Effect of enzymes, hydrocolloids, and emulsifiers on qualities of dough and bread made from whole grain wheat flour

by

Lauren Renae Tebben

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Approved by:

Major Professor
Yonghui Li

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Abstract

Despite the associated health benefits of whole grains, consumption of whole grain products remains far below the recommended levels. Whole grain wheat flour has gained considerable attention as a breadmaking ingredient due to its nutritional and health benefits. Compared to white bread, whole wheat bread has a small loaf volume and hard crumb texture, creating unique challenges for the baking industry and for consumer acceptability. Dough conditioners and bread improvers within the classes of enzymes, emulsifiers, and hydrocolloids have been widely studied in white pan bread, but less information has been published on their use in whole wheat bread. The objective of this research was to determine effects of common enzymes, emulsifiers, and hydrocolloids on whole wheat bread properties, with a focus on dough physical and rheological properties, loaf volume, bread texture, and staling.

Bread was prepared from whole wheat flour following AACC method 10-10.03. Enzymes (α-amylase, cellulase, glucose oxidase, maltogenic amylase, xylanase), emulsifiers (DATEM, polysorbate 80, soy lecithin, SSL, sucrose esters), and hydrocolloids (CMC, guar gum, HPMC, sodium alginate, xanthan gum) were added individually at three levels. Vital wheat gluten (VWG) was added as an additional, separate treatment at 2.5% (fwb) in the enzyme study. Dough rheological properties were determined by farinograph and mixograph. For the emulsifiers and hydrocolloids, additional dough properties were measured by the SMS/Chen-Hoseney stickiness test and the Kieffer rig uniaxial extensibility test. Specific volume was measured for fresh bread, and moisture content, texture profile analysis (TPA), and crumb structure were analyzed the following day. Moisture content and TPA were measured again after 3 and 7 days of storage at 22 °C to determine changes associated with staling. Effect on starch retrogradation was quantified by differential scanning calorimentry (DSC) after the 7 days.

Hydrocolloids increased the water absorption and tended to decrease the stability of the dough, whereas enzymes had minimal effect on dough properties. Each enzyme and hydrocolloid increased specific loaf volume for at least one of the usage levels tested ($P < 0.01$). Of the emulsifiers, only polysorbate 80 and soy lecithin significantly increased loaf volume. Xanthan gum and HPMC resulted in the largest loaf volume among the hydrocolloids. Xylanase at the medium and high levels produced the greatest increase in loaf volume among the enzyme treatments, which also lead to the greatest reduction in fresh bread hardness. No enzyme was as effective as VWG at increasing loaf volume. VWG, maltogenic amylase, xylanase, HPMC, and xanthan gum reduced the rate of bread firming over 7 days. Sucrose esters and polysorbate 80 were the most effective anti-staling agents among the emulsifiers. DSC analysis revealed that maltogenic amylase nearly eliminated the endothermic peak for recrystallized amylopectin, showing this enzyme's strong ability to reduce retrogradation in bread.

This study demonstrated the specific application of enzymes, emulsifiers, and hydrocolloids in whole wheat bread to increase loaf volume and decrease initial crumb hardness and bread staling, which may help improve the sensory appeal of whole wheat bread and ultimately increase whole grain consumption.

Table of Contents

List of Figures

List of Tables

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1 Introduction

Whole wheat bread has increased nutritional and health benefits compared to white pan bread, due to fiber, vitamins, minerals, and phytochemicals in the bran and germ. Choosing whole wheat bread over white bread is a simple way for consumers to increase their whole grain intake, as recommended by the Dietary Guidelines for Americans. However, whole wheat bread has some unique technical challenges compared to white pan bread, including different dough handling properties, a smaller loaf volume, and a harder crumb texture. Improvers are used extensively in the baking industry to alter the physical properties of dough and bread. Most of the literature available is focused on the use of improvers in white dough and bread. Less information is available about their specific application to whole wheat systems.

The objectives of this research were to determine the individual effects of five improvers each from the classes of enzymes, hydrocolloids, and emulsifiers in whole wheat dough and bread. The primary objective was to find the improvers that produced the greatest increase in loaf volume. An increase in loaf volume generally leads to a decrease in initial crumb firmness, but another major objective of this work was to determine which improvers decreased the rate of crumb firming in whole wheat bread. Rheological and textural properties of the dough with added improvers were also evaluated to determine changes in dough handling properties.

Chapter 2 of this thesis provides an overview of the literature on the use of improvers in whole wheat dough and bread. Chapters 3, 4, and 5 describe the complete research studies performed for enzymes, hydrocolloids, and emulsifiers, respectively, in whole wheat dough and bread. The study in chapter 3 also included the addition of vital wheat gluten as a separate treatment. Chapter 6 gives overall conclusions from this thesis work, as well as recommendations for future studies.

2 Literature Review: Improvers and functional ingredients in whole wheat bread and their effects on dough properties and bread quality[1]

Abstract

Despite the associated health benefits of whole grains, consumption of whole grain products remains far below recommended levels. Whole wheat bread is often associated with many distinctive attributes such as low loaf volume, firm and gritty texture, dark and rough crust and crumb appearance, bitter flavor, and reduced shelf-life. There is a need to improve its quality and sensory characteristics so as to increase consumer appeal and, ultimately, increase the intake of whole wheat bread. The inclusion of various ingredients improves dough and bread properties. This review examines the effects of enzymes, emulsifiers, hydrocolloids, and oxidants on the properties of whole wheat bread and dough, with particular attention to effects on loaf volume and hardness. Wheat gluten and other plant materials are also discussed. Gaps in the research into whole wheat bread are identified, and future research needs are recommended.

Xylanase reduces the water absorption of whole wheat flour and increases loaf volume and crumb softness by hydrolyzing ararbinoxylans. α-amylase can be beneficial under certain conditions. Phytase may activate endogenous α-amylase. G4-amylase is promising but needs validation by further research on its effect on loaf volume, crumb hardness, and staling. Vital wheat gluten overcomes many of the challenges of whole wheat bread production and is found in

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the majority of commercial whole wheat breads. Emulsifiers DATEM and SSL can improve the volume, texture and staling profile of whole wheat bread. Several types of improvers are generally needed in combination to provide the greatest improvement to whole wheat dough and bread.

Keywords: Whole wheat; Dough; Bread; Enzyme; Emulsifier; Hydrocolloid; Oxidant

Highlights

- Whole wheat bread often has low volume, firm texture, and fast staling.
- Specific enzymes and emulsifiers have major improving effects.
- Vital wheat gluten plays a critical role in the production of whole wheat bread.
- Combining functional ingredients is recommended for most comprehensive improvement.

2.1 Introduction

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

Whole wheat flour produces dough and bread with characteristic differences compared to refined wheat flour. Effects associated with whole wheat bread production and their causes have been reviewed (Doblado-Maldonado, Pike, Sweley, & Rose, 2012; Gan, Ellis, Vaughan, & Galliard, 1989; Heiniö et al., 2016) and include low loaf volume, increased crumb hardness, coarse texture, darker color, and distinctive flavor and aroma. These attributes may not be appealing to consumers accustomed to white bread, which is made from refined flour.

Reasons suggested for the effects of non-endosperm components on bread quality are fiber-gluten interactions (Noort, van Haaster, Hemery, Schols, & Hamer, 2010); dilution of

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

The physical and chemical effects of the bran and germ necessitate some degree of formula and process modifications as compared to white bread. Water absorption must be increased. Vital wheat gluten, dough conditioners such as oxidizing agents, emulsifiers, and enzymes, as well as shortening and mold inhibitors are often added or their concentration is increased compared to white bread formulations (Dubois & Vetter, 1987). Phenolic compounds

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

Wheat flour mills as well as bread manufacturers may add a variety of amounts of non-endosperm components to refined wheat flours. For example, some products consist of various amounts of bran combined with endosperm but without the germ, thus creating "germ-free and bran-rich flours." However, in order for the product to be labelled whole grain, it must include all parts of the caryopsis – the endosperm, germ, and bran – in the same proportions as are present in the intact kernel (AACC International, 1999). The effects of wheat bran presence in bread have been recently reviewed and summarized (Hemdane, Jacobs, Dornez, Verspreet, Delcour, & Courtin, 2016), and many publications exist on the use of improvers in reconstituted dough systems, where ground bran is added back to refined wheat flour. In contrast, this review

focuses mainly on improvers and other functional ingredients in whole wheat dough and bread, rather than the deleterious effects of endogenous wheat components. Furthermore, it covers studies that used whole wheat flour rather than refined flour to which bran was added. Within the whole wheat bread system, there is a need to improve the quality and sensory aspects to increase consumer appeal and therefore increase the intake of whole grain bread. For breads that are inherently firmer, such as whole wheat bread, softer breads achieve higher scores for overall acceptability (Armero & Collar, 1996a). With this in mind, this review gives particular attention to crumb hardness and loaf volume, which is a strong contributor to hardness. In this review, a very large space has been given to enzymes, especially amylase, phytase, and xylanase. Other sections introduce specific emulsifiers, hydrocolloids, oxidants, and other functional ingredients such as vital wheat gluten and miscellaneous flours.

2.2 Enzymes

The use of enzymes in commercial applications has increased in recent years as consumers demand bakery products with more natural-sounding ingredients. Various types of enzymes can be used as alternatives to chemical improving agents, such as some hydrocolloids and emulsifiers, and those types used in bakery applications can all be declared by the single word “enzymes,” a term which many consumers perceive as natural and clean label compared to additives labeled by their chemical name. Many enzymes occur naturally in flour, but several enzymes are added, specifically for their beneficial effects on dough and bread characteristics. Consequences include increased dough handling and hydration, improved volume and/or crumb texture, reduced rate of staling, or improved nutritional qualities. Enzyme activity is affected by several factors including temperature, pH, water activity, and enzyme concentration. Commonly

used exogenous enzymes include xylanase, phytase, and amylases. Table 2.1 presents the major findings that have been published on the uses of enzymes in whole wheat dough and bread.

2.2.1 α-Amylase

α-Amylase is an endo-hydrolosate that catalyzes the hydrolysis of α-1,4-glycosidic bonds of starch polymers, producing low molecular weight polysaccharides and dextrins. β-Amylase decreases the molecular size/weight of these polysaccharides by cleaving the disaccharide maltose from the non-reducing end. Unlike higher glucose polysaccharides, maltose is fermentable by yeast. The resulting increase in fermentable sugars has a positive effect on yeast fermentative activity, which along with gas retention is a fundamental element of bread production. An increase in fermentative gas production, combined with the ability of the dough to retain that gas, leads to an increase in loaf volume. In 60:40 blends of refined flour and whole wheat flour, α-amylase improved the gas retention capacity of the dough, increased specific loaf volume, and decreased crumb hardness and staling rate (Matsushita et al., 2017). Remarkably, the hardness of the whole wheat-supplemented bread prepared with α-amylase was lower than that of the refined wheat control after 3 d of storage, demonstrating this enzyme's promise for improving the shelf life of whole wheat bread, which often has a shorter shelf life than refined wheat bread. The decrease in hardness and staling achieved by α-amylase is due to both the increase in low molecular weight saccharides and the increase in specific volume. The low molecular weight products of starch hydrolysis are not available for retrogradation, and these smaller saccharides also delay the retrogradation of gelatinized starch (Matsushita et al., 2017). Furthermore, those saccharides interfere with starch-protein interactions in the aging bread, which decreases firming. α-amylase retains its activity early in baking and is capable of

degrading gelatinized starch, and this partially decomposed starch has a low rate of retrogradation.

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

Other researchers have examined the use of malt flour in whole wheat bread rather than adding purified α-amylase. Malt flour is commonly used as an enzyme supplement because it is rich in α-amylase, and it also contains maltose, minerals, proteins, and flavor compounds. These components modify the color, flavor, and moisture retention of the bread (Boz, Karaoglu, Kotancilar, & Gercekaslan, 2010). However, the effect of malt flour depends on flour quality (Hruskova, Svec, & Kucerova, 2003). The addition of 2% malt flour in whole wheat dough decreased the resistance to extension, suggesting a weaker dough (Boz et al., 2010). Extensibility and water absorption were increased by malt flour. Malt flour also lowered the dough energy as measured by the extensograph. Stickiness, adhesion, and stringiness measured using the SMS/Chen-Hoseney stickiness rig on a texture analyzer were all increased by malt addition, indicating that the dough may be more difficult to handle with this additive. A subsequent study (Boz & Karaoglu, 2013) reported that 2% malt flour provided only marginal improvement to the

general acceptability sensory score of whole wheat bread. The score for crumb grain decreased, and the aroma was rated no differently than the control bread. Loaf volume showed a small but significant increase compared to the control, but crumb firmness was not significantly improved. Based on this study, malt flour provides only marginal improvement to whole wheat bread, and other improvers may be needed in addition to the malt in order to produce a more acceptable product.

2.2.2 G4-amylase

Bae, Lee, Yoo, and Lee (2014) studied the effect of a maltotetraose-producing enzyme (G4-amylase) in whole wheat dough and bread. This enzyme produces a high concentration of maltooligosaccharides, which show high moisture retention and antiretrogradation properties. This enzyme decreased the water absorption and increased the dough development time but did not affect dough stability as measured by Mixoglab. Enzymatic breakdown of damaged starch during mixing likely affected the dough hydration and mixing properties (Bae et al., 2014). During heating and cooling in the Mixolab, the torque values were lower for the enzyme-treated doughs compared with the control, especially during starch gelatinization, stability of gelatinized starch granules, and starch retrogradation. Enzymatic hydrolysis and continual shearing led to the breakdown of gelatinized starch granules and accounted for the decreased torque observed during the temperature hold at 90°C (C3 to C4). The effect on tensile properties of the dough were evaluated using a texture analyzer. G4-amylase increased dough extensibility (E) at higher (0.08 and 0.12 BMK) addition levels but did not affect the maximum resistance to extension (R_{max}). These data suggested that the viscous nature of the dough became more dominant due to the G4-amylase. This agreed with the results of dynamic viscoelastic analyses, which showed that G' was reduced to a greater extent than G" by G4-amylase activity.

Addition of G4-amylase significantly increased the specific volume of the loaves, up to 1.2 times greater than the control. The change in volume was attributed to the enzyme-mediated increase in fermentable sugars, which increases yeast fermentative activity. In this case, gas retention capacity was sufficient to support the increased gas production, and a larger dough volume during proofing was the result. The observed changes in dough rheology, specifically, the more dominant viscous character, may have allowed greater oven spring. Supplementation with α-amylase also increased the specific volume, but not to the same extent as the highest level of G4-amylase tested.

The G4-amylase significantly decreased the initial bread firmness. Firming after 7d storage was also reduced up to 31%, suggesting that the enzyme decreased the rate of retrogradation. The positive effect on retrogradation was predicted based on the low final torque value obtained in the Mixolab analysis of the dough, which is an indicator starch depolymerization. The authors proposed several mechanisms to explain the antiretrogradation effect. The depolymerization of starch might inhibit the extent of starch recrystallization, and the highly hygroscopic maltooligosaccharides may prevent intermolecular starch interactions by holding onto water molecules (Bae et al., 2014). The size of the maltooligosaccharides may allow them to interfere with hydrogen bonding between starch chains due to steric hindrance (Bae et al., 2014; Min et al., 1998).

2.2.3 Amyloglucosidase

Amyloglucosidase, also known as glucoamylase, catalyzes the release of glucose molecules from the non-reducing ends of oligo- and polysaccharides such as starch and can act on both α-1,4 and α-1,6 glycosidic bonds. Amyloglucosidase is functional in whole wheat bread formulations, which benefit from an increase in sugars both for improving the sweetness and

increasing yeast activity. Oftentimes sweeteners such as honey, molasses, corn syrup, and brown sugar are added to the formulation, but amyloglucosidase creates extra glucose in the dough, which aids in fermentation and crust color via Maillard browning. Depending on the usage level, amyloglucosidase either decreased or increased the resistance to extension of whole wheat dough (Altinel & Ünal, 2017a,b). The change was significant only after the dough had rested for 135 min, suggesting that the enzyme requires an extended time to have effect. As in the control, the resistance increased with resting time. Hydration of the dough may be delayed with amyloglucosidase addition, since the reaction catalyzed by the enzyme competes with flour for water. This delay was reflected by the increased dough resistance (Altinel & Ünal, 2017a,b). Amyloglucosidase also decreased moisture loss during baking of whole wheat bread, which was considered a positive feature, but specific loaf volume was decreased (Altinel & Ünal, 2017b).

2.2.4 Cellulase

Cellulase catalyzes the hydrolysis of cellulose, a component of cell walls. Cellulases could find special relevance in whole grain applications, as whole grains have a higher concentration of cellulosic material than do refined grain products. However, cellulase did not significantly alter the farinographic or extensographic properties of whole wheat dough, or the specific volume or final moisture content of whole wheat bread (Altinel & Ünal, 2017b). Cellulase increased the moisture loss of whole wheat bread during baking. After hydrolysis of cellulose in the cell wall of the bran, water absorbed by the bran was released, leading to greater moisture loss from the dough as it baked. Cellulase decreased the firmness of bread made with whole grain waxy wheat but did not affect the specific volume (Hung, Maeda, Fujita, & Morita, 2007).

2.2.5 Glucose oxidase

Glucose oxidase is an enzyme that catalyzes the oxidation of glucose into gluconic acid and hydrogen peroxide. Altinel and Ünal (2017a) reported that glucose oxidase did not significantly affect the farinographic properties of whole wheat or white flour dough. Dough made from whole wheat flour has a higher resistance to extension than dough from white flour due to interactions between gluten and the nonstarch polysaccharides in the bran (Altinel & Ünal, 2017a). Addition of glucose oxidase decreased the resistance to extension of whole wheat dough to a level similar to that of white dough (Altinel & Ünal, 2017a,b). This change was attributed to the dough weakening effect of hydrogen peroxide, which is produced during the reaction catalyzed by glucose oxidase (Altinel & Ünal, 2017b). In white flour dough, the effect on dough resistance was minimal (Altinel & Ünal, 2017a). The extensibility of white or whole wheat dough was not significantly affected by glucose oxidase. The enzyme decreased the energy of whole wheat and white dough, as measured by the extensograph. Therefore, glucose oxidase can decrease the energy required for handling the dough during bread production (Altinel & Ünal, 2017b). According to a study by Yang et al. (2014), glucose oxidase added to whole wheat flour produced a stiffer dough with increased elastic (G') and viscous (G") moduli. Whole wheat dough has a lower elasticity than does white flour dough, so increasing the stiffness of the dough is generally undesirable.

Altinel and Ünal (2017a,b) found that glucose oxidase significantly increased the specific volume of whole wheat bread, which was related to the changes in dough rheology, including decreased resistance to extension. This bread was prepared without additional improvers or added gluten, so the improving effect of the oxidative enzyme was clearly seen compared to the results of da Silva et al. (2016), who used gluten and diacetyl tartaric esters of monoglyceride

(DATEM) in all formulas and did not observe any significant effects on bread prepared with glucose oxidase. Glucose oxidase did not change the volume of white bread significantly. White bread had a greater volume than that of whole wheat bread both with and without enzymes (Altinel & Ünal, 2017a).

2.2.6 Laccase

Laccase in an enzyme which oxidizes phenolic compounds such as arabinoxylan and could therefore prove especially beneficial in whole wheat bread, but to date it has only been studied in white bread. In dough from refined wheat, laccase has been shown to increase dough strength and stability, decrease stickiness, increase loaf volume, and improve crumb structure and softness (Minussi, Pastore, & Durán, 2002).

2.2.7 Lipase

The use of lipases in wheat-based food systems including bread and cakes has been previously reviewed (Gerits, Pareyt, Decamps, & Delcour, 2014). Several types of lipases may be used by the food industry, and they are classified into three general categories: triacylglycerol lipases (the "real" lipases), phospholipases, and galactolipases (Gerits et al., 2014). Colakoglu and Özkaya (2012) studied the effects of two lipases on the farinograph, extensograph, and texture properties of dough and compared them to the effect of DATEM. Overall, whole wheat dough was less responsive to the additives than was white dough. Lipase had a hardening effect on the dough, as suggested by a decrease in the softening degree and increase in dough hardness. Both lipases decreased dough stickiness, which could increase the machinability of whole wheat dough. Generation of surface active compounds from the hydrolysis of polar and nonpolar lipids has been suggested as the mechanism behind lipase functionality in dough (Colakoglu & Özkaya, 2012). The authors suggested that other mechanisms are also at play. Exogenous lipases

may increase the activity of endogenous lipases. The monoglycerides liberated by both groups of lipases are able to bind with gluten proteins and decrease their hydrophobicity, leading to changes in dough properties. Binding of gluten by liberated monoglycerides may affect the interactions between gluten and starch. Altinel and Ünal (2017b) reported that farinographic and extensographic properties were minimally affected by addition of lipase. This enzyme decreased the specific loaf volume of whole wheat bread, which was attributed to the destabilizing effect of free fatty acids that are released upon lipid hydrolysis.

Colakoglu and Özkaya (2012) also used DSC to study the thermal properties of dough prepared with lipases and DATEM. The most significant finding was that the lipases increased the melting enthalpy and decreased the onset temperature for the dissociation of the amylose-lipid complex. A greater amount of amylose-lipid complex is generally considered beneficial, because it is associated with a decrease in starch retrogradation. The two lipases affected these parameters to different extents, but both had a greater effect than did DATEM. This difference indicates that the two enzymes do not have the same activity in the dough system and that the products from each enzyme may interact differently with amylose.

Phospholipases catalyze the cleavage of phospholipids. This enzyme is reported to improve the elasticity and extensibility of dough and increase loaf volume (Inoue & Ota, 1986). Phospholipase added with hemicelluase improved the rheology of whole wheat dough by decreasing the resistance to extension and resistance/extensibility ratio. An increased specific loaf volume was attained, presumably because the enzymes allowed for greater expansion of the dough (Altinel & Ünal, 2017b). Similar function has been observed in refined wheat dough and bread (Inoue & Ota, 1986).

2.2.8 Phytase

Phytase reduces the phytate content in whole grain products and has therefore often been studied for its nutritional benefits (Haros, Rosell, & Benedito, 2001b; Porres, Etcheverry, Miller, & Lei, 2001; Sanz Penella, Collar, & Haros, 2008). It may also improve the loaf volume and softness of whole wheat bread.

2.2.8.1 Effect of phytase on dough properties

Most studies focus on the phytate content in dough and bread, rather than the physical and handling properties of the dough upon phytase addition. Haros, Rosell, and Benedito (2001a) reported that fungal phytase decreased the proof time of whole wheat dough; up to a 24% reduction for 2500 μL/100g flour was achieved. Sanz Penella et al. (2008) found that phytase slightly decreased the farinograph water absorption of wheat dough with added bran. Phytase did not significantly affect dough development time (DDT) or dough stability, but it did increase the drop time, indicating the ability to delay overmixing (Sanz Penella et al., 2008). Phytase did not affect the properties evaluated by rheofermentometer. However, this study only evaluated one level of phytase addition.

2.2.8.2 Effect of phytase on bread properties

Haros et al. (2001a) used fungal phytase to increase specific loaf volume. It produced a continuous increase over a range of 25 to 2500 μL phytase/100 g flour. Crumb firmness decreased with increasing phytase addition, with a maximum decrease of 28%. The authors explained the positive effects of phytase by an activation of endogenous α-amylase in flour. Phytase liberates divalent calcium ions from phytate complexes, allowing them to be utilized by α-amylase. The effect of the phytate-calcium complex on α-amylase was demonstrated by an *in vitro* study. The authors concluded that phytase helps to overcome the inhibitory action of phytic

acid on α-amylase activity, which in turn allows for the improvement in the bread as seen in this study. In a separate study, Haros, Rosell, and Benedito (2001b) found no effect on specific volume of whole wheat bread due to phytase addition. Further, Rosell, Santos, Penella, and Haros (2009) observed a significant decrease in specific volume for conventionally prepared whole wheat bread prepared with phytase. They attributed the contradictory results to differences in flour composition and amount of endogenous α-amylase, although they did not test this hypothesis. Rosell et al. (2009) also tested fungal phytase in whole wheat bread prepared by different methods, including freezing and parbaking. They found that the enzyme led to a non-significant increase in specific volume for frozen dough, but no change in specific volume of par-baked bread. Furthermore, they observed that phytase decreased the hardness of bread from frozen dough but did not affect the hardness of bread from conventional or par-bake methods.

2.2.9 Transglutaminase

Transglutaminase reinforces the gluten network primarily by catalyzing the formation of protein-protein cross-linkages that modify the dough structure and bread properties (Collar & Bollaín, 2005). Collar and colleagues also reported that transglutaminase improved sensory and textural characteristics of bread. The improvement was even greater when both transglutaminase and α-amylase were added to the dough (Collar, Bollaín, & Angioloni, 2005; Collar & Bollaín, 2005). However, the beneficial effects were greater in white bread than whole wheat bread (Collar et al., 2005). Transglutaminase alone had a negative effect on the initial hardness (Collar & Bollaín, 2005; Collar et al., 2005) and loaf volume (Collar et al., 2005) of whole wheat bread, but other sensory characteristics were improved (Collar et al., 2005). Transglutaminase exhibited beneficial and synergistic effects with α-amylase, but this effect was more prominent in white bread than in whole wheat (Collar & Bollaín, 2005). Although transglutaminase retarded the

staling kinetics in white bread, this effect was not observed for whole wheat bread (Collar & Bollaín, 2005). Grausgruber et al. (2008) used a commercial enzyme supplement consisting of transglutaminase, α-amylase, and xylanase to increase the loaf volume and decrease the firmness of whole grain einkorn wheat bread. Addition of emulsifiers further improved the volume and decreased hardness. Einkorn, a diploid variety of soft wheat, has characteristically weak gluten and therefore benefited from the strengthening effect of transglutaminase. In dough and bread systems with strong gluten, the added strength may result in undesirable hardening of the dough and bread. Caution has arisen surrounding the use of microbial transglutaminase in recent years. Some research suggests the enzyme-induced crosslinking of gluten proteins may play a role in the onset of Celiac disease (Lerner & Matthias, 2015), although this is debated (Heil et al., 2017).

2.2.10 Xylanase and other hemicellulases

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

The non-starch polysaccharides present in the cells walls of bran and germ are one of the reasons for the poor breadmaking quality of whole wheat flour (Autio, 2006). During dough mixing, arabinoxylans compete with gluten for water (Labat, Rouau, & Morel, 2002; Li et al., 2012). Xylanases hydrolyze the xylanase backbone of water-unextractable arabinoxylan,

reducing their molecular size and water-holding capacity (Gruppen, Kormelink, & Voragen, 1993). This allows for greater gluten hydration, which results in better gluten matrix development and breadmaking ability.

2.2.10.1 Effect of xylanase on dough properties

Xylanase addition to whole wheat formulas reduces its water absorption while maintaining dough consistency similar to that of the whole wheat control (Driss, Bhiri, Siela, Bessess, Chaabouni, & Ghorbel, 2013; Ghoshal, Shivhare, & Banerjee, 2013; Shah, Shah, & Madamwar, 2006). Xylanase is reported to release free water, decreasing the amount of water that must be added to the dough. Conversely, one study of whole wheat dough reported that a blend of hemicellulases consisting mainly of endoxylanase did not produce any significant change in the farinographic properties of whole wheat dough (Altinel & Ünal, 2017b). Higher proof height occurred in xylanase-supplemented dough. This change was attributed to more complete gluten hydration resulting from the transfer of water from pentose molecules to protein. Xylanase activity results in a higher concentration of fermentable sugars in the dough, resulting in a higher rate of fermentation (Driss et al., 2013; Shah et al., 2006). Gas retention capacity was improved by hemicellulase activity (Matsushita et al., 2017). Hemicellulases (primarily endoxylanase) decreased the resistance to extension of whole wheat dough. It softened the dough, with lower levels only producing significant changes after 135 min of resting compared to a higher level of enzymes which were effective sooner (45 min) (Altinel & Ünal, 2017b). Yang et al. (2014) found that xylanase decreased the storage (G′) and loss (G″) modulus (i.e., elastic and viscous behavior, respectively) of whole wheat dough. Increasing the level of xylanase increased tan δ. This indicated a shift to more dominant elastic character.

2.2.10.2 Effect of xylanase on loaf volume

Increased loaf volume upon xylanase addition has been demonstrated by several studies (Altinel & Ünal, 2017b; Driss et al., 2013; Ghoshal et al., 2013; Jaekel, da Silva, Steel, & Chang, 2012; Kumar & Satyanarayana, 2014; Shah et al., 2006). Many explanations for the positive effect on volume have been put forth. Addition of xylanase decreases the water absorption of the flour, leading to better gluten hydration and network formation and hence higher dough rise during fermentation (Ghoshal et al., 2013; Jaekel et al., 2012; Shah et al., 2006). The dough requires less formula water due to the release of free water following addition of hydrolyzing enzymes (Martínez-Anaya & Jiménez, 1998). Kumar and Satyanarayana (2014) reported higher amounts of reducing sugars and soluble protein in bread prepared with xylanase. Further, the transfer of water from pentoses to gluten can lead to restructuring of the gluten network as the dough ferments, allowing for greater rise and larger bread volume (Ghoshal et al., 2013). Improvement in loaf volume could also be resulted from hydrolyzed (lower molecular weight) hemicellulose that is less able to interfere with gluten network formation (Matsushita et al., 2017). Altinel and Ünal (2017b) suggested that volume increased due to the conversion of water-unextractable arabinoxylan into water-extractable arabinoxylan, which improves gas retention capacity in the dough. These authors reported an increase in volume for both white and whole wheat loaves upon hemicellulase addition, so the effect was not specific to whole grain systems (Altinel & Ünal, 2017a). However, in whole wheat bread but not in white bread, hemicellulase activity decreased moisture loss during baking (Altinel & Ünal, 2017a,b). The greater moisture retention was accredited to the creation of a viscous solution formed by the increase in water-extractable arabinoxylan. The more viscous aqueous phase reduced the amount of water that was lost as the bread baked. Jaekel et al. (2012) observed increasing loaf volume as xylanase dose was increased from 0 to 8 g/100 kg flour, then a decrease at 12 g/100 kg flour. At the highest

addition level, the dough had the largest proof volume but collapsed during baking. Therefore, optimization of enzyme usage level is important, and fermentation conditions may need to be adjusted to prevent loaf instability due to over proofing. The levels of xylanase tested by da Silva, Almeida, and Chang (2016) did not increase the volume of whole wheat bread significantly. In this case, the presence of DATEM and vital wheat gluten in all treatments likely overshadowed any improvement due to xylanase. Another factor influencing the effect of any enzyme is the variation in activity and action pattern due to enzyme source, purity, and specificity. Two xylanases (Xyl1 and Xyl2) produced by *Trichoderma stromaticum* affected the volume and texture of whole wheat bread differently as a result of different enzyme composition (Carvalho et al., 2017). Xyl1 was a mixture of three xylanases that worked together to improve loaf volume (Carvalho et al., 2017). Grausgruber et al. (2008) evaluated α-amylase, xylanase, and transglutaminase in whole grain einkorn wheat bread. Xylanase, alone or in combination with α-amylase or with α-amylase and transglutaminase, led to slight but significant increases in loaf volume. When combined with emulsifiers, the effect on loaf volume was further increased, suggesting a synergistic effect between enzymes and emulsifiers.

