Krog et al (1989) explained that “retrogradation is a physical change of a starch amylose and amylopectin from a swollen, gel-like state to a more crystalline state and is probably related to the undesirable increase in crumb firmness during bread storage.” Although some studies have shown that gluten and lipids also play important roles in the staleness, starch retrogradation is the main factor responsible for the observed increase in crumb firmness during bread storage (Bollain 2005, Hosenev 1998, Selomulyo and Zhou 2006). Bread made from frozen dough usually has a shorter shelf life than if it were made with a non-frozen dough. “The main effect of frozen storage indicated a consequent decrease in moisture contents of bread baked from day 0 and until 60 days after frozen storage” (Asghar et al 2006). Some articles demonstrate that long storage periods are associated with a decrease in final product volume. Giannou and Tzia (2007) found that frozen dough sample quality degraded rapidly during the first months of frozen storage but then stabilized and remained stable for up to after 9 months of storage.

Bread storage conditions, particularly temperature, affect the staling rate. According to Pylers’s (1988) study, “breads remained fresh when stored at 60°C (140°F) or higher; became half stale at 40°C (104°F); nearly stale at 30°C (86°F); stale at 17°C (63°F); and very stale at 0°C (32°F). It remained fresh when stored a -7 to -184°C (14 to -300°F).”

To reduce the bread staling rate, additives such as enzymes have been suggested for inclusion in dough formulations. Bacterial α -Amylase can have an antistaling effect (Hug-Iten et al 2001, Gil et al 1999, Pylers 1988). However, it retains its activity not only during starch gelatinization but also at the final internal temperature reached during baking (Pylers 1988). High levels of bacterial α -Amylase stopped bread firming that developed during five days of storage after baking (Martin and Hosenev 1991) but also caused a sticky and gummy bread crumb

texture (Conn et al 1950). β -Amylase was also effective for reducing the rate of bread firmness, but it cannot fully stop the firming (Martin and Hosney 1991). The newer generation of amylase, maltogenic α -Amylase, provides clear antifirming effects and is able to maintain the elastic recovery levels during bread storage. It turns out to be an ideal antistaling enzyme in bread (Hug-Iten et al 2003). Bollain and his coworkers (2005) concluded that starch and non-starch enzymes can provide enhancement of fresh quality and/or inhibition of staling. Fiszman et al (2005) pointed out that “fungal enzyme preparations with high endoxylanase, β -xylosidase, and α -L-arabinosidase activities have delayed bread staling considerably without affecting porosity or loaf volume.”

Texture and Image Analysis

Texture Analysis

The firmness of baked foods is important because it directly affects the consumers' perception. Because bread firmness increase is mainly caused by staling, firmness is often used as a measure of bread staling. It has been determined successfully by using instruments such as the LFRA Texture Analyzer (Fig. 2.8) or TA-XT2 Texture Analyzer (Fig. 2.9) in a static compression mode (Bollain et al 2005). The texture measurement is an objective method for measuring the staling rate. AACC International Method 74-09 (AACC 2000) provides a standard method for bread staling based on force-deformation measurement of firmness.

The LFRA Texture Analyzer was developed prior to the TA-XT2 Texture Analyzer. However, it provides a similar measurements. The LFRA Texture Analyzer is usually operated to measure force at a specified compression distance. Using this instrument the test is simple and rapid. The TA-XT2 Texture Analyzer provides a three dimensional (force, distance, and time) product analysis. When connected to a PC running Stable Micro Systems XT.RA Dimension software package, it allows the user to read and analyze the data via the PC program. Therefore, though more expensive, the TA-XT2 Texture Analyzer is commonly used today for research.



Figure 2.8 LFRA Texture Analyzer

(<http://www.hwashin.net/products/products.php?Pcate=19&cate=153&uid=257>)

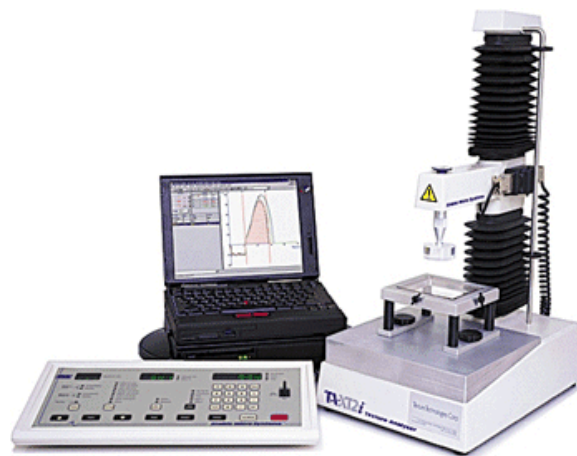


Figure 2.9 TA-XT2 Texture Analyzer

(<http://www.texturetechnologies.com/>)

Image Analysis

Digital image analysis (DIA) is an efficient and objective method for defining bread quality. It operates by measuring bread grain cell characteristics. Numerous researchers have adopted image analysis systems for bread crumb scoring (Rogers et al 1995, Sapirstein et al 1994, Bertrand et al 1992, van Duynhoven et al 2003, Zayas 1993, Zghal et al 1999). Before the method was developed, bread scoring as determined by a human eyes was subjective, could be imprecise, and time consuming. Rogers et al (1995) outlined two problems with natural bread crumb scoring systems: the absence of an everlasting record and the subjective nature of the results. Published studies have compared digital image analysis and bread quality definitions. Sapirstein and coworkers (1994) pointed out that the electronic image analysis method is completely objective, rapid, and precise. In their study, a PC vision system was employed to determine bread crumb cells. Bertrand et al (1992) noted that consumers are often deeply influenced by the appearance of bread crumb when purchasing products and that bakery products which have a fine structure are better appreciated by consumers (van Duynhoven et al, 2003). Therefore, an objective bread quality analysis method is an essential tool. Van Duynhoven et al (2003) describe “the extraction of crumb features from video images by a mathematical method based on a two dimensional Haar transform, spatial and spectral.” Moreover, van Duynhoven et al (2003) showed “an example of the joint deployment of magnetic resonance imaging and image analysis procedures for the assessment of gas cell development and anisotropy in the growth of the dough during proofing.” Zayas (1993) utilized the Kontron image processing system (IPS) for conducting image analysis. An image analyzer was used to describe the relationship between bread crumb density and bread crumb grain (Zghal et al 1999).

Recently, a high efficiency image analyzer, known as “C-Cell[®]”, has been developed specifically for crumb structure evaluation. C-Cell was developed by CCFRA (Campden and Chorleywood Food Research Association, Station Road, Chipping Campden, Gloucestershire, GL55 6LD, UK). The instrument is claimed to be able to evaluate bread and the visual grain quality of any yeast leavened product. The camera specification is designed with 1296 × 1026 pixels and a 182 × 143 mm field of view. The provided software (C-Cell Software) is intended

to analyze 48 different slice data properties and 6 imaging (raw, brightness, cell, elongation, shape, and volume) (Fig. 2.10) parameters automatically (Whitworth et al 2004). Using this instrument, each sample can be measured in a few seconds.

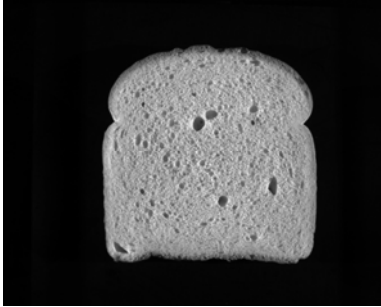

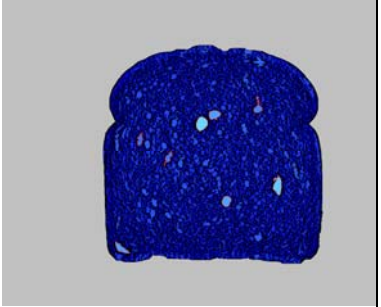
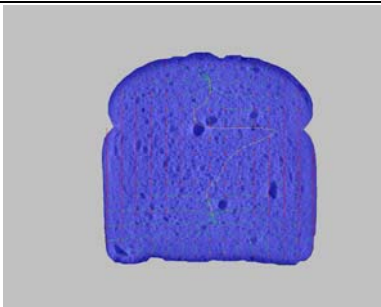

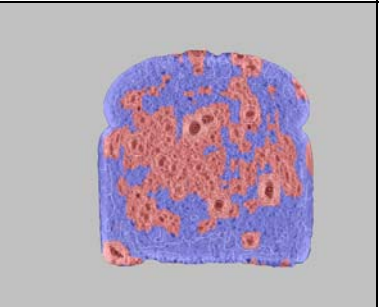
		
Raw	Brightness	Cell
		
Elongation	Shape	Volume

Figure 2.10 Six processed images from the C-Cell imaging system, from the same slice of bread.

CHAPTER 3 - Materials

A hard wheat bread flour was supplied by ConAgra Mills, Omaha, NE, USA. The flour was unmalted, but bleached and enriched. Flour protein content was determined with a combustion type (Leco) nitrogen analyzer. It contained 12.72% protein (AACC method 46-30) and 0.52% ash (AACC method 08-01), 14% MB. The flour moisture was 11.99% measured by oven (AACC method 44-15A). In these studies, instant dry yeast was used due to its stability during storage, as compared with compressed yeast. The yeast samples were provided by Lesaffre Yeast Corporation, Milwaukee, WI, USA. The rest of the ingredients were from the commercial market. For the enzyme treatment tests, enzyme samples (fungal endoxylanase, bacterial hemicellulase, and lipase) were obtained from the DSM Food Specialties USA, Inc. The standardized activity of the fungal endoxylanase (HSP 6000 BG), the bacterial hemicellulase (BXP 5000 BG), and the lipase (L 80000 A) were 6000 EDX/g, 5000 NBXU/g, and 80000 PLI/g \pm 5 %, respectively. The commercial (DSM Food Specialties USA, Inc) cost of fungal endoxylanase is \$28/kg, hemicellulase is \$22/kg, and lipase is \$240/kg.

CHAPTER 4 - Methods

Dough formulation

The following base formula was used for all baking test experiments : 100% flour (12.72% protein), 63% water, 4% sugar, 3% nonfat dry milk (NFDM), 3% all purpose shortening, 2% salt, 1.5% instant dry yeast, 1% vital wheat gluten (VWG), and 0.5% sodium stearoyl-2- lactylate (SSL) (Tab. 4.1). The base formula was modified from the student lab manual and my personal experience as a professional baker. Variations for oxidant type and level studies, 20 ppm of potassium bromate (based on flour weight), and 150 ppm (based on flour weight) of ascorbic acid were added to the doughs as oxidants. For the enzyme treatment experiments, 75ppm of hemicellulase, 35 ppm of endoxylanase, and 40 ppm of lipase were added to the individual dough formulas. The amounts of enzyme added were based upon the specification sheets supplied with the enzyme samples and provided by DSM Food Specialties USA, Inc.

Table 4.1 Base Dough formula. For test purpose, potassium bromate, ascorbic acid, and enzymes were added at various levels. *VWG = Vital Wheat Gluten. **NFDM = Non-Fat Dry Milk.

Ingredients	%
HRW Flour (12.7% Protein)	100
VWG*	1
Water (0°C)	63
Instant Dry Yeast	1.5
NFDM**	3
Shortening(All purpose)	3
SSL	0.5
Sugar	4
Salt	2
Total	178

Dough Rheological Tests

Mixograph

The mixograph test method was based on AACC Approved Methods 54-40A (AACC 2000). The 10 gram mixing bowl (National Manufacturing Division of TMCO, Lincoln, NE) was used in this measurement. Sixty-two percent water absorption was added to the testing formula. For the variation treatments, 150 ppm of ascorbic acid (based on the flour weight) and 75 ppm of hemicellulase (based on the flour weight) were added to the test.

Alveograph

The alveograph test method was obtained using AACC Approved Methods 54-30A (AACC 2000). (Model Alveographe NG, Chopin, France). Sixty-two percent water absorption was added to the testing formula. For the variation treatments, 150 ppm of ascorbic acid (based on the flour weight) and 75 ppm of hemicellulase (based on the flour weight) were added to the test.

Procedure for frozen dough preparation

Dry ingredients, excluding the yeast, salt, and sugar, were first weighed together in a steel bowl (Fig. A.1). An A-200 Hobart mixer (The Hobart MFG. CO., Troy, Ohio) equipped with a McDuffee Bowl and two-pronged fork from National Manufacturing CO, Lincoln, NE was used for all tests. The jacketed mixing bowl temperature was maintained at 6 °C (43°F) by a circulating refrigerated water bath (Fisher Scientific, Inc. Pittsburgh PA 15219 U.S.A.) (Fig. A.2 & A.3). The dough mixing procedure was a no-time method with delayed sugar and salt addition. First, all ingredients were placed in the mixing bowl excluding the yeast, salt, and sugar and mixed 15 seconds in low speed (#1). Then yeast was added and mixed for another 15 seconds. Second, change the mixing speed to #2 and mix the dough for three and half minutes. Third, add the salt and sugar into the dough and mix for 30 seconds in low speed. Then the mixing speed was changed to #2 and the dough was mixed to optimum based on a skilled baker's

experience. Each batch had a total dough weight of 1700 grams. The target dough temperature was set at $19^{\circ}\text{C} \pm 1^{\circ}\text{C}$ ($66^{\circ}\text{F} \pm 2^{\circ}\text{F}$) and measured in the mixing bowl immediately after mixing was completed (Fig. A.4). This was a no-time dough method, and immediately following mixing, the dough was divided into three 540 ± 1 gram each pieces (Fig. A.5 & A.6) without any fermentation. After manually rounding, the dough balls were allowed to rest for 5 minutes at room temperature, 21°C (70°F) (Fig. A.7). Then, each dough ball was sheeted individually with a sheeter/ molder (Oshikiri Machinery Ltd, Fujisawa, Kanagawa, Japan), rolling into a loaf shape (21.5cm long & 5.4cm in diameter) (Fig. A.8). The dough pieces were then placed on a perforated sheet pan (Fig. A.9).