2.2.10.3 Effect of xylanase on crumb hardness and staling

Xylanase has also been shown effective at decreasing the initial hardness and rate of staling (increase in hardness with storage) of whole wheat bread. Crumb moisture content and loaf specific volume are key factors affecting bread firmness. Shah et al. (2006) observed a 77% reduction in hardness upon xylanase addition. Moisture content in the baked loaf was increased by 125%. Driss et al. (2013) also reported a reduction in hardness. Ghoshal et al. (2013) similarly observed that xylanase decreased the hardness of fresh and stored bread and also increased the moisture retention. Ghoshal et al. (2013) used Avrami analysis to relate firmness with time to

starch crystallization. They concluded that xylanase reduced the formation and growth of starch crystals, a conclusion based on a reduction in limiting firmness values and the Avrami constant (n) for crystal shape and growth. The authors suggested that xylanase action reduced the rate of staling in bread as defined by the change from the amorphous to crystalline state given by the Avrami equation. In white bread substituted with whole wheat flour, hemicellulases (including xylanase) improved loaf specific volume and decreased the rate of bread staling, presumably by the degradation of arabinoxylan. Hydrolysis of insoluble arabinoxylans creates smaller polysaccharides which interfere with starch-protein interactions and thus inhibit staling (Matsushita et al., 2017). Jaekel et al. (2012) reported that xylanase levels of 4 and 8 g/100 kg flour significantly decreased the hardness of whole wheat bread at day 1 and day 7 of storage at room temperature. Addition of 12 g/100 kg flour did not significantly lower the hardness values, but that concentration also produced lower loaf volume than did intermediate levels of xylanase. The authors concluded that 8 g/100 kg flour was the optimal usage level in their study. Unlike the previously mentioned reports, this study did not find significant changes to bread moisture content due to enzyme addition. da Silva et al. (2016) found that intermediate levels of xylanase combined with higher levels of oxidizing agents generally decreased the hardness of whole wheat bread. The lower hardness values corresponded with a higher moisture content (da Silva et al., 2016). The authors concluded that to be effective, the level of xylanase must be optimized and should be used in combination with oxidants. Grausgruber et al. (2008) reported that loaf hardness of einkorn wheat bread decreased significantly upon addition of xylanase with or without α-amylase and transglutaminase. Addition of emulsifiers along with the enzymes further decreased hardness.

2.2.10.4 Effect of xylanase on crumb sensory characteristics

Ghoshal et al. (2013) reported significant improvements to the organoleptic properties of whole wheat bread when prepared with xylanase. Specific improvements determined by descriptive panel included smoother texture; decreased stickiness; more uniform cell structure; and better aroma, taste, and color. These attributes were rated higher than those of the control for the bread when it was fresh and after 7 days of storage, indicating that xylanase can help provide a more sensorily acceptable product over the bread's shelf life. Shah et al. (2006) reported improvements to the following sensory attributes of whole wheat bread upon xylanase supplementation: aroma, taste, color and appearance of crust, color of crumb, symmetry, baking uniformity, overall texture, and grain. Kumar and Satyanarayana (2014) reported an improved crumb structure for xylanase supplemented bread. Whole wheat bread prepared with xylanase received higher scores in all sensory attributes evaluated by Driss et al. (2013).

2.3 Emulsifiers

Emulsifiers used in bread baking generally serve the functions of dough strengthening and/or crumb softening. Dough strengthening is the result of increased interactions with the proteins in the dough. Crumb softening may also be referred to as antistaling and occurs via interaction between the emulsifier and the starch. Some commonly used emulsifiers in bread formulations are DATEM, sodium stearoyl lactylate (SSL), polysorbates, mono- and diglycerides, various monoglyceride derivatives, lecithin, and sucrose esters. Polysorbates refer to the fatty acid esters of ethoxylated sorbitan; polysorbate 60 is also known as polyoxyethylene sorbitan monostearate, and polysorbate 80 as polyoxyethylene sorbitan monooleate. DATEM, SSL, and polysorbate act as dough strengtheners (Stampfli & Nersten, 1995). Reported effects of emulsifiers on whole wheat dough and bread properties are summarized in Table 2.2.

2.3.1 Effect of emulsifiers on dough properties

Mettler and Siebel (1993) studied the effect of two emulsifiers and two hydrocolloids on the properties of dough (e.g., final proof time, fermentation stability) and bread made from whole wheat flour using response surface methodology. Both final proof time and fermentation stability are determined by Maturograph®. The final proof time is defined as the time from the start of the final proof to the first drop of the maturogram after the maximum. It is claimed to indicate the time needed for optimum fermenting maturity. Fermentation stability is related to the final proof time and shows the time tolerance that the loaf has to be placed into oven to achieve a consistent bread volume. Mono- and diglycerides decreased the final proof time, while DATEM increased the final proof time as measured by Maturograph. Thus, mono- and diglycerides allowed the dough to rise faster, whereas DATEM produced a slower rise. A faster rise during proofing is typically desirable in commercial production (Hrušková, Švec, & Jirsa, 2006). As fermentation continues beyond the optimum final proof time, the dough will lose volume (Mettler & Siebel, 1995). DATEM increased the fermentation stability, allowing for a larger window of time during which the dough can be moved from the proof stage to baking and still attain optimum loaf volume. In other words, dough with a higher fermentation stability is more tolerant to overproofing (Hrušková et al., 2006). In contrast to the findings of Mettler & Seibel (1993), DATEM decreased fermentation stability and final proof time in a study by Armero and Collar (1996b). Both studies reported that dough elasticity was improved by DATEM, and also to a lesser extent by mono- and diglycerides (Armero & Collar, 1996b; Mettler & Seibel, 1993). Elasticity is an important property in commercial baking, as it allows the dough to sustain and recover from deformations caused by mechanical handling. A greater elasticity generally corresponded to an increase in dough height (Mettler & Seibel, 1993). Another emulsifier, SSL,

has been shown to increase the tolerance for a high water absorption in reconstituted whole wheat dough and improved the subjective dough handling properties (Lai et al., 1989).

2.3.2 Effect of emulsifiers on loaf volume

An increase in loaf volume is one property associated with dough strength. In the study by Mettler and Siebel (1993), DATEM increased the specific volume of the baked loaf. The increased loaf volume could be attributed to an increase in dough elasticity, a property which allows for greater dough deformation without rupture and generally correlated to increasing dough height (Mettler & Seibel, 1993). Galliard and Collins (1988) reported that DATEM improved the loaf volume of whole wheat bread prepared by the Chorleywood Bread Process. A substantial synergistic effect was observed when DATEM was used in combination with the oxidizers ascorbic acid, dehydroascorbic acid, or potassium bromate. DATEM and oxidants both improve the gas holding ability of dough during proofing and baking. This assists with final loaf volume because whole wheat doughs are typically less stable and more prone to collapse than white doughs (Galliard & Collins, 1988). Lai, Davis, and Hoseney (1989) developed an optimum whole wheat bread formula and procedure that involved soaking the bran and shorts before reconstitution and adding lipoxygenase and vital wheat gluten to the dough. Their study also showed that, at 0.5% (fb), certain emulsifiers improved loaf volume by 7-13%. SSL and DATEM led to the greatest increase. Ethoxylated monoglycerides, succinylated monoglycerides, and lecithin also significantly increased loaf volume; polysorbate and monoglycerides did not significantly improve loaf volume (Lai et al., 1989). Indrani and Rao (1992a) found that the following emulsifiers all improved loaf volume of whole wheat bread: DATEM, SSL, soy lecithin, polyoxyethylene sorbitan monostearate (polysorbate-60), polyoxyethylene sorbitan monopalmitate (polysorbate-40), and glycerol-monostearate. All emulsifiers in this study were

tested at 0.5% (fb). In another study by the same authors, 0.5% SSL increased the loaf volume of whole wheat bread produced by three methods - straight, sponge and dough, and mechanical dough development (Indrani & Rao, 1992b). This level of SSL provided a greater improvement to loaf volume for straight and sponge and dough methods than did the other ingredients tested in the study, which included the oxidants potassium bromate and ascorbic acid, and increasing amounts of sugar, fat, and yeast. However, for mechanical dough development, higher amounts of yeast or fat, or addition of 200 ppm ascorbic acid, led to higher loaf volume than did 0.5% SSL (Indrani & Rao, 1992b), showing that the effect of improvers will vary based on processing conditions. In a study by Armero and Collar (1996a), none of the emulsifiers tested, which included monoglycerides (0.3%), DATEM (0.3%), and SSL (0.5%), increased the specific volume of whole wheat or white bread.

Grausgruber, Miesenberger, Schoenlechner, and Vollmann (2008) studied the effect of emulsifiers and enzymes in bread from whole grain einkorn wheat. Einkorn is a soft, diploid wheat species with high protein content but low gluten strength and inferior rheological properties (Grausgruber et al., 2008). Improvements to whole grain einkorn products may provide similar or greater improvement for regular whole wheat bread. The addition of 0.4% DATEM increased the loaf volume by almost ten percent, but addition of 0.6% monoglycerides did not significantly change loaf volume.

2.3.3 Effect of emulsifiers on crumb hardness and staling

Mettler and Siebel (1993) found that DATEM exhibited antistaling properties. The rate at which crumb firmness increased with storage was reduced. The authors attributed the reduction in firmness primarily to the increase in loaf volume. Grausgruber et al. (2008) also reported a decrease in hardness for whole grain einkorn wheat bread upon addition of either 0.4% DATEM

or 0.6% monoglycerides. The ability of SSL to decrease the firming rate of whole wheat bread over 4 days of storage was demonstrated by Indrani and Rao (1992a) at an addition level of 0.5%. In the same study, individual addition of 0.5% DATEM, SSL, soy lecithin, polysorbate-60, polysorbate-40, and glycerol-monostearate each improved the softness of whole wheat bread as scored by a 6-person sensory panel (Indrani & Rao, 1992a).

Armero and Collar (1996a) found that DATEM and SSL acted as crumb softeners in whole wheat bread but not in white bread. A softening effect can be the result of a decrease in the surface area of resistant material, which is in turn related to increased loaf volume, and/or a decrease in the resistance of the material. Because DATEM and SSL did not affect specific volume, the softening effect in this case was due to a reduction in material resistance. Monoglycerides did not decrease the crumb firmness in white or whole wheat bread.

2.3.4 Effect of emulsifiers on crumb sensory characteristics

DATEM and mono- and diglycerides tended to improve the crumb structure of whole wheat bread (Mettler & Seibel, 1993). DATEM decreased the sensorily-evaluated elasticity of the bread, which was attributed to the thinning of cell walls that accompanied a high loaf volume (Mettler & Seibel, 1993). Indrani and Rao (1992a) reported a slight improvement to crumb grain score for breads prepared with soy lecithin, polysorbate 40, SSL, and DATEM, although the criteria used to calculate the score were not described. Another study by the same authors reported that 0.5% SSL improved the crumb grain score for whole wheat bread prepared by three different methods, with the greatest improvement to the score in the sponge and dough method (Indrani & Rao, 1992b). The crumb was described as fine and uniform. Grausgruber et al. (2008) found that DATEM and monoglycerides did not significantly affect the size or number of air cells in whole grain einkorn wheat bread when they were used singly. However, pore size and

area were significantly increased when both emulsifiers were added together in combination with a cocktail of enzymes. The cell size and area were increased and the number of cells decreased when the emulsifiers and enzymes were further combined with gluten. A greater number of large air cells is generally considered a lower quality crumb, but these breads also had a larger volume and less firm crumb than did the control einkorn wheat bread. Volume and firmness may be considered the greater priority, but it is still important to note that various additives will often improve some quality parameters while diminishing others.

DATEM was shown to increase the crumb grain of whole wheat bread but not white bread, based on sensory panel scores. SSL increased the eatability score of both varieties of bread but also decreased crumb elasticity. Monoglycerides improved the eatability score of whole wheat bread only (Armero & Collar, 1996a).

2.4 Hydrocolloids

Hydrocolloids can be used to improve dough performance, act as antistaling agents, preserve dough and bread quality for frozen dough or par-baked bread, improve the sensory quality of bread, and compensate for low protein or high fiber in various types of flours (Ferrero, 2017). Crumb softening due to locust bean gum, xanthan gum, and sodium alginate has been demonstrated in white bread (Davidou, Le Meste, Debever, & Bekaert, 1996). Because most hydrocolloids are hydrophilic, their use in breadmaking requires an increase in formula water (Ferrero, 2017; Mettler & Seibel, 1993; Sudha & Rao, 2009; Zannini, Waters, & Arendt, 2014). The hydration capacity and consequently the amount of additional water depends on the type of hydrocolloid and its structural and chemical properties. Several of the mechanisms involved with hydrocolloid interactions in the dough and bread system have been reviewed by Ferrero (2017).

The following sections describe the effects of hydrocolloids in whole wheat systems, and these findings are summarized in Table 2.3.

2.4.1 Effect of hydrocolloids on dough properties

In a response surface study of hydrocolloids and emulsifiers, guar gum and carboxymethylcellulose (CMC) decreased the final proof time of whole wheat bread dough (Mettler & Seibel, 1993). Armero and Collar (1996b) reported a similar effect for CMC on whole wheat dough, but not for white dough. Guar gum combined with DATEM increased the fermentation stability, but the effect was mainly attributed to DATEM (Mettler & Seibel, 1993). Both CMC and guar gum decreased the elasticity of whole wheat dough, which tended to correspond to a reduction in dough proof height. In this respect, CMC and guar gum had a negative effect. Despite this, increasing concentrations of guar gum did provide a slight increase in the volume of the baked bread. CMC decreased the resistance to extension in both whole wheat and white dough (Armero & Collar, 1996b).

Zannini et al. (2014) evaluated whole wheat dough prepared with xanthan gum, hydroxypropyl methylcellulose (HPMC), and dextran (0.5 to 5%, fb) through farinograph and extensograph analysis. Addition of each hydrocolloid was positively correlated with water absorption. HPMC possessed the greatest water-binding capacity. Increasing levels of these hydrocolloids increased dough development time (DDT) by slowing the rate of gluten hydration. In contrast to HPMC and xanthan gum, lower levels of dextran actually decreased DDT compared to the control because this hydrocolloid had a more rapid hydration rate. Dough stability was negatively correlated with hydrocolloid presence. This decrease was attributed to a disruption to the gluten network caused by the hydrocolloids. No effect on mixing tolerance index was observed for hydrocolloid addition. Of the three hydrocolloids, xanthan gum increased

dough elasticity, measured as the width of the farinograph curve at peak consistency. The authors explain that this effect is undesirable, because it indicates that the (R_{50}/E) viscoelastic ratio is changed. HPMC and xanthan gum significantly increased the R_{50}/E ratio, a value which compares the resistance to constant deformation (R_{50}) to the extensibility (E) after 50 mm of stretching by the extensograph. HPMC mainly reduced elasticity, whereas xanthan gum affected both elasticity and resistance. However, Armero and Collar (1996b) found that HPMC led to an increase in dough elasticity as measured by maturograph. HPMC also increased the proof height and decreased resistance to extension. Armero and Collar tested the HPMC at 0.3% (fb), whereas the levels used by Zannini et al. ranged from 0.5 to 5.0% (fb). Dextran also caused a small but non-significant increase in the R_{50}/E ratio primarily by reducing the elasticity. This study measured the gas retention coefficient using a Rheofermentometer®. HPMC presence was positively correlated with the retention coefficient in whole wheat dough, whereas dextran and xanthan gum tended to decrease the retention coefficient. The negative effect of xanthan gum may be caused by its anionic properties, which prevent the positively charged gluten proteins from forming an elastic film. Dextran was suggested to physically interfere with the gas-liquid interface and ability of the dough to expand (Zannini et al, 2014). Sudha and Rao (2009) reported that HPMC increased the water absorption of whole wheat dough without changing the dough development time. In contrast to Zannini et al. (2014), dough stability increased slightly. HPMC decreased resistance to extension, and extensibility decreased slightly from 124 to 122 mm, but statistically significance was not reported. HPMC addition lowered the pasting temperature of the starch by decreasing available water and interaction between the hydrocolloid and starch. Peak viscosity and cold paste viscosity were also decreased (Sudha & Rao, 2009). Therefore, HPMC appears to interfere with the starch gelatinization and subsequently retrogradation, which

may help provide anti-staling effects in baked products. SEM examination of dough showed that HPMC addition formed a more continuous gluten network presumable by allowing for greater gluten mobility. The starch-gluten matrix was more condensed and compact in the HPMC-treated dough, with greater incorporation of starch granules into the matrix. In the dough without HPMC, the starch appeared more as distinct granules, not embedded in the matrix. The more cohesive dough structure may explain the positive effect of HPMC on loaf volume and other bread characteristics reported in other studies.

2.4.2 Effect of hydrocolloids on loaf volume

Mettler and Seibel (1993) demonstrated the ability of guar gum to increase the specific volume of whole wheat bread when tested at constant, medium levels of CMC and mono- and diglycerides. The effect was greater as the level of DATEM also increased. Zannini et al. (2014) found that HPMC, xanthan gum, and dextran produced non-significant decreases in specific volume. In the case of HPMC, increased gas retention coefficient did not translate to an increased loaf volume. The authors propose that xanthan gum may limit dough extension due to strong interactions with gluten. A reduced dough volume was also observed for xanthan and dextran addition. The usage level may have also been too high. Levels of hydrocolloids as low as 0.1% have been shown to improve the volume of white bread (Guarda, Rosell, Benedito, & Galotto, 2004). Also, the water absorption and mixing time may not have been optimized for baking. These parameters were based on farinograph data which does not always translate well to breadmaking requirements. Armero and Collar (1996a) reported that HPMC increased the specific volume of both white and whole wheat bread, while CMC did not increase the loaf volume of either variety of bread.

2.4.3 Effect of hydrocolloids on crumb hardness and staling

Both CMC and guar gum have been shown to reduce the staling rate of whole wheat bread, defined as the increase in firmness as a function of storage time (Mettler & Seibel, 1993). Guar gum required more formula water addition, and it decreased crumb elasticity to a greater extent than CMC did. Nevertheless, statistical analysis revealed that increase in volume was more important than water addition for a reduction in firmness (Mettler & Seibel, 1993). Therefore, a hydrocolloid should also effectively increase the loaf volume if a reduction in hardness is desired. The ability to absorb more water is not useful if the dough cannot also expand. Zannini et al. (2014) reported that dextran and HPMC resulted in a non-significant decrease in initial loaf hardness and delay in staling. The small effect on hardness is likely related to the lack of volume improvement in this study. Textural improvement from HPMC was explained by a higher gas retention during dough fermentation and increased water absorption (Zannini et al., 2014). The improved gas retention may result from a stabilization effect by HPMC at the gas-dough interface (Guarda et al., 2004; Zannini et al., 2014). Air cell stabilization allows for greater expansion during fermentation and also for an even distribution of small gas cells (Zannini et al., 2014), which could improve the texture of the baked bread. Xanthan gum increased loaf hardness in the same study by Zannini et al. (2014). One possible reason for the firmness is that xanthan gum thickens the cell walls of the bread crumb (Rosell, Rojas, & Benedito de Barber, 2001; Zannini et al., 2014). However, Zannini et al. (2014) reported that 0.5 or 1% addition of xanthan gum, HPMC, or dextran all resulted in significantly decreased cell wall thickness, so other factors must be considered when explaining loaf hardness. HPMC softened the crumb of both white and whole wheat bread, but CMC was ineffective in this regard for both white and whole wheat (Armero & Collar, 1996a). The decreased firmness could be due

to the increase in volume alone, which decreases the surface area of resistant material. HPMC may also decrease the crumb firmness by decreasing the material resistance to compression.

2.4.4 Effect of hydrocolloids on crumb sensory characteristics

Guar gum has been shown to decrease the crumb elasticity of whole wheat bread, possibly due to the high amount of water required when this gum is present (Mettler & Seibel, 1993). Zannini et al. (2014) found that xanthan gum, HPMC, and dextran generally did not affect the number and size of air cells in whole wheat bread. However, any of the three hydrocolloids added at 0.5 or 1.0% decreased cell wall thickness. Thinner cell walls are generally desired because they help create a smoother texture. HPMC improved the crumb elasticity, "eatability," and overall acceptability of whole wheat bread but not white bread (Armero & Collar, 1996a). It improved the scores for crumb grain and crumb structure in both whole wheat and white bread. Although CMC improved the elasticity and eatability scores in white bread, this hydrocolloid did not significantly affect any of the sensory related scores for whole wheat bread (Armero & Collar, 1996a).

2.5 Oxidants

Oxidants are added to increase dough strength mainly by forming disulfide bonds through the oxidation of free sulfhydryl groups on the gluten proteins (Stauffer, 1990b). Oxidants may also increase dough elasticity, improve handling tolerance, and increase ovenspring and final loaf volume (Stauffer, 1990b). Examples of oxidants include ascorbic acid, potassium bromate, potassium iodate, calcium peroxide, and azodicarbonamide (ADA). Certain synthetic oxidants, including potassium bromate and ADA, have come under scrutiny due to potential health effects and are banned in several countries outside of the U.S., such as Australia, Singapore, and many countries in Europe (Gelroth, Sanders, Cogswell, & Zvaners, 2009; Ye, Wang, Sang, & Liu,

2011). Natural oxidants such as ascorbic acid and oxidizing enzymes, e.g. glucose oxidase (see enzymes section of this paper), are increasingly used as replacements. Ascorbic acid is a reducing agent, but is oxidized to the dehydroascorbic acid form early during mixing, and this form acts as an oxidant in dough (Dong & Hoseney, 1995). Reducing compounds from wheat bran may counteract the effects of oxidants in whole wheat dough. Therefore, oxidizing agents are less effective in whole wheat systems compared to those in refined wheat flour and typically must be used at higher levels. The published results of oxidant use in whole wheat breadmaking are summarized in Table 2.4.

2.5.1 Effect of oxidants on dough properties

Potassium bromate increased the farinograph DDT of whole wheat flour produced by disc and stone milling (Indrani & Rao, 1992c). Ascorbic acid increased the DDT for flour from hammer, disc, stone, and roller mills. Both oxidants increased the dough stability time but to varying degrees for the differently milled flours. The dough consistency was minimally affected as measured by the mixing tolerance index. Slight increases in the valorimeter value were also observed with either potassium bromate or ascorbic acid, indicating an increase in dough strength. Mixograph and extensograph analyses also suggested stronger doughs upon oxidant addition. Minimal effect on peak height and weakening angle were observed in the mixograph curves. Extensograph characteristics showed higher resistance and lower extensibility, suggesting the oxidants improved the gas retention ability of the dough (Indrani & Rao, 1992c). Rosehip added as a source of ascorbic acid has also been shown to increase the resistance to extension and decrease the extensibility of whole wheat dough (Boz & Karaoglu, 2013). Rosehip also decreased the water absorption of the dough, and it increased the dough energy determined by extensograph. The Chen-Hoseney stickiness test revealed a slight increase in stickiness but a

decrease in adhesion and stringiness upon rosehip addition (Boz & Karaoglu, 2013). As for other dough conditioners, oxidizing agents must be used at the correct level. Under-oxidation makes the dough overly weak, extensible, soft and sticky, and the finished product has decreased volume, uneven grain, weak crust, and poor symmetry. Over-oxidation creates an overly tight and dry dough, which leads to a denser finished product with holes, coarse texture, and lower volume.

2.5.2 Effect of oxidants on loaf volume

Galliard and Collins (1988) demonstrated that ascorbic acid improved the loaf volume of whole wheat bread prepared by the Chorleywood process (mechanical dough development). Dehydroascorbic acid was more effective at increasing volume when the dough was mixed under reduced pressure and hence reduced O_2 availability, but a difference between the effects of these two oxidants was not observed under atmospheric pressure. Indrani and Rao (1992b) observed a slight increase in loaf volume due to 100 or 200 ppm ascorbic acid in whole wheat bread prepared by the sponge and dough or straight dough methods. The greatest effect of ascorbic acid was observed for 200 ppm in the mechanical dough system. In this case, the specific volume increased by 21% compared to the control. Mechanical dough development is known to require a high level of oxidants (Stauffer, 1990a). An emulsifier was also needed to produce optimal loaf volume using this production method. Addition of potassium bromate alone showed little effect on loaf volume for any of the three methods. The largest improvement in volume and bread quality in this study was obtained by the sponge and dough method with 20 ppm potassium bromate, 0.5% SSL, and an increased fat level (Indrani & Rao, 1992b). This study illustrates that the effect of improvers varies based on breadmaking techniques. Therefore, it is important to tailor ingredients to the specific processing method. In another study by the same authors,

ascorbic acid was more effective at increasing loaf volume than was potassium bromate (Indrani & Rao, 1992c). The improvement depended on the type of milling utilized to produce the flour. Hammer and roller mills produced higher quality flours than disc and stone mills, and the flours from hammer and roller mills were more responsive to the oxidants. Ascorbic acid was tested in whole wheat bread by da Silva et al. (2016). The usage level did not significantly increase loaf volume. The authors acknowledged that the use of vital wheat gluten in the base formula probably prevented any noticeable effects from the improvers, because the control itself had a good volume. They did find that high levels of oxidants were needed to see improvement from xylanase addition to whole wheat bread (da Silva et al., 2016). Rosehip, added to whole wheat bread as a source of ascorbic acid, increased the specific volume and decreased the firmness of fresh bread and bread stored for two days (Boz & Karaoglu, 2013).

2.5.3 Effect of oxidants on crumb characteristics

Potassium bromate did not significantly affect the crust and crumb characteristics or sensory score of whole wheat bread prepared by three methods (Indrani & Rao, 1992b). A slight improvement to the crumb grain score was reported for ascorbic acid addition to bread prepared by sponge and dough or mechanical dough development methods, but the basis of this sensory scoring was not defined (Indrani & Rao, 1992b). Rosehip, a source of ascorbic acid, improved the sensory scores for crumb grain, texture, crumb color, aroma, and general acceptability of whole wheat bread (Boz & Karaoglu, 2013).

2.6 Other functional ingredients

Other ingredients which improve the quality of whole wheat dough and/or bread that do not fit in the categories previously discussed are presented in Table 2.5 and discussed below.

2.6.1 Vital wheat gluten

Vital wheat gluten is a very important, if not critical, ingredient in whole wheat bread formulations (Day, Augustin, Batey, & Wrigley, 2006). Supplementation with vital gluten is almost ubiquitous in whole wheat formulations because bran and germ dilute the amount of gluten in the flour. The addition of vital gluten provides strength, dough elasticity and gas retention which help to counteract the negative effects of bran on loaf volume. Gluten plays a key role in all stages of breadmaking, including mixing, proofing, and baking (Ortolan & Steel, 2017). It is commonly added to weak wheat flours to improve breadmaking quality, in high fiber breads where the gluten is diluted, or in products requiring additional strength and gas retention such as hamburger buns or frozen dough (Esteller, Pitombo, & Lannes, 2005; Ortolan & Steel, 2017; Rosell & Gómez, 2007). High levels of vital gluten are not uncommon in the baking industry, for example, 10% (fb) vital gluten in whole wheat bread (Maningat, Bassi, & Hesser, 1994). The amounts evaluated in published studies of whole wheat bread are more modest and do not necessarily reflect the full benefits that are achieved when vital gluten is used at the higher levels used by bakeries. It is also important to note that the quality of the wheat gluten determines the effectiveness (Ortolan & Steel, 2017). Lai et al. (1989) used 2% vital wheat gluten to improve the strength of reconstituted whole wheat dough made from soaked bran and shorts. The gluten also improved the shape and crumb structure of the resulting loaf (Lai et al., 1989). Indrani and Rao (1992a) found that a 2% addition of vital gluten significantly increased the loaf volume of whole wheat bread. Boz et al. (2010) reported that vital gluten added to whole wheat dough increased dough water absorption, extensibility, resistance to extension, dough energy, stickiness, and adhesion. Addition of 2.5% vital gluten increased the loaf volume, decreased the crumb hardness, and increased the cohesiveness and springiness of the crumb (Boz

& Karaoglu, 2013). Firmness was lower than control both for fresh bread and after 2 days of storage at room temperature.

2.6.2 Legume flours

Legumes may be added to whole grain bread in order to improve the nutritional quality of the product, but the effect on the technological quality attributes is often detrimental. Legume flours which maintain enzyme activity may prove beneficial. Enzyme-active soy flour was used by Lai et al. (1989) as a source of lipoxygenase to improve whole wheat bread. Indrani and Rao (1992a) reported a small but significant increase in specific volume due to 0.5% addition of enzyme-active soy flour.