Freezing conditions and storage time

Freezing used an air blast system (Enersyst Development Co., Dallas, TX.) at -20°C (-4°F) (Fig. A.10). Dough pieces were placed in the air blast until the dough's core reached -5 to -8°C (18 to 23°F), (about 45-50 minutes exposure). After freezing, the dough pieces were packed into plastic bags (Fig. A.11). The doughs were then stored at -18 to -20°C (-4 to 0°F) for 1 day or 4, 8, or 12 week intervals before thawing for a baking test.

Figures A.1 to A.11 show the procedures for frozen dough preparation.

Thawing and Proofing

Individual loaf pans ($4.5 \times 10.5 \times 3$ inch) (Fig. A.12 & A.13) were greased, and each dough piece paned. Before baking, the dough pieces were thawed for 16 to 18 hours in a retarder (walk-in cooler) at 3 to 4°C (37 to 39°F) and 90% relative humidity (Fig. A.14 & A.15). Thawed dough pieces were placed at room temperature conditions until their core temperature reached 18°C (64°F). The doughs were then moved into a proof cabinet (Adamatic Inc., Eatontown, NJ) (Fig. A.16) maintained at 40°C (104°F) and 70% relative humidity, and proofed to a height two cm above the pan (Fig. A.17). Depending on the treatment, proofing time

required ranged from 70 to 110 minutes. Table B.1 shows the proofing time data of bake test – set one. Table B.2 shows the proofing time data of bake test –set two.

Baking and Cooling

Doughs were baked for 22 minutes at 210°C (410°F) in a gas fired reel oven (Reed Oven Co.) (Fig. A.18 & A.19), and cooled under room conditions for about 60 to 75 minutes until the loaf core reached 32-43°C (90-110°F). After cooling (Fig. A.20), the loaves were packed in plastic bags (Fig. A.21) prior to subsequent texture and image analysis.

Figures A.12 to A.21 show the whole process for the process after the frozen storage. The frozen dough preparation process is outlined in Figure 4.1.

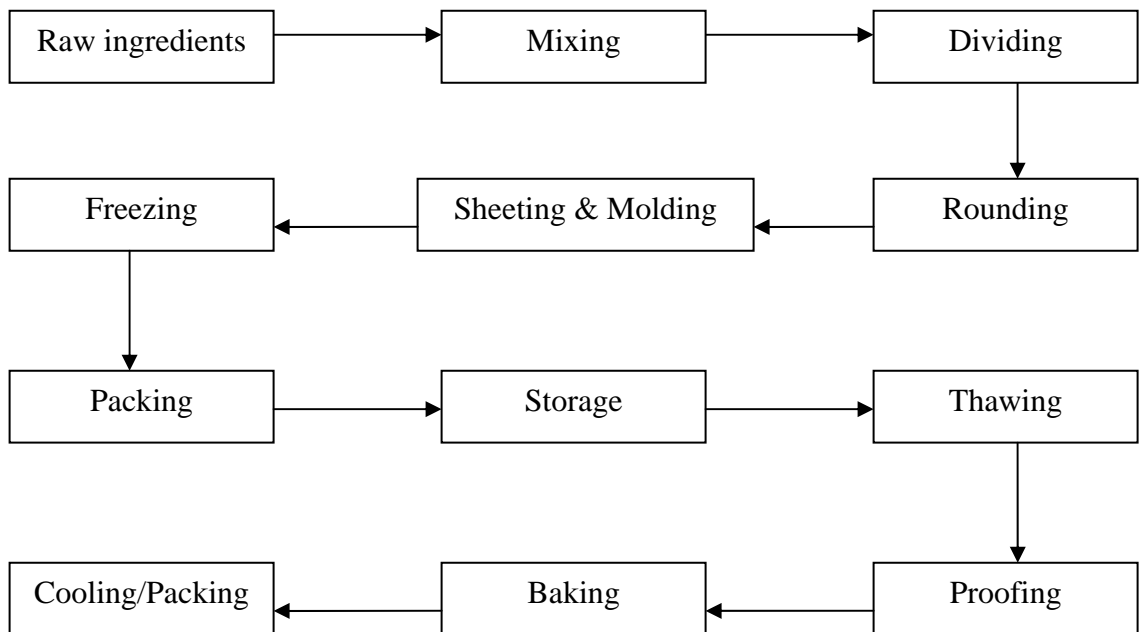


Figure 4.1 Process flow chart for frozen dough preparation and baking.

Specific loaf volume measurement

Loaf weight and loaf volume were measured at 60 to 75 minutes after baking. Loaf volumes were determined with a rapeseed displacement volume meter (AACC 10-05 2000) (Fig. A.22 & A.23). Specific volume was calculated as equation (1).

$$\text{Specific Volume (SV)} = \frac{\text{Loaf volume (ml)}}{\text{Loaf weight (gram)}} \quad (1)$$

Texture and Image Analysis

Bread texture was measured by the Voland-Stevens-LFRA Texture Analyser (Brookfield Engineering Lab. Inc. Middle Boro, MA, USA) (Fig. A.24) based on AACC method 74-09, and test type was set to normal, penetration distance 6 mm, and speed set at 2.0 mm/sec. The acrylic plastic probe was 25 mm in diameter and 32 mm long. For this test, the probe was depressed 6 mm (25%) into a 25 mm thick sample slice. The bread samples were sliced to 13 mm (0.5 inches) thickness and stocked as 2 slices when measuring. The samples were assessed at 1, 2, and 3 days after baking. The test results were determined as the average of 6 slices for each loaf sampled. These 6 slices were chosen from the fourth and fifth slice from each end of the loaf and from the middle slices.

Image analysis of loaf crumb structure was conducted by C-Cell (Calibre Control International Ltd. Warrington WA4 4ST, UK) (Fig. A.26 to A.29). The instrument was connected to a PC running C-Cell software version 2. The samples were measured 24 hours after baking.

For sample preparation, the loaf was sliced using a rotary slicer (electric food slicer model 640, Chef'sChoice[®] International) (Fig. A.25) to 13mm thickness. Three of the slices

were selected and measured per loaf sample. These 3 slices were chosen from the fourth slice from each end of the loaf and from the middle slice. Before starting to measure the samples, a calibration board was placed into the sample drawer to adjust the instrument. Then, each slice was placed in the center of the sample drawer for measurement. The slice picture was taken with a black background. The C-Cell software can provide data on 48 different slice properties including slice area (mm²), slice brightness, wall thickness (mm), and cell diameter (mm) that will be considered in the discussion.

Statistical Analysis

Dough rheological tests, bake test set two, and bread staling test data were collected in duplicate. The other test data were collected in triplicate. The data were evaluated by analysis of variance (ANOVA) with SAS computer software (version 9.1, SAS Institute, Inc., Cary, NC, USA). A significance level of $P < 0.05$ was applied throughout the analysis.

CHAPTER 5 - Results and Discussion

Dough Rheological Tests

Many physical and chemical factors can affect a dough's rheological behavior during and after mixing. Reducing or oxidizing agents and enzyme treatments are some good chemical examples. Oxidizing agents and enzyme treatments can affect a dough's viscoelasticity. The purpose of this rheological test is to know the effect of AA and hemicellulase upon dough viscoelasticity during mixing and proofing.

Individual tests were done in duplicate. Table 5.1 shows the test data. Figures 5.1 to 5.8 show one example of each treatment by Mixograph or Alveograph measurements. Figures 5.1 to 5.4 show the effects of using AA alone and in combination with hemicellulase by the Mixograph measurements in duplicate. As compared with dough that had no additive, the doughs after being treated either with AA or hemicellulase did not show any evident change in their Rheology. This is because the Mixograph test was run at about 25 °C with 10 minutes mixing time. Based on this temperature or mixing time, hemicellulase probably did not have enough time to be fully activated. However, the combination of AA and hemicellulase did show some affect on the dough's viscoelasticity, as compared with the dough that had no additives.

Figures 5.5 to 5.8 show the results from the Alveograph tests for these same four samples in duplicate. Alveograph results obviously showed stronger or weaker gluten networks after being treated with either AA or hemicellulase (Fig 5.6 & Fig 5.7). As compared with the dough without any additives, hemicellulases are able to make the dough less viscoelastic and slack if used alone; oxidizing agents can strength gluten by reconstructing disulfide bonds during dough mixing. Therefore, after adding AA into the dough, the dough's extensibility decreased and tenacity increased. The combination of AA and hemicellulase also showed increased dough's viscoelasticity. Alveograph test time is about 30 minutes for each treatment and at 30 °C, which allows AA or hemicellulase to have grater effect on the doughs.

Table 5.1 Dough rheological test results from the Mixograph and the Alveograph. Each tabulated point is the average for the tests. The Mixograph and the Alveograph tests were each done in duplicate. For both the Mixograph and Alveograph tests, the flour protein was about 12.72 % and the water absorption was 62 %, based on flour weight.

	Mixo Peak Time (min)	Mixo Value (%)	Mixo Width (%)	Alveo P (mm)	Alveo L (mm)	Alveo Work (%-min)
No additives	4.49	49.30	20.60	85	105	312.0
Ascorbic Acid (AA)	4.02	49.85	27.41	108	92	375.5
Hemicellulase (HC)	4.04	49.49	22.93	85	123	344.5
AA+HC	3.82	47.85	22.24	113	73	335.5

Mixograph

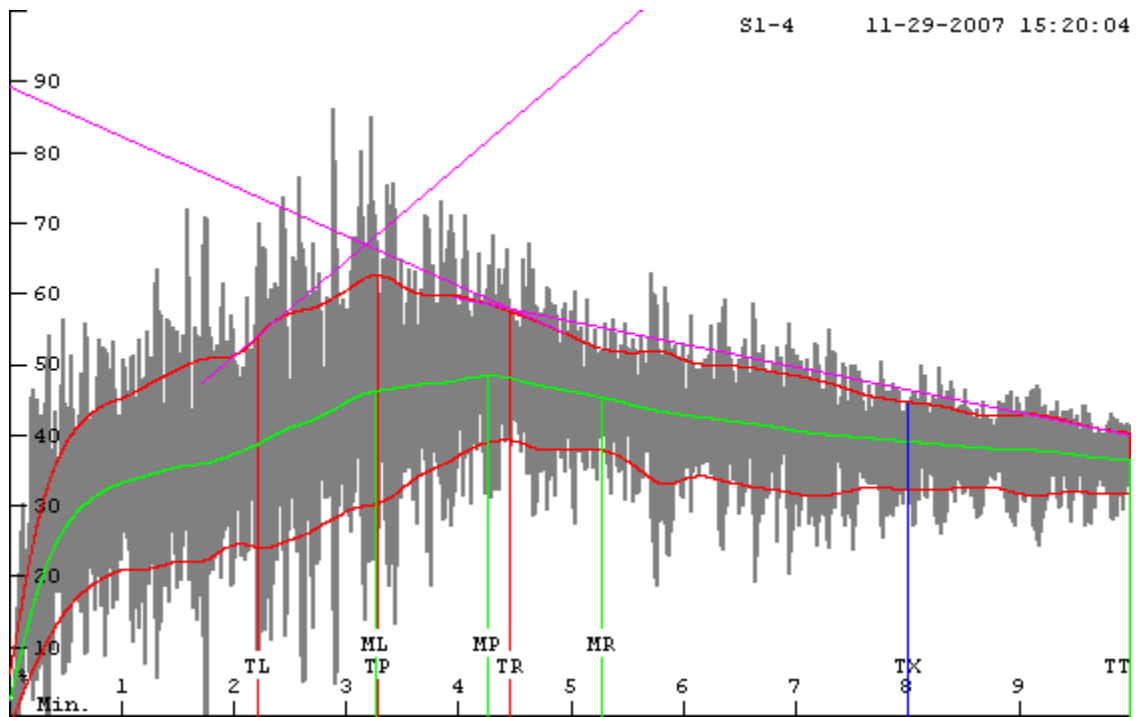


Figure 5.1 Mixogram for commercial bread flour without dough additive.