2.6.3 Defatted Cephalaria syriaca flour

Boz et al. (2010) examined the effect of defatted *Cephalaria syriaca* flour (DCSF) in whole wheat dough. This plant is considered as both a weed and an additive to increase dough strength in Turkey. In this study, DCSF decreased water absorption, dough stickiness, adhesion, and stringiness. Dough energy and resistance to extension were increased, and extensibility was decreased, indicating a stronger dough (Boz et al., 2010). The increased dough strength led to changes in bread characteristics, including increased specific loaf volume (Boz & Karaoglu, 2013). Crumb firmness was decreased for both fresh and stored bread, a change attributed to the larger volume and improvement in crumb structure. Cohesiveness, springiness, and chewiness, determined by TPA, increased in the DCSF-supplemented bread. The volume and texture improvements suggest that DCSF can be used to improve the sensory appeal of whole wheat bread.

2.7 Conclusions and opportunities

Researchers and the baking industry look to improve the dough handling properties, loaf volume, texture, sensory, and shelf-life of whole wheat bread, to increase both production efficiency and consumer acceptance of whole wheat products. Addition of functional ingredients is the easiest way to modify the dough and bread properties because it does not require special equipment, intensive employee training, or extended amounts of time. These ingredients can usually be added directly to the flour along with the other ingredients prior to dough mixing. Much research has demonstrated the nutritional value and health benefits of whole wheat, but there remains a disparity between recommended and actually consumed levels of whole grain products. Sensory properties including hardness play a large role in consumers' decision to choose white vs. whole wheat bread. Hardness is just one aspect that can easily be improved by certain functional ingredients such as enzymes and emulsifiers.

Various improvers have demonstrated an ability to improve whole wheat dough physical and rheological properties and increase loaf volume and decrease the initial firmness and staling rate of whole wheat bread. Typical effects of improvers in whole wheat dough and bread are presented in Table 2.6. Xylanase, α-amylase, G4-amylase, and gluten significantly benefit whole wheat dough handling and bread quality. Other improvers including glucose oxidase, phytase, DATEM, SSL have also shown varying success in improving the characteristics of whole wheat dough or bread. Bread made with whole wheat flour was more responsive to certain additives than was white bread, especially regarding the effect of HPMC and α-amylase on sensory characteristics related to physical properties. Furthermore, the emulsifiers DATEM and SSL decreased the hardness of whole wheat bread only.

There is still much room for additional research on the application of improvers of all types to whole wheat bread. Inconsistent effects of some ingredients were reported in different studies, and more research is needed to better define the specific effects of these functional ingredients. In addition, more studies are necessary to determine the effect of improvers when they are used in combination with other ingredients of the same type and of different types. Although hydrocolloids have been studied extensively in gluten-free baking applications, research on their use in whole wheat bread is limited. It is also necessary to better understand, at the molecular level, how these improvers interact with whole wheat flour constituents during dough development and bread-making to better guide product development.

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2.9 Tables

Table 2.1 Effects of enzymes on dough properties, loaf volume, crumb hardness and crumb staling of whole wheat bread

Enzyme	**Effects on dough**	**Effect on loaf volume**	**Effect on crumb hardness**	**Effect on crumb staling**	**Reference**
α-amylase		Increased	Decreased		Armero & Collar (1996a)
α-amylase		No effect	No effect		Grausgruber et al. (2008)
α-amylase	Decreased water absorption; increased mixing tolerance index; increased dough height; increased proof time; increased gas production; decreased gas retention coefficient				Sanz Penella et al. (2008)
α-amylase (from malt flour)	Increased water absorption; increased extensibility; decreased resistance to extension; increased stickiness, adhesion, and stringiness				Boz et al. (2010)
α-amylase (from malt flour)		Increased	No effect		Boz & Karaoglu (2013)
α-amylase		Increased			Bae et al. (2014)
α-amylase + xylanase		Increased	Decreased		Grausgruber et al. (2008)
α-amylase + xylanase +		Increased	Decreased		Grausgruber et al. (2008)

transglutaminase					
Amylase (G4-amylase)	Decreased water absorption; increased dough development time; increased viscous characteristics	Increased	Decreased	Decreased	Bae et al. (2014)
Amyloglucosidase	Increased or decreased resistance to extension (dependent on dose)	Decreased			Altinel & Ünal (2017a,b)
Cellulase		No effect	Decreased		Hung et al. (2007)
Cellulase	No effect	No effect			Altinel & Ünal (2017b)
Glucose oxidase	Increased stiffness				Yang et al. (2014)
Glucose oxidase		No effect	Decreased when higher glucose oxidase levels combined with ascorbic acid + xylanase		da Silva et al. (2016)
Glucose oxidase	Decreased resistance to extension; decreased extensograph dough energy	Increased			Altinel & Ünal (2017a,b)
Lipase	Increased dough hardness; decreased stickiness				Colakoglu & Özkaya (2012)
Lipase	No effect	Decreased			Altinel & Ünal (2017b)
Phytase	Decreased proof time	Increased	Decreased		Haros et al. (2001a)
Phytase	Slightly decreased water absorption				Sanz Penella et al. (2008)
Phytase		Decreased (conventional	Decreased (frozen dough); no effect		Rosell et al. (2009)

		breadmaking); no effect (frozen dough; par-baked)	(conventional; par-baked)		
Transglutaminase			Increased		Collar and Bollaín (2005)
Transglutaminase		Decreased	Increased		Collar et al. (2005)
Xylanase	Decreased water absorption; increased proof height	Increased	Decreased		Shah et al. (2006)
Xylanase		Increased	Decreased		Grausgruber et al. (2008)
Xylanase		Increased	Decreased		Jaekel et al. (2012)
Xylanase	Decreased water absorption; increased proof height	Increased	Decreased		Driss et al. (2013)
Xylanase	Decreased water absorption; increased proof height	Increased	Decreased	Decreased	Ghoshal et al. (2013)
Xylanase		No effect			Bae et al. (2014)
Xylanase	Increased proof height	Increased			Kumar & Satyanarayana (2014)
Xylanase		No effect	Decreased when intermediate xylanase levels combined with oxidants		da Silva et a. (2016)
Xylanase	Decreased resistance to extension	Increased			Altinel & Ünal (2017a,b)
Xylanase		Increased (Xyl1); no effect (Xyl2)	Decreased (Xyl1); increased (Xyl2)		Carvalho et al. (2017)

Table 2.2 Effects of emulsifiers on dough properties, loaf volume, crumb hardness and crumb staling of whole wheat bread

Emulsifier	**Effects on dough**	**Effect on loaf volume**	**Effect on crumb hardness**	**Effect on crumb staling**	**Reference**
DATEM		Increased			Lai et al. (1989)
DATEM		Increased	Decreased		Indrani & Rao (1992a)
DATEM	Increased proof time; increased fermentation stability; increased dough elasticity; increased dough height	Increased		Decreased	Mettler & Seibel (1993)
DATEM		No effect	Decreased		Armero & Collar (1996a)
DATEM	Decreased fermentation stability; decreased final proof time; increased elasticity				Armero & Collar (1996b)
DATEM		Increased	Decreased		Grausgruber et al. (2008)
Ethoxylated monoglycerides		Increased			Lai et al. (1989)
Glycerol monostearate		Increased	Decreased		Indrani & Rao (1992a)
Lecithin		Increased			Lai et al. (1989)
Monoglycerides		No effect			Lai et al. (1989)
Monoglycerides	Decreased proof time; slightly increased dough elasticity				Mettler & Seibel (1993)
Monoglycerides		No effect	No effect		Armero & Collar (1996a)
Monogylcerides		No effect	Decreased		Grausgruber et al. (2008)
Polysorbate		No effect			Lai et al. (1989)
Polysorbate 40		Increased	Decreased		Indrani & Rao (1992a)

Polysorbate 60		Increased	Decreased		Indrani & Rao (1992a)
Soy lecithin		Increased	Decreased		Indrani & Rao (1992a)
SSL	Improved dough handling properties	Increased			Lai et al. (1989)
SSL		Increased	Decreased	Decreased	Indrani & Rao (1992a)
SSL		Increased			Indrani & Rao (1992b)
SSL		No effect	Decreased		Armero & Collar (1996a)
Succinylated monoglycerides		Increased			Lai et al. (1989)

Note: DATEM (diacetyl tartaric esters of monoglyceride), SSL (sodium stearoyl lactylate)

Table 2.3 Effects of hydrocolloids on dough properties, loaf volume, crumb hardness and crumb staling of whole wheat bread

Hydrocolloid	Effects on dough	Effect on loaf volume	Effect on crumb hardness	Effect on crumb staling	Reference
CMC	Decreased proof time; decreased dough elasticity	No effect		Decreased	Mettler & Seibel (1993)
CMC		No effect	No effect		Armero & Collar (1996a)
CMC	Decreased resistance to extension; decreased final proof time				Armero & Collar (1996b)
Dextran	Dose-dependent effect on dough development time; decreased dough stability	No effect	No effect		Zannini et al. (2014)
Guar gum	Decreased proof time; decreased dough elasticity; decreased dough height	Increased		Decreased	Mettler & Seibel (1993)
HPMC		Increased	Decreased		Armero & Collar (1996a)
HPMC	Increased elasticity; increased proof height; decreased resistance to extension				Armero & Collar (1996b)
HPMC	Increased dough development time; decreased dough stability; decreased dough elasticity	No effect	No effect		Zannini et al. (2014)
Xanthan Gum	Increased dough development time; decreased dough stability; increased dough elasticity	No effect	No effect		Zannini et al. (2014)

Note: CMC (carboxymethylcellulose), HPMC (hydroxypropyl methylcellulose)

Table 2.4 Effects of oxidants on dough properties, loaf volume, crumb hardness and crumb staling of whole wheat bread

Oxidant	**Effects on dough**	**Effect on loaf volume**	**Effect on crumb hardness**	**Effect on crumb staling**	**Reference**
Ascorbic acid		Increased (mechanical dough development)			Galliard and Collins (1988)
Ascorbic acid		Increased (dependent on breadmaking method)			Indrani & Rao (1992b)
Ascorbic acid	Increased dough development time; increased dough stability; slightly increased dough strength; increased resistance to extension; decreased extensibility	Increased or no effect (dependent on milling type)			Indrani & Rao (1992c)
Ascorbic acid (from rosehip)	Decreased water absorption; increased resistance to extension; decreased extensibility; slightly increased stickiness	Increased	Decreased		Boz & Karaoglu (2013)
Ascorbic acid		No effect	Decreased when higher ascorbic acid levels combined with glucose oxidase + xylanase		da Silva et a. (2016)
Potassium bromate		No effect			Indrani & Rao (1992b)
Potassium bromate	Increased dough development time; increased dough stability; slightly increased dough strength; increased resistance to extension; decreased extensibility	Increased or no effect (dependent on milling type)			Indrani & Rao (1992c)

Table 2.5 Effects of other functional ingredients on dough properties, loaf volume, crumb hardness and crumb staling of whole wheat bread

Ingredient	**Effects on dough**	**Effect on loaf volume**	**Effect on crumb hardness**	**Effect on crumb staling**	**Reference**
Vital wheat gluten	Increased dough strength				Lai et al. (1989)
Vital wheat gluten		Increased			Indrani & Rao (1992a)
Vital wheat gluten	Increased water absorption, extensibility, resistance to extension, dough energy, stickiness, and adhesion				Boz et al. (2010)
Vital wheat gluten		Increased	Decreased		(Boz & Karaoglu, 2013)
Enzyme active soy flour	Decreased rest time (sponge and dough process)	Increased			Lai et al. (1989)
Enzyme active soy flour		Increased			Indrani & Rao (1992a)
Defatted *Cephalaria syriaca* flour	Decreased water absorption, dough stickiness, adhesion, and stringiness; increased dough energy, resistance to extension, and dough strength				Boz et al. (2010)
Defatted *Cephalaria syriaca* flour		Increased	Decreased		(Boz & Karaoglu, 2013)

Table 2.6 Improving effects of typical ingredients in whole wheat dough and bread

Ingredient	**Overall impact level**	**Observed effects**
α-amylase	Major	Increased loaf volume, decreased crumb hardness and staling
G4-amylase	Major	Increased loaf volume, decreased crumb hardness and staling (one study). Needs further research.
Vital wheat gluten	Major	Increased dough strength, increased loaf volume, improved sensory characteristics
Xylanase	Major	Decreased water absorption, increased loaf volume, decreased crumb hardness and staling, improved sensory characteristics
DATEM	Moderate	Increased loaf volume, decreased crumb hardness, improved sensory characteristics
Glucose oxidase	Moderate	Increased dough strength, decreased dough resistance to extension, increased loaf volume
Phytase	Moderate	Dependent on enzyme strain and flour composition. Activation of endogenous α-amylase can lead to increased loaf volume, decreased crumb hardness
SSL	Moderate	Increased loaf volume, decreased crumb hardness, improved sensory characteristics
Amyloglucosidase	Minor	Various effect on dough strength, slight decrease in loaf volume
Ascorbic Acid	Minor	Increased dough strength, increased loaf volume
Cellulase	Minor	Decreased crumb hardness
HPMC	Minor	Increased loaf volume, decreased crumb hardness, improved sensory characteristics
Lipase	Minor	Dough hardening, decreased loaf volume
Transglutaminase	Minor	Decreased loaf volume, increased crumb hardness, improved sensory characteristics

Note: DATEM (diacetyl tartaric esters of monoglyceride), SSL (sodium stearoyl lactylate), HPMC (hydroxypropyl methylcellulose)

3 Individual effects of enzymes and vital wheat gluten on whole wheat dough and bread properties

Abstract

Enzymes have been widely studied in white pan bread, but less information has been published on their use in whole wheat bread. The objective of this research was to determine effects of five enzymes on whole wheat bread properties, with a focus on loaf volume, bread texture, and staling. Bread was prepared from whole wheat flour following AACC method 10-10.03. Enzymes (conventional α-amylase, cellulase, glucose oxidase, maltogenic α-amylase, and xylanase) were added at three levels based on the minimum, maximum, and 50% greater than the maximum recommendations provided by the manufacturer. Vital wheat gluten (VWG) was added as an additional, separate treatment at 2.5% (fwb). Dough rheological properties were determined by farinograph and mixograph. Specific volume was measured for fresh bread, and moisture content, texture profile analysis (TPA), and crumb structure were analyzed the following day. Moisture content and TPA were measured again after 3 and 7 days of storage at 22 °C to determine changes associated with staling. Effect on starch retrogradation was quantified by differential scanning calorimetry (DSC) after the 7 days. Enzymes had minimal effect on water absorption and mixing time for whole wheat dough. Each enzyme increased specific loaf volume for at least one of the usage levels tested ($P < 0.01$). Among the enzyme treatments, the greatest loaf volume was seen for xylanase at the medium and high levels. No enzyme was as effective as VWG at increasing loaf volume. Enzymes did not significantly change cell structure, except for a slight increase in cell wall thickness ($P < 0.05$) and cell diameter ($P < 0.01$) for the high level of maltogenic α-amylase. The greatest reduction in fresh bread hardness was obtained for the high level of xylanase. VWG, maltogenic α-amylase, and

xylanase reduced the rate of bread firming over 7 days. Conventional α-amylase, cellulase, and maltogenic α-amylase decreased starch retrogradation at day 7 as measured by DSC ($P < 0.01$). Maltogenic α-amylase nearly eliminated the endothermic peak for recrystallized amylopectin, showing this enzyme's strong ability to reduce retrogradation in bread. This study demonstrated the specific application of enzymes in whole wheat bread to increase loaf volume and decrease initial crumb hardness and bread staling, which may help improve the sensory appeal of whole wheat bread and ultimately increase whole grain consumption.

Keywords: Bread; Whole wheat; Enzymes; Staling; Gluten; Dough properties; α-amylase, Cellulase; Glucose oxidase; Maltogenic α-amylase; Xylanase

3.1 Introduction

Enzymes can provide a wide range of functions related to dough conditioning and bread improvement. They are clean label alternatives to other types of improvers. Most of the enzymes used in bakery applications are hydrolases, including various types of amylase, cellulase, lipase, protease, and endoxylanase. Oxidoreductases, such as glucose oxidase and lipoxygenase, and a specific transferase, transglutaminase, are also used in the baking industry and produce a strengthening effect on the dough (Joye et al., 2009, Zhang et al., 2013). α-Amylases degrade starch polymers and are used to aid fermentation, increase loaf volume, improve texture, and decrease staling of bread (Goesaert & Slade et al., 2009; van der Maarel et al., 2002). Endoxylanases cleave the xylan backbone of arabinoxylan, a non-starch polysaccharide found in cell walls, which modifies the functionality of arabinoxylan and improves dough handling, oven spring, loaf volume, crumb structure, and shelf life of bread (Butt et al., 2008; Courtin &

Delcour, 2002). Cellulase is used to increase loaf volume, improve texture, and decrease staling (Haros et al., 2002).

Enzymes have been widely studied in white pan bread, but less information has been published on their use in whole wheat bread, which has a smaller loaf volume and harder crumb texture compared to white bread. For example, although cellulase is industrially promoted for whole wheat bread applications, current literature does not report any significant effect on dough properties or loaf volume (Altinel & Ünal, 2017b; Hung et al., 2007). We have previously reviewed the literature on enzymes in whole wheat dough and bread (Tebben et al., 2018). Xylanase has consistently shown an overall beneficial effect on loaf volume and crumb hardness. Conventional α-amylase has also generally been shown to increase loaf volume, but the results of that and other enzymes in whole wheat bread have been inconsistent or not well studied. Vital wheat gluten is often added to whole wheat bread formulations to improve dough handling properties and loaf volume, at levels that may reach or exceed 10% (Maningat et al., 1994) but limited literature is available on its comprehensive effects on whole wheat dough and bread properties.

The objective of this research was to determine the individual effects of vital wheat gluten and five enzymes (conventional α-amylase, cellulase, glucose oxidase, maltogenic α-amylase, and fungal endoxylanase) on whole wheat dough and bread properties, with a focus on loaf volume, bread texture, and staling. The specific enzymes were selected to cover a range of activities. Cellulase and xylanase were chosen due to their action on cell wall material, which is present in high amounts in whole wheat flour. The strengthening effect of glucose oxidase was expected to benefit whole wheat dough, in which the bran and germ tend to weaken the gluten network. Conventional α-amylase is one of the most commonly used enzymes for bread making,

and maltogenic α-amylase was selected for its anti-staling properties. These enzymes are also commercially available and utilized in bakeries.

3.2 Materials and Methods

3.2.1 Materials

Whole wheat flour (13.5% moisture content, 13.85% protein) was kindly supplied by Mennel Milling Company (Fostoria, OH). Fungal α-amylase (Fungamyl 4000 SG; 4408 FAU-F/g), cellulase (Celluclast BG; 3705 EGU/g), glucose oxidase (Gluzyme Mono 10000 BG; 30,000 GODU/g), maltogenic α-amylase (Novamyl 10000 BG; 11,084 MANU/g), and fungal endoxylanase (Pentopan Mono BG; 2829 FXU-W/g) were kindly supplied by Novozymes North America (Franklinton, NC). Vital wheat gluten (Whetpro® 80, 80% protein) was obtained from ADM (Decatur, Illinois). Food grade calcium propionate was obtained from Niacet Corporation (Niagara Falls, New York). Instant yeast, sucrose, sodium chloride, and shortening were obtained from a local supermarket.

3.2.2 Dough preparation and properties

Each of the five enzymes was evaluated in whole wheat dough at three levels: low, medium, and high. The “low” level corresponded to the lower recommended dose provided by the manufacturer, the “medium” level was the upper recommended dose provided by the manufacturer, and the “high” level was 50% greater than the upper recommended dose. The amounts of each enzyme corresponding to the low, medium, and high levels were as follows: α-amylase (1.2, 12.5, and 18.75 ppm), cellulase (70, 130, and 195 ppm), glucose oxidase (2.5, 15, and 22.5 ppm), maltogenic α-amylase (10, 100, and 150 ppm), and xylanase (20, 50, and 75 ppm). Vital wheat gluten was added as an additional treatment at 2.5% (fwb). A control dough without enzymes or vital wheat gluten was also prepared for all analyses.

3.2.2.1 Mixograph analyses

Dough mixing properties were determined by a 10 g mixograph (National Manufacturing, Lincoln, NE) and MixSmart software according to AACCI Method 54-40.02. Flour (10g, 14% moisture basis), water, and enzyme or gluten, when tested, were mixed in a 10 g mixograph bowl at 22 °C.

3.2.2.2 Farinograph analyses

Farinograph dough properties were measured with a 50 g doughLAB (Perten Instruments North America, Springfield, IL), following AACCI Method 54.70.01 for High-Speed Mixing Rheology of Wheat Flour. The following parameters were determined: water absorption (percentage (fwb) of water required to reach a dough consistency of 500 Farinograph Units (FU)), dough development time (DDT; time for dough to reach peak consistency), stability (time for the top curve to reach peak resistance and to fall below peak resistance), and mixing tolerance index (MTI; the difference in FU from the top of the curve at peak mixing time to the top of the curve five minutes after the peak mixing time).

3.2.3 Bread making

Bread was baked following the AACC method for straight-dough bread-making (AACC International 10-10.03) without any of the optional ingredients but with the addition of 0.3 g calcium propionate. Two grams of instant yeast (Bellarise Red) was used instead of active dry yeast, and water was added as determined by mixograph analysis. Enzymes or gluten were added to the formulation according to the experimental design. Weight was measured and volume was determined by rapeseed displacement (AACC International 10-05.01) immediately after baking. Upon cooling, bread was transferred to polyethylene bags. The following day, bread was sliced into 15 mm thick slices for further analysis. Three replicates of each treatment were prepared

over three separate days of baking.

3.2.3.1 Evaluation of crumb structure

The central slice of each loaf was photographed using a C-Cell Bread Imaging System (Calibre Control International Ltd., Appleton, Warrington, UK). Each image was analyzed by the provided software to quantify the number of cells, cell wall thickness, and cell diameter.

3.2.3.2 Texture properties

Crumb texture was analyzed by texture profile analysis (TPA) using a TA-XT Plus Texture Analyzer (Stable Micro Systems, Godalming, Surrey, UK) equipped with a 30 kg load cell. Single slices of bread (15 mm) were compressed twice at 1.00 mm/s to 50% strain with a 0.049 N trigger force and 1 s pause between compressions. Parameters recorded were hardness, resilience, cohesion, springiness, and chewiness. Two of the central slices from each loaf were analyzed. TPA was performed after storage for 1, 3, and 7 d under ambient conditions (22 °C) to determine the effect of enzymes on the textural changes in whole wheat bread. To evaluate the effect of enzymes on firming, linear regression was used to determine the slope of the increase in crumb hardness during storage.

3.2.3.3 Moisture content

Following TPA, a sample of the crumb (~1 g) from each slice was dried at 105 °C for 3 h in a convection oven. Samples were allowed to cool for 45 min in a desiccator before weighing. Moisture content was determined for bread after storage for 1, 3, and 7 d.

3.2.3.4 Retrogradation

Thermal phase transitions of bread stored for 7 d were conducted using a Q200 differential scanning calorimeter (DSC) (TA Instruments, New Castle, DE). The instrument was calibrated with indium as a standard. Approximately 20 mg of bread crumb was weighed into a

stainless steel pan and hermetically sealed. Samples were heated from 0-150 °C at 10 °C/min under nitrogen atmosphere with a gas flow rate of 50 mL/min. The onset temperature (T_o), peak temperature (T_p), and melting enthalpy (ΔH, joules/g) were determined with TA Universal Analysis software for the endothermic peaks around 60 °C and 115-120 °C. These two peaks corresponded to the melting of retrograded amylopectin and the amylose-lipid complex, respectively. Two replicates were performed per treatment.

3.2.4 Statistical analysis

Treatment means were compared to the control using Dunnett's test in SAS Studio 3.7 (SAS Institute Inc., Cary, NC). Significance was defined at $p < 0.05$.

3.3 Results and Discussion

3.3.1 Dough Properties

3.3.1.1 Mixograph analyses

3.4 Water absorption remained constant for all enzymes tested (Tables

Table 3.1). The midline peak value and peak width were similar to the control for all enzyme treatments, indicating that dough strength during the test was not substantially altered. This is expected given that the optimal temperature for the enzymes used is higher than room temperature, and the mixograph peak occurs after only four to five minutes of mixing, resulting in little enzyme activity during this test. Addition of 2.5% gluten to the dough increased water absorption from 70% to 75% (fwb). Gluten protein absorbs approximately twice its weight in water (Day et al., 2006). Dough strength was increased by gluten addition as indicated by the increase in peak value and peak width.

3.4.1.1 Farinograph analyses

3.5 Farinograph tests showed some variation in water absorption between the enzyme treatments, whereas mixograph water absorbance remained constant (Tables

Table 3.1). The different results could be due to the different nature of the two instruments and their capacities. The farinograph operates at 30 °C, whereas the mixograph operates at room temperatures, or about 22 °C. The higher temperature of the farinograph would produce more enzyme activity compared to the enzyme activity during the mixograph test. The farinograph used a 50 g bowl, and the software calculated the precise water absorption down to the tenth of a percent, whereas the mixograph used a 10 g bowl and the water absorption was based on operator judgment of the resulting graph. The two instruments also have very different mixing actions. The farinograph showed that conventional α-amylase decreased the water absorption compared to control, in accordance with work by Sanz Penella and colleagues (2008) for dough with added bran. The hydrolysis of damaged starch reduces its water holding capacity, and can increase the viscosity of the dough. The conventional α-amylase also decreased DDT and somewhat decreased stability and increasing MTI, suggesting a greater likelihood of over mixing. Maltogenic α-amylase and cellulase showed similar trends for decreasing stability and increasing MTI. Conversely, glucose oxidase increased stability and decreased MTI, suggesting a strengthening effect on the dough and making it less prone to over mixing. An increase in dough stiffness from glucose oxidase in whole wheat dough has been reported (Yang et al., 2014). Xylanase showed a slight tendency to increase absorption, which is contrary to some published studies (Driss et al., 2013; Ghoshal et al., 2013; Shah et al., 2006). The hydrolysis of arabinoxylan reduces its water-holding capacity and releases free water, which decreases the

amount of water that must be added to form a properly hydrated dough (Gruppen et al., 1993; Shah et al., 2006).

3.5.1 Bread Properties

3.5.1.1 Loaf volume

Addition of 2.5% vital wheat gluten produced the greatest increase in loaf volume. All enzymes tested significantly increased the loaf volume for at least one of the levels tested, but not to the same extent as gluten (Table 3.2, Figure 3.1, Figure 3.2). In the case of maltogenic α-amylase and xylanase, all three levels significantly increased volume compared to control. Maltogenic α-amylase produces primarily α-maltose through the hydrolysis of α-(1–4) glycosidic bonds within the starch polymer; it is believed act as an endo-enzyme, but also has exo-action especially at higher temperatures (Goesaert & Slade et al., 2009). In white pan bread, the effect of maltogenic α-amylase on loaf volume has been inconsistent (Goesaert & Leman et al., 2009; Gomes-Ruffi et al., 2012; Purhagen et al., 2011). Published studies on maltogenic α-amylase in whole wheat or bran-supplemented bread have not reported the effect on loaf volume (Bollaín et al., 2005; Giménez et al., 2007). Our studies showed an increase in volume of 7.6-10.6% due to maltogenic α-amylase, depending on the dose. Yeast will preferentially ferment glucose followed by fructose, which are the two products of sucrose hydrolysis. Once the concentration of glucose and fructose is diminished, yeast activates its maltase and maltose permease enzymes, allowing the organism to hydrolyze and ferment maltose (Sluimer, 2005). The bread formula used here contained 6% sucrose (fwb), so it seems unlikely that the yeast would have needed additional sources of fermentable sugars. Further, the maltogenic α-amylase utilized for this study has an optimal temperature range of 140-160 °F (60-70 °C), so the enzyme would have limited activity during fermentation and proofing, and therefore assert minimal influence on yeast activity during

these stages. Instead, the increase in loaf volume due to α-amylase may be related to a decrease in dough viscosity during starch gelatinization, hence prolonging oven rise (Goesaert & Slade et al., 2009). This reasoning could explain the increased loaf volume observed for both conventional α-amylase, which generates low molecular weight α-dextrins and oligosaccharides of varying length, and for maltogenic α-amylase. The dextrins and oligosaccharides produced from the hydrolysis by conventional α-amylase are further hydrolyzed into maltose by endogenous β-amylase.

The present study demonstrated an improvement to loaf volume upon cellulase addition, contrary to previously reported findings for whole wheat bread (Altinel & Ünal, 2017a). However, cellulase and xylanase have been shown to increase the volume of white pan bread (Haros et al., 2002). The present study also demonstrated increased loaf volume due to xylanase, consistent with several other reports for whole wheat bread (Altinel & Ünal, 2017; Carvalho et al., 2017; Ghoshal et al., 2013; Jaekel et al., 2012; Shah et al., 2006). Cellulase and xylanase are both types of hemicellulase, a group of enzymes that hydrolyze nonstarch polysaccharides (Sluimer, 2005). The result is a reduction in dough elasticity, which improves processing tolerance but also creates a slacker and possibly stickier dough; loaf volume is improved due to increased gas retention (Sluimer, 2005). Cellulase acts on cellulose, a polymer of β-1,4-linked glucose units, whereas xylanase acts on the backbone of arabinoxylan, xylan, which is polymer of β-1,4-linked xylose units. Both cellulose and xylose are found in cell walls and are present in higher amounts in whole wheat flour compared to refined flour due to the inclusion of bran and germ. Several mechanisms behind the ability of xylanase to increase in loaf volume have been suggested. Hydrolysis of arabinoxylan causes a redistribution of water to gluten, allowing for greater hydration of gluten and a subsequent increase in the gluten volume. The increase in the

gluten fraction volume imparts extensibility and allows for greater oven spring (Maat et al., 1992). Improved gas retention capacity has been reported for whole wheat-supplemented dough with xylanase addition, leading to an increase in loaf volume (Matsushita et al., 2017). Matsushita and colleagues (2017) suggested that when xylanase hydrolyzes nonstarch polysaccharides, the resulting short chain saccharides are less able to interfere with gluten network formation. The conversion of water-unextractable arabinoxylan into water-extractable arabinoxylan could also improve the gas retention capacity of whole wheat dough and lead to the increase in loaf volume (Altınel & Ünal, 2017b).