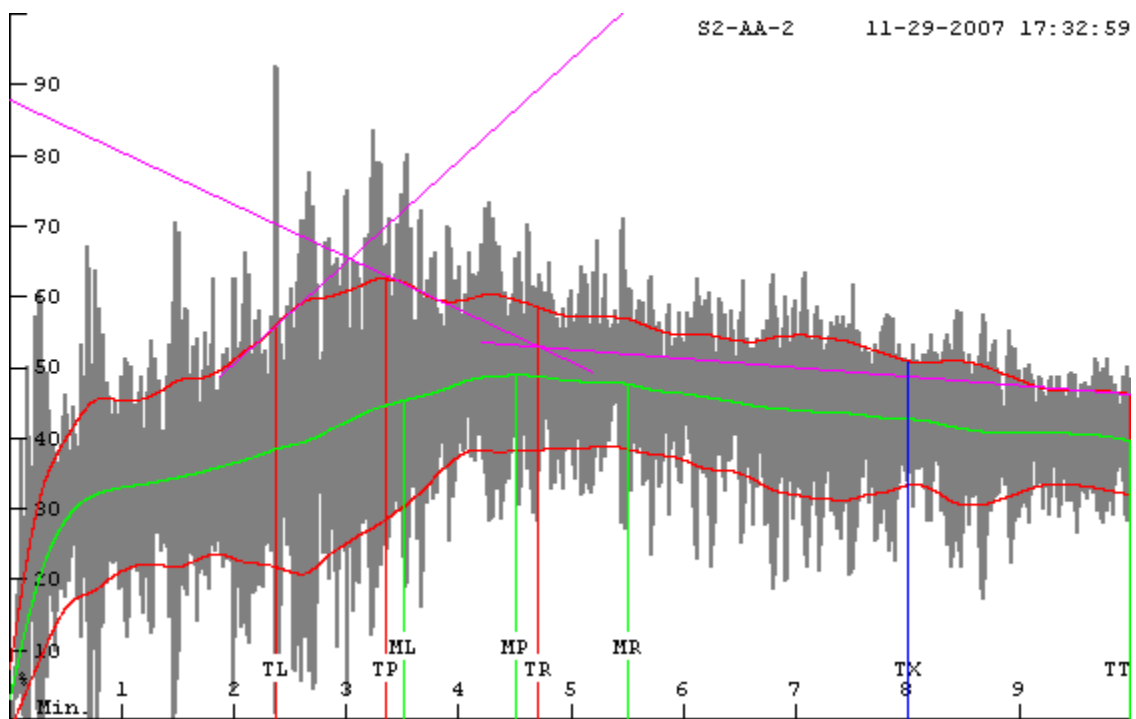


Figure 5.2 Mixogram for commercial bread flour with the oxidant, ascorbic acid.

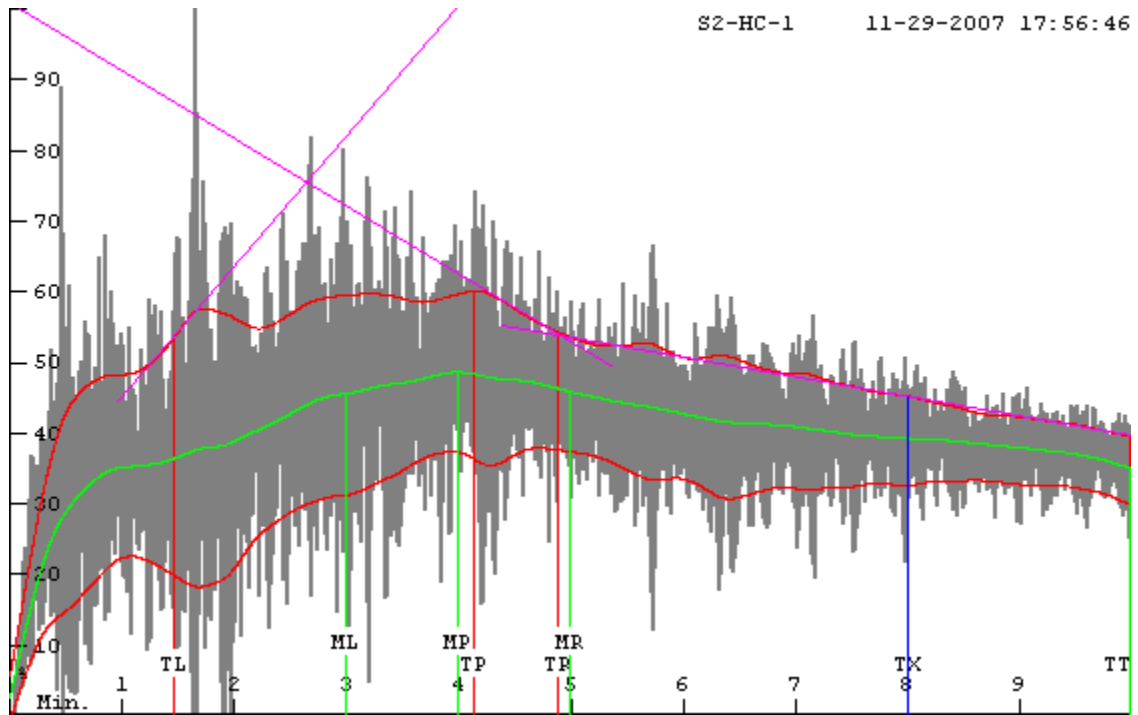


Figure 5.3 Mixogram for commercial bread flour with enzyme treatment, hemicellulase.

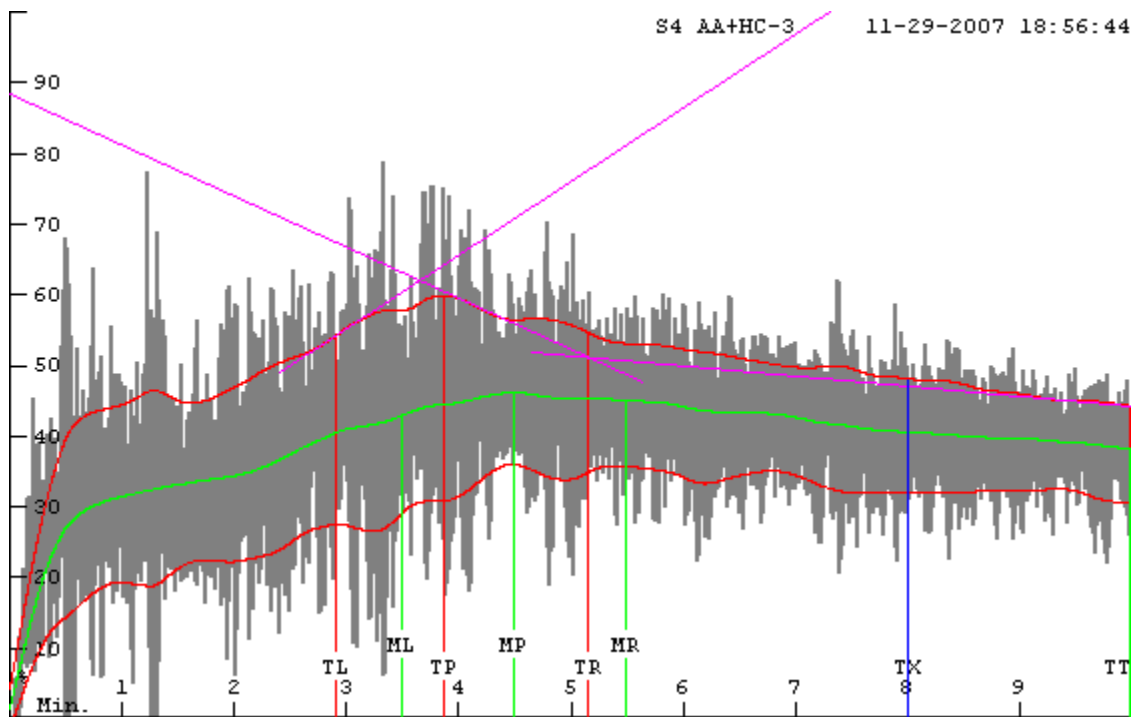


Figure 5.4 Mixogram for commercial bread flour with both dough additives, ascorbic acid and hemicellulase.

Alveograph

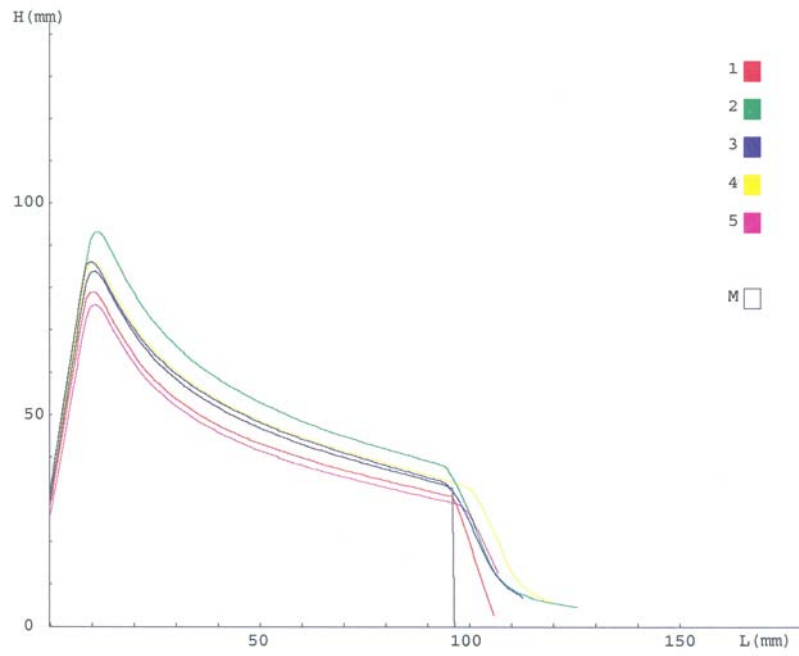


Figure 5.5 Alveograph for commercial bread flour without dough additive.

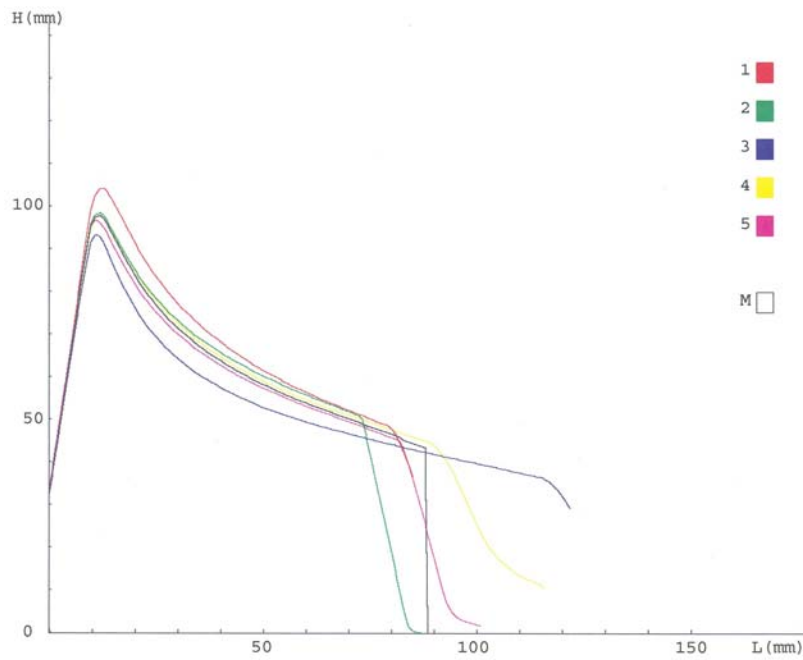


Figure 5.6 Alveograph for commercial bread flour with the oxidant, ascorbic acid.

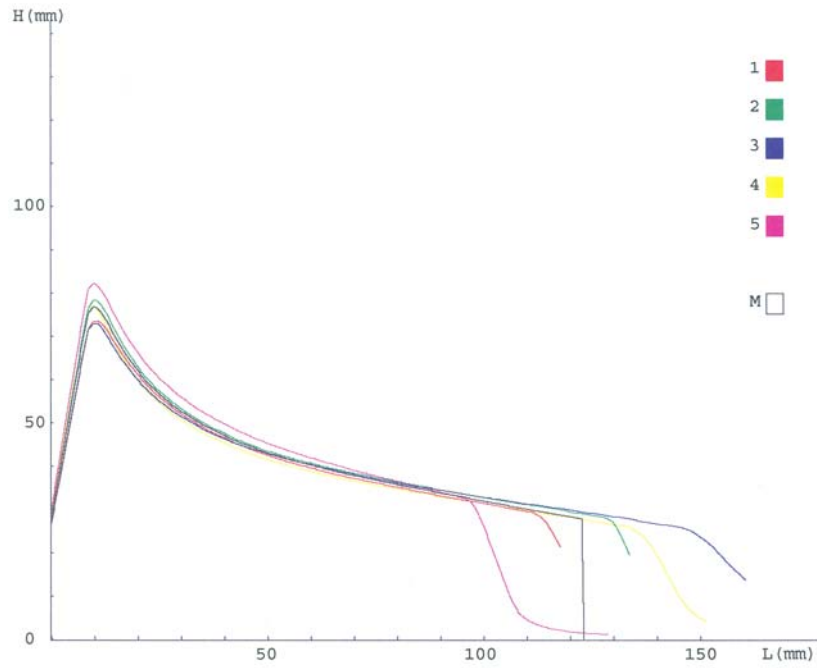


Figure 5.7 Alveogram for commercial bread flour with the enzyme treatment, hemicellulase.

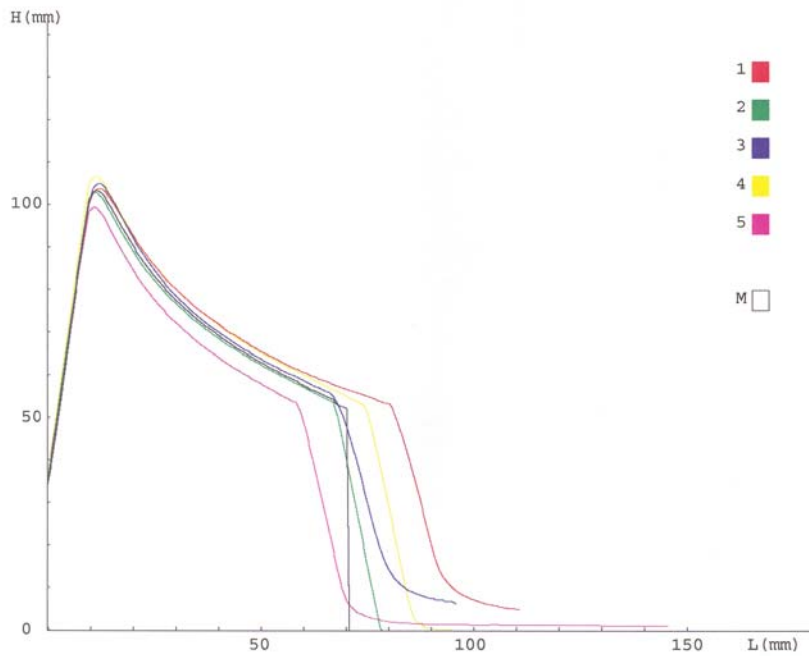


Figure 5.8 Alveogram for commercial bread flour with both dough additives, ascorbic acid and hemicellulase.

**The effects of bromate, ascorbic acid, and hemicellulase/endoxylanase on
bread specific volume**

Set One

Table 5.2 Average specific loaf volumes (SV) from nine loaf samples (three doughs, three loaves from each), three days baking in triplicate. The baking test was done one week after the frozen dough was produced. Superscripts A, B, C, and D are significantly different at $P < 0.05$ from each other superscript group.

	Bromate	AA*	Lipase	Hemi-cellulase	Bromate + AA*	Bromate + Lipase	Bromate + Hemi-cellulase	AA* + Lipase	AA* + Hemi-cellulase
Average SV (ml/gram)	4.894 ^B	4.854 ^{BC}	4.470 ^D	4.882 ^B	5.343 ^A	4.714 ^{BCD}	5.219 ^A	4.610 ^{CD}	5.238 ^A
STDEV	0.104	0.180	0.077	0.177	0.221	0.078	0.146	0.099	0.163
CV, %	2.119	3.716	1.723	3.635	4.134	1.665	2.789	2.154	3.104

* AA = Ascorbic Acid.

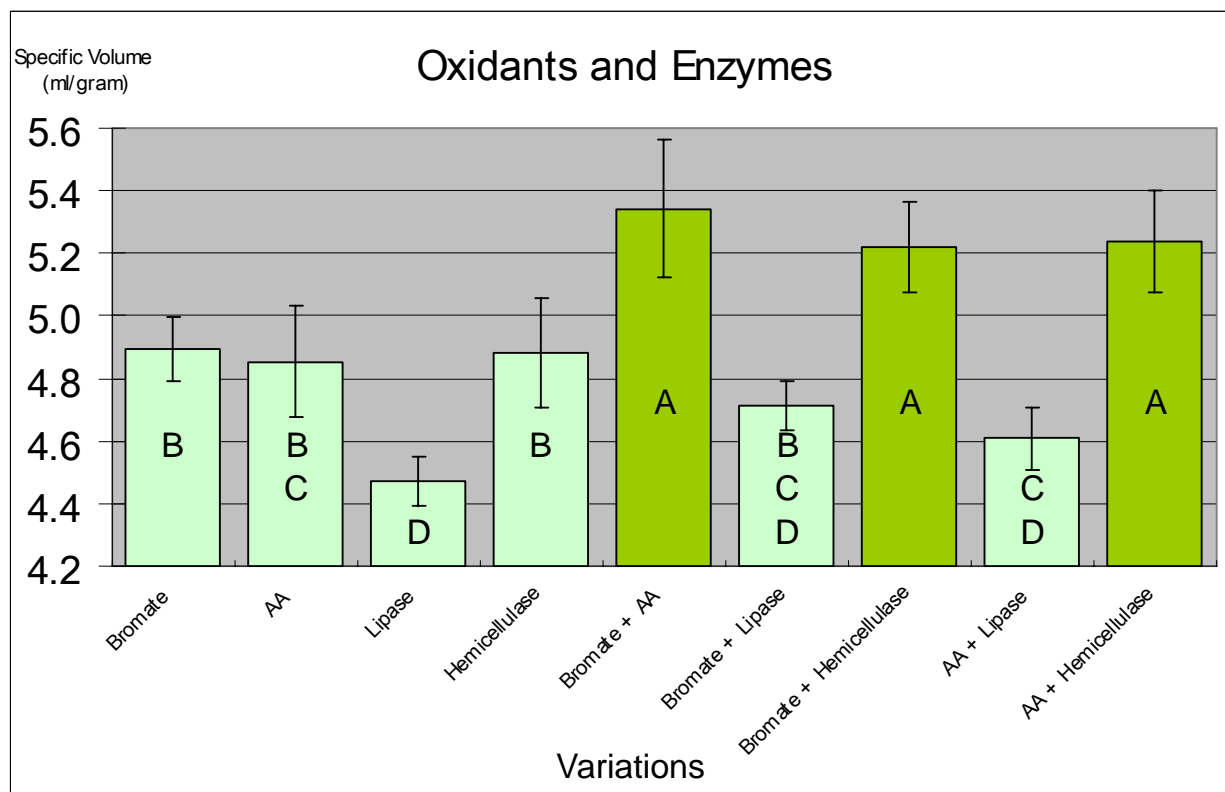


Figure 5.9 Each bar is the average specific loaf volume from nine loaves (three doughs, three loaves from each), three loaves in each of triplicate experiments. The doughs were baked one week after the frozen dough was produced. Groups A, B, C, and D proved to be significantly different from each other at $P < 0.05$.

The effect of the oxidants, bromate and ascorbic acid, on bread volume

The purpose of the oxidants, potassium bromate and ascorbic acid (AA), is to improve bread quality and final product volume (Cauvain and Young 2001). From Table 5.2 and Figure 5.9, the results obviously show that using AA alone does not increase bread loaf volume very much. Even though potassium bromate is a well-known strong oxidant, using potassium bromate alone does not increase final product volume as much as the combination of potassium bromate and AA because these two oxidants have different reaction rates. According to Pylar (1988), potassium bromate has a slow reaction rate and acts during oven time. AA is an intermediate reaction oxidant and acts during dough mixing and proofing. Therefore, using either one of them alone does not benefit frozen dough quality. The combination of potassium bromate and AA provides the best loaf volume in this test set. Similar experimental results were

found in Inoue and Bushuk's (1991) study. The authors pointed out that, as compared with using AA alone, the combination of potassium bromate and AA strengthened the doughs and improved the baking potential of frozen dough.

The effect of oxidants and enzymes used alone and their combinations on bread volume

Enzymes are able to increase dough quality as measured by final volume they but also make the dough less viscoelastic and slack. Hence, industrial as well as academic researchers include them in combination with other dough additives (Morita et al 1997). This experiment used lipase and hemicellulase either alone or combined with potassium bromate or AA. The results showed that using either lipase or hemicellulase alone did not increase bread loaf volume, as compared with the combination of bromate and AA, partially because an enzyme alone made the dough weaker. On the other hand, using a combination with either potassium bromate or hemicellulase did increase the final loaf volume significantly as compared with using each of these additives alone (See Table 5.2 & Figure 5.9). The final specific loaf volume was the lowest when using lipase alone or in combination with either potassium bromate or AA. That means that, as used here, the lipase had no benefit in bread making. Based on our discussion in "Lipase", lipase can have either positive or negative effects on bread doughs. The experimental results could be affected, based on the processing condition, the sources of lipase, and its concentration. This test used the concentration of 40 ppm of lipase alone or in combination with either bromate or AA. This concentration may not be the very best concentration to benefit the dough quality, however, when used in combination.

Moreover, Figure 5.9 also shows that, for specific loaf volume, the combination of hemicellulase either combined with potassium bromate or AA showed no significant difference at $P < 0.05$ as compared with the combination of potassium bromate and AA. Thus, either the combination of potassium bromate and hemicellulase or the combination of AA and hemicellulase can be a replacement for the traditional combination of potassium bromate and AA in frozen dough making.

Potassium bromate is known to be functional in improving dough strength, but its use has been banned in many countries. Replacing potassium bromate with hemicellulase in this combination has potentially higher acceptance by consumers. Recently, consumers tend to

prefer natural bread without additives (Morita 1997). In this combination, AA is well-known as vitamin C, and hemicellulase is a safe natural dough improver additive. The mechanism is different from the way that potassium bromate functions, but the final result benefits the dough similarly. Therefore, hemicellulase can be used for a bromate replacement when it is combined with AA.

Set Two

Table 5.3 Average specific loaf volumes (SV) from six loaf samples (two doughs, three loaves from each), two days baking in duplicate. The baking test was done one week after the frozen dough was produced. Superscripts A, B, C, and D are significantly different at $P < 0.05$ from each other superscript group.

	Control	Bromate	AA	Hemi-cellulase	Endo-xylanase	Bromate + AA	Bromate + Hemi-cellulase	Bromate + Endo-xylanase	AA + Hemi-cellulase	AA + Endo-xylanase
Average SV (ml/gram)	4.805 ^B	4.762 ^B	4.746 ^{BC}	4.420 ^D	4.572 ^{CD}	5.043 ^A	5.015 ^A	4.812 ^A	4.870 ^{AB}	4.912 ^{AB}
STDEV	0.044	0.211	0.345	0.103	0.147	0.071	0.201	0.143	0.065	0.233
CV, %	0.920	4.438	7.273	2.332	3.221	1.416	4.007	2.962	1.337	4.751

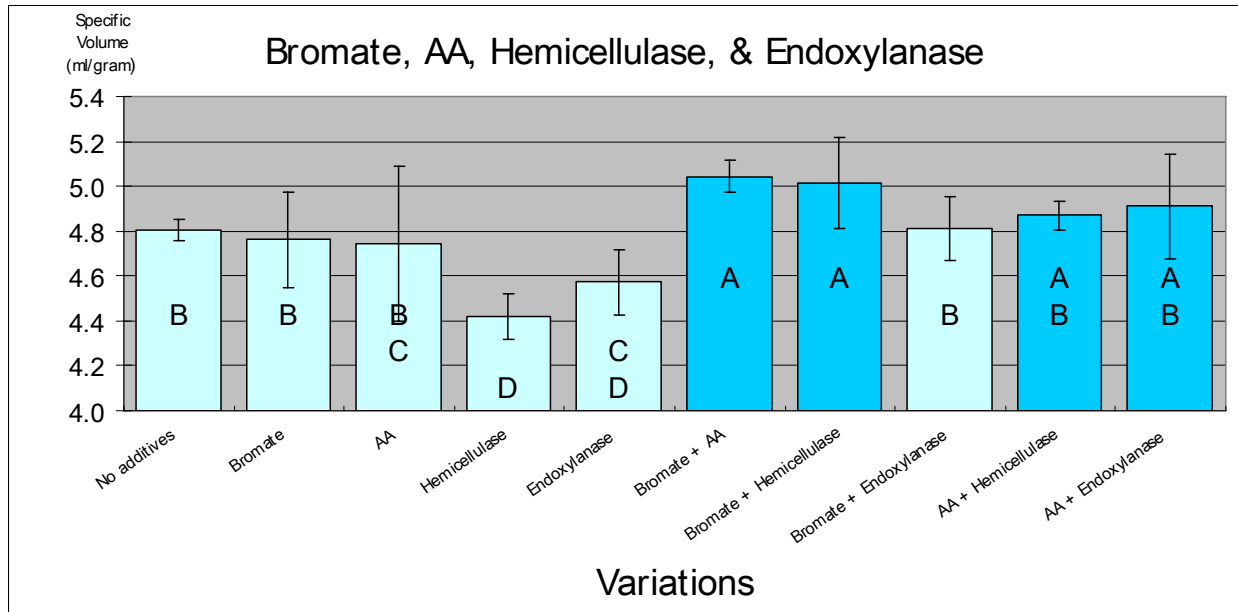


Figure 5.10 Average specific loaf volumes from six loaf samples (two doughs, three loaves from each), two days baking in duplicate. The baking test was done one week after the frozen dough was produced. Superscripts A, B, C, and D are significantly different at $P < 0.05$ from each other superscript group.

The results from “set two” are similar to those from set one. The baking test was done one week after the frozen dough was produced. Hemicellulose is a minor fraction (~3%) of wheat flour. One of its major constituents is arabinoxylan. Endoxylanase is able to hydrolyze the linkage between two xylose units in the xylan backbone resulting in reduction of the length of the backbone (Bakezyme HSP 6000 BG application data sheet).

Figure 5.10 shows that using hemicellulase or endoxylanase alone decreased bread volume significantly, as compared with the control. This is because the enzyme can make yeast dough slack and less elastic when used alone. Because using these enzymes alone cannot benefit frozen dough quality, using an enzyme-oxidant combination is often recommended. As we observed in test set one, using potassium bromate or AA alone cannot increase loaf volume by very much. However, when used in combination, the volume increase was significant at $P < 0.05$.