Glucose oxidase improved loaf volume in the present study at the lowest dose tested. This enzyme oxidizes glucose into gluconic acid and hydrogen peroxide. Hydrogen peroxide activates endogenous peroxidase, promoting the oxidative gelation of water-soluble arabinoxylans via ferulic acid oxidation (Garcia et al., 2004, Joye et al., 2009). Additionally, hydrogen peroxide indirectly promotes covalent crosslinking between gluten molecules (Garcia et al., 2004, Joye et al., 2009). It can lead to a stiffer dough (Yang et al., 2014), so care must be taken not to overdose this enzyme, as was demonstrated here by a trend of decreasing volume as the level of glucose oxidase was increased. The production of hydrogen peroxide leads to a drying effect on dough, which increases the water absorption of the flour (Miller et al., 2008). The dough samples with the medium and high levels of glucose oxidase were likely insufficiently hydrated, since the water absorption was kept constant for all three levels of glucose oxidase tested. Increasing the water along with the increase in enzyme dosage may have led to significant volume increases, rather than decreases.

3.5.1.2 Crumb structure

Enzymes did not affect the crumb structure of whole wheat bread, based on C-cell measurements for number of cells, cell wall thickness, and cell diameter (Table 3.3, Figure 3.4). The one exception was the highest dose of maltogenic α-amylase, which produced a small but significant increase in cell wall thickness and cell diameter, suggesting a somewhat coarser crumb structure compared to the control. In contrast, others have reported that alpha-amylase but not maltogenic α-amylase produced a coarser crumb structure in white pan bread (Goesaert & Leman et al., 2009).

3.5.1.3 Textural properties of bread

Supplementation with gluten and the high levels of cellulase, maltogenic α-amylase, and xylanase decreased crumb hardness and chewiness as measured on the first day after baking (Table 3.4). The highest level of glucose oxidase increased crumb hardness, which can be attributed to the decrease in loaf volume. The enzymes produced little effect on the other Day 1 TPA parameters, except for maltogenic α-amylase, which decreased crumb resilience, cohesion, springiness, and chewiness. Although gluten produced the highest loaf volume, it did not result in the lowest hardness value at Day 1. That value was reduced to the control loaf, however, in accordance with other work on vital wheat gluten in whole wheat bread (Boz & Karaoglu, 2013). Loaf volume is a major contributor to hardness, but the nature of the crumb material is also involved (Armero & Collar, 1996a). The lowest hardness value at Day 1 was obtained with the highest dose of xylanase produced, with a hardness of 2.50 N compared to 3.18 N for the control bread. Several other researchers have reported significant reductions in crumb hardness for whole wheat bread supplemented with xylanase (Driss et al., 2013; Ghoshal et al., 2013; Jaekel et al., 2012; Shah et al., 2006). The textural change is most often attributed to the increase in volume. A reduction in starch crystallization and crystal growth, based on Avrami analysis, has

also been suggested (Ghoshal et al., 2013). In the case of cellulase, Haros et al. (2002) suggested that starch retrogradation does not explain the reduction in initial hardness and crumb firming.

Conventional α-amylase showed a trend for decreasing hardness over the three days on which TPA was measured, but the difference from control hardness was significant only on Day 3 and for the medium level. Conventional α-amylase is commonly used to improve loaf volume, and a decrease in crumb hardness for whole wheat bread has been reported (Armero & Collar, 1996a; Matsushita et al., 2017), and it has been shown to decrease hardness and firming in white bread (Armero & Collar, 1996a; Goesaert & Slade et al., 2009; Hug-Iten et al., 2003). Conventional α-amylase is an endo-enzyme that acts on damaged starch and gelatinized starch. This action reduces the molecular weight of the polymers and weakens the starch networks present in the final loaf, which can contribute to a decrease in crumb firmness (Goesaert & Slade et al., 2009). Additionally, dextrins of intermediate size inhibit crumb firming by interfering with crosslinking between remnants of starch granules and protein fibrils (Martin & Hoseney, 1991).

Although staling involves changes in several quality parameters including moisture migration and loss, loss of aroma, and textural changes (Hug-Iten et al., 2003), perhaps the most important characteristic of staling is an increase in crumb hardness over time, which is also referred to as firming. Table 3.4 displays the rate of firming as defined by the slope of the increase in hardness during storage. The plot of this firming data is shown in Figure 3.4. A pronounced decrease in firming rate was obtained for gluten and maltogenic α-amylase at the medium and high levels, and xylanase exhibited a slight decreasing effect. Maltogenic α-amylase is generally used in bread formulations for its anti-staling effect, which is mostly accomplished by the hydrolysis of amylopectin side chains, thus preventing retrogradation (Goesaert & Slade et al., 2009). Amylopectin retrogradation may result in crumb firming due to the immobilization

of water within the crystal structure. That water is consequently unavailable to plasticize the gluten network (Goesaert & Slade et al., 2009). By limiting the formation of amylopectin crystallites, maltogenic α-amylase allows more water to remain available as a plasticizer, thus leading to a decrease in firming. The anti-firming effect of maltogenic α-amylase may also be attributed to modifications of the amylose fraction (Hug-Iten et al., 2003). Overall, the exact mechanisms of bread staling and their impact on crumb firming remain unclear (Fadda et al., 2014).

3.5.1.4 Moisture content

Addition of enzymes did not alter the moisture content of whole wheat bread compared to the control (Table 3.2). The same water absorption was used for the enzyme treatments and the control. Therefore, enzymes did not improve moisture retention. Loaves supplemented with vital wheat gluten did have higher moisture contents than the control, but the dough was prepared with 75% water absorption, compared to 70% for all other doughs.

3.5.1.5 Starch retrogradation

Based on DSC analysis (Table 3.5, Figure 3.5), the highest level of conventional α-amylase and all levels of maltogenic α-amylase decreased amylopectin retrogradation, with a greater decrease observed for maltogenic α-amylase, which is in accordance with published studies (Goesaert & Leman et al., 2009b; Hug-Iten et al., 2003). The highest level of xylanase and the medium level of cellulase also decreased the amount of amylopectin retrogradation, but to a lesser extent than the amylases. Xylanase and cellulase have been shown to slow crumb firming of white bread and decrease amylopectin retrogradation in flour-water samples, but the exact mechanism of these actions was not elucidated (Haros et al., 2002). Xylanase hydrolyzes arabinoxylans, so it is unclear how that action reduces amylopectin retrogradation. Overall, the

peak temperature for the melting of retrograded amylopectin was not altered by enzymes. The highest dose of maltogenic α-amylase decreased the melting temperature compared to control, but was still within the range of 50-70 °C given by the literature (Hug-Iten et al., 2003). The peak itself was almost nonexistent, demonstrating that maltogenic α-amylase effectively hindered the retrogradation of amylopectin. Maltogenic α-amylase degrades starch polymers predominantly by releasing maltose, and it does so mainly on the side chains of amylopectin (Goesaert & Slade et al., 2009). The shortening of side chains inhibits amylopectin retrogradation, shown here by the decrease in melting enthalpy of peak 1 on the DSC thermogram. Conventional α-amylase acts on the internal bonds of starch molecules and reduces their molecular weight. Conventional α-amylase has less of an effect on the amylopectin side chains, which are the sections involved in retrogradation (Goesaert & Slade et al., 2009). Thus, conventional α-amylase is not as effective as maltogenic α-amylase at decreasing retrogradation.

A decrease in amylopectin retrogradation, indicated by peak 1 melting enthalpy (Table 3.5), did not always correspond with lower crumb hardness on Day 7 or a decrease in crumb firming rate (Table 3.4 and Table 3.5). For example, treatment with 2.5% vital wheat gluten decreased Day 7 hardness and the rate of crumb firming, but did not produce a significant change in peak 1 melting enthalpy. Conversely, the high level of α-amylase and the medium level of cellulase decreased peak 1 melting enthalpy, but did not significantly affect Day 7 crumb hardness or the rate of crumb firming. These findings support the understanding that crumb firming is not synonymous with amylopectin retrogradation (Fadda et al., 2014).

The melting enthalpy for the amylose-lipid complex, which occurs at the second endothermic peak of the DSC thermogram, was only altered by the medium and high levels of maltogenic α-amylase. Extensive hydrolysis of amylose into maltose units could explain the

decrease in the ability of amylose to complex with endogenous flour lipids. In contrast to the present work, Hug-Iten et al. reported that conventional but not maltogenic α-amylase decreased slightly the melting enthalpy of the amylose-lipid complex (2003). It has been reported that the amylose-lipid complexes do not change as the bread stales (Davidou et al., 1996; Hug-Iten et al., 2003).

3.6 Conclusions

All enzymes tested, which included conventional α-amylase, cellulase, glucose oxidase, maltogenic α-amylase, and xylanase showed promise at improving the quality of whole wheat bread by increasing the loaf volume for at least one of the three levels tested. The greatest improvement in loaf volume due to enzymes was 13%, which was obtained with the highest dose of xylanase. This enzyme also showed a trend of decreasing crumb hardness and slowing the rate of crumb firming. In terms of anti-staling, maltogenic α-amylase was the most effective of the treatments, decreasing the hardness on Days 3 and 7, slowing the rate of crumb firming, and decreasing amylopectin retrogradation. Somewhat surprisingly, maltogenic α-amylase also significantly increased loaf volume. The enzymes had minimal effect on dough mixing properties and are considered clean label alternatives to other types of bread improvers. Although none of the enzymes tested singly improved loaf volume of whole wheat bread to the same extent as 2.5% vital wheat gluten, future studies could examine the combination of different types of enzymes, such as xylanase and amylases, in order to further increase loaf volume while also decreasing staling.

3.7 References

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3.8 Tables

Table 3.1 Rheological properties for whole wheat dough with added vital wheat gluten or enzymes

Treatment	Mixograph Water Absorption (%, fb)	Mixograph Peak Time (min)	Midline Peak Value (%)	Midline peak width (%)	Farinograph Water Absorption[a]	Dough development time (min)	Stability[b] (min)	MTI[c] (FU)
Control	70	4.05	38.2	22.3	75.0	5.7	7.9	38.2
Gluten 2.5%	75	4.39	44.0	25.6	79.9	6.2	8.6	36.7
α-amyl low	70	3.94	37.3	21.1	74.3	4.8	7.3	37.7
α-amyl med	70	4.34	35.5	21.3	72.3	4.9	7.5	39.8
α-amyl high	70	4.72	36.3	21.6	72.2	4.6	6.8	45.9
cel low	70	4.35	38.2	24.2	75.0	5.5	6.9	49.0
cel med	70	4.31	38.4	27.9	75.1	4.8	7.2	36.7
cel high	70	4.31	38.0	24.8	75.2	5.8	6.8	49.5
GOX low	70	4.26	38.8	24.0	75.5	5.6	7.3	42.3
GOX med	70	4.40	38.3	24.0	75.4	6.6	8.5	39.3
GOX high	70	4.40	38.4	24.7	75.2	6.3	10	33.7
m amyl low	70	4.20	38.4	24.6	74.2	5.8	7.6	39.8
m amyl med	70	3.85	38.8	27.6	74.1	5.9	7.7	40.8
m amyl high	70	4.34	38.3	22.9	74.1	4.6	6.7	43.4
xyl low	70	4.35	38.6	24.2	75.5	5.4	7.7	36.7
xyl med	70	4.40	38.0	25.1	75.6	6.3	7.6	42.3
xyl high	70	4.09	39.3	23.3	75.8	5.4	7.2	38.2

[a]Water absorption corrected for target peak resistance and actual flour moisture content

[b]Stability: Difference between arrival and departure times (time for top curve to reach peak resistance and to fall below peak resistance)

[c]MTI: The difference in Farinograph Units (FU) from the top of the curve at peak mixing time to the top of the curve five minutes after the peak mixing time.

Table 3.2 Weight, volume, specific volume, and moisture content for whole wheat bread with added vital wheat gluten or enzymes

Treatment	Proof Ht (cm)	Wt (g)	Vol (cm^3)	Specific Vol (cm^3/g)	Day 1 Moisture Content (% wb)	Day 3 Moisture Content (% wb)	Day 7 Moisture Content (% wb)
Control	7.3	152.98	660±5	4.31	45.01	44.58	40.98
Gluten 2.5%	8.4***	156.45***	787±18***	5.03***	46.30***	46.11***	42.93***
α-amyl low	7.2	152.96	665±10	4.35	45.07	44.58	40.92
α-amyl med	7.5	151.70	705±10***	4.65***	45.07	44.58	40.56
α-amyl high	7.1	152.13	698±20**	4.59**	44.97	44.48	40.31
cel low	7.3	152.11	683±10	4.49	45.25	44.78	40.81
cel med	7.6	150.81	705±13***	4.67***	45.10	44.65	40.41
cel high	7.5	151.29	723±3***	4.78***	45.12	44.59	40.42
GOX low	7.7	151.39	697±13**	4.60**	45.26	44.67	40.71
GOX med	7.8	152.72	667±6	4.37	45.33	44.92	41.64
GOX high	7.6	153.82	648±13	4.22	45.18	44.96	41.81
m amyl low	7.8	150.65*	713±13***	4.74***	45.29	44.90	40.85
m amyl med	8.0*	151.58	710±17***	4.69***	45.20	44.86	40.90
m amyl high	7.9*	150.30*	730±5***	4.86***	45.28	44.93	40.80
xyl low	7.9	151.25	720±0***	4.76***	45.19	44.73	40.91
xyl med	8.0**	149.90**	742±8***	4.95***	45.20	44.72	40.55
xyl high	8.1**	150.67*	748±13***	4.97***	45.21	44.82	40.81

All means were compared to control. Level of significance indicated by * = 0.05 to 0.01, ** = 0.01 to 0.001, and *** = 0.001 and lower.
Loaves were prepared in triplicate. Moisture content is average of six replicates.

Table 3.3 Crumb structure analysis for whole wheat bread with added vital wheat gluten or enzymes

Treatment	Number of Cells	Cell Wall Thickness (mm)	Cell Diameter (mm)
Control	3412±102	0.420±0.002	1.908±0.045
Gluten 2.5%	3496±76	0.427±0.006	2.061±0.071
α-amyl low	3417±161	0.420±0.007	1.918±0.102
α-amyl med	3356±152	0.425±0.005	1.996±0.045
α-amyl high	3410±166	0.423±0.012	1.978±0.082
cel low	3475±33	0.420±0.004	1.896±0.027
cel med	3397±101	0.423±0.001	1.966±0.083
cel high	3422±104	0.426±0.002	2.003±0.075
GOX low	3311±4	0.425±0.001	1.960±0.019
GOX med	3427±219	0.421±0.011	1.952±0.108
GOX high	3378±57	0.416±0.005	1.848±0.056
m amyl low	3356±79	0.429±0.003	2.004±0.044
m amyl med	3352±20	0.431±0.006	2.053±0.054
m amyl high	3246±58	0.435±0.003*	2.138±0.088**
xyl low	3406±97	0.425±0.003	2.017±0.044
xyl med	3449±94	0.428±0.005	2.068±0.088
xyl high	3437±63	0.427±0.006	2.066±0.108

All means were compared to control. Level of significance indicated by * = 0.05 to 0.01, ** = 0.01 to 0.001, and *** = 0.001 and lower. Means are the average of three replicates.

Table 3.4 Texture profile analysis of whole wheat bread with added vital wheat gluten or enzymes after 1d storage at 22 °C, and change in hardness after 3 and 7d storage

Treatment	Hardness, N	Resilience, %	Cohesion	Springiness, %	Chewiness, N	Day 3 Hardness, N	Day 7 Hardness, N	Slope: rate of firming	R^2
Control	3.18	34.73	0.714	94.70	2.152	4.60	7.10	0.649	0.906
Gluten 2.5%	2.65*	36.73	0.727	96.60*	1.864*	3.71***	5.56*	0.481	0.973
α-amyl low	3.19	34.82	0.713	94.82	2.153	4.30	7.06	0.653	0.950
α-amyl med	2.82	33.37	0.706	94.51	1.884	3.95*	6.61	0.636	0.930
α-amyl high	2.93	32.52	0.703	94.08	1.930	4.16	6.72	0.634	0.872
cel low	3.06	34.79	0.711	95.39	2.076	4.24	7.30	0.715	0.879
cel med	2.91	35.15	0.711	95.59	1.981	4.17	7.01	0.686	0.922
cel high	2.66*	35.32	0.723	95.68	1.840*	3.81**	6.65	0.671	0.925
GOX low	3.10	34.86	0.710	95.86	2.109	4.45	7.18	0.682	0.924
GOX med	3.20	35.75	0.734	95.63	2.227	4.66	7.64	0.740	0.860
GOX high	3.73**	32.50	0.688	95.15	2.440*	5.35**	8.64	0.819	0.940
m amyl low	3.00	34.63	0.708	95.21	2.020	3.76**	6.64	0.622	0.820
m amyl med	2.90	31.83**	0.690	93.90	1.880*	3.28***	4.71***	0.309	0.544
m amyl high	2.72*	29.20***	0.668**	92.14***	1.676***	3.21***	4.31***	0.265	0.734
xyl low	2.84	34.48	0.709	94.79	1.910	3.92*	6.35	0.587	0.921
xyl med	2.70*	35.40	0.716	95.17	1.840*	3.78**	6.67	0.700	0.858
xyl high	2.50***	35.71	0.719	95.52	1.718***	3.56***	6.06	0.597	0.912

All means were compared to control. Level of significance indicated by * = 0.05 to 0.01, ** = 0.01 to 0.001, and *** = 0.001 and lower. Six replicates were analyzed per treatment.

Table 3.5 Retrogradation parameters for whole wheat bread with added vital wheat gluten or enzymes stored at 22°C

Treatment	Tm_1 onset (°C)	Tm_1 peak (°C)	ΔH_1 (J/g)	Tm_2 onset (°C)	Tm_2 peak (°C)	ΔH_2 (J/g)
Control	52.04	66.14	2.71	104.75	120.14	1.080
Gluten 2.5%	51.29	65.70	2.72	103.82	117.15*	0.781
α-amyl low	51.90	67.01	2.55	104.70	120.25	1.065
α-amyl med	51.83	67.59	2.36	105.21	120.47	1.155
α-amyl high	52.45	66.10	2.05***	104.75	118.34	0.822
cel low	52.40	66.36	2.47	105.08	119.89	1.007
cel med	52.42	67.83	2.11**	103.89	120.31	0.969
cel high	53.58	68.10	2.41	104.90	120.83	1.150
GOX low	52.34	66.55	2.73	105.26	119.84	0.893
GOX med	50.96	65.67	2.38	105.32	119.15	0.881
GOX high	51.01	65.86	2.58	104.91	118.86	0.921
m amyl low	51.55	66.49	2.08***	104.00	119.38	0.851
m amyl med	52.49	66.57	0.63***	103.33	116.80**	0.463**
m amyl high	52.63	55.47***	0.08***	105.85	118.06	0.337***
xyl low	51.37	66.82	2.62	105.07	120.27	1.117
xyl med	51.04	66.49	2.39	105.28	118.90	0.832
xyl high	51.96	66.22	2.28*	104.70	119.96	0.933

All means were compared to control. Level of significance indicated by * = 0.05 to 0.01, ** = 0.01 to 0.001, and *** = 0.001 and lower. Tests were performed in duplicate.

3.9 Figures

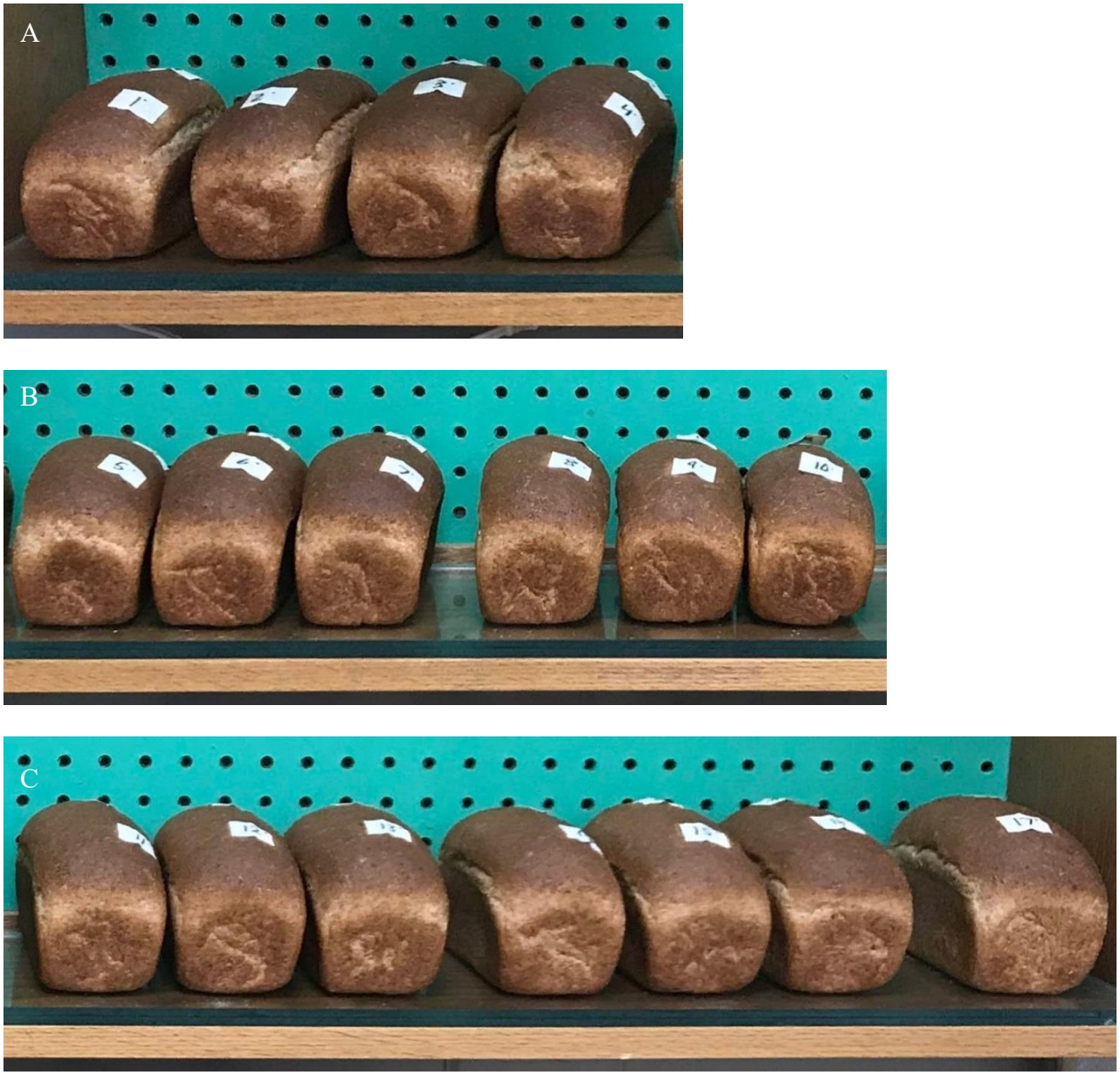


Figure 3.1 A-C Representative loaves of whole wheat bread with added gluten or enzymes.
A: Control; conventional α-amylase low, med, high; B: cellulase low, med, high; glucose oxidase low, med, high; C: maltogenic α-amylase low, med, high; xylanase low, med, high; 2.5% vital wheat gluten

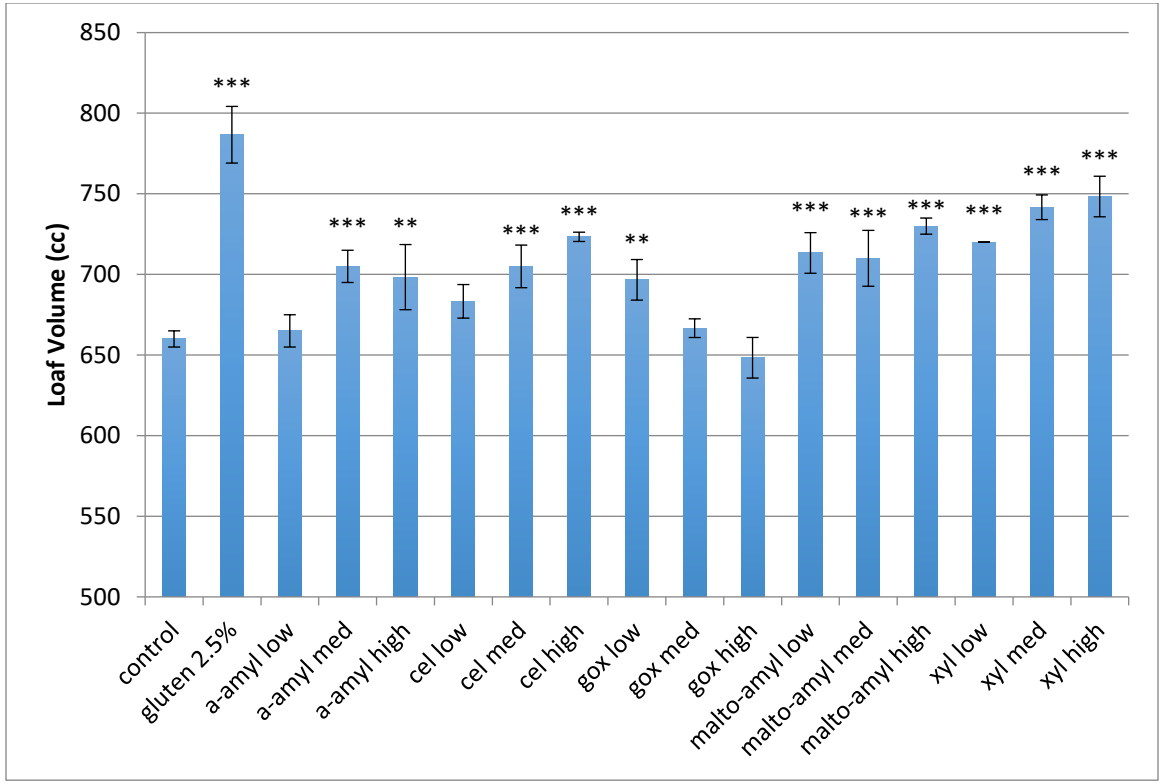
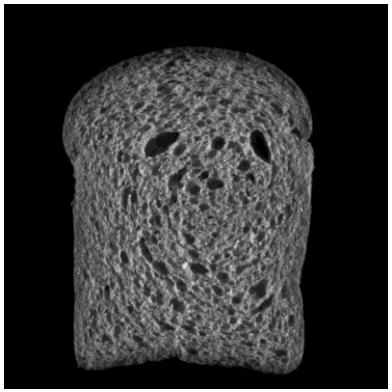
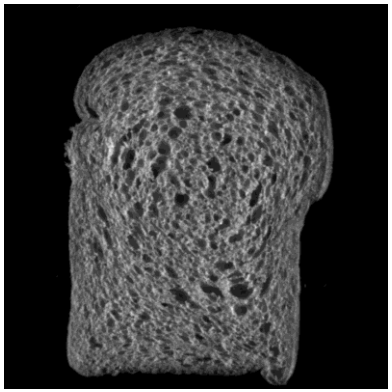
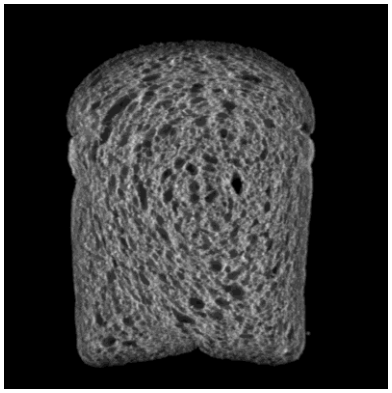
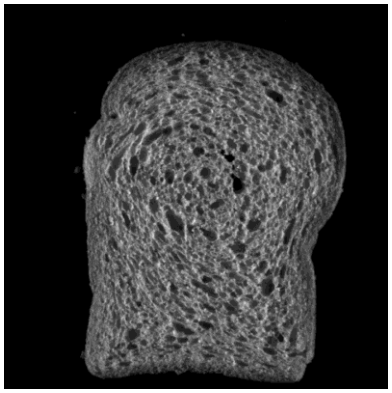


Figure 3.2 Loaf volume of whole wheat bread with added vital wheat gluten or enzymes
All means were compared to control. Level of significance indicated by * = 0.05 to 0.01, ** = 0.01 to 0.001, and *** = 0.001 and lower. Loaves were prepared in triplicate.