Based on the average of the tests (See Table 5.3), the combination of potassium bromate and AA provided the best final loaf volume. In the other three combination treatments, potassium bromate and hemicellulase, AA and hemicellulase, and AA and endoxylanase showed no significant differences in their final bread volume (See Figure 5.10). Therefore, these three combination treatments can be a replacement for the additional combination, potassium and AA, in frozen dough making.

The effect of bromate, ascorbic acid, and hemicellulase on stored bread

Staling of bread during storage

Bread firmness during storage was measured by the Volland-Stevens-LFRA Texture Analyser. The results are shown in Figure 5.11 and 5.12. Each figure shown is for frozen dough stored in the freezer (-20°C) for 1 day, or 4, 8, or 12 weeks after the dough was produced. Each point on the graphs is the average of two loaves (twelve slices), one loaf for each duplicate experiment. There were no statistically significant differences ($P < 0.05$) among the bread specific volumes for the different frozen dough storage time. Firmness for the three treatments was similar one day after baking. After two or three days storage time at room temperature (20 - 23°C), the reference, which contained bromate and AA, had increased its staling rate rapidly, as compared with the other two treatments. This is because the enzyme is able to slow bread's staling rate. Starch begins staling immediately after bread baking. Starch staling is a natural phenomenon. If an anti-staling dough additive is added to the dough formula, the bread staling rate can be reduced. A similar experiment was done by Morita et al (1997). They concluded that the bread containing hemicellulase alone and hemicellulase with calcium stearoyl-2-lactylate (CSL) had slightly increased in softness during its storage. Figure 5.11 and 5.12 obviously show that the dough containing hemicellulase delayed firmness development during bread storage. In other words, hemicellulase is able to extend bread shelf life when used in frozen dough making. This is because, during dough mixing and proofing, hemicellulase broke down hemicelluloses, so that the gluten can have better gas retention. It resulted in a better final bread volume and reduced staling rate.

Not only dough additives, but also the frozen storage time, can influence bread staleness. Figure 5.11 and 5.12 also show that, as the freezing storage time increases, the bread firmed faster. Comparing the different storage times, the dough which was stored for four weeks increased its firmness over that for one day storage. Likewise, comparing the storage time of eight weeks with four weeks in all treatments, the shelf life was on average one day less for the eight weeks bread. After eight weeks storage, however, the staling rate of the baked bread did

not show great evidence of increasing. Conversely, as frozen storage time increases, the tripeptide glutathione and the number of yeast dead cells normally increases, which influenced the dough viscoelasticity (Wolt and D'apponia 1984a). Therefore, longer storage time reduces oven spring during bread baking.

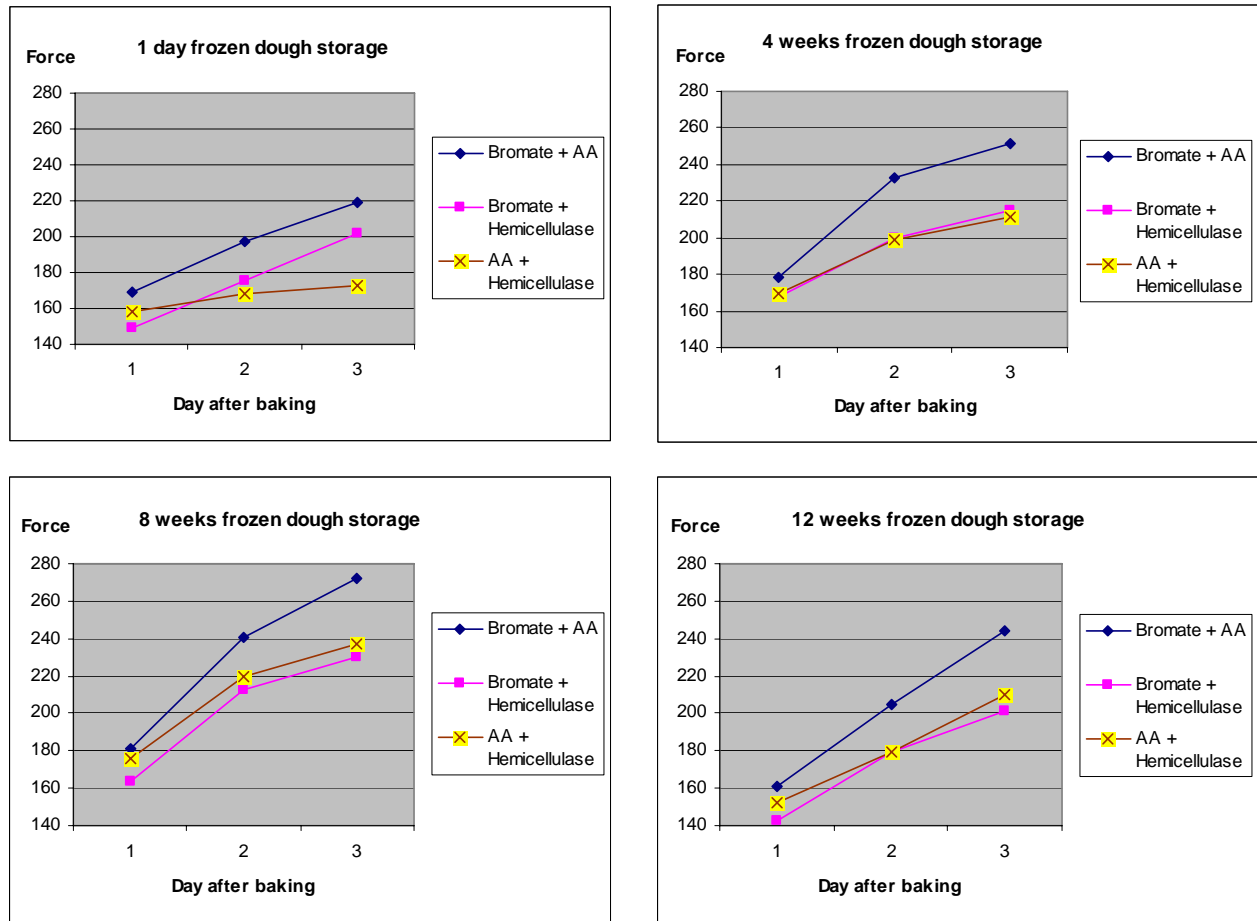


Figure 5.11 Bread firmness at 1, 2, and 3 days after baking.

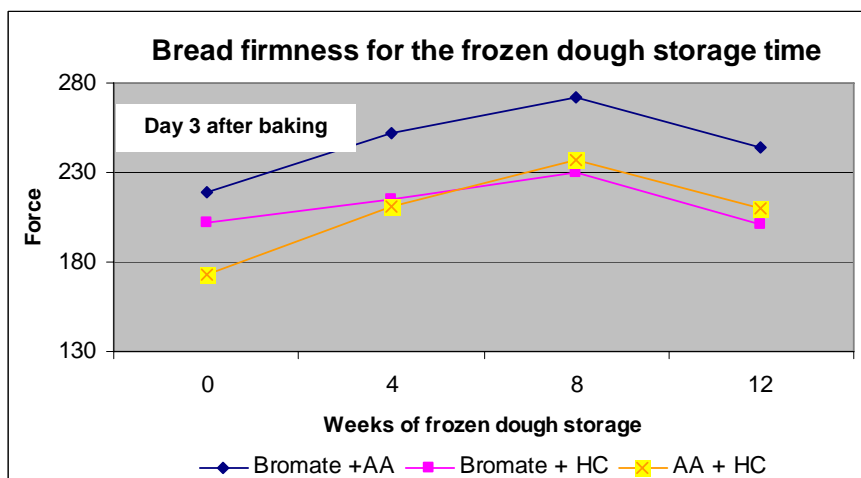
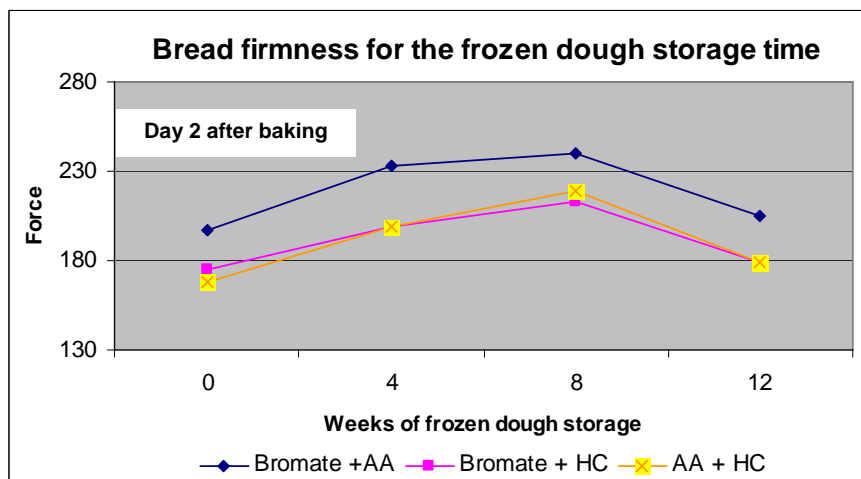
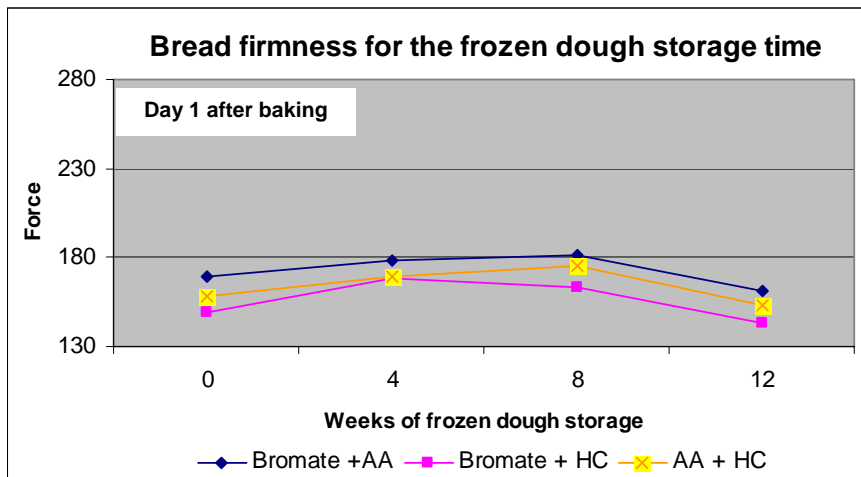


Figure 5.12 Bread firmness at 1, 2, and 3 days after baking.

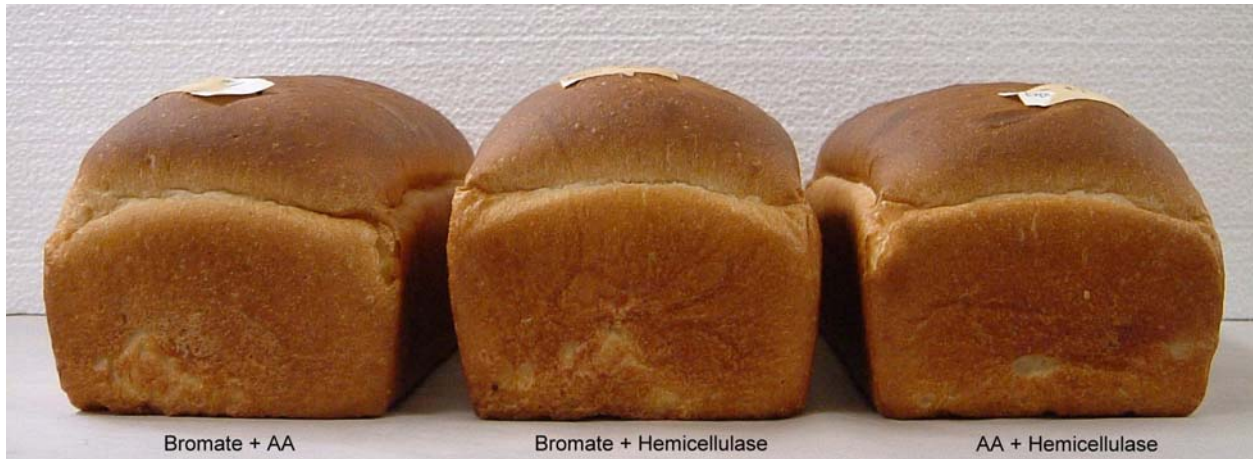


Figure 5.13 Bread sample pictures. The sample from left to right is the combination of bromate and AA, bromate and hemicellulase, and AA and hemicellulase.