Control


Gluten


α-amyl low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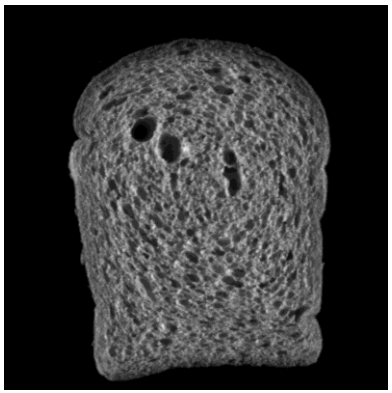
α-amyl high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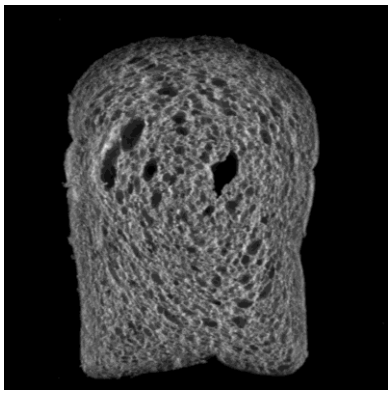
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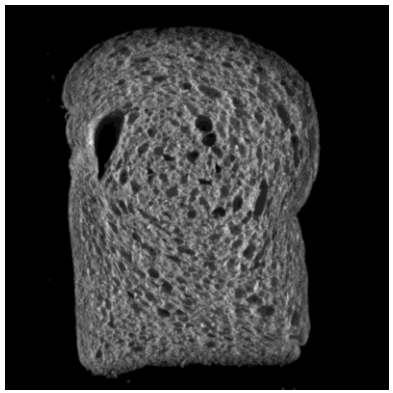
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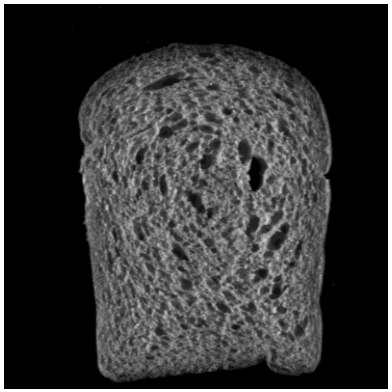
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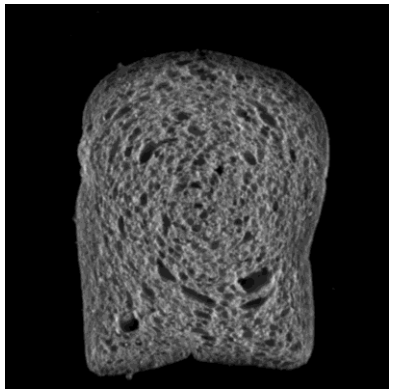
GOX med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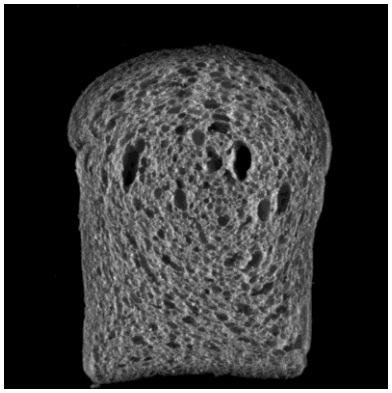
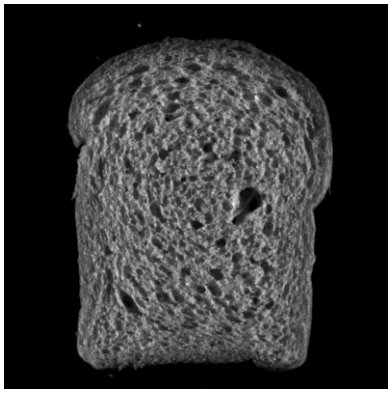

m amyl low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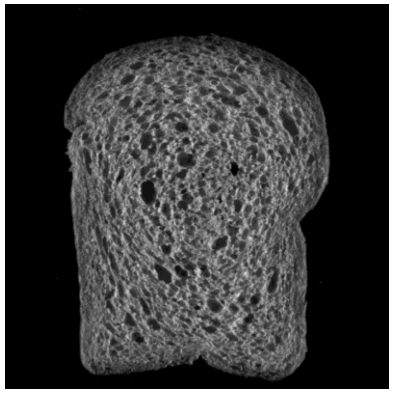
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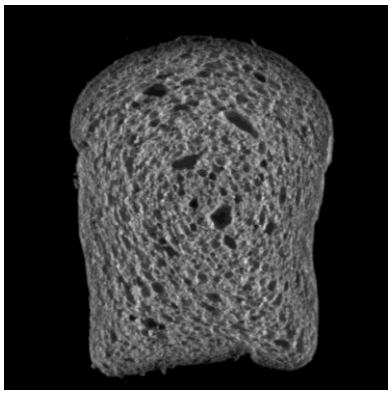
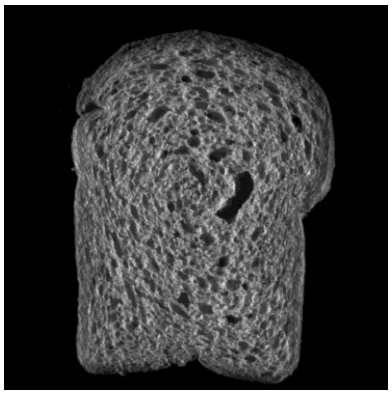

xyl low


xyl med

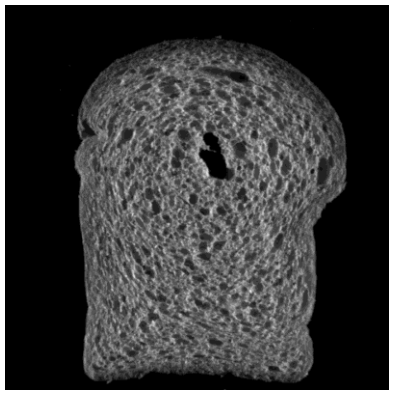

xyl high

Figure 3.3 Representative C-cell images of whole wheat bread with added gluten or enzymes

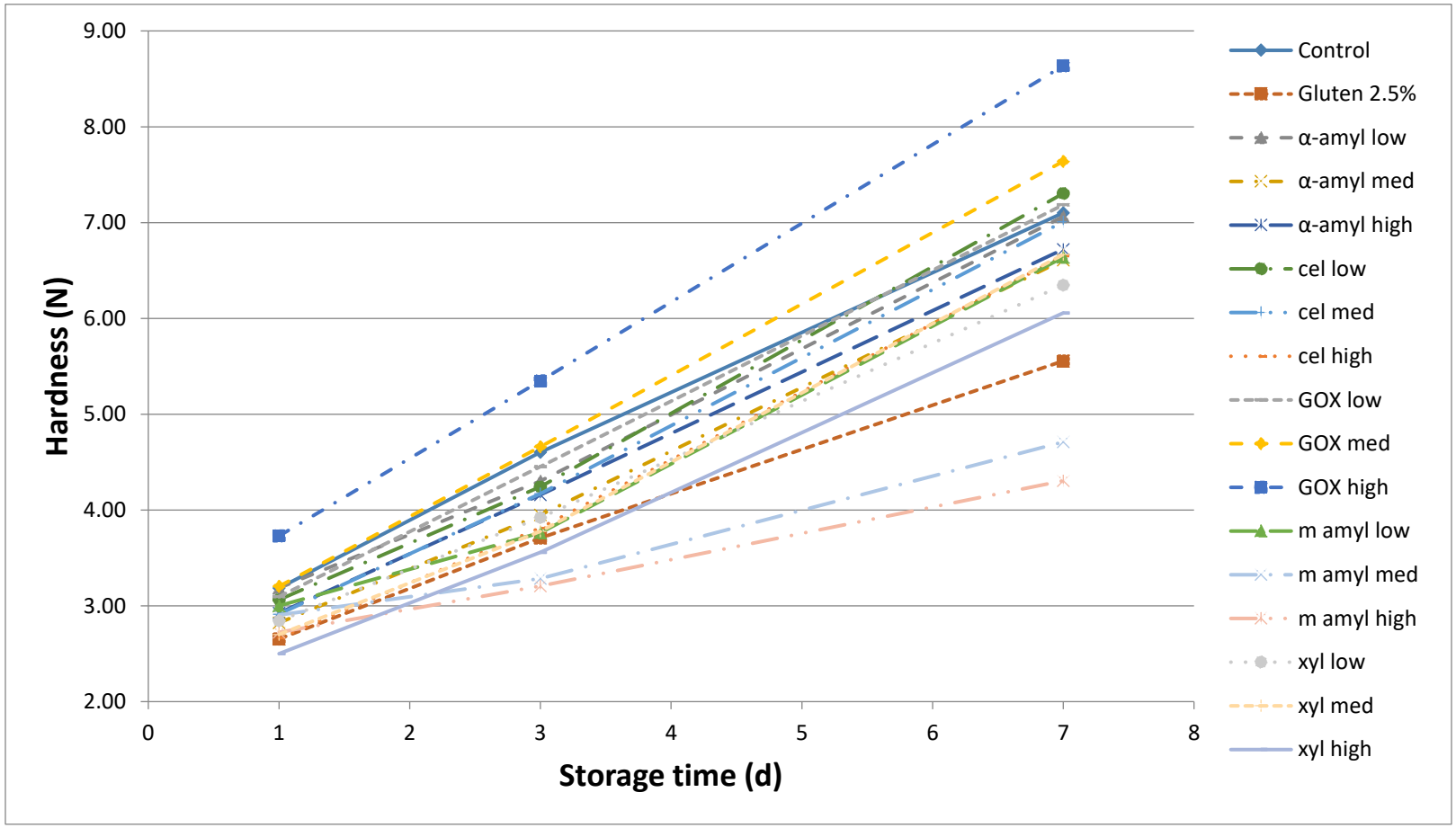


Figure 3.4 Increase in crumb hardness with time for whole wheat bread with added vital wheat gluten or enzymes

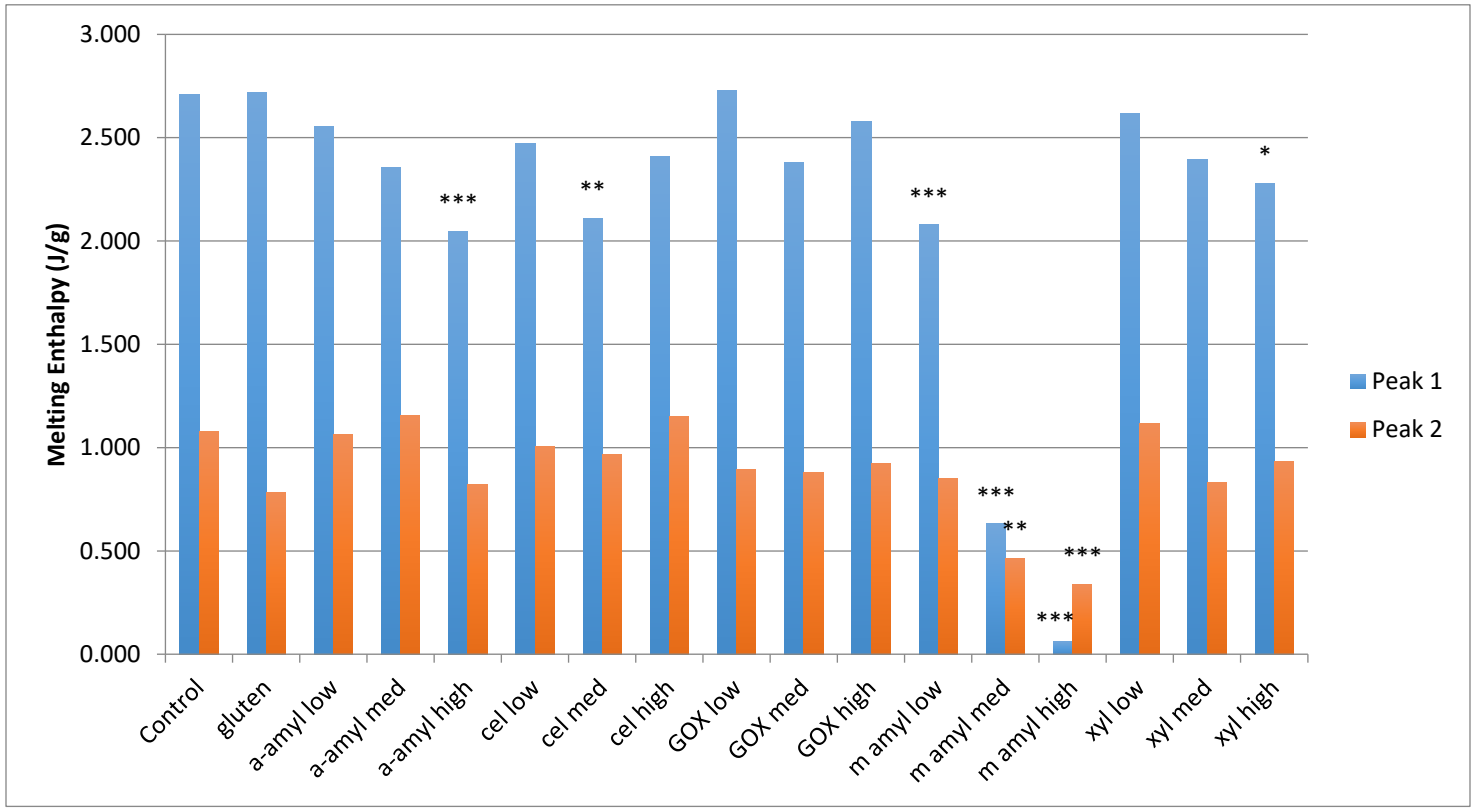


Figure 3.5 Melting enthalpies from DSC thermograms of bread stored for 7 days
Level of significance indicated by * = 0.05 to 0.01, ** = 0.01 to 0.001, and *** = 0.001 and lower. Tests were performed in duplicate.

4 Effects of hydrocolloids on whole wheat dough and bread properties

Abstract

Hydrocolloids can be used to improve dough handling and bread quality and retard staling. The strengthening effect of hydrocolloids is particularly beneficial to bread from whole wheat flour and other flours of low gluten quality. The objective of this research was to determine the effects of five hydrocolloids on whole wheat dough and bread properties, with a focus on loaf volume, bread texture, and staling. Bread was prepared from whole wheat flour following AACC method 10-10.03. Hydrocolloids (carboxymethyl cellulose (CMC), guar gum, hydroxypropyl methylcellulose (HPMC), sodium alginate, and xanthan gum) were added at 0.25, 0.5, and 1.0% fwb. Dough properties were determined by farinograph, mixograph, Chen-Hoseney stickiness test, and Kieffer rig uniaxial extensibility. Specific volume was measured for fresh bread, and moisture content, texture profile analysis (TPA), and crumb structure were analyzed the following day. Moisture content and TPA were measured again after 3 and 7 days of storage at 22 °C to determine changes associated with staling. Effect on starch retrogradation was quantified by differential scanning calorimetry (DSC) after the 7 days. Hydrocolloids increased water absorption and mixing time for whole wheat dough. All hydrocolloids except for CMC increased specific loaf volume for at least one of the usage levels tested ($P < 0.01$), with minimal change to crumb structure. HPMC (all levels) and the medium level of xanthan gum produced the greatest increase in specific loaf volume. The high level of guar gum and medium level of HPMC reduced crumb hardness on Day 1 ($P < 0.01$). On Day 7, only HPMC and xanthan gum at the medium level resulted in a crumb that was less hard than the control. HPMC, sodium alginate, and xanthan gum delayed staling as measured as the rate of hardness increase

during storage. No significant changes in amylopectin retrogradation or amylose-lipid complexation were observed, although xanthan gum showed a trend toward decreasing formation of the complex. HPMC is recommended as the most favorable hydrocolloid to increase loaf volume and delay staling of whole wheat bread.

Keywords: Bread; Whole wheat; Dough properties; Staling; Hydrocolloids; Carboxymethyl cellulose (CMC); Guar gum; Hydroxypropyl methylcellulose (HPMC); Alginate; Xanthan gum

4.1 Introduction

Hydrocolloids, or gums, are high molecular weight polymers that are hydrophilic and form gels or highly-viscous suspensions in water-based systems. Most are polysaccharides, but the group also includes proteins, namely gelatin (Saha & Bhattacharya, 2010). Hydroxyl groups allow these molecules to interact with and bind water. In foods, hydrocolloids are used to modify texture and viscosity, and can be broadly classified as thickeners or gel formers (Saha & Bhattacharya, 2010). Hydrocolloids come from several sources, including seeds, plant exudates or cell wall material, seaweed, cellulose derivatives, microbial fermentation products, and modified starches (Ferrero, 2017; Saha & Bhattacharya, 2010). Hydrocolloids are used extensively in gluten-free bakery products to provide a certain degree of strength, stability, and viscoelasticity in the absence of a gluten network (Anton & Artfield, 2008). In wheat-based bakery applications, hydrocolloids increase water absorption and modify dough properties, provide stability to frozen dough and par-baked bread, and, in the final product, increase loaf volume, improve crumb texture, increase moisture retention, and retard staling (Ferrero, 2017; Kohajdová & Karovičová, 2009). The specific results depend on the structure of the hydrocolloid. The effects of hydrocolloids in whole wheat dough and bread have been previously

reviewed (Tebben et al., 2018), but the literature lacks a comprehensive study of the unique effects of multiple hydrocolloids on both dough and bread properties.

Five hydrocolloids were chosen to represent a range of structures and sources: CMC (carboxymethyl cellulose) and HPMC (hydroxypropyl methylcellulose) (cellulose derivatives), guar gum (galacto-mannan, from guar beans), sodium alginate (seaweed extract), and xanthan gum (product of bacterial fermentation). The five hydrocolloids were evaluated individually at three levels. The objectives of this research were to determine the specific effects of each hydrocolloid on the physical properties of dough and bread made with whole wheat flour, with the aim of increasing loaf volume and decreasing staling.

4.2 Materials and Methods

4.2.1 Materials

Whole wheat flour (13.5% moisture content, 13.85% protein) was kindly supplied by Mennel Milling Company (Fostoria, OH). Food grade CMC, guar gum, HPMC (Methocel® F50 Hydroxypropyl Methylcellulose), and sodium alginate were purchased online. Xanthan gum from *Xanthomonas campestris* was obtained from Sigma-Aldrich (St. Louis, MO, USA). Food grade calcium propionate was obtained from Niacet Corporation (Niagara Falls, New York). Instant yeast, sucrose, sodium chloride, and shortening were obtained from a local supermarket.

4.2.2 Dough preparation and properties

Each of the five hydrocolloids were evaluated in whole wheat dough at three levels: 0.25, 0.5, and 1.0% (fwb), with the exception of sodium alginate, which was only evaluated at the low (0.25% fwb) and medium (0.5% fwb) levels. A suitable dough could not be formed with the high dose (1.0% fwb). A control dough without hydrocolloids was also prepared for all analyses.

4.2.2.1 Mixograph analyses

Dough mixing properties were determined by a mixograph (National Manufacturing, Lincoln, NE). Flour (10 g, 14.0% moisture basis), water, and hydrocolloid, when tested, were mixed in a 10 g mixograph bowl at 22 °C. The water absorption and mixing time as determined by midline peak time were used to prepare all samples for the remaining dough tests.

4.2.2.2 Farinograph analyses

Farinograph dough properties were measured with a 50 g doughLAB (Perten Instruments North America, Springfield, IL), using AACCI Method 54-70.01 and extending the length of the test to 12 min beyond the peak resistance or until the top of the curve fell below 500 FU, whichever was later. The following parameters were determined: water absorption (percentage (fwb) of water required to reach a dough consistency of 500 Farinograph Units (FU)), dough development time (DDT; time for dough to reach peak consistency), stability (time for the top curve to reach peak resistance and to fall below peak resistance), and mixing tolerance index (MTI; the difference in FU from the top of the curve at peak mixing time to the top of the curve five minutes after the peak mixing time).

4.2.2.3 Chen-Hoseney stickiness test

Dough stickiness was analyzed using a TA-XT Plus Texture Analyzer (Stable Micro Systems, Godalming, Surrey, UK) equipped with a 30 kg load cell. A SMS/Chen-Hoseney Dough Stickiness Rig and 25 mm perspex cylinder probe were used for the test as described by Huang and Hoseney (1999). Six replicates were performed for each dough, and each dough was prepared in duplicate. Parameters recorded were stickiness (in N), work of adhesion (in N.s), and cohesiveness (in mm).

4.2.2.4 Kieffer rig uniaxial extensibility

Uniaxial extensibility was measured using the Kieffer dough and gluten extensibility rig

on the TA-XT Plus Texture Analyzer. Approximately 10 g of prepared dough was pressed in the lubricated Teflon molder and allowed to rest at 22 °C for 30 min. A strip of dough was removed from the molder and clamped between the plates of the Kieffer rig before each test. A test speed of 3.3 mm/sec and trigger force of 0.049 N were used. Resistance to extension (R_{max}, in N) and extensibility (E_{Rmax}, in mm) were recorded as the peak force and the distance at the peak force, respectively. Nine strips per dough were tested, and each dough was prepared in duplicate.

4.2.3 Bread making

Bread was baked following the AACC method for straight-dough bread-making (AACC International 10-10.03). Modifications included the addition of 0.3 g calcium propionate and use of 2 g of instant yeast (Bellarise Red) instead of active dry yeast, and none of the optional ingredients were used. Water and mixing times were based off of mixograph analysis and optimized through preliminary baking trials. Hydrocolloids were added to the formulation according to the experimental design at 0.25, 0.5, and 1.0% (fwb), again with the exception of sodium alginate, which was only added at the low and medium levels. Volume was determined by rapeseed displacement (AACC International 10-05.01) and weight were measured immediately after baking. Upon cooling, bread was transferred to polyethylene bags. The following day, bread was sliced into 15 mm thick slices for further analysis. Three replicates of each treatment were prepared over three separate days of baking.

4.2.3.1 Evaluation of crumb structure

The central slice of each loaf was photographed using a C-Cell Bread Imaging System (Calibre Control International Ltd., Appleton, Warrington, UK). Each image was analyzed by the provided software to quantify the number of cells, cell wall thickness, and cell diameter.

4.2.3.2 Texture properties

Crumb texture was analyzed by texture profile analysis (TPA) using the TA-XT Plus Texture Analyzer. Single slices of bread (15 mm) were compressed twice at 1.00 mm/s to 50% strain with a 0.049 N trigger force and 1 s pause between compressions. Parameters recorded were hardness, resilience, cohesion, springiness, and chewiness. Two of the central slices from each loaf were analyzed. TPA was performed after storage for 1, 3, and 7 d under ambient conditions (22 °C) to determine the effect of hydrocolloids on changes to crumb texture.

4.2.3.3 Moisture content

Following TPA, a sample of the crumb (~1 g) from each slice was dried at 105 °C for 3 h in a convection oven. Samples were allowed to cool for 45 min in a desiccator before weighing. Moisture content was determined for bread after storage for 1, 3, and 7 d.

4.2.3.4 Retrogradation

Thermal phase transitions of bread stored for 7 d were conducted using a Q200 differential scanning calorimeter (DSC) (TA Instruments, New Castle, DE). The instrument was calibrated with indium as a standard. Approximately 20 mg of bread crumb was weighed into a stainless steel pan and hermetically sealed. Samples were heated from 0-150 °C at 10 °C/min under nitrogen atmosphere with a gas flow rate of 50 mL/min. The onset temperature (T_o), peak temperature (T_p), and melting enthalpy (ΔH, joules/g) were determined with TA Universal Analysis software for the endothermic peaks around 60 °C and 115-120 °C. These two peaks corresponded to the melting of retrograded amylopectin and the amylose-lipid complex, respectively. Two replicates were performed per treatment.

4.2.4 Statistical analysis

Treatment means were compared to the control using Dunnett's test in SAS Studio 3.7 (SAS Institute Inc., Cary, NC). Significance was defined at $p < 0.05$.

4.3 Results and Discussion

4.3.1 Dough Properties

4.3.1.1 Mixograph analyses

The mixograph properties for each treatment are shown in Table 4.1. All hydrocolloids increased the water absorption (WA) of the dough, as expected due to their hydrophilic nature and high hydration capacity (Ferrero, 2017). Xanthan gum produced the greatest increase in WA. With the exception of HPMC, higher amounts of hydrocolloids and consequently greater amounts of water increased the peak time. That is, the dough took a longer time to develop. Mixograph properties for dough prepared with hydrocolloids have generally not been widely reported in the literature, most likely due to the somewhat subjective nature of the test – the operator must determine the ideal WA based on his or her interpretation of the mixograph curve, which is one of the most challenging tasks in mixograph operation (Ohm & Chung, 1999). The WA is typically estimated based on protein and moisture content of the wheat (AACC International Method 54-40.02; Ohm and Chung, 1999), but adjustments for added ingredients are determined empiracally. In tests of only flour and water, mixograph WA significantly correlates with baking WA (Ohm & Chung, 1999). A comparison of mixograph and baking WA and mix times for the hydrocolloids treatments are presented in Table 4.1. Baking WA followed the same trend as mixograph WA, but the former was often increased to achieve a better dough and/or higher loaf volume.

The midline peak value and midline peak width are both indicators of dough strength, and have been correlated with loaf volume (Ohm & Chung, 1999). Increasing levels of CMC, alginate, and xanthan gum decreased the midline peak value, whereas guar gum increased midline peak value, and HPMC had little effect on this parameter. However, HPMC did increase

the midline peak width, suggesting a modest strengthening effect.

4.3.1.2 Farinograph analyses

Both the type and amount of hydrocolloid influenced the farinograph parameters (Table 4.1). Except for the low and medium levels of guar gum, all hydrocolloids increased the Farinograph WA. Linlaud and collegaues (2009) similarly found that xanthan gum produces a large increase in water absorption, whereas guar gum does not. Rosell and colleagues (2001) also demonstrated that HPMC, alginate, and xanthan gum increased the water absorption in white dough. They found that HPMC increased WA to the greatest extent, similar to the present work. The degree of increase in WA depends on the chemical structure of the hydrocolloid, particularly the number of hydroxyl groups, which interact with water through hydrogen bonds (Rosell et al., 2001).

In accordance with the aforementioned study (Rosell et al., 2001) xanthan gum increased DDT, which is the time for the dough to reach peak resistance, while HPMC did not affect this parameter. Guar gum also increased DDT in the whole wheat dough, as reported for white dough (Linlaud et al., 2009). CMC was shown here to increase DDT, and alginate resulted in only a modest increase, in contrast to the findings of Rosell et al. (2001). The specific type of alginate could explain the differences in our findings compared to published data. Alginates can be derived from several species of algae, which leads to variation among the chemical makeup of the product (Kohajdová & Karovičová, 2009).

CMC, HPMC, alginate, and xanthan gum all decreased stability and increased MTI (i.e., the drop in dough consistency five minutes after the peak resistance is reached). Alginate had the most pronounced effect in this regard. Therefore, all of these doughs exhibited a greater breakdown compared to the control dough. In contrast, alginate and xanthan both increased

stability and decreased MTI in white dough (Rosell et al., 2001). Another published study found that the hydrocolloids xanthan gum, locust bean gum, and high-methoxyl pectin increased the stability of white dough in the absence of salt, but that the hydrocolloids actually decrased dough stability when 2% NaCl was also added (Linlaud et al., 2009). The authors suggested a negative interaction effect between the salt and hydrocolloids. In a similar way, the hydrocolloids may have a negative interaction effect with ions or other components in the bran and germ, leading to the decreased stability of whole wheat dough.

For guar gum, the medium level increased stability and decreased MTI, indicating a stronger dough that is more tolerant to overmixing, similar to its effect in white dough (Linlaud, N. E. et al., 2009). These beneficial effects were removed at the high level of guar gum, possibly because the higher amount of gum required more water, creating a more viscous or weaker dough.

4.3.1.3 Dough stickiness

Dough handling properties, including textural attributes such as stickiness, are important to industrial bakery settings. Most of the hydrocolloids did not significantly alter the parameters measured by the Chen-Hoseney stickiness test, namely dough stickiness, work of adhesion, and cohesiveness (Table 4.2). Tests of xanthan gum and HPMC, at levels up to 0.112 and 0.348 g/100g flour, respectively, also did not find significant effects on dough stickiness (Collar et al., 1999). The present study did find exceptions for the high levels of CMC and HPMC, which increased dough stickiness by ca. 12 and 14%, respectively, compared to the control. Ahmed & Thomas (2018) reported that, in general, neither xanthan gum nor guar gum significantly increased the stickiness of brown wheat flour/β-glucan composite dough, although increases were observed for mid-range levels after holding times of 60 and 90 min. It may be expected that

addition of hydrocolloids and the resultant increase in water would increase dough stickiness, but the strong interaction between the water and hydrocolloids appeared to mitigate such an effect by limiting the amount of unbound water. Our results suggest that when the water absorption and mixing time are optimized, a negative influence on dough textural properties can be minimized. This conclusion agrees with the knowledge that for nonsticky dough flour, increasing either the water absorption or the mix time will increase dough stickiness (Chen & Hoseney, 1995). Overmixing breaks down gluten proteins, which may increase stickiness either through a weakening of the gluten network and/or by reducing the water holding capacity of the gluten. Excess water may increase stickiness due to a weakening of the dough or by increasing surface stickiness (Chen & Hoseney, 1995).

4.3.1.4 Kieffer rig uniaxial extensibility

The Kieffer rig functions as a small scale Brabender extensograph and measures unixial extension, with the advantages of a constant amount of dough being deformed, small scale deformations that are more relevant to the deformations occuring during fermentation, and the measurements of force in Newtons (Dunnewind et al., 2004). Except for xanthan gum, the addition of hydrcolloids did not significantly alter resistance to extension (R_{max}) or extensibility (E_{Rmax}) of the whole wheat dough (Table 4.2). Contrary to our findings using the Kieffer rig, Armero and Collar (1996b) reported that CMC and HPMC decreased resistance to extension of whole wheat dough as measured by extensograph. Extensograph measurements on white dough taken after 45 min of resting showed that addition of 0.5% (fwb) alginate, HPMC, and xanthan decreased resistance at 50 mm of extension by ca. 29, 39, and 6.5%, respectively (Rosell et al., 2001). Extensibility was increased by alginate (6%), HPMC (13%), and xanthan (11%). The differences may be due to the differences in resting time and the variation between the conditions

of the two tests. The high level of xanthan gum increased R_{max} by ca. 49% and decreased E_{Rmax} by ca. 51%, which suggests a stiffer dough. The medium level of xanthan gum also decreased E_{Rmax} by ca. 28% compared to control. Collar et al. (1999) found quadratic effects of xanthan gum on resistance to extension, but reported that this effect held no practical relevance. The amount of xanthan gum used in that study was also almost ten-fold smaller than the high level used in the present work, however.

4.3.2 Bread Properties

4.3.2.1 Specific volume

With the exception of CMC, all hydrocolloids increased specific volume of the whole wheat bread for at least one of the levels evaluated (Table 4.3, Figure 4.1). The findings for CMC and guar gum are in accordance with published works on whole wheat bread (Armero & Collar, 1996a; Mettler & Seibel, 1993). The most noticeable improvements were for the medium level of xanthan gum (ca. 13% increase) and all levels of HPMC (ca. 10.5-11% increase). In white dough, a comparison of alginate, xanthan, and HPMC found that 0.1% xanthan and HPMC both produced a similar increase to specific volume (Guarda et al., 2004). The increase due to alginate was not significant. Volume improvements can be related to increases in dough development and gas retention (Mettler & Seibel, 1993; Rosell et al., 2001). Alveograph tests of HPMC, xanthan, and alginate in white dough have shown that these hydrocolloids strengthen dough and improve the balance of elastic resistance and extensibility (Rosell et al., 2001). These hydrocolloids also improved dough stability during fermentation, preventing loss in dough volume over long fermentation periods. Guarda et al. (2004) reported that increasing the hydrocolloid addition from 0.1 to 0.5% provided an additional increase in loaf volume for HPMC but not for xanthan. In contrast, our work showed that increasing the amount of

hydrocolloid further improved the specific volume for xanthan gum but not for HPMC. The present work used a greater increase in water absorption for increasing amounts of xanthan gum compared to Guarda et al., which likely allowed for the volume increase between the low and medium levels of xanthan gum.

The ability of hydrocolloids to improve loaf volume are often attributed to a strengthening effect on the gluten network and an improvement in gas retention. The exact mechanisms behind these effects are not well defined and vary between different types of hydrocolloids. Several attempts have been made to clarify the interactions between hydrocolloids and wheat flour constituents. The specific interactions depend on the type and level of hydrocolloid (Bárcenas et al., 2009; Linlaud et al., 2011; Ribotta et al., 2005). The interaction with especially gluten, the main structural component of bread, is of particular interest in explaining the effect of hydrocolloids on loaf volume. Such interactions include hydrogen bonding, in the case of neutral hydrocolloids like guar gum, and noncovalent linkages between amide groups of gluten and the hydroxyl groups of anionic hydrocolloids like xanthan gum and alginate (Linlaud et al., 2011; Ribotta et al., 2005). Hydrocolloids have been shown to alter the secondary structure of gluten proteins (Linlaud et al., 2011), which affects the gluten network. For example, SEM visualization of dough microstructure suggested that guar gum promoted a more integrated gluten network (Linlaud et al., 2009). In the case of HPMC, once hydrocolloid gelation occurs, it strengthens the gluten network and may partially replace protein within the network. This modification of the protein and integration into the structural network may explain the beneficial effects on loaf volume and other quality aspects of bread (Rosell & Foegeding, 2007). Due to an abundance of hydroxyl groups, xanthan gum interacts strongly with gluten and hence limits dough extension (Rosell et al., 2001; Zannini et al., 2014). However, ^{1}H NMR

relaxation assays show that xanthan gum increases molecular mobility of dough, suggesting a less rigid gluten-hydrocolloid-water network, and FT-Raman analysis indicated a less ordered structure in the gluten network (Linlaud et al., 2011). SDS-PAGE revealed non-covalent crosslinking of gliadin proteins in the presence of xanthan gum, forming large, soluble aggregates. Xanthan gum also promoted the formation of a more entangled network (Linlaud et al., 2011), which was related to the more elastic characteristics of dough supplemented with xanthan gum (Linlaud et al., 2009).

4.3.2.2 Crumb structure

The hydrocolloids tested did not significantly affect crumb structure of the bread (Table 4.4), except for a modest increase in number of cells for the medium level of HPMC, and an increase in cell diameter for the medium level of xanthan gum. Zannini et al. (2014) also found that xanthan gum and HPMC had no effect on number of cells or cell size, but did report a decrease in cell wall thickness. The difference could be due to the different bread-making methods or the type of HPMC used.

4.3.2.3 Textural properties of bread

TPA of bread on Day 1 revealed mostly non-significant reductions in crumb hardness as a result of hydrocolloids, except for significant reductions due to the high level of guar gum and the medium level of HPMC (Table 4.5). The initial softening effect of HPMC in whole wheat bread has been previously reported (Armero & Collar, 1996a; Zannini et al., 2014). The other textural parameters measured by TPA were also largely unaffected by hydrocolloid addition. The treatments that produced the largest loaf volume were not always the ones with the lowest values for crumb hardness, reinforcing the fact that although loaf volume is a major contributor to firmness (Armero, E. & Collar, 1998; Mettler & Seibel, 1993), gas retention capacity and

increased water absorption of dough (Zannini et al., 2014) and the specific nature of the crumb also play a role in the resistance of crumb to compression (Armero & Collar, 1996a).

Differences in crumb hardness became more apparent with storage time. The increase in hardness with the high level of xanthan at Day 3 could be caused by the low loaf volume compared to the low and medium treatments of the same gum (Armero & Collar, 1998). The increased hardness can also be caused by a lack of water for plasticizing the gluten network (Goesaert et al., 2009), since the water absorption was the same for the high and medium levels of xanthan gum. HPMC, alginate, and the low and medium levels of xanthan gum all showed a trend for decreasing the rate of staling, based on the rate of increase in crumb hardness over time. The anti-staling effect of HPMC could result from its ability to hinder interactions among the other components in the crumb by enveloping them in a polymer network (Barcenas & Rosell, 2005) and by its preferential binding to starch, which influences the interactions among lipid, starch, and gluten (Collar et al., 1998). Xanthan gum and alginate soften bread crumb by interfering with starch-gluten interactions (Davidou et al., 1996). Alginate decreases the gelatinization temperature of starch, which allows a longer window during which amylases can act on the starch (Rojas et al., 1999). Amylases are commonly added either by malted barley flour or from fungal sources, and prolonging their action would contribute to an anti-staling effect. Furthermore, amylograph analysis revealed that alginate increased the formation of the amylose-lipid complex, which is associated with a softening of the crumb (Rojas et al. 1999). Water retention capacity and starch interactions have also been proposed to explain the softening effects of hydrocolloids (Collar et al., 1998; Guarda et al., 2004).

4.3.2.4 Moisture content

Hydrocolloids increased crumb moisture content of fresh bread (Table 4.3), in accordance with Guarda et al. (2004). An increase in moisture content of the fresh bread was expected, since all of the hydrocolloids increased the water absorption of the dough to varying degrees due to their high water-binding capacities. None of the treatments displayed significant differences in moisture content compared to control after 7 days of storage, although the trend for increasing moisture content with hydrocolloids was still observed. Guarda et al. (2004) reported increased moisture retention in bread supplemented with hydrocolloids.

4.3.2.5 Starch retrogradation

The present work did not reveal any significant changes to the endothermic transitions of bread crumb due to hydrocolloid addition (Table 4.6). These results suggest that the hydrocolloids did not modify starch retrogradation, at least not consistently or significantly. These findings are in contrast with certain published studies on the use of hydrocolloids as anti-staling agents in white bread. In white bread, HPMC retarded staling by decreasing the retrogradation index (Barcenas & Rosell, 2005). When used in combination with high ester pectin, xanthan gum reduced the amount of amylose-lipid complex, which would promote bread staling (Collar et al., 1999). In whole wheat flatbread, which has a lower moisture content than pan bread, guar gum decreased the extent of amylopectin retrogradation (Shaikh et al., 2008). DSC analysis of wheat starch gels with either guar or xanthan gum found that xanthan gum decreased starch retrogradation (Biliaderis et al., 1997). Our previous work with xanthan gum in whole wheat bread found a decrease in amylose-lipid complexation, whereas amylopectin retrogradation was not changed (Tebben & Li, 2019). The current study did reveal a trend for decreasing amylose-lipid complexation, although this change was not significant at the 0.05 significance level ($p = 0.0838$ for medium and $p = 0.1134$ for high levels of xanthan). According

to Davidou et al. (1996), alginate, xanthan gum, and locust bean gum had little effect on starch retrogradation or amylose-lipid complexation in white bread, although alginate did reduce somewhat the retrogradation of amylopectin under certain storage conditions. The differences observed in the present study compared to certain literature could be due to the specific nature of whole wheat pan bread compared to the other systems such as white bread, flat bread, or flour or starch gels, or to the variation in the structure of the hydrocolloids. The present findings support the view that amylopectin retrogradation is one of many aspects of bread staling, and is not the only cause of crumb firming (Davidou et al., 1996; Hug-Iten et al., 2003; Martin et al., 1991a).

4.4 Conclusions

Guar gum, HPMC, sodium alginate, and xanthan gum all effectively increased loaf volume of whole wheat bread without substantial alterations in crumb structure. CMC was not an effective hydrocolloid for improving the loaf volume or hardness and staling of whole wheat bread. HPMC, sodium alginate, and xanthan gum decreased the rate of crumb firming but did not alter amylopectin retrogradation. HPMC at 0.5% fwb is recommended as the ideal hydrocolloid for whole wheat bread, promoting an increase in volume and decrease in crumb firmness and staling. HPMC reduced the mixing time and did not substantially alter water absorption of the dough, unlike xanthan gum. With any hydrocolloid, care must be taken to optimize the water absorption and mix time of the dough to the particular type and level of the improver in order to benefit loaf volume and crumb texture. This study examined the individual effects of hydrocolloids. For further improvement to loaf volume, future work should examine the combination of hydrocolloids with emulsifiers or enzymes.

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4.6 Tables

Table 4.1 Farinograph and mixograph properties of control and hydrocolloid-supplemented whole wheat dough compared to water absorption and mix times used for baking tests

Treatment	Farinograph WA	DDT (min)	Stability (min)	MTI (FU)	Mixograph WA (%, fb)	Mixograph Peak Time (min)	Midline Peak Value (%)	Midline peak width (%)	Baking WA (%, fb)	Baking Mix Time (min)
Control	75.6	5.5	8.2	33.7	71	4.67	57.74	31.47	71	4.42
CMC low	78.0	5.6	5.5	53.6	71	4.60	59.86	35.72	72	4.33
CMC med	79.7	6.1	5.3	53.6	71.5	4.97	56.90	25.73	73	4.50
CMC high	82.3	6.4	4.2	67.8	73	6.24	55.03	26.14	75	5.25
guar low	75.8	7.3	7.9	44.9	71	3.96	48.32	25.57	72	3.83
guar med	75.8	7.1	8.9	27.5	71.5	4.39	53.64	28.76	73	4.25
guar high	77.2	7.0	8.4	33.2	73	4.39	56.64	27.48	75	4.33
HPMC low	78.0	5.0	6.5	43.4	71	4.16	55.10	24.76	72	3.83
HPMC med	79.7	5.4	6.5	42.8	71.5	4.32	56.99	31.82	73	3.67
HPMC high	84.5	5.6	5.4	50.0	73	4.25	56.62	30.64	73	3.50
alginate low	77.7	5.4	5.4	55.1	72	5.36	58.56	29.03	73	4.25
alginate med	79.5	5.8	3.9	63.2	73	6.84	53.23	25.12	75	4.50
xanthan low	77.8	6.1	7.2	40.3	72	4.53	54.52	23.32	72	4.03
xanthan med	79.5	6.2	5.5	46.9	74	6.08	48.96	20.03	76	6.00
xanthan high	81.7	9.0	6.2	58.1	76	6.53	44.88	23.85	76	6.75

Stability: Difference between arrival and departure times (time for top curve to reach peak resistance and to fall below peak resistance)
MTI: The difference in Farinograph Units (FU) from the top of the curve at peak mixing time to the top of the curve five minutes after the peak mixing time.

Table 4.2 Textural properties of control and hydrocolloid-supplemented whole wheat dough

Treatment	Stickiness[a] (N)	Work of adhesion[a] (N.s)	Dough cohesiveness[a] (mm)	R_{max}[b] (N)	E_{Rmax}[b] (mm)
Control	0.329±0.035	0.038±0.014	2.40±0.73	0.171±0.011	27.03±4.52
CMC low	0.345±0.040	0.047±0.018	2.95±0.76	0.173±0.011	32.19±4.35
CMC med	0.330±0.017	0.036±0.009	2.25±0.46*	0.168±0.015	31.53±3.88
CMC high	0.396±0.060*	0.060±0.026	3.01±0.83	0.181±0.017	31.03±2.69
guar low	0.309±0.038	0.028±0.017	1.90±0.72	0.162±0.011	24.17±2.09
guar med	0.323±0.025	0.033±0.008	2.45±0.49	0.164±0.013	24.33±3.32
guar high	0.315±0.044	0.030±0.012	2.14±0.67	0.171±0.013	26.01±2.29
HPMC low	0.333±0.034	0.034±0.015	2.15±0.72	0.177±0.008	25.89±3.00
HPMC med	0.339±0.024	0.033±0.009	1.88±0.41	0.172±0.012	25.47±2.37
HPMC high	0.366±0.028**	0.043±0.011	2.13±0.43	0.173±0.015	28.82±1.93
alginate low	0.301±0.039	0.033±0.012	2.50±0.95	0.176±0.011	26.67±2.80
alginate med	0.308±0.022	0.028±0.005	2.20±0.59	0.178±0.013	26.68±4.33
xanthan low	0.295±0.032	0.030±0.010	2.18±0.75	0.165±0.011	23.12±3.36
xanthan med	0.285±0.025	0.027±0.009	1.87±0.54	0.173±0.014	17.98±2.30***
xanthan high	0.315±0.033	0.032±0.009	1.83±0.45	0.247±0.016***	12.34±0.76***

[a]All means were compared to the control from the same day the treatment was analyzed. Level of significance indicated by * = 0.05 to 0.01, ** = 0.01 to 0.001, and *** = 0.001 and lower.

[a]Stickiness, work of adhesion, and dough cohesiveness as measured by the SMS/Chen-Hoseney dough stickiness test. Means for each treatment are the averages of 12 replicates.

[b]R_{max} (resistance to extension) and E_{Rmax} (extensibility) as measured by Kieffer dough and gluten extensibility test. Means for each treatment are the averages of 18 replicates.

Table 4.3 Weight, volume, specific volume, and moisture content of control and hydrocolloid-supplemented whole wheat bread

Treatment	Proof Ht (cm)	Wt (g)	Vol (cm^3)	Specific Vol (cm^3/g)	Increase in specific vol vs. control	Day 1 Moisture Content (% wb)	Day 3 Moisture Content (% wb)	Day 7 Moisture Content (% wb)
Control	7.5	152.87	633	4.15		46.15	45.84	42.17
CMC low	7.6	153.26	658	4.30	3.67%	46.42**	46.17***	41.71
CMC med	7.8	153.42	663	4.33	4.34%	46.73***	46.51***	41.84
CMC high	7.7	154.29	655	4.25	2.45%	46.97***	46.81***	43.54
guar low	7.5	152.76	663	4.34	4.78%	46.35*	46.13**	42.49
guar med	8.0*	152.79	678*	4.44*	7.13%	46.68***	46.50***	42.54
guar high	7.9	155.11	683**	4.41	6.33%	47.19***	46.92***	42.92
HPMC low	8.0*	152.07	697***	4.58***	10.54%	46.54***	46.27***	42.65
HPMC med	8.0**	153.79	707***	4.60***	10.87%	46.76***	46.44***	41.87
HPMC high	7.9	153.47	703***	4.60***	11.09%	46.60***	46.34***	41.77
alginate low	8.1**	153.41	693**	4.52**	9.03%	46.98***	46.53***	42.22
alginate med	8.1**	155.82**	692**	4.44*	7.09%	47.38***	47.23***	43.51
xanthan low	7.9	153.03	697***	4.55**	9.84%	46.51***	46.21***	42.69
xanthan med	8.4***	154.80	723***	4.67***	12.76%	47.70***	47.40***	44.29
xanthan high	8.1**	155.93**	620	3.98	-4.06%	47.63***	47.42***	43.69

All means were compared to control. Level of significance indicated by * = 0.05 to 0.01, ** = 0.01 to 0.001, and *** = 0.001 and lower. Loaves were prepared in triplicate. Moisture content is average of six replicates.

Table 4.4 Crumb structure analysis of control and hydrocolloid-supplemented whole wheat bread

Treatment	Number of Cells	Cell Wall Thickness (mm)	Cell Diameter (mm)
Control	3223	0.423	1.95
CMC low	3242	0.422	1.97
CMC med	3238	0.425	1.99
CMC high	3209	0.422	1.98
guar low	3248	0.426	1.99
guar med	3270	0.424	1.97
guar high	3292	0.425	2.02
HPMC low	3306	0.428	2.06
HPMC med	3417*	0.424	2.00
HPMC high	3293	0.430	2.08
alginate low	3269	0.429	2.06
alginate med	3171	0.428	2.03
xanthan low	3328	0.425	2.05
xanthan med	3359	0.428	2.12*
xanthan high	3205	0.419	1.87

All means were compared to control. Level of significance indicated by * = 0.05 to 0.01. Means are the average of three replicates.

Table 4.5 Texture profile analysis of control and hydrocolloid-supplemented whole wheat bread after 1d storage at 22 °C, and change in hardness after 3 and 7d storage

Treatment	Hardness, N	Resilience, %	Cohesion	Springiness, %	Chewiness, N	Day 3 Hardness, N	Day 7 Hardness, N	Slope: rate of firming	R^2
Control	3.63	35.4	0.715	95.2	2.46	4.97	8.14	0.758	0.808
CMC low	3.32	34.9	0.715	95.6	2.26	4.35	7.44	0.699	0.832
CMC med	3.56	34.7	0.711	95.9	2.43	4.88	8.26	0.792	0.898
CMC high	3.42	33.9*	0.698*	95.2	2.27	4.82	7.90	0.749	0.875
guar low	3.58	35.8	0.715	95.0	2.43	4.92	8.29	0.792	0.689
guar med	3.53	35.8	0.715	95.6	2.40	4.69	7.49	0.667	0.779
guar high	3.05*	35.7	0.714	96.0	2.02**	4.26*	6.98	0.658	0.853
HPMC low	3.23	35.9	0.718	95.7	2.22	4.45	6.92	0.615	0.803
HPMC med	2.91**	36.5	0.722	95.2	2.00**	4.12**	6.27*	0.557	0.864
HPMC high	3.17	36.2	0.720	95.7	2.18	4.42	7.07	0.652	0.882
alginate low	3.57	36.0	0.717	95.8	2.45	4.53	6.63	0.512	0.858
alginate med	3.89	35.2	0.708	95.2	2.61	5.33	7.53	0.599	0.776
xanthan low	3.20	35.7	0.718	95.7	2.18	4.29*	6.85	0.614	0.848
xanthan med	3.31	36.7*	0.723	96.4	2.29	3.94***	6.13**	0.482	0.719
xanthan high	4.04	35.3	0.710	96.1	2.74	5.94**	8.55	0.737	0.756

All means were compared to control. Level of significance indicated by * = 0.05 to 0.01, ** = 0.01 to 0.001, and *** = 0.001 and lower. Six replicates were analyzed per treatment.

Table 4.6 Retrogradation parameters for whole wheat bread with added hydrocolloids stored at 22 °C

Treatment	Tm_1 onset (°C)	Tm_1 peak (°C)	ΔH_1 (J/g)	Tm_2 onset (°C)	Tm_2 peak (°C)	ΔH_2 (J/g)
Control	51.59	65.98	2.52	100.02	118.07	1.71
CMC low	51.19	64.70	2.19	96.05	115.24	1.31
CMC med	50.51	62.71	2.04	96.81	113.88	0.93
CMC high	50.35	63.96	2.77	96.50	114.76	1.21
guar low	50.94	64.22	2.45	99.72	116.75	1.25
guar med	50.70	64.03	2.63	96.71	117.24	1.51
guar high	50.94	64.69	2.23	97.59	115.21	1.28
HPMC low	51.59	65.35	2.69	98.25	118.06	1.36
HPMC med	50.79	64.00	2.52	99.90	117.89	1.80
HPMC high	50.46	63.98	2.81	96.71	116.50	1.37
alginate low	51.21	64.66	2.48	97.77	115.75	1.44
alginate med	51.43	65.54	2.56	101.40	119.02	1.32
xanthan low	50.93	64.53	2.57	96.28	116.08	1.72
xanthan med	51.26	63.12	2.10	98.75	114.46	0.74
xanthan high	51.20	64.09	2.26	98.43	113.86	0.80

All means were compared to control. Tests were performed in duplicate. No significant differences were found in comparisons of treatment means to control.

4.7 Figures

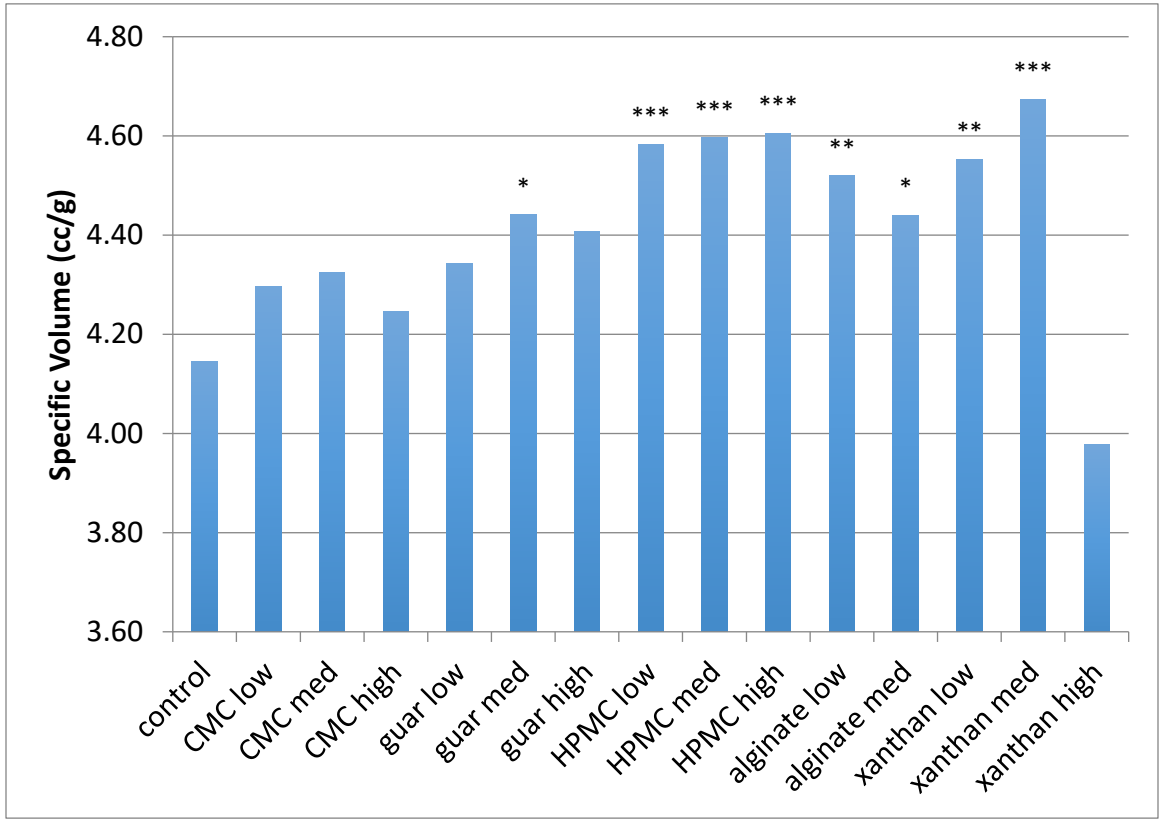

Figure 4.1 Specific volume of whole wheat bread with added hydrocolloids.
Hydrocolloids were added on fwb at levels low = 0.25%, med = 0.5%, high = 1.0%. All means were compared to control. Level of significance indicated by * = 0.05 to 0.01, ** = 0.01 to 0.001, and *** = 0.001 and lower. Loaves were prepared in triplicate.

5 Effects of emulsifiers on whole wheat dough and bread properties

Abstract

Emulsifiers are used in the baking industry to improve dough handling, increase loaf volume, and improve the crumb and textural properties of bread. Whole wheat bread has a smaller loaf volume and harder texture than white bread, so it is expected to benefit from the dough-strengthening and crumb-softening effects of emulsifiers. The objective of this research was to determine the effects of five emulsifiers on whole wheat dough and bread properties, with a focus on loaf volume, bread texture, and staling. Emulsifiers (diacetyl tartaric acid esters of mono- and diglycerides (DATEM), polysorbate 80, sodium stearoyl lactylate (SSL), soy lecithin, and sucrose esters) were added individually to whole wheat flour at 0.2, 0.5, and 1.0% fwb. Dough rheology and texture were determined by farinograph, mixograph, Chen-Hoseney stickiness test, and Kieffer rig uniaxial extensibility. Bread was prepared following AACC method 10-10.03. Specific volume was measured for fresh bread. Moisture content and TPA were measured after 1, 3, and 7 days of storage at 22 °C. Crumb structure was measured by C-cell image analysis on Day 1. Differential scanning calorimetry (DSC) was used to measure changes to the starch fraction after the 7 days. Emulsifiers produced only minimal changes to dough rheological properties. SSL increased dough stability, but sucrose esters and polysorbate 80 decreased this parameter. Sucrose esters, polysorbate 80, and SSL led to small but significant decreases in dough stickiness. DATEM, sucrose esters, and SSL increased the resistance to extension, whereas soy lecithin and polysorbate 80 increased dough extensibility. Soy lecithin and polysorbate 80 were the only emulsifiers to significantly increase loaf volume compared to control. It appears that the dough-strengthening effect from some emulsifiers was not beneficial

to the loaf volume of whole wheat bread. An increase in amylopectin retrogradation was observed for some treatments but was not related to an increase in crumb hardness. Sucrose esters, polysorbate 80, and SSL increased the amount of amylose-lipid complex. These three emulsifiers also produced the lowest crumb hardness on Day 7 and decreased the rate of crumb firming.

Keywords: Bread; Whole wheat; Dough properties; Staling; Emulsifiers; Surfactants; Diacetyl tartaric acid esters of mono- and diglycerides (DATEM); Lecithin; Polysorbate 80; Sodium stearoyl lactylate (SSL); Sucrose esters

5.1 Introduction

Emulsifiers are a group of compounds with both hydrophilic and lipophilic moieties. They are also called surface active agents, or surfactants. Emulsifiers are one of three sources of lipids in the bread making process, along with native flour lipids and shortening/margarine (Pareyt et al., 2011). In bread, emulsifiers contribute beneficial effects beyond their ability to stabilize systems that are thermodynamically unstable by lowering the surface tension at hydrophobic-hydrophilic interfaces (Kohajdová et al., 2009). Some of these benefits include improving dough handling properties, increasing dough strength and stability, improving gas retention of dough, increasing loaf volume, improving the sensory characteristic of bread including creating a finer crumb structure, decreasing crumb firmness, and delaying staling (Pareyt et al., 2011; Stampfli & Nersten, 1995).

Emulsifiers used in the baking industry can broadly be divided into two categories: dough strengtheners, which interact primarily with gluten and improve loaf volume, and crumb softeners, which interact primarily with starch (Stampfli & Nersten, 1995). Some emulsifiers exhibit both of the aforementioned properties. Examples of dough strengtheners are diacetyl

tartaric acid esters of mono- and diglycerides (DATEM), polysorbate, sodium stearoyl lactylate (SSL), and sucrose esters. Examples of crumb softeners include mono- and diglycerides, lecithin, and SSL (Stampfli & Nersten, 1995; Stampfli et al., 1996). Emulsifiers are also classified by their electrostatic charge. DATEM and SSL are anionic, lecithin is amphoteric, and monoglycerides, sucrose esters, and polysorbate are nonionic (Stampfli & Nersten, 1995). Soy lecithin is considered a clean-label ingredient and therefore will likely become a more common replacement for other emulsifiers due to increasing consumer demand for ingredient lists that are free from additives they do not recognize.

The objectives of this study were to determine the effects of emulsifiers on whole wheat dough properties and on the quality of fresh and aged whole wheat bread. Five of the most common emulsifiers were chosen for this study to represent both dough strengtheners and crumb softeners. More dough strengtheners were selected because a primary goal was the increase in loaf volume. The emulsifiers used were soy lecithin, DATEM, sucrose esters, polysorbate 80, and SSL.

5.2 Materials and Methods

5.2.1 Materials

Whole wheat flour (11.8% moisture content, 15.8% protein) was kindly supplied by Mennel Milling Company (Fostoria, OH). Polysorbate 80, soy lecithin, sodium stearoyl-2-lactylate, and sucrose esters were purchased online (modernistpantry.com). DATEM was obtained from Profood International. Food grade calcium propionate was obtained from Niacet Corporation (Niagara Falls, New York). Instant yeast, sucrose, sodium chloride, and shortening were obtained from a local supermarket.

5.2.2 Dough properties

Each of the five emulsifiers was evaluated in whole wheat dough three levels: 0.2, 0.5, and 1.0% (fwb). A control dough without emulsifiers was also prepared for all analyses.

5.2.2.1 Farinograph

Farinograph dough properties were measured with a 50 g doughLAB (Perten Instruments North America, Springfield, IL), using the 10 min AACCI Method 54-70.01 and extending the test duration as necessary to 12 min beyond the peak resistance or until the top of the curve fell below 500 FU, whichever was later. The following parameters were determined: water absorption (WA; percentage (fwb) of water required to reach a dough consistency of 500 Farinograph Units (FU)), dough development time (DDT; time for dough to reach peak consistency), stability (time for the top curve to reach peak resistance and to fall below peak resistance), and mixing tolerance index (MTI; the difference in FU from the top of the curve at peak mixing time to the top of the curve five minutes after the peak mixing time).

5.2.2.2 Mixograph analyses

Dough mixing properties were determined by a mixograph (National Manufacturing, Lincoln, NE). Flour (10 g, 14.0% moisture basis), water, and emulsifier, when tested, were mixed in a 10 g mixograph bowl at 22 °C. The water absorption and mixing time as determined by midline peak time were used to prepare all samples for the remaining dough tests.