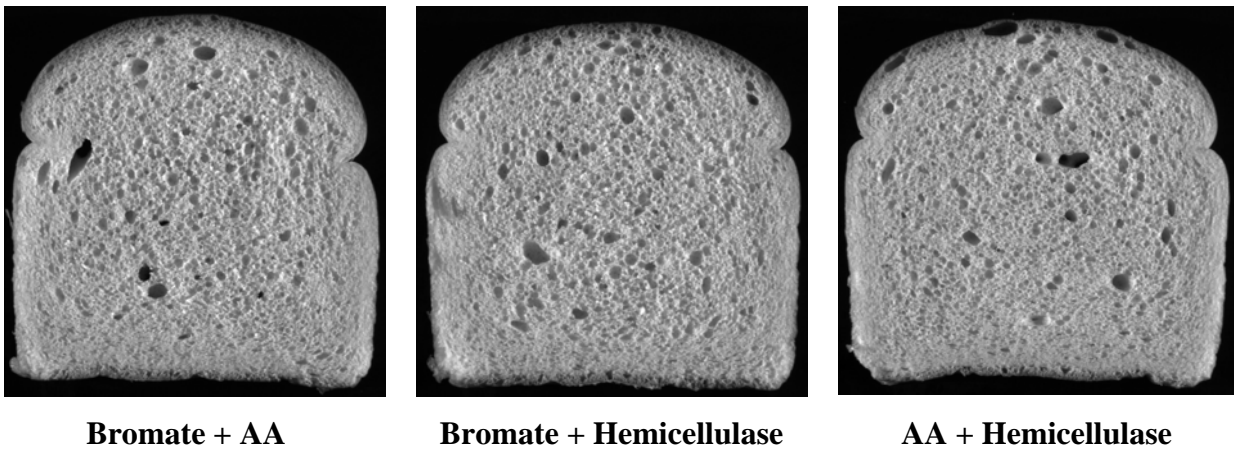


Figure 5.14 Final product crosssection. The sample from left to right is the combination of bromate and AA, bromate and hemicellulase, and AA and hemicellulase. The pictures were taken by C-Cell.

Slice Area

Table 5.4 Average of slice area (mm²). The data was measured by C-Cell. The bread was evaluated one day after the dough was baked. Four different frozen storage times, 1 day, or 4, 8, or 12 weeks, are shown in each column. Three slices were chosen from one loaf in each duplicate.

Variation	Frozen dough storage time			
	1 day	4 weeks	8 weeks	12 weeks
Bromate + AA				
Average	8922	8971	8635	8778
St Dev	299	156	173	252
CV, %	3.35	1.74	2.01	2.87
Bromate + HC				
Average	8932	8882	8790	8612
St Dev	285	155	133	308
CV, %	3.20	1.75	1.52	3.58
AA + HC				
Average	8881	8894	8437	8789
St Dev	330	85	74	99
CV, %	3.71	0.96	0.88	1.13

The three treatments which are shown in Table 5.4 showed no significant differences at $P < 0.05$ in their slice area (mm²) between these three different treatments and various storage times. The frozen dough samples were stored in the freezer (-18°C to -20°C) for one day, or four, eight, or twelve weeks before the bake test. This result was in agreement with the test results shown in Table 5.2 and Figure 5.9.

These three different treatments provided the statistically same final loaf volume for each. Therefore, following this result, either the combination of potassium bromate and HC or

the combination of AA and HC can be a replacement for the combination of potassium bromate and AA in frozen dough making.

Slice Brightness

Table 5.5 Average of slice brightness (0 (dark) – 255(white)). The data was measured by C-Cell. The bread was evaluated one day after the dough was baked. Four different frozen storage times, 1 day, or 4, 8, or 12 weeks, were shown in each column. Three slices were chosen from one loaf in each duplicate.

	Frozen dough storage time			
Variation	1 day	4 weeks	8 weeks	12 weeks
Bromate + AA				
Average	148.77	146.32	145.22	145.20
St Dev	1.320	0.259	1.061	1.320
CV, %	0.89	0.18	0.73	0.91
Bromate + HC				
Average	145.77	142.10	141.52	141.98
St Dev	5.563	0.330	1.673	2.145
CV, %	3.82	0.23	1.18	1.51
AA + HC				
Average	144.27	143.82	141.43	143.22
St Dev	0.236	3.182	1.603	0.306
CV, %	0.16	2.21	1.13	0.21

From Table 5.5, the results obviously show that there are two factors affecting the crumb brightness. One is the oxidant treatment; the other one is the frozen storage time. When comparing these three treatments, the samples which contained potassium bromate had the highest crumb brightness. This may be partially because of some decoloration of lipids and pigments caused by oxidants, especially potassium bromate. When adding potassium bromate to the dough, it oxidized proteins to strength the gluten network. Therefore, the final baked product had a finer and more uniform cell grain. In general then, the finer cells reflect a lighter crumb

color since the surface does not have the large, dark holes. Even though hemicellulase is able to induce finer grain cells (Hammond 1994), its function is limited and its function is not as strong as potassium bromate, especially after a long prior frozen storage.

Table 5.5 also shows that, as frozen storage time increased, the crumb brightness decreased. The frozen dough loses its strength gradually during frozen storage as the time increased from 1 day to 12 weeks. The dough requires a longer proofing time and results in a coarser texture for the bread crumb. Hence, any larger and uneven cell grain reflects a gray or dark crumb color rather than white or bright.

Cell Diameter

Table 5.6 Average of cell diameter (mm). The data was measured by C-Cell. The bread was evaluated one day after the dough was baked. Four different frozen storage times, 1 day, or 4, 8, or 12 weeks, are shown in each column. Three slices were chosen from one loaf in each duplicate.

	Frozen dough storage time			
Variation	1 day	4 weeks	8 weeks	12 weeks
Bromate + AA				
Average	1.463	1.532	1.555	1.688
St Dev	0.036	0.030	0.031	0.042
CV, %	2.48	1.99	2.00	2.51
Bromate + HC				
Average	1.469	1.620	1.602	1.745
St Dev	0.044	0.149	0.010	0.055
CV, %	3.02	9.20	0.63	3.18
AA + HC				
Average	1.514	1.573	1.671	1.728
St Dev	0.014	0.147	0.012	0.039
CV, %	0.90	9.35	0.71	2.26

In the case of the slice brightness study, we mentioned that the frozen storage time could affect the bread crumb texture. Table 5.6 shows this quantitatively. As frozen storage time increases, the bread tends to have a coarser texture. The results appear to be compared with the size of the cell diameters. Comparing the cell diameter from time to time for the same treatment; it significantly increased at $P < 0.05$. This phenomenon was shown for each different treatment. Since frozen dough storage time increased, the amount of the tripeptide glutathione released from dead yeast cells probably increased. The protein network tends to weaken. Therefore, during dough proofing, small gas cells coalesce into larger gas cells.

Comparing the differences for each treatment, based on the same storage time, the three samples showed no significant difference in their cell diameter at $P < 0.05$. Hemicellulase was also used for a bread experiment in 1997 by Morita et al. The researchers concluded that “the addition of hemicellulase did not change the mean diameter of gas cells distinctly.” Our results are in agreement with theirs.

Therefore, based on this experiment, we can conclude that the frozen storage time for a dough has a larger effect than does treatment with a dough additive.

Wall Thickness

Table 5.7 Average of wall thickness (mm). The data was measured by C-Cell. The bread was evaluated one day after the dough was baked. Four different frozen storage times, 1 day, or 4, 8, or 12 weeks, are shown in each column. Three slices were chosen from one loaf in each duplicate.

	Frozen dough storage time			
Variation	1 day	4 weeks	8 weeks	12 weeks
Bromate + AA				
Avg	0.397	0.405	0.409	0.420
St Dev	0.004	0.004	0.002	0.007
CV	0.951	0.931	0.461	1.684
Bromate + HC				
Avg	0.403	0.414	0.413	0.423
St Dev	0.000	0.010	0.004	0.007
CV	0.000	2.505	0.856	1.560
AA + HC				
Avg	0.402	0.411	0.419	0.421
St Dev	0.002	0.015	0.001	0.003
CV	0.411	3.553	0.281	0.671

From Table 5.7, the results show that based on the same treatment, as frozen dough storage time increased from one day to twelve weeks, crumb wall thickness increased. These results relate to the discussions in “Cell Diameter.” Longer frozen dough storage time provides weaker gluten structure. During dough proofing, small gas cells coalesce into larger gas cells. Therefore, the grain cell wall becomes thicker.

However, based on the same frozen dough storage time, bread crumb wall thickness did not show evident difference among the three treatments. Therefore, we can conclude that the

frozen storage time for a dough has a larger effect on bread wall thickness than does treatment with dough additive.

Correlation coefficient, R, between various slice properties across all three treatments

Table 5.8 One day frozen storage before baking test.

Slice Properties	SV (ml/gram)	Slice Area (mm ²)	Slice Bright- Ness	Cell Diameter (mm)	Wall Thick- ness (mm)	Firm- ness (1 day) (gram)	Firm- ness (2 day) (gram)	Firm- ness (3 day) (gram)
Specific Volume (SV) (ml/gram)	1							
Slice Area (mm ²)	0.19	1						
Slice Brightness	0.89	0.62	1					
Cell Diameter (mm)	-0.47	-0.96	-0.82	1				
Wall Thickness (mm)	-1.00	-0.21	-0.90	0.48	1			
Firmness(day 1) (gram)	0.94	-0.16	0.68	-0.14	-0.93	1		
Firmness(day 2) (gram)	0.92	0.57	1.00	-0.78	-0.92	0.72	1	
Firmness(day 3) (gram)	0.69	0.85	0.94	-0.96	-0.70	0.39	0.92	1

Table 5.9 Four weeks frozen storage before baking test.

	SV (ml/gram)	Slice Area (mm ²)	Slice Bright- Ness	Cell Diameter (mm)	Wall Thick- ness (mm)	Firm- ness (1 day) (gram)	Firm- ness (2 day) (gram)	Firm- ness (3 day) (gram)
Specific Volume (SV) (ml/gram)	1							
Slice Area (mm ²)	1.00	1						
Slice Brightness	0.96	0.96	1					
Cell Diameter (mm)	-0.91	-0.91	-0.99	1				
Wall Thickness (mm)	-0.99	-0.99	-0.99	0.96	1			
Firmness(day 1) (gram)	1.00	1.00	0.94	-0.89	-0.98	1		
Firmness(day 2) (gram)	0.99	0.99	0.90	-0.83	-0.95	0.99	1	
Firmness(day 3) (gram)	0.98	0.98	0.88	-0.81	-0.94	0.99	1.00	1

Table 5.10 Eight weeks frozen storage before baking test.

	SV (ml/gram)	Slice Area (mm ²)	Slice Bright- Ness	Cell Diameter (mm)	Wall Thick- ness (mm)	Firm- ness (1 day) (gram)	Firm- ness (2 day) (gram)	Firm- ness (3 day) (gram)
Specific Volume (SV) (ml/gram)	1							
Slice Area (mm ²)	-0.07	1						
Slice Brightness	0.99	0.09	1					
Cell Diameter (mm)	-0.71	-0.65	-0.82	1				
Wall Thickness (mm)	-0.71	-0.65	1.00	1.00	1			
Firmness(day 1) (gram)	0.84	-0.60	0.74	-0.22	-0.22	1		
Firmness(day 2) (gram)	0.99	-0.17	0.97	-0.64	-0.64	0.89	1	
Firmness(day 3) (gram)	1.00	-0.09	0.98	-0.70	-0.70	0.85	1.00	1

Table 5.11 Twelve weeks frozen storage before baking test.

	SV (ml/gram)	Slice Area (mm ²)	Slice Bright- Ness	Cell Diameter (mm)	Wall Thick- ness (mm)	Firm- ness (1 day) (gram)	Firm- ness (2 day) (gram)	Firm- ness (3 day) (gram)
Specific Volume (SV) (ml/gram)	1							
Slice Area (mm ²)	0.57	1						
Slice Brightness	0.97	0.76	1					
Cell Diameter (mm)	-0.99	-0.69	-1.00	1				
Wall Thickness (mm)	-0.90	-0.87	-0.98	0.96	1			
Firmness(day 1) (gram)	0.90	0.87	0.98	-0.96	-1.00	1		
Firmness(day 2) (gram)	0.99	0.45	0.92	-0.96	-0.83	0.83	1	
Firmness(day 3) (gram)	1.00	0.62	0.98	-1.00	-0.93	0.93	0.98	1

Tables 5.8 to 5.11 show R, the correlation coefficient, between various slice properties across all three treatments. The data is shown in Table B.13.

The correlation between specific volume (SV) and the other slice properties was high for most properties. For unknown reasons, the data for the “one day frozen” samples did not follow the trend. The reasons may be caused by unstable test conditions including changing room temperature and relative humidity. The high correlations between SV and the other slice properties may be used to predict the measurement results for the C-Cell and the Voland-Stevens Texture Analyzer.

The overall data showed that the values for slice area were partly correlated with the other slice property values. For unknown reasons, the data at four weeks storage time showed high correlation, but not at eight or twelve weeks.

Slice brightness showed a good inverse correlation with other slice properties including cell diameter. Consequently, the other measurements may predict the crumb brightness volume. For example, cell diameter is usually affected by slice brightness. The higher SV provided better crumb brightness since the higher loaf volume had in general finer cells and thinner cell walls. It reflects a lighter crumb color since the surface does not contain the large and dark holes. Conversely, larger cell diameter normally has thicker cell walls and reflects a darker crumb color.

The results also showed that the average correlation R between firmness for one to three days was high. The value was especially high between the second and the third days. Therefore, firmness value in the first two days may predict its development in the third day.

CHAPTER 6 - Conclusions

In this study, several enzymes were combined with the oxidants potassium bromate or AA to replace the combination of potassium bromate with AA in frozen dough making. The test results showed that the additional combination of potassium bromate and AA provided greater specific volume for frozen dough bread. The test results also showed that the combination of potassium bromate and AA, and the combination of AA and hemicellulase/endoxy lanase, are able to improve frozen dough quality. Their final bread volume showed that these combinations can be a replacement for the combination of potassium bromate and AA, since their final bread volumes were not significantly different.