5.2.2.3 Chen-Hoseney stickiness test

Dough stickiness was analyzed using a TA-XT Plus Texture Analyzer (Stable Micro Systems, Godalming, Surrey, UK) equipped with a 30 kg load cell. A SMS/Chen-Hoseney Dough Stickiness Rig and 25 mm perspex cylinder probe were used for the test as described by Huang and Hoseney (1999). At least four replicates were performed for each dough, and each

dough was prepared in duplicate. The test was used to determine dough stickiness (in N), work of adhesion (N.s), and dough strength/cohesiveness (in mm).

5.2.2.4 Kieffer rig uniaxial extensibility

Uniaxial extensibility was measured using the Kieffer dough and gluten extensibility rig on the TA-XT Plus Texture Analyzer. Approximately 10 g of prepared dough was pressed in the lubricated Teflon molder and allowed to rest at 22°C for 30 min. A strip of dough was removed from the molder and clamped between the plates of the Kieffer rig before each test. A test speed of 3.3 mm/sec and trigger force of 0.049 N were used. Resistance to extension (R_{max}, in N) and extensibility (E_{Rmax}, in mm) were recorded as the peak force and the distance at the peak force, respectively. Five or six strips per dough were tested, and each dough was prepared in duplicate.

5.2.3 Bread making

Bread was baked following the AACC method for straight-dough bread-making (AACC International 10-10.03), with the addition of 0.3 g calcium propionate and without any of the optional ingredients. Two grams of instant yeast (Bellarise Red) was used instead of active dry yeast. Emulsifiers were added to the formulation according to the experimental design at low, medium, and high levels corresponding to 0.2, 0.5, and 1.0% (fwb). Mixing time and amount of water were based on mixograph data, except for mixing times for the SSL treatments, which were reduced to 6, 7, and 8 min for the low, med, and high levels, respectively, based on preliminary baking tests. Immediately after baking, volume was determined by rapeseed displacement (AACC International 10-05.01), and the weight was measured. Upon cooling, bread was transferred to polyethylene bags. The following day, bread was sliced into 15 mm thick slices for further analysis. Four replicates of each treatment were prepared over four separate days of baking.

5.2.3.1 Evaluation of crumb structure

The central slice of each loaf was photographed using a C-Cell Bread Imaging System (Calibre Control International Ltd., Appleton, Warrington, UK). Each image was analyzed by the provided software to quantify the number of cells, cell wall thickness, and cell diameter.

5.2.3.2 Texture properties

Crumb texture was analyzed by texture profile analysis (TPA) using the TA-XT Plus Texture Analyzer. Single slices of bread (15 mm) were compressed twice at 1.00 mm/s to 50% strain with a 0.049 N trigger force and 1 s pause between compressions. Parameters recorded were hardness, resilience, cohesion, springiness, and chewiness. Two of the central slices from each loaf were analyzed. TPA was performed after storage for 1, 3, and 7 d under ambient conditions (22 °C) to determine the effect of emulsifiers on the textural changes in whole wheat bread.

5.2.3.3 Moisture content

Following TPA, a sample of the crumb (~1 g) from each slice was dried at 105 °C for 3 h in a convection oven. Samples were allowed to cool for 45 min in a desiccator before weighing. Moisture content was determined for bread after storage for 1, 3, and 7 d.

5.2.3.4 Retrogradation

Thermal phase transitions of bread stored for 7 d were conducted using a Q200 differential scanning calorimeter (DSC) (TA Instruments, New Castle, DE). The instrument was calibrated with indium as a standard. Approximately 20 mg of bread crumb was weighed into a stainless steel pan and hermetically sealed. Samples were heated from 0-150 °C at 10 °C/min under nitrogen atmosphere with a gas flow rate of 50 mL/min. The onset temperature (T_o), peak temperature (T_p), and melting enthalpy (ΔH, joules/g) were determined with TA Universal

Analysis software for the endothermic peaks around 60 °C and 115-120 °C. These two peaks corresponded to the melting of retrograded amylopectin and the amylose-lipid complex, respectively. Two replicates were performed per treatment.

5.2.4 Statistical analysis

Treatment means were analyzed using Tukey's multiple comparisons test in SAS Studio 3.7 (SAS Institute Inc., Cary, NC). Significance was defined at $p < 0.05$.

5.3 Results and Discussion

5.3.1 Dough Properties

5.3.1.1 Farinograph analyses

Emulsifiers did not noticeably affect farinograph WA, although the high levels of sucrose esters and polysorbate 80 did increase the WA somewhat, and SSL produced a slight decrease in WA (Table 5.1). DDT was also relatively unaffected by emulsifier addition, except for a small decrease due to polysorbate 80 and an increase with the high level of SSL. SSL has been shown to increase DDT of dough from weak flour, but not from strong flour (Indrani & Rao, 2003). In white dough, neither DATEM nor lecithin influenced DDT (Stampfli et al., 1996). DATEM, sucrose esters, and polysorbate 80 decreased stability and increased MTI, indicating a faster breakdown of the dough. This effect was most noticeable for polysorbate 80. Conversely, SSL exhibited a strengthening effect on the dough by increasing stability and decreasing MTI, which is consistent with published results for white dough (Indrani & Rao, 2003; Ravi et al., 2000). DATEM and polysorbate 80 were expected to increase stability, based on studies of white dough (Ding & Yang, 2013; Indrani & Rao, 2003; Ravi et al., 2000; Stampfli et al., 1996). Increasing stability is attributed to the interaction of the ionic surfactants with protein, lipids, and starch in the flour (Indrani & Rao, 2003). The reverse effect in whole wheat dough may be due to

different interactions with wheat components not present in white flour dough. More research is needed to clarify this supposition and determine the interactions involved.

5.3.1.2 Mixograph analyses

Soy lecithin and DATEM increased WA in the mixograph tests, whereas the other emulsifiers did not affect WA (Table 5.1). The emulsifiers tended to increase mixograph peak time (Table 5.1, Figure 5.1). The greatest increase in peak time was seen for SSL, which was consistent with the farinograph results for DDT. In white dough, SSL has been shown to increase midline peak time, while sucrose esters did not influence midline peak time (Lang et al., 1992). DATEM, SSL, sucrose esters, and polysorbate are generally recognized as dough strengtheners (Stampfli & Nersten, 1995), and would therefore be expected to increase the mix time.

5.3.1.3 Stickiness

The results of the Chen-Hoseney stickiness test are displayed in Table 5.2. Sucrose esters (1%), polysorbate 80 (0.5%), and SSL (1%) significantly decreased dough stickiness, work of adhesion, and cohesiveness compared to the control. DATEM increased dough cohesiveness, consistent with the work by Armero and Collar (1997). Adhesiveness as measured by TPA for white dough found that lectihin, DATEM, polysorbate 60, and SSL all decreased adhesion (Indrani & Rao, 2003). Reports on the effect of emulsifiers on dough stickiness are limited and show mixed results. In whole wheat dough, DATEM has increased (Patil & Arya, 2016) and shown no effect on stickiness (Colakoglu & Özkaya, 2012). In white dough, DATEM decreased stickiness (Colakoglu & Özkaya, 2012), and it had no effect on stickiness in sourdough (Armero & Collar, 1997). SSL has shown either an increase in stickiness (Patil & Arya, 2016) or no effect (Armero & Collar, 1997). In white dough, sucrose esters did not impact stickiness (Sangnark & Noomhorm, 2004), but the present study revealed that this emulsifier decreased dough stickiness.

Some of the differences among the literature may be due to the composition of the flour used, beyond the white/whole wheat designation, as well as the exact makeup of the emulsifiers, and the water absorption and mixing procedures employed by the different researchers. Clearly, the effect of emulsifiers on the textural properties of dough is highly dependent on the conditions in which it is used.

5.3.1.4 Kieffer rig uniaxial extensibility

Based on the Kieffer rig test for uniaxial extensibility, DATEM (1%), sucrose esters (0.5 and 1%) and all levels of SSL increased the resistance to extension (R_{max}) (Table 5.2). Soy lecithin (0.5%), and the upper levels of polysorbate 80 increased the extensibility of the dough, based on the distance at peak resistance, similar to results for white dough (Ding & Yang, 2013; Stampfli et al., 1996). Results of the Kieffer test for white dough have shown that DATEM and SSL increased resistance to extension, but the effect on extensibility has been mixed (Aamodt et al., 2003; Gómez et al., 2013). An increase in R_{max} is another indicator of the dough strengthening effect. The extensograph is another method for evaluating the unixial extensibility of dough. The strengthening effect of various emulsifiers including DATEM, polysorbate 60, and SSL has been well documented based on extensograph tests for white dough (Ding & Yang, 2013; Indrani, & Rao, 2003; Kenny et al., 1999; Ravi et al., 2000; Stampfli et al., 1996). Scanning electron microscopy of dough revealed that DATEM created a more continuous gluten network that enwrapped the starch granules (Ding & Yang, 2013). Polysorbate 60 resulted in a tight, thick gluten film. The authors wrote that DATEM interacts with gluten primarily through hydrophobic interactions, whereas polysorbate 60 interacted mainly through hydrogen bonding and resulted in crosslinks among gluten proteins (Ding & Yang, 2013). However, previous data from IR spectroscopy suggested that DATEM interacts with starch and gluten via hydrogen

bonds (Hähnel et al., 1995). Nonetheless, the chemical makeup of emulsifiers and hence the differences in interactions with the gluten network affect the dough-strengthening properties. Dough strenghteners and crumb softeners bind to different regions of the gluten polymers, and the former bind more strongly than the latter (Pareyt et al., 2011). It is believed that the lipophilic tails of emulsifers attach to hydrophobic regions of gluten proteins (Armero & Collar, 1998). The negatively charged regions of the emulsifiers then create more electrostaic interactions with gluten proteins, increasing protein aggregation (Pareyt et al., 2011) and resulting in the dough-strengthening effect (Armero & Collar, 1998). Emulsifiers with a high hydrophilic-lipophilic balance, such as DATEM and SSL, would produce more of these electrostatic interactions and therefore are better strengtheners than emulsifers with a low hydrophilic-lipophilic balance, such as lecithin. However, it has also been suggested that DATEM and SSL increase DDT by shielding the electrostatic charges of gluten (Pareyt et al., 2011). Lecithin has a lesser effect on extensograph parameters than the other emulsifiers (Indrani & Rao, 2003). A higher value for resistance to extension is considered a beneficial attribute for breadmaking, and it has been positively correlated with loaf volume (Kenny et al., 1999), although this parameter alone cannot be used to predict loaf volume. Strain hardening and resistance to deformation are also important factors in determining the baking quality of a flour (Kokelaar et al., 1996).

5.3.2 Bread Properties

5.3.2.1 Loaf volume

The different emulsifiers had varying effects on loaf volume (Table 5.3, Table 5.2). The level of emulsifier used did not substantially affect volume. Of the five emulsifiers tested in this study, only polysorbate 80 and soy lecithin significantly (p-value < 0.05) improved loaf volume compared to the control (**Figure 5.2**). Holding all other variables constant, loaves made with

polysorbate 80 were 77 cc larger (12% increase) on average compared to control, and loaves with lecithin were an average of 46 cc larger (7% increase) than control. Sucrose esters increased loaf volume by an average of 41 cc, although a wider standard deviation prevented significance at the 0.05 level.

Improvement in loaf volume due to emulsifiers is generally related to their dough-strengthening ability. Other mechanisms by which emulsifiers increase loaf volume include increasing gas retention, which leads to improved oven spring, and increasing the beneficial action of shortening by aiding in the dispersion of such lipids (Pareyt et al., 2011). Unexpectedly, the emulsifiers that exhibited dough-strengthening properties in this study were not the ones that significantly improved loaf volume. For example, DATEM, sucrose esters, and SSL all increased resistance to extension (Table 5.2). The levels of these emulsifiers may have produced too strong of a dough strengthening effect, hindering expansion of the dough during proofing and the early stages of baking when oven spring occurs. DATEM and SSL are two of the most common dough strengtheners in bread making, and most research reports an increase in loaf volume with these additives for both white and whole wheat bread (Armero & Collar, 1996b; Gómez, M. et al., 2004; Indrani & Rao, 1992a; Lai et al., 1989; Mettler & Seibel, 1993). However, arabinoxylans in whole wheat flour interact with gluten in a way that produces a higher resistance to extension than white dough (Altinel & Ünal, 2017a). Therefore, an increase in resistance to extension may not be desirable for whole wheat dough if the goal is to improve loaf volume. Rather, a decrease in resistance to extension has been shown to increase loaf volume for whole wheat bread (Altinel & Ünal, 2017a, 2017b). The dough-strengthening emulsifiers may necessitate a greater increase in baking water absorption than what was used in the present study. The water absorptions used for baking were the same as the mixograph water absorptions (Table 5.1). Models of dough

properties and loaf volume as a function of water absorption and SSL suggest that high levels of both are required for good loaf volume of whole wheat bread (Lai et al., 1989). When comparing the results of emulsifiers in the literature, it should be noted that variations in the fatty acid composition (Helmerich & Koehler, 2005; Köhler & Grosch, 1999) as well as the fermentation or proof time (Gómez, M. et al., 2004) will contribute to the differences among studies.

The two emulsifiers that significantly increased loaf volume, lecithin and polysorbate 80, also increased extensibility of the dough without significantly decreasing resistance to extension (Table 5.2). The greater extensibility likely allowed for greater expansion during proofing and oven spring (Bae et al., 2014). Other factors are also involved. Lecithin is composed largely of polar lipids, mainly phospholipids and glycolipids, plus smaller concentrations of nonpolar lipids and nonlipid material (Selmair & Koehler, 2009). Phospholipids have a weakening effect on dough during mixing, but exert their beneficial effects during proofing and baking, stabilizing the gas bubble interface and increasing gas holding capacity (Helmerich & Koehler, 2005). The minor phospholipids in lecithin, not phosphatidylcholine, have the greatest effect on baking performance, and recombinant mixtures of phospholipids can be more effective than crude and defatted lecithins (Helmerich & Koehler, 2005). The composition and ratio of the phospholipids, rather than the total concentration of phospholipids, are the primary determinants of the effect on loaf volume. Saturated fatty acids are believed to be more beneficial than unsaturated fatty acids. Besides phospholipids, the glycolipids in lecithin also influence functionality, reportedly by directly or indirectly stabilizing the liquid film lamellae at the interface of the dough liquor and gas cells (Selmair & Koehler, 2009).

5.3.2.2 Moisture content

The emulsifiers that increased water absorption of the dough, DATEM and soy lecithin, increased moisture content of bread on Day 1 and Day 7 compared to the control (Table 5.3). The other emulsifiers decreased moisture content of the bread, which was expected because the doughs were prepared with 70% water absorption (fwb) compared to 70% for the control. A similar trend was observed for the moisture loss from the crumb from Day 1 to Day 7: DATEM and soy lecithin appeared to help with moisture retention during storage, while sucrose esters, polysorbate 80, and SSL had the opposite effect (Table 5.3, Figure 5.3). SSL, polysorbate 60, and mono- and diglycerides have been shown to increase moisture migration from crumb to crust (Pisesookbunterng & D'Appolonia, 1983).

5.3.2.3 Crumb structure

Emulsifiers did not significantly increase the number of cells in the crumb of whole wheat bread compared to the control, although minor differences were observed between treatments (Table 5.4, Figure 5.4, Figure 5.5). Sucrose esters, polysorbate 80, and SSL increased the cell wall thickness compared to control. Sucrose esters and polysorbate 80 also increased cell diameter. Objective measurements of cell structure for emulsifier-supplemented whole wheat bread is not well reported, but subjective evaluations generally report small improvements in the sensory characteristics of the crumb (Armero & Collar, 1996a; Indrani & Rao, 1992a, 1992b; Mettler & Seibel, 1993). An improvement in crumb grain is related to the ability of emulsifiers to increase air incorporation during mixing, or to increase the number of air cells without an increase in the total volume of air incorporated (Pareyt et al., 2011). The creation and stabilization of smaller air cells is due to a decrease in surface tension or by preventing proteins or lipids from disrupting the lamella lining the gas cells (Pareyt et al., 2011). The lack of significant change in cell number may be due to chemical or physical destabilizing or interacting

effects of the bran and germ in whole wheat flour. Polysorbate 60 has been shown to form a tight, thick gluten network compared to dough without emulsifier (Ding & Yang, 2013). A similar result for polysorbate 80, sucrose esters, and SSL may explain the thickening of cell walls observed in the present study.

5.3.2.4 Textural properties of bread

After 1 day of storage, only polysorbate 80 and the highest level of sucrose esters led to a significant decrease in crumb hardness compared to the control (Table 5.5). Soy lecithin showed a trend for increasing resilience and springiness. Other textural changes included a decrease in resilience, springiness, and/or chewiness for at least one level of sucrose esters, polysorbate 80, and SSL. Very low springiness values, when coupled with low values for crumb hardness, indicate bread that falls apart quickly during chewing, which is usually considered undesirable. The reductions observed for these related texture parameters were not severe and probably would not reduce eating quality of the bread.

A major cause of the initial reduction in crumb hardness is most likely the increase in loaf volume, which creates a less dense crumb that is more readily compressed. After 3 and 7 days of storage, all treatments had lower crumb hardness values than the control, although this effect was not significant for all cases (Table 5.5). On day 7, the lowest hardness value was obtained for 1% polysorbate 80, which had a 35% reduction compared to the control. This treatment as well as 1% sucrose esters gave the most drastic reduction in the rate of crumb firming, displaying substantial anti-staling effects (Table 5.5, Figure 5.6). Among all 15 treatments, only 1% addition of SSL reduced the extent of amylopectin retrogradation (Table 5.6), so it seems unlikely that this is a major cause of the reduction in crumb hardness and rate of firming. In fact, some of the treatments had an increase in amylopectin retrogradation along with a decrease in

crumb hardness at Day 7. This was the case for 1% polysorbate 80, the treatment with the greatest reduction in crumb firmness.

A reduction in initial crumb hardness for whole wheat bread has been reported for DATEM (Armero, E. & Collar, 1996a; Indrani & Rao, 1992a), lecithin (Indrani & Rao, 1992a), polysorbates (Indrani & Rao, 1992a), and SSL (Armero & Collar, 1996a; Indrani & Rao, 1992a), and decrease in crumb staling has been reported for DATEM (Mettler & Seibel, 1993) and SSL (Indrani & Rao, 1992a). Most of the studies on the anti-staling effects of emulsifiers are based on white bread, but they provide valuable insight into the proposed mechanisms of these effects. Primary reasons for a reduction in fresh bread firmness are increased loaf volume and a finer crumb structure (Gómez, M. et al., 2004). Furthermore, emulsifiers interact with starch and hence delay water absorption and starch swelling, which produces a softer initial texture (Pareyt et al., 2011). Amylose complexed with emulsifiers, such as monoglycerides, does not gel during baking and hence cannot recrystallize, hence reducing the firmness of fresh bread, since amylose recrystallization occurs within the first 24 h of storage (Stampfli & Nersten, 1995).

Several mechanisms may produce the decrease in crumb firming over time obtained through emulsifier supplementation. The binding of emulsifiers with starch granules prevents increases moisture migration from crumb to crust, presumably by preventing its redistribution from gluten to starch as the bread ages, which contributes to the anti-staling effect, as was demonstrated for SSL and mono- and diglycerides (Pisesookbunterng & D'Appolonia, 1983). Sucrose esters can form complexes with proteins and with starch, and these interactions may lead to the anti-staling effect (Kohajdová et al., 2009). Lecithin exerts an anti-staling effect by complexing with amylose, as well as preventing amylopectin retrogradation due to the lysophospholipids present in lecithin (Gómez, M. et al., 2004). In this study, the most effective

crumb softeners actually gave loaves with a lower moisture content compared to the control. However, this finding does not imply that a higher moisture content results in greater crumb firming. The distribution of water within the crumb, along with several other factors, are important to the textural properties (Stampfli & Nersten, 1995).

Despite a non-significant decrease in loaf volume, SSL decreased crumb hardness on Days 3 and 7 and reduced the rate of crumb firming. The effectiveness of SSL to reduce the rate of staling is attributed to its ability to form complexes with both amylose and amylopectin (Stampfli & Nersten, 1995). SSL supplementation decreases the amount of water soluble starch, a substance that creates a rigid matrix between gluten and starch granules within cell walls (Shaikh et al., 2008). A decrease in soluble starch therefore leads to a softer crumb. Additionally, SSL reduces the ability of starch to absorb water, either by chemically binding to starch or by physically shielding it. Therefore, more water is available to hydrate the gluten network, allowing it to remain flexible (Shaikh et al., 2008). Dough strengtheners including SSL may also result in crumb softening due to the denaturation or change in configuration of gluten protein (Pisesookbunterng & D'Appolonia, 1983).

5.3.2.5 Starch retrogradation

Among all of the treatments, only the highest level of SSL significantly reduced the amount of retrograded amylopectin after 7 days of storage, as measured by the first endothermic peak in the DSC thermogram (Table 5.6, Figure 5.7, Figure 5.8). One level each of DATEM, sucrose esters, polysorbate 80, and SSL actually increased amylopectin retrogradation. The emulsifiers did not change the peak melting temperature of recrystallized amylopectin, although there were minor decreases in the onset temperature. Rao and colleagues (1992) found that 0.5% sucrose esters and SSL individually reduced amylopectin retrogradation, whereas Xu and

colleagues (1992) reported that SSL inhibited amylopectin retrogradation while DATEM and sucrose esters did not. The prevention of retrogradation is related to the ability of an emulsifier to interact with starch. SSL and monoglycerides are generally considered the most effective anti-staling agents (Pareyt et al., 2011), and SSL has the greatest affinity for binding to starch among SSL, polysorbate 40, and mono- and diglycerides (Pisesookbunterng & D'Appolonia, 1983).

Regarding the second peak, which is centered around 114-120 °C and corresponds to the dissociation of the amylose-lipid complex (Davidou et al., 1996), soy lecithin and DATEM decreased the peak temperature and melting enthalpy compared to the control. Conversely, the high levels of sucrose esters, polysorbate 80, and SSL increased the amount of amylose-lipid complex. In bread made with a blend of white flour and resistant maize starch, individual addition of SSL, polysorbate 80, or DATEM did not alter the amount of amylose-lipid complex after 1 and 7 days of storage, although significant increasing effects were found for the combination of SSL and DATEM (Gómez, A. et al., 2013). An increase in the amount of amylose-lipids complex has been correlated with softer crumb, although other authors have found no such relationship (Gray & Bemiller, 2003; Pareyt et al., 2011). The complex is also believed to decrease the extent of amylopectin retrogradation, because the complexed amylose cannot co-crystallize with amylopectin (Davidou et al., 1996).

5.4 Conclusions

Overall results of dough tests showed that DATEM, sucrose esters, and SSL exhibited dough-strengthening activity. Somewhat surprisingly, none of those emulsifiers significantly increased loaf volume compared to the control. Rather, soy lecithin and polysorbate 80 were the only emulsifiers to produce a significant improvement in loaf volume versus the control. It appears that an increase in dough strength, as determined by resistance to extension, was not

necessarily beneficial to the loaf volume of whole wheat bread. However, the effects of emulsifiers on dough and bread are expected to vary with differences in the flour, dough recipe, and production method. All of the emulsifiers reduced the rate of staling as measured by crumb firming over 1 week, with the most effective treatments being 1% of either sucrose esters or polysorbate 80. Neither initial crumb hardness nor loaf volume seemed to be an indicator of hardness on Day 7 or of the rate of firming. Additionally, no clear relationship was found between amylopectin retrogradation and crumb hardness or rate of firming.

The mechanisms behind the anti-staling activities of these emulsifiers in whole wheat bread has yet to be completely understood. Future work may examine the proposed interactions with starch and gluten, and how those influence bread staling. Furthermore, the interactions between emulsifiers and the components of wheat bran and germ should be investigated, in order to explain the differences in effects on whole wheat dough and bread compared to reported results in dough and bread made from refined flour.

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5.6 Tables

Table 5.1 Farinograph and mixograph properties for whole wheat dough with added emulsifiers

Treatment	Farinograph WA (%, fwb)	Development time (min)	Stability (min)	MTI (FU)	Mixograph WA (%, fwb)	Mixograph Peak Time (min)
Control	73.6	10	10.5	33.2	71	5.20
0.2% soy lecithin	73.6	10	10.1	37.7	73	5.45
0.5% soy lecithin	73.2	10	11.4	35.2	74	5.76
1% soy lecithin	72.9	10	11.2	33.2	75	5.88
0.2% DATEM	73.5	10	9.6	39.3	71	5.65
0.5% DATEM	73.6	10	9.3	41.8	72	6.00
1% DATEM	73.7	9.8	9.2	42.3	74	6.38
0.2% sucrose esters	73.2	9.7	9.4	41.8	70	5.59
0.5% sucrose esters	73.2	10	9.3	46.4	70	6.08
1.0% sucrose esters	75.0	8.8	7.7	46.4	70	5.71
0.2% polysorbate 80	73.5	9.1	8.8	44.9	70	5.32
0.5% polysorbate 80	73.3	8.5	7.3	45.4	70	5.82
1% polysorbate 80	75.1	9.2	6.2	57.1	70	4.75
0.2% SSL	73.1	10	11.0	24	70	7.47
0.5% SSL	72.0	12	14.1	16	70	9.43
1% SSL	72.0	19	18.6	9	69	9.79

Stability: Difference between arrival and departure times (time for top curve to reach peak resistance and to fall below peak resistance)
MTI: The difference in Farinograph Units (FU) from the top of the curve at peak mixing time to the top of the curve five minutes after the peak mixing time.

Table 5.2 Textural properties for whole wheat dough with added emulsifiers

Treatment	Stickiness[b] (N)	Work of adhesion[b] (N.s)	Dough cohesiveness[b] (mm)	R_{max}[c] (N)	E_{Rmax}[c] (mm)
Control	0.359±0.037 abc	0.042±0.012 bcde	2.51±0.72 bcd	0.287±0.039 def	27.25±5.34 defg
0.2% soy lecithin	0.372±0.062 a	0.049±0.019 abc	2.84±0.69 abc	0.254±0.027 ef	32.30±4.54 abcd
0.5% soy lecithin	0.357±0.036 abcd	0.044±0.009 abcd	2.43±0.35 bcd	0.274±0.029 ef	34.60±5.77 ab
1% soy lecithin	0.368±0.015 ab	0.039±0.005 bcde	2.23±0.38 cde	0.258±0.047 ef	30.67±5.69 bcde
0.2% DATEM	0.343±0.019 abcde	0.052±0.013 ab	3.41±0.88 a	0.299±0.034 cde	28.87±5.91 bcdefg
0.5% DATEM	0.331±0.028 abcde	0.046±0.011 abc	2.90±0.57 abc	0.296±0.030 def	31.05±6.66 bcde
1% DATEM	0.352±0.050 abcd	0.057±0.023 a	3.22±0.77 ab	0.353±0.040 b	24.34±1.45 fg
0.2% sucrose esters	0.341±0.019 abcde	0.035±0.007 cdef	2.43±0.58 bcd	0.334±0.018 bcd	28.05±3.31 cdefg
0.5% sucrose esters	0.319±0.021 cdef	0.026±0.005 efg	1.89±0.55 def	0.350±0.025 b	25.06±3.24 efg
1.0% sucrose esters	0.280±0.016 f	0.018±0.003 g	1.50±0.35 ef	0.368±0.018 b	23.92±1.60 g
0.2% polysorbate 80	0.326±0.024 abcdef	0.029±0.006 defg	1.89±0.26 def	0.296±0.030 def	31.46±4.23 bcd
0.5% polysorbate 80	0.312±0.015 def	0.028±0.004 defg	1.94±0.37 def	0.257±0.018 ef	38.03±3.98 a
1% polysorbate 80	0.324±0.022 bcdef	0.020±0.003 fg	1.27±0.22 f	0.250±0.013 f	33.94±4.21 abc
0.2% SSL	0.364±0.036 abc	0.045±0.006 abc	2.79±0.52 abc	0.346±0.049 bc	30.57±3.82 bcdef
0.5% SSL	0.338±0.021 abcde	0.039±0.010 bcde	2.56±0.57 bcd	0.374±0.038 b	32.59±3.73 abcd
1% SSL	0.302±0.015 ef	0.020±0.006 fg	1.44±0.46 ef	0.536±0.058 a	28.02±2.92 cdefg

[a]Different letters within the same column indicate values are significantly different ($p < 0.05$). At least 8 replicates were analyzed per treatment for stickiness test. At least 11 replicates were analyzed per treatment for the Kieffer test.
[b]Stickiness, work of adhesion, and dough cohesiveness as measured by the SMS/Chen-Hoseney dough stickiness test
[c]R_{max} (resistance to extension) and E_{Rmax} (extensibility) as measured by Kieffer dough and gluten extensibility test

Table 5.3 Weight, volume, specific volume, and moisture content for whole wheat bread with added emulsifiers

Treatment	Wt (g)	Vol (cm^3)	Specific Vol (cm^3/g)	Day 1 Moisture Content (% wb)	Day 3 Moisture Content (% wb)	Day 7 Moisture Content (% wb)	Moisture loss between Day 1 and 7 (%)
Control	155.84±0.99	651±16 cde	4.18±0.13 cd	44.44±0.25 cde	44.15±0.40 abc	40.37±0.55 cd	9.16
0.2% soy lecithin	158.19±1.28	686±35 abcd	4.34±0.25 abcd	44.86±0.33 abc	44.54±0.39 ab	41.38±0.33 ab	7.76
0.5% soy lecithin	157.93±0.75	719±27 abc	4.55±0.18 abcd	45.09±0.21 a	44.54±0.84 ab	41.38±0.24 ab	8.23
1% soy lecithin	160.03±0.82	686±19 abcd	4.29±0.13 bcd	45.11±0.24 a	44.86±0.41 a	41.69±0.27 a	7.58
0.2% DATEM	155.90±0.96	664±25 bcde	4.26±0.18 bcd	44.36±0.18 def	43.99±0.50 bcd	40.31±0.61 cd	9.13
0.5% DATEM	156.88±0.83	660±23 bcde	4.21±0.14 bcd	44.53±0.15 bcd	44.07±0.39 bcd	40.83±0.48 bc	8.32
1% DATEM	159.51±0.39	649±11 de	4.07±0.07 d	44.92±0.12 ab	44.65±0.37 ab	41.43±0.56 ab	7.79
0.2% sucrose esters	153.99±1.25	671±34 abcde	4.36±0.25 abcd	43.95±0.20 fg	43.56±0.28 cde	39.40±0.54 ef	10.35
0.5% sucrose esters	153.50±0.57	701±30 abcd	4.57±0.21 abcd	43.77±0.41 gh	43.55±0.27 cde	39.15±0.49 ef	10.55
1.0% sucrose esters	155.15±0.91	705±29 abcd	4.54±0.21 abcd	43.89±0.21 fgh	43.38±0.60 de	39.55±0.51 def	9.87
0.2% polysorbate 80	153.50±0.72	721±19 ab	4.70±0.14 ab	44.04±0.34 efg	43.67±0.39 cde	39.39±0.58 ef	10.56
0.5% polysorbate 80	152.89±1.04	740±23 a	4.84±0.18 a	43.85±0.58 gh	43.45±0.58 cde	39.08±0.54 ef	10.86
1% polysorbate 80	154.29±0.46	724±23 ab	4.69±0.16 abc	43.95±0.22 fg	43.41±0.45 cde	39.12±0.50 ef	11.00
0.2% SSL	153.75±1.20	649±44 de	4.22±0.32 bcd	44.02±0.15 efg	43.75±0.22 cde	39.81±0.49 de	9.56
0.5% SSL	153.48±0.82	640±35 de	4.17±0.25 d	43.91±0.19 fg	43.68±0.16 cde	39.85±0.37 de	9.25
1% SSL	153.68±1.14	626±37 e	4.08±0.27 d	43.43±0.15 h	42.99±0.14 e	38.92±0.56 f	10.39

[a]Different letters within the same column indicate values are significantly different ($p < 0.05$). Four loaves were prepared per treatment. Moisture content is average of eight replicates.