However, using hemicellulase/endoxy lanase alone weakened the dough and could not benefit the loaf volume. Since using enzyme alone cannot improve frozen dough quality, making an enzyme with an oxidant is often recommended. The test results also showed that, as frozen storage time increased, bread staling rate increased. Frozen storage time also affected the bread crumb texture, wall thickness, and brightness. As frozen storage time passes, the breads tend to have coarser textures, thicker cell walls, and darker crumb colors.

These test results can be applied to current frozen dough making technology. Using these non-synthetic dough additives may improve frozen dough quality the same as using chemical dough additives.

For future work, more enzyme combinations with different oxidants may be applied to the test. Also, different levels of enzyme concentration may be tested experimentally. This approach may provide a mean to achieve better synergistic effects in the final bread loaf.

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Appendix A - Pictures

The pictures of the frozen dough preparation process



Figure A.1 Raw Ingredients



Figure A.2. Water bath & Mixer. Mixing bowl temperature maintained at 6 °C by a circulating refrigerated water bath.



Figure A.3 Dough mixing



Figure A.4 Dough temperature measured after mixing in mixing bowl.



Figure A.5 Dough dividing.



Figure A.6 Dough Scaling. Individual dough pieces were scaled at 540 gram.



Figure A.7 Manual Rounding of individual dough pieces.



Figure A.8 Sheeter/ Molder. After 5 minutes floor time, each dough piece was sheeted and molded by the Oshikiri equipment.



Figure A.9 Molded dough pieces slice on a perforated sheet pan



Figure A.10 Air impingement blast freezer operating at -20 °C.



Figure A.11 Frozen Dough in final Packaging.



Figure A.12 Pan Greasing.



Figure A.13 Panning



Figure A.14 Dough placed in cart with cover.



Figure A.15 Slow thawing in retarder at 3 to 4 °C (37 to 39°F) and 98% humidity for 16 – 18 hours.



Figure A.16 Proofing Cabinet. Doughs were proofed in proofing cabinet at 40°C (104°F) and 70% humidity.



Figure A.17 Dough proofed to 2cm height over the pan.



Figure A.18 Reel oven



Figure A.19 Baking. The doughs were baked for 22 minutes at 210°C (410°F) in a reel oven.



Figure A.20 Cooling under room conditions.



Figure A.21 Final Product Packaging



Figure A.22 Volume Meter



Figure A.23 Single loaf was placed in sample holder.



Figure A.24 Voland-Stevens instrument with bread slice sample.



Figure A.25 Rotary Slicer



Figure A.26 The C-Cell instrument.



Figure A.27 Sample drawer



Figure A.28 Calibration board

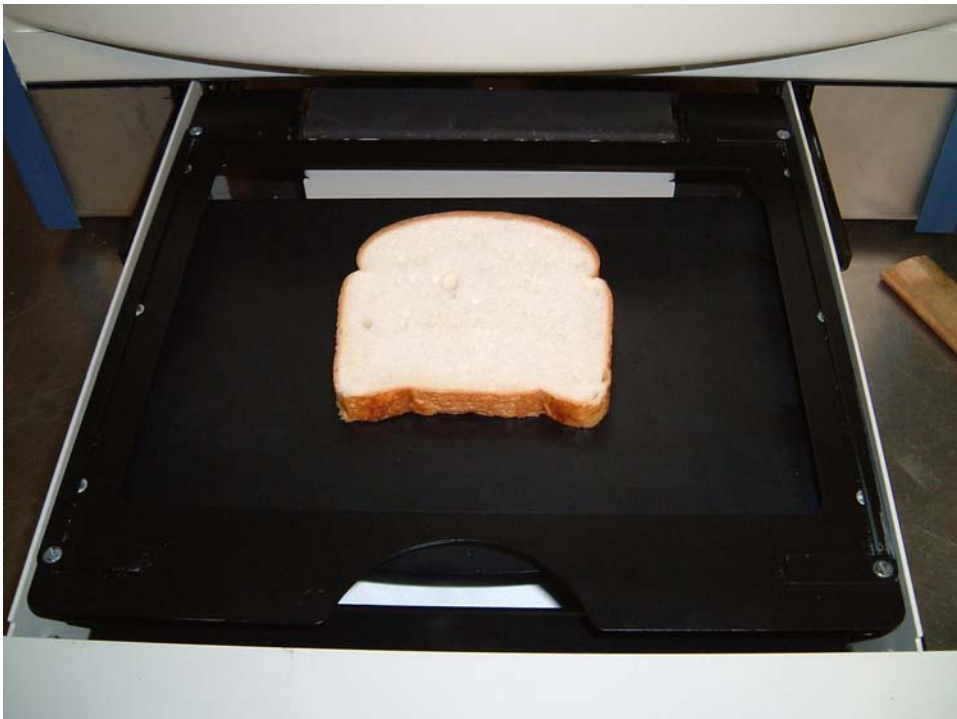


Figure A.29 Sample placed in drawer

Appendix B - Tables & Figures

Table B.1 Dough proof time (min) of the bake test – set one. The proof cabinet condition was set at 100° F/ RH 80%. The data was collected in triplicate.

		Test Variations (Proof time in minutes)								
Re- plicate	Actual Condition	Bromate	AA	Lipase	HC	Bromate + AA	Bromate + Lipase	Bromate + HC	AA + Lipase	AA + HC
1 st	95 °F/ RH 90%	108	86	96	73	96	110	118	118	114
2 nd	100 °F/ RH 70 %	90	95	95	95	90	95	80	88	88
3 rd	102 °F/ RH 70%	80	87	100	95	95	95	90	95	95
STDEV		14.19	4.93	2.65	12.70	3.21	8.66	19.70	15.70	113.45
Average		93	89	97	88	94	100	96	100	99
CV		15.31	5.52	2.73	14.49	3.43	8.66	20.52	15.64	13.59

Table B.2 Dough proof time (min) of the bake test – set two. The proof cabinet condition was set at 100° F/ RH 80%. The data was collected in duplicate.

		Test Variations (Proof time in minutes)									
Re- plicate	Actual Condition	No additives	Bromate	AA	HC	Endo- xylanase	Bromat + AA	Bromate + HC	Bromate + Endo- xylanase	AA + HC	AA + Endo- xylanase
1 st	110 °F/ RH 70%	82	65	67	69	79	76	90	82	90	78
2 nd	104 °F/ RH 70%	80	78	78	75	78	80	80	75	75	78
STDEV		1.41	9.19	7.78	4.24	0.71	2.83	7.07	4.95	10.61	0
Average		81	72	73	72	79	78	85	79	83	78
CV		1.75	12.86	10.73	5.89	0.90	3.63	8.32	6.31	12.86	0

Staling – Volland-Stevens Texture Analyzer

Table B.3 One day storage.

Staling (1 day)	Crumb Firmness		
Treatments	Day 1	Day 2	Day 3
Bromate + AA	169	197	219
Bromate + Hemicellulase	150	176	202
AA + Hemicellulase	159	168	173

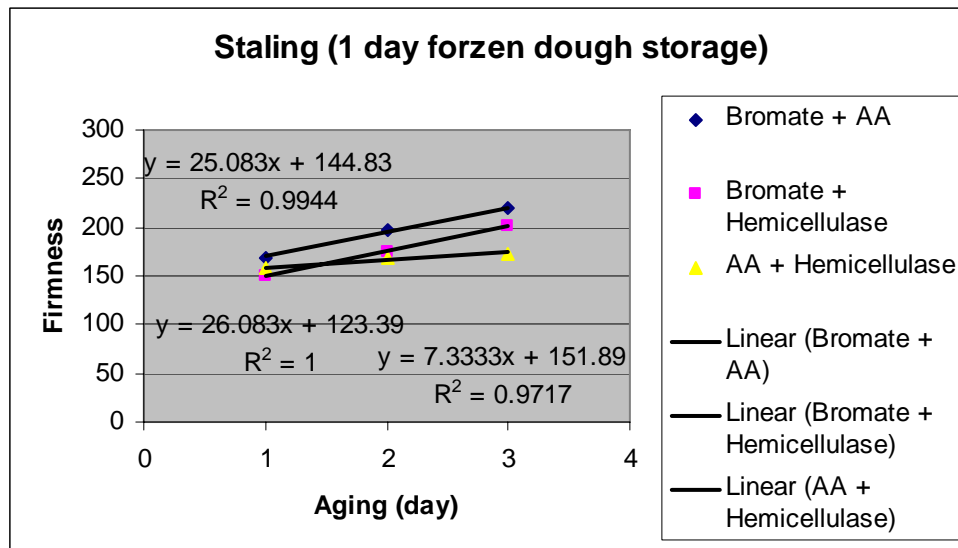


Figure B.1 One day storage.

Table B.4 Four weeks storage.

Staling (4 weeks)	Crumb Firmness			
	Treatments	Day 1	Day 2	Day 3
Bromate + AA		179	233	252
Bromate + Hemicellulase		168	200	215
AA + Hemicellulase		169	199	212

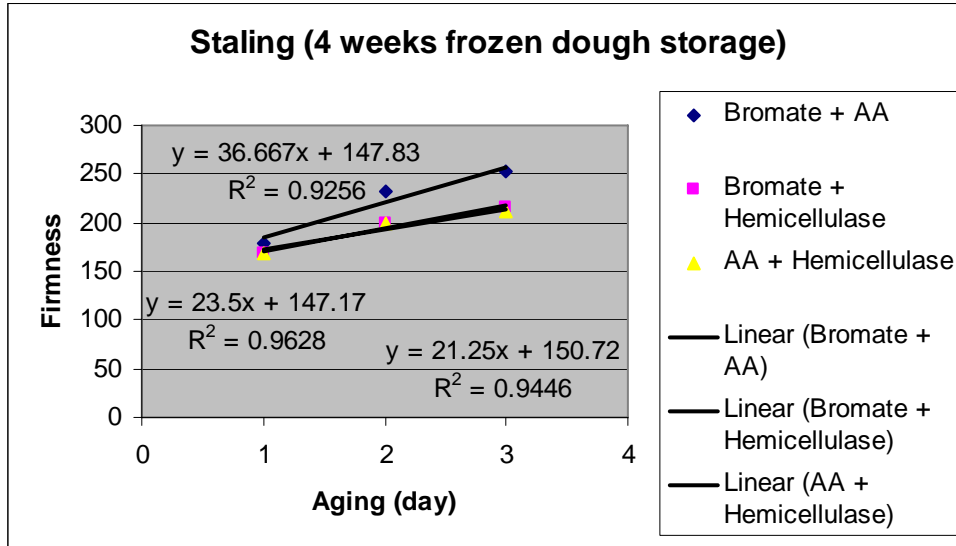


Figure B.2 Four weeks storage.

Table B.5 Eight weeks storage.

Staling (8 weeks)	Crumb Firmness			
	Treatments	Day 1	Day 2	Day 3
Bromate + AA		182	241	272
Bromate + Hemicellulase		164	213	230
AA + Hemicellulase		176	220	237

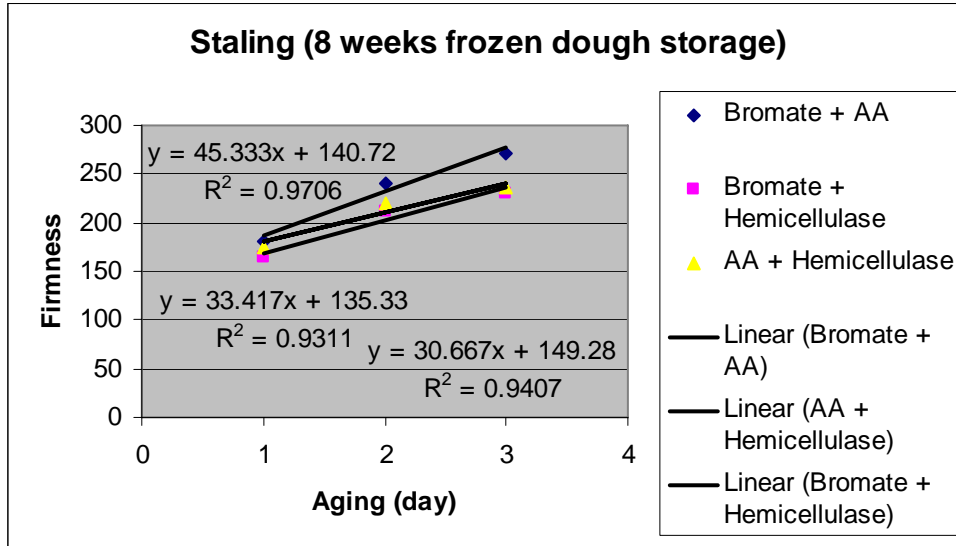


Figure B.3 Eight weeks storage.

Table B.6 Twelve weeks storage.

Staling (12 weeks)	Crumb Firmness			
	Treatments	Day 1	Day 2	Day 3
Bromate + AA		161	205	244
Bromate + Hemicellulase		143	179	201
AA + Hemicellulase		153	179	210

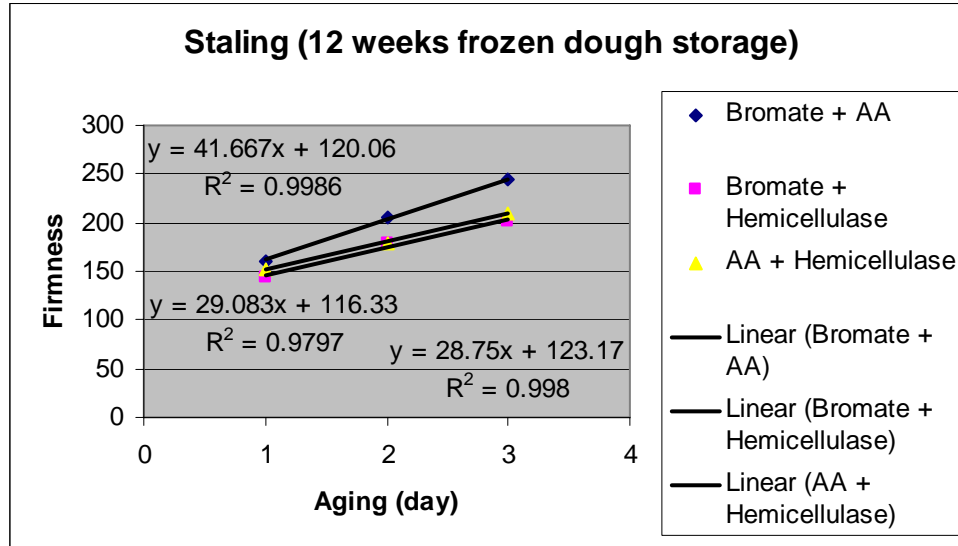


Figure B.4 Twelve weeks storage.

C-Cell data

Table B.7 One day storage (1st Run)

Slice Name	Slice Area / (mm²)	Slice Brightness	Cell Diameter / (mm)	Wall Thickness / (mm)
Bromate 1-1	8589	150.7	1.619	0.411
Bromate 1-2	9002	145.1	1.340	0.383
Bromate -3	8541	147.7	1.507	0.404
Avg	8711	147.833	1.489	0.399
St Dev	253	2.802	0.140	0.015
CV	2.910	1.896	9.431	3.649
Bromate + AA 2-1	8413	143.0	1.540	0.408
Bromate + AA 2-2	9108	141.6	1.404	0.392
Bromate + AA 2-3	8668	140.9	1.556	0.408
Avg	8730	141.833	1.500	0.403
St Dev	352	1.069	0.084	0.009
CV	4.027	0.754	5.568	2.294
AA + HC 3-1	8542	145.0	1.548	0.407
AA + HC 3-2	8816	143.5	1.386	0.389
AA + HC 3-3	8585	144.8	1.580	0.406
Avg	8648	144.433	1.505	0.401
St Dev	147	0.814	0.104	0.010
CV	1.704	0.564	6.912	2.525

Table B.8 One day storage (2nd Run)

Slice Name	Slice Area / (mm²)	Slice Brightness	Cell Diameter / (mm)	Wall Thickness / (mm)
Bromate 1-1	8633	149.3	1.473	0.396
Bromate 1-2	9122	149.3	1.340	0.384
Bromate -3	9645	150.5	1.499	0.402
Avg	9133	149.700	1.437	0.394
St Dev	506.095	0.693	0.085	0.009
CV	5.541	0.463	5.934	2.326
Bromate + AA 2-1	8809	143.5	1.556	0.407
Bromate + AA 2-2	8847	144.1	1.439	0.397
Bromate + AA 2-3	8809	141.9	1.532	0.404
Avg	8822	143.167	1.509	0.403
St Dev	21.939	1.137	0.062	0.005
CV	0.249	0.794	4.095	1.274
AA + HC 3-1	9063	143.7	1.482	0.402
AA + HC 3-2	9371	144.4	1.456	0.393
AA + HC 3-3	8907	144.2	1.634	0.414
Avg	9114	144.100	1.524	0.403
St Dev	236.113	0.361	0.096	0.011
CV	2.591	0.250	6.309	2.614

Table B.9 Four weeks storage (1st Run)

Slice Name	Slice Area / (mm²)	Slice Brightness	Cell Diameter / (mm)	Wall Thickness / (mm)
Bromate 1-1	8765	147.9	1.523	0.407
Bromate 1-2	9361	146.0	1.404	0.392
Bromate -3	9117	144.5	1.603	0.408
Avg	9081.000	146.133	1.510	0.402
St Dev	299.626	1.704	0.100	0.009
CV	3.299	1.166	6.631	2.228
Bromate + AA 2-1	8840	143.4	1.564	0.413
Bromate + AA 2-2	9219	142.4	1.431	0.398
Bromate + AA 2-3	8917	141.2	1.548	0.409
Avg	8992.000	142.333	1.514	0.407
St Dev	200.322	1.102	0.073	0.008
CV	2.228	0.774	4.795	1.910
AA + HC 3-1	8718	142.7	1.564	0.410
AA + HC 3-2	9149	146.7	1.377	0.394
AA + HC 3-3	8995	148.8	1.465	0.399
Avg	8954.000	146.067	1.469	0.401
St Dev	218.406	3.099	0.094	0.008
CV	2.439	2.122	6.370	2.041

Table B.10 Four weeks storage (2nd Run)

Slice Name	Slice Area / (mm²)	Slice Brightness	Cell Diameter / (mm)	Wall Thickness / (mm)
Bromate 1-1	8461	147.6	1.572	0.407
Bromate 1-2	9368	146.2	1.499	0.406
Bromate -3	8751	145.7	1.588	0.410
Avg	8860.000	146.500	1.553	0.408
St Dev	463.220	0.985	0.047	0.002
CV	5.228	0.672	3.055	0.511
Bromate + AA 2-1	8627	142.2	1.716	0.420
Bromate + AA 2-2	9092	140.9	1.672	0.418
Bromate + AA 2-3	8598	142.5	1.787	0.426
Avg	8772.333	141.867	1.725	0.421
St Dev	277.219	0.850	0.058	0.004
CV	3.160	0.599	3.364	0.988
AA + HC 3-1	8305	141.3	1.701	0.420
AA + HC 3-2	9087	141.4	1.556	0.411
AA + HC 3-3	9109	142.0	1.773	0.434
Avg	8833.667	141.567	1.677	0.422
St Dev	457.971	0.379	0.111	0.012
CV	5.184	0.267	6.592	2.749

Table B.11 Eight weeks (1st Run)

Slice Name	Slice Area / (mm²)	Slice Brightness	Cell Diameter / (mm)	Wall Thickness / (mm)
Bromate 1-1	8779	145.5	1.580	0.412
Bromate 1-2	9207	145.0	1.540	0.407
Bromate -3	8285	147.4	1.611	0.413
Avg	8757.000	145.967	1.577	0.411
St Dev	461.394	1.266	0.036	0.003
CV	5.269	0.867	2.257	0.783
Bromate + AA 2-1	9109	142.4	1.716	0.420
Bromate + AA 2-2	9075	142.9	1.448	0.398
Bromate + AA 2-3	8468	142.8	1.619	0.414
Avg	8884.000	142.700	1.594	0.411
St Dev	360.667	0.265	0.136	0.011
CV	4.060	0.185	8.511	2.769
AA + HC 3-1	8042	142.6	1.679	0.423
AA + HC 3-2	8772	143.4	1.687	0.417
AA + HC 3-3	8653	141.7	1.672	0.414
Avg	8489.000	142.567	1.679	0.418
St Dev	391.659	0.850	0.008	0.005
CV	4.614	0.597	0.447	1.096

Table B.12 Eight weeks (2nd Run)

Slice Name	Slice Area / (mm²)	Slice Brightness	Cell Diameter / (mm)	Wall Thickness / (mm)
Bromate 1-1	8155	146.0	1.556	0.412
Bromate 1-2	8828	144.7	1.386	0.392
Bromate -3	8553	142.7	1.657	0.420
Avg	8512.000	144.467	1.533	0.408
St Dev	338.368	1.662	0.137	0.014
CV	3.975	1.151	8.934	3.535
Bromate + AA 2-1	8275	140.5	1.687	0.424
Bromate + AA 2-2	8898	140.1	1.482	0.403
Bromate + AA 2-3	8912	140.4	1.657	0.420
Avg	8695.000	140.333	1.609	0.416
St Dev	363.798	0.208	0.111	0.011
CV	4.184	0.148	6.883	2.683
AA + HC 3-1	8366	139.7	1.759	0.431
AA + HC 3-2	8823	140.9	1.580	0.410
AA + HC 3-3	7963	140.3	1.649	0.418
Avg	8384.000	140.300	1.663	0.420
St Dev	430.282	0.600	0.090	0.011
CV	5.132	0.428	5.430	2.526

Table B.13 Twelve weeks (1st Run)

Slice Name	Slice Area / (mm²)	Slice Brightness	Cell Diameter / (mm)	Wall Thickness / (mm)
Bromate 1-1	9050	146.3	1.657	0.416
Bromate 1-2	9183	146.3	1.580	0.408
Bromate -3	8635	145.8	1.738	0.421
Avg	8956	146.133	1.658	0.415
St Dev	286	0.289	0.079	0.007
CV	3.192	0.198	4.764	1.580
Bromate + AA 2-1	8519	141.9	1.766	0.422
Bromate + AA 2-2	9093	146.7	1.694	0.421
Bromate + AA 2-3	8879	141.9	1.657	0.412
Avg	8830	143.500	1.706	0.418
St Dev	290	2.771	0.055	0.006
CV	3.285	1.931	3.250	1.317
AA + HC 3-1	8807	143.8	1.642	0.414
AA + HC 3-2	8914	142.4	1.766	0.423
AA + HC 3-3	8858	142.8	1.694	0.421
Avg	8860	143.000	1.701	0.419
St Dev	53.519	0.721	0.062	0.005
CV	0.604	0.504	3.661	1.127

Table B.14 Twelve weeks (2nd Run)

Slice Name	Slice Area / (mm²)	Slice Brightness	Cell Diameter / (mm)	Wall Thickness / (mm)
Bromate 1-1	8826	143.3	1.752	0.428
Bromate 1-2	8587	146.4	1.588	0.411
Bromate -3	8387	143.1	1.815	0.436
Avg	8600	144.267	1.718	0.425
St Dev	220	1.850	0.117	0.013
CV	2.556	1.283	6.820	3.004
Bromate + AA 2-1	8262	141.0	1.787	0.428
Bromate + AA 2-2	8735	140.0	1.716	0.420
Bromate + AA 2-3	8186	140.4	1.849	0.435
Avg	8394	140.467	1.784	0.428
St Dev	297	0.503	0.067	0.008
CV	3.544	0.358	3.730	1.755
AA + HC 3-1	8717	140.7	1.862	0.433
AA + HC 3-2	8890	145.9	1.564	0.410
AA + HC 3-3	8550	143.7	1.842	0.427
Avg	8719	143.433	1.756	0.423
St Dev	170	2.610	0.167	0.012
CV	1.950	1.820	9.486	2.818

Data for Specific Volume, C-Cell, and Volland-Stevens Tests

Table B.15 Average specific loaf volumes (SV) from nine loaves (three doughs, three replicate bake from each). The average C-Cell test results from two loaves, three slices from each. The Volland-Stevens-FLRA Texture Analyzer firmness test results from two loaves, six slices from each.

Test Instrument	Slice Properties	Variations		
		Bromate + AA	Bromate + HC	AA + HC
Rapeseed displacement volume meter	Avg Specific Volume (SV) (ml/gram)	5.34	5.22	5.24
C-Cell	Slice Area (1 day) (mm ²)	8922	8932	8881
	Slice Area (4 wks) (mm ²)	8971	8882	8894
	Slice Area (8 wks) (mm ²)	8635	8790	8437
	Slice Area (12 wks) (mm ²)	8778	8612	8789
	Slice Brightness (1 day)	148.77	145.77	144.27
	Slice Brightness (4 wks)	146.32	142.10	143.82
	Slice Brightness (8 wks)	145.22	141.52	141.43
	Slice Brightness (12 wks)	145.2	141.98	143.22
	Cell Diameter (1 day) (mm)	1.463	1.469	1.514
	Cell Diameter (4 wks) (mm)	1.532	1.620	1.573
	Cell Diameter (8 wks) (mm)	1.555	1.602	1.671
	Cell Diameter (12 wks) (mm)	1.688	1.745	1.728
	Wall thickness (1 day) (mm)	0.397	0.403	0.402
	Wall thickness (4 wks) (mm)	0.405	0.414	0.411
	Wall thickness (8 wks) (mm)	0.409	0.413	0.419
Wall thickness (12 wks) (mm)	0.420	0.423	0.421	

Table B.15 (Continue)

Test Instrument	Slice Properties	Variations		
		Bromate + AA	Bromate + HC	AA + HC
Voland-Stevens-LFRA Texture Analyser	Firmness(1 day-day 1) (gram)	169	150	159
	Firmness(1 day-day 2) (gram)	197	176	168
	Firmness(1 day-day 3) (gram)	219	202	173
	Firmness(4 wks-day 1) (gram)	179	168	169
	Firmness(4 wks-day 2) (gram)	233	200	199
	Firmness(4 wks-day 3) (gram)	252	215	212
	Firmness(8 wks-day 1) (gram)	182	164	176
	Firmness(8 wks-day 2) (gram)	241	213	220
	Firmness(8 wks-day 3) (gram)	272	230	237
	Firmness(12 wks-day 1) (gram)	161	143	153
	Firmness(12 wks-day 2) (gram)	205	179	179
	Firmness(12 wks-day 3) (gram)	244	201	210