Table 5.4 Crumb structure analysis of whole wheat bread with added emulsifiers

Treatment	Number of Cells	Cell Wall Thickness (mm)	Cell Diameter (mm)
Control	3873±84 abc	0.403±0.002 c	1.64±0.06 c
0.2% soy lecithin	3953±232 ab	0.410±0.005 bc	1.70±0.06 abc
0.5% soy lecithin	3987±196 a	0.410±0.006 bc	1.74±0.10 abc
1% soy lecithin	3932±231 ab	0.408±0.008 bc	1.67±0.08 bc
0.2% DATEM	3793±114 abc	0.409±0.005 bc	1.71±0.07 abc
0.5% DATEM	3731±152 abc	0.411±0.005 bc	1.70±0.09 abc
1% DATEM	3735±40 abc	0.410±0.003 bc	1.67±0.03 bc
0.2% sucrose esters	3586±199 abc	0.418±0.002 ab	1.76±0.04 abc
0.5% sucrose esters	3596±138 abc	0.421±0.003 ab	1.82±0.06 abc
1.0% sucrose esters	3527±119 bc	0.428±0.004 a	1.88±0.06 a
0.2% polysorbate 80	3863±109 abc	0.416±0.004 abc	1.79±0.08 abc
0.5% polysorbate 80	3761±100 abc	0.419±0.005 ab	1.83±0.07 ab
1% polysorbate 80	3683±57 abc	0.419±0.004 ab	1.85±0.10 ab
0.2% SSL	3682±301 abc	0.412±0.005 bc	1.75±0.09 abc
0.5% SSL	3467±224 c	0.417±0.007 ab	1.78±0.05 abc
1% SSL	3453±295 c	0.417±0.010 ab	1.71±0.11 abc

[a]Different letters within the same column indicate values are significantly different ($p < 0.05$). Four replicates were analyzed per treatment.

Table 5.5 Texture profile analysis of control and emulsifier-supplemented whole wheat bread after 1d storage at 22 °C

Treatment	Hardness, N	Resilience, %	Cohesion	Springiness, %	Chewiness, N	Day 3 Hardness, N	Day 7 Hardness, N	Slope: rate of firming	R^2
Control	4.07±0.55 ab	33.43±1.45 bcd	0.697±0.013 abc	94.86±0.77 abcd	2.69±0.30 a	5.65±0.56 a	9.04±0.75 a	0.83	0.93
0.2% soy lecithin	3.73±0.49 abcd	35.17±1.93 ab	0.707±0.016 ab	95.61±1.10 ab	2.51±0.26 abc	5.23±0.49 ab	8.10±0.77 abcd	0.73	0.91
0.5% soy lecithin	3.32±0.50 bcdef	37.18±1.29 a	0.724±0.014 a	96.16±1.03 a	2.30±0.29 abcde	4.78±0.47 abc	7.50±0.63 bcde	0.69	0.92
1% soy lecithin	3.95±0.45 abc	35.88±1.39 ab	0.707±0.014 ab	96.16±0.84 a	2.68±0.25 a	5.42±0.41 a	8.38±0.85 abc	0.74	0.91
0.2% DATEM	3.89±0.54 abcd	33.46±1.74 bcd	0.700±0.015 ab	94.50±1.05 abcd	2.57±0.29 ab	5.36±0.37 a	8.93±0.73 ab	0.85	0.94
0.5% DATEM	3.83±0.58 abcd	33.63±1.91 bcd	0.700±0.018 ab	95.13±1.13 abc	2.54±0.33 ab	5.49±0.38 a	8.55±0.94 abc	0.78	0.90
1% DATEM	4.22±0.65 a	32.30±1.69 cdef	0.682±0.018 bcd	94.38±0.43 abcd	2.71±0.37 a	5.57±0.33 a	8.66±0.59 abc	0.75	0.93
0.2% sucrose esters	3.59±0.47 abcd	32.43±1.62 cd	0.702±0.014 ab	93.79±0.68 bcde	2.36±0.27 abcd	5.01±0.75 abc	8.33±0.97 abc	0.80	0.89
0.5% sucrose esters	3.27±0.64 bcdef	29.73±1.30 fg	0.685±0.012 bcd	92.00±1.10 ef	2.00±0.31 defg	4.21±0.51 cde	7.45±1.38 bcde	0.71	0.80
1.0% sucrose esters	3.02±0.61 def	27.14±1.15 gh	0.670±0.011 cde	90.99±0.69 f	1.83±0.34 efg	3.84±0.47 de	6.24±0.71 ef	0.55	0.85
0.2% polysorbate 80	3.09±0.24 cdef	33.79±1.09 bc	0.708±0.011 ab	94.61±0.53 abcd	2.07±0.14 cdefg	4.40±0.25 bcd	7.52±0.73 abcde	0.75	0.95
0.5% polysorbate 80	2.67±0.42 ef	32.42±1.37 cde	0.708±0.018 ab	94.72±0.91 abcd	1.79±0.27 fg	3.90±0.46 de	6.71±0.56 def	0.68	0.93
1% polysorbate 80	2.55±0.27 f	29.76±1.03 efg	0.687±0.011 bc	93.16±0.97 de	1.63±0.16 g	3.48±0.26 e	5.90±0.55 f	0.57	0.94
0.2% SSL	3.75±0.50 abcd	31.04±2.19 def	0.693±0.028 bc	93.36±1.94 cde	2.41±0.21 abcd	5.07±0.77 abc	8.69±1.22 abc	0.84	0.86
0.5% SSL	3.62±0.45 abcd	26.48±0.84 h	0.658±0.007 de	90.16±1.09 f	2.14±0.24 bcdef	4.40±0.55 bcd	7.53±0.82 abcde	0.66	0.87
1% SSL	3.48±0.47 abcde	23.31±1.63 i	0.648±0.023 e	88.24±1.62 g	1.98±0.19 defg	4.32±0.58 cde	7.40±1.15 cdef	0.67	0.83

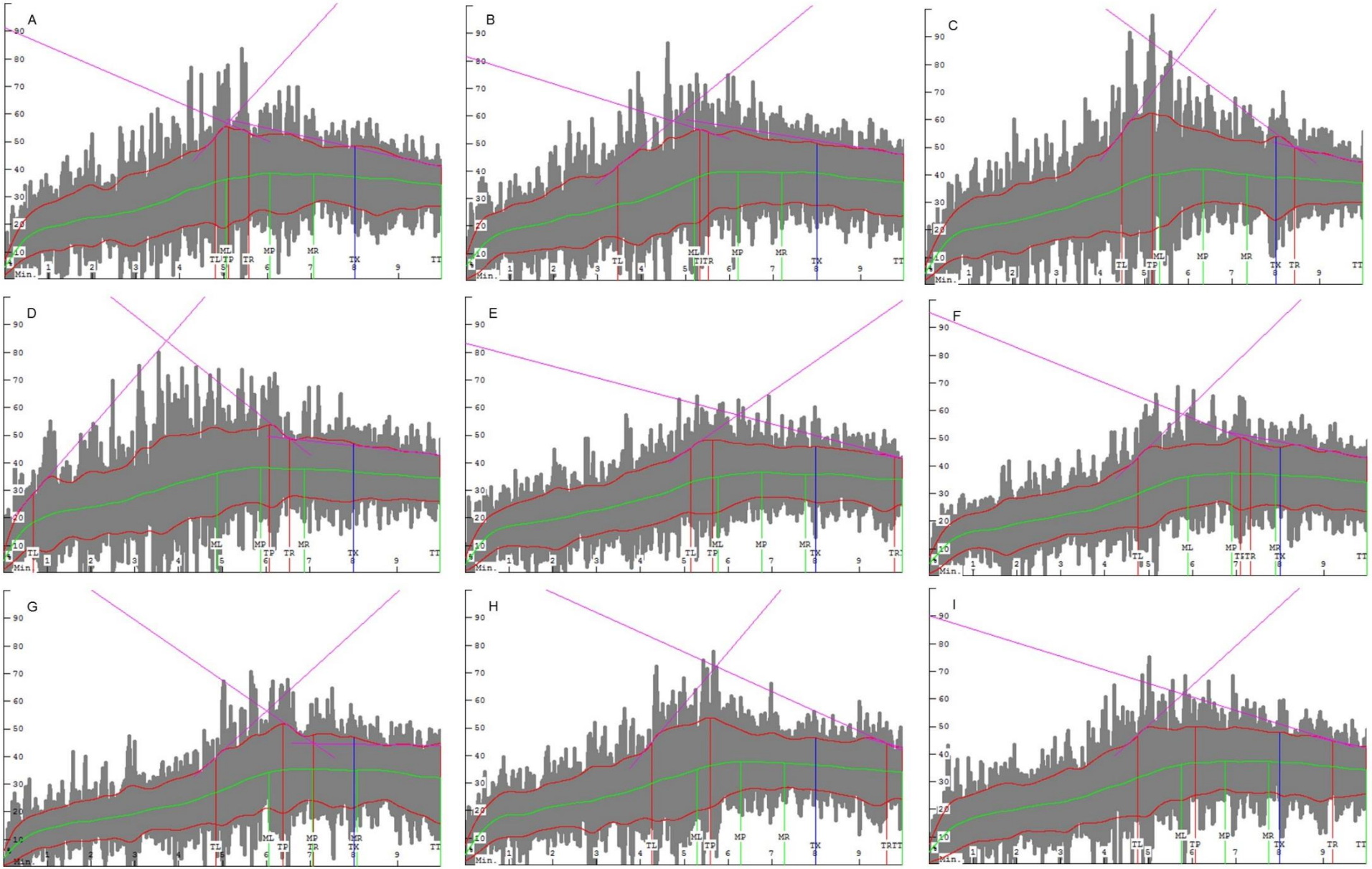
[a]Different letters within the same column indicate values are significantly different ($p < 0.05$). Eight replicates were analyzed per treatment.

Table 5.6 Retrogradation parameters for control and emulsifier-supplemented whole wheat bread stored at 22 °C

Treatment	Tm_1 onset (°C)	Tm_1 peak (°C)	ΔH_1 (J/g)	Tm_2 onset (°C)	Tm_2 peak (°C)	ΔH_2 (J/g)
Control	53.53 a	65.50	1.356 de	101.63 bc	119.70 a	1.520 def
0.2% soy lecithin	52.77 ab	64.43	1.642 cd	101.38 bc	116.38 abcd	1.192 efg
0.5% soy lecithin	52.20 b	63.63	1.735 bcd	98.93 c	114.44 d	0.931 g
1% soy lecithin	52.73 ab	64.55	1.585 cde	99.08 c	114.74 bcd	1.143 fg
0.2% DATEM	52.70 ab	65.23	1.504 cde	103.03 bc	118.29 abcd	1.193 efg
0.5% DATEM	52.08 b	63.31	1.821 bc	98.93 c	114.57 cd	1.011 g
1% DATEM	52.62 ab	65.14	1.591 cde	99.81 c	114.60 bcd	1.145 efg
0.2% sucrose esters	52.55 ab	65.01	1.581 cde	102.94 bc	119.05 ab	1.439 def
0.5% sucrose esters	52.76 ab	66.25	2.121 ab	106.65 ab	119.75 a	1.674 cd
1.0% sucrose esters	52.74 ab	66.56	1.593 cde	106.32 ab	120.35 a	2.418 b
0.2% polysorbate 80	52.44 ab	64.94	1.731 bcd	101.71 bc	119.78 a	1.554 de
0.5% polysorbate 80	52.40 ab	64.39	1.689 cd	103.20 abc	119.53 a	1.626 d
1% polysorbate 80	52.33 ab	64.62	1.816 bc	101.96 bc	120.25 a	2.068 bc
0.2% SSL	51.99 b	65.54	2.366 a	103.95 abc	118.94 abc	1.646 d
0.5% SSL	52.29 ab	64.39	1.221 ef	106.54 ab	119.66 a	2.142 b
1% SSL	52.50 ab	66.34	0.859 f	108.48 a	119.96 a	2.929 a

[a]Different letters within the same column indicate values are significantly different ($p < 0.05$). Tests were performed in duplicate.

5.7 Figures



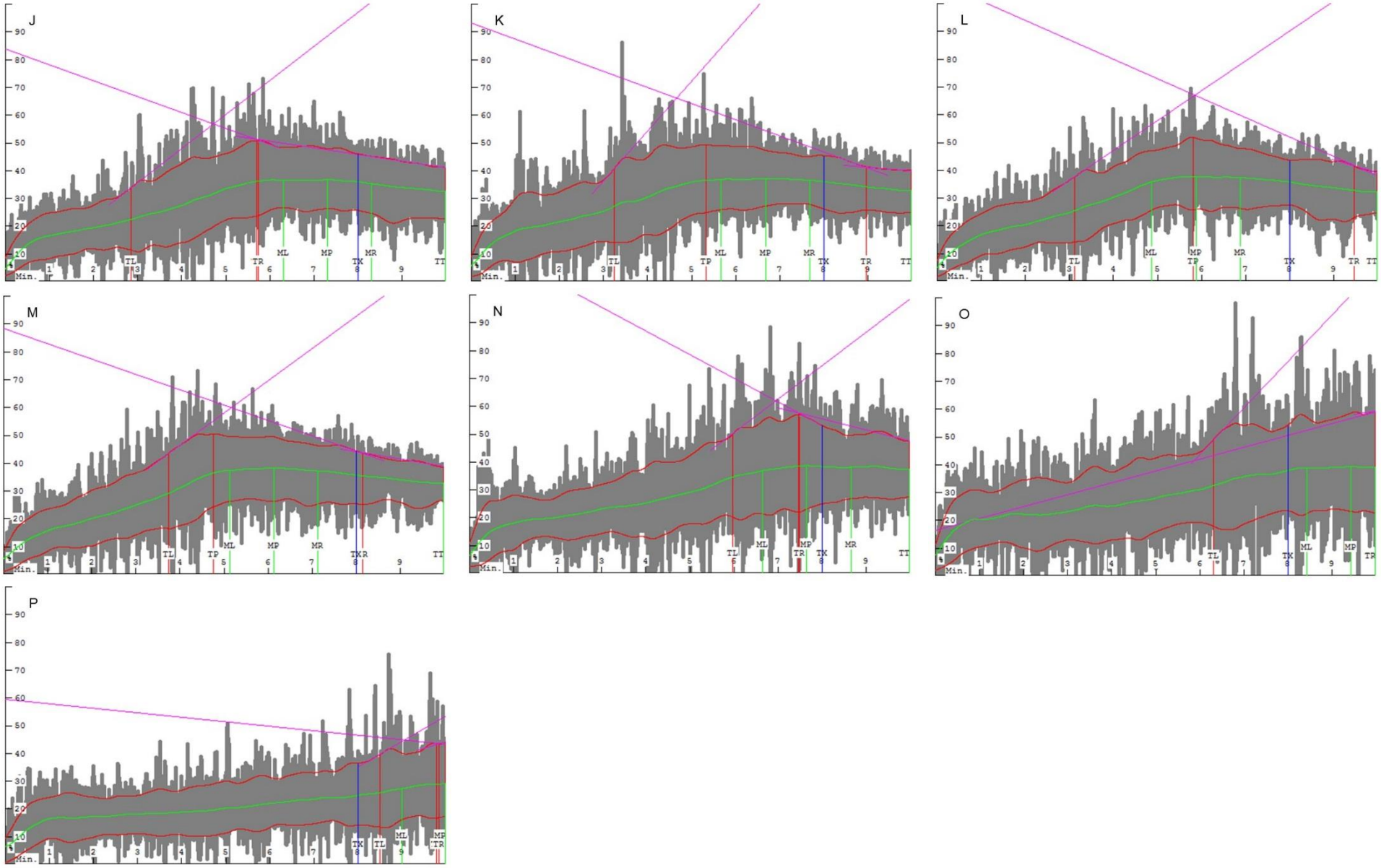


Figure 5.1A-P. Typical mixographs of whole wheat dough with different types and levels of emulsifiers.
A: control, B: 0.2% soy lecithin, C: 0.5% soy lecithin, D: 1.0% soy lecithin, E: 0.2% DATEM, F: 0.5% DATEM, G: 1.0% DATEM, H: 0.2% sucrose esters, I: 0.5% sucrose esters, J: 1.0% sucrose esters, K: 0.2% polysorbate 80, L: 0.5% polysorbate 80, M: 1.0% polysorbate 80, N: 0.2% SSL, O: 0.5% SSL, P: 1.0% SSL

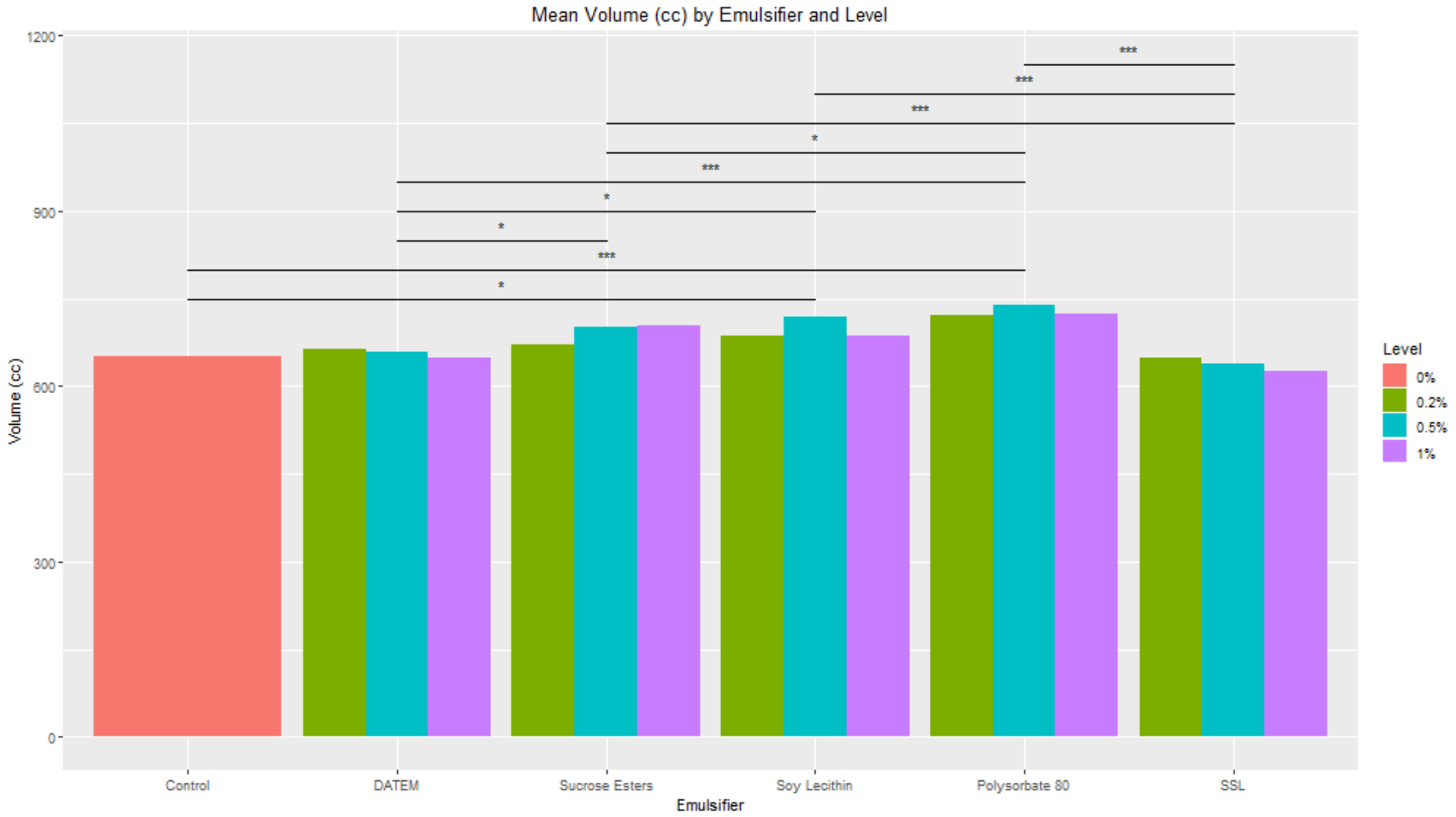


Figure 5.2 Effect of emulsifier type on loaf volume of whole wheat bread.
Significance testing was done between emulsifiers and does not include comparisons for emulsifiers across levels. Significance of comparisons indicated by * = 0.05 to 0.01, ** = 0.01 to 0.001, and *** = 0.001 and lower

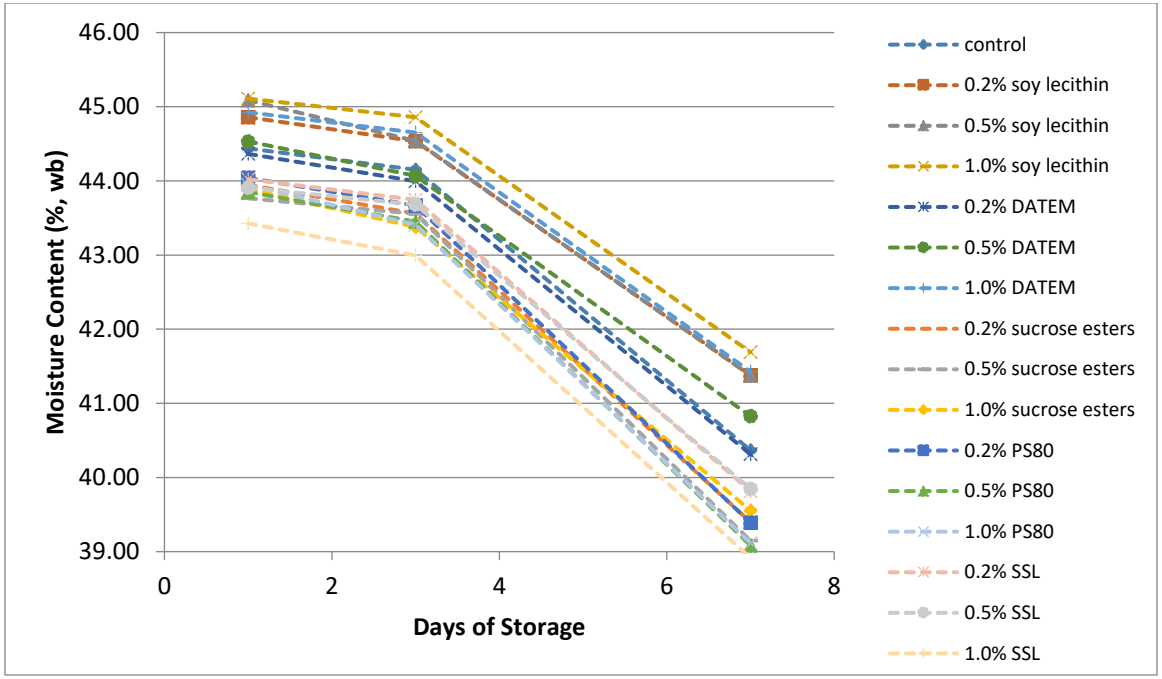


Figure 5.3 Change in moisture content over 7 days of storage for whole wheat bread with added emulsifiers

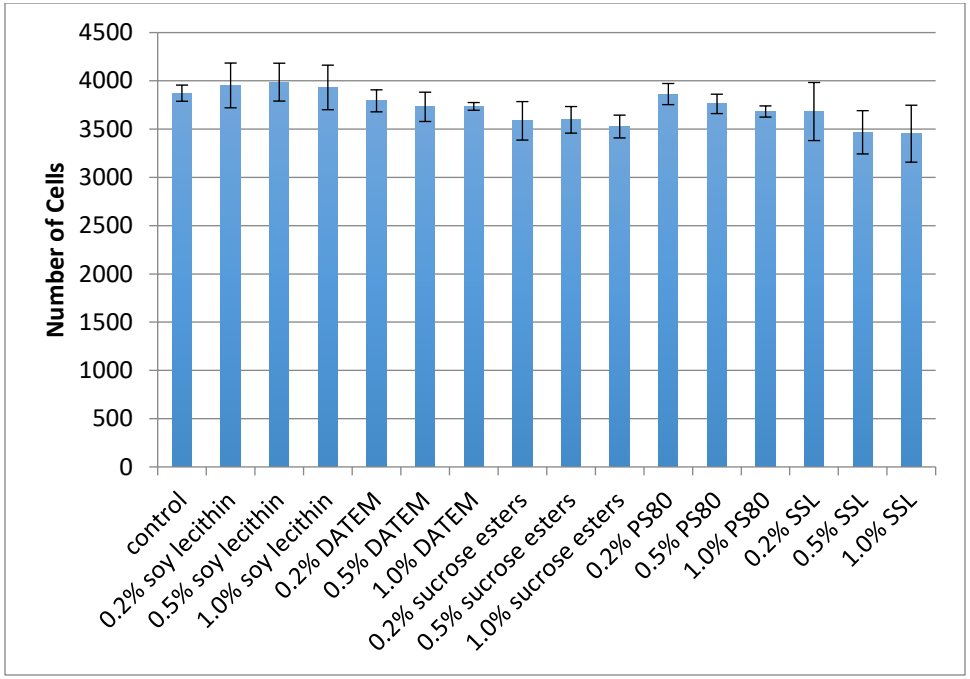


Figure 5.4 Number of cells for whole wheat bread with added emulsifiers as measured by C-cell image analysis on central slice

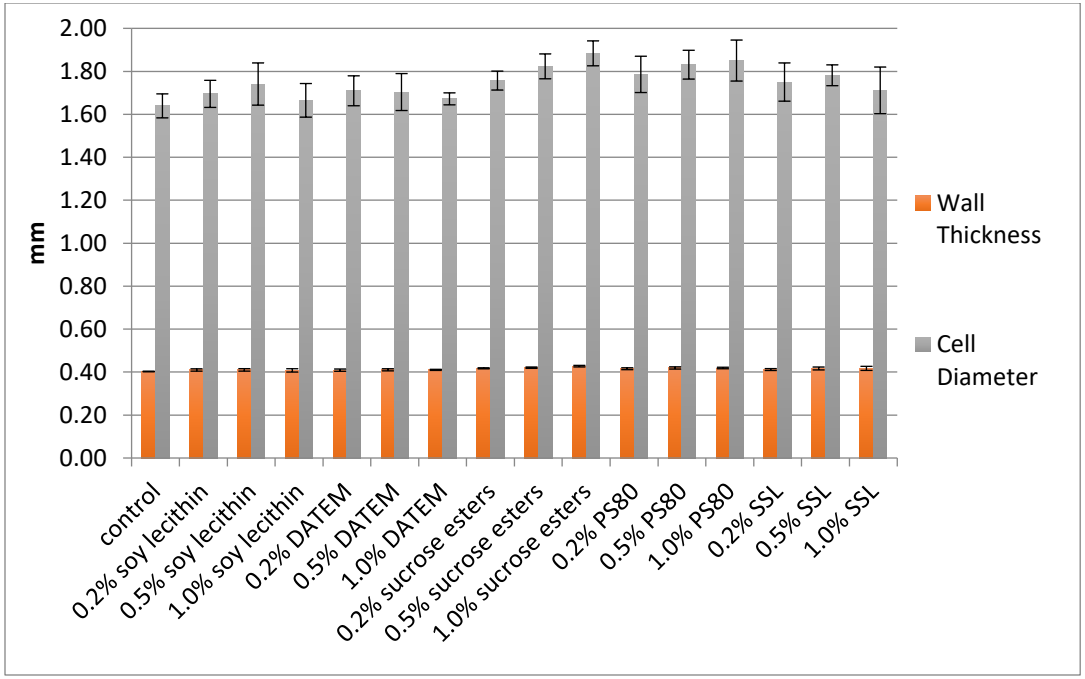


Figure 5.5 Cell wall thickness and cell diameter for whole wheat bread with added emulsifiers as measured by C-cell image analysis on central slice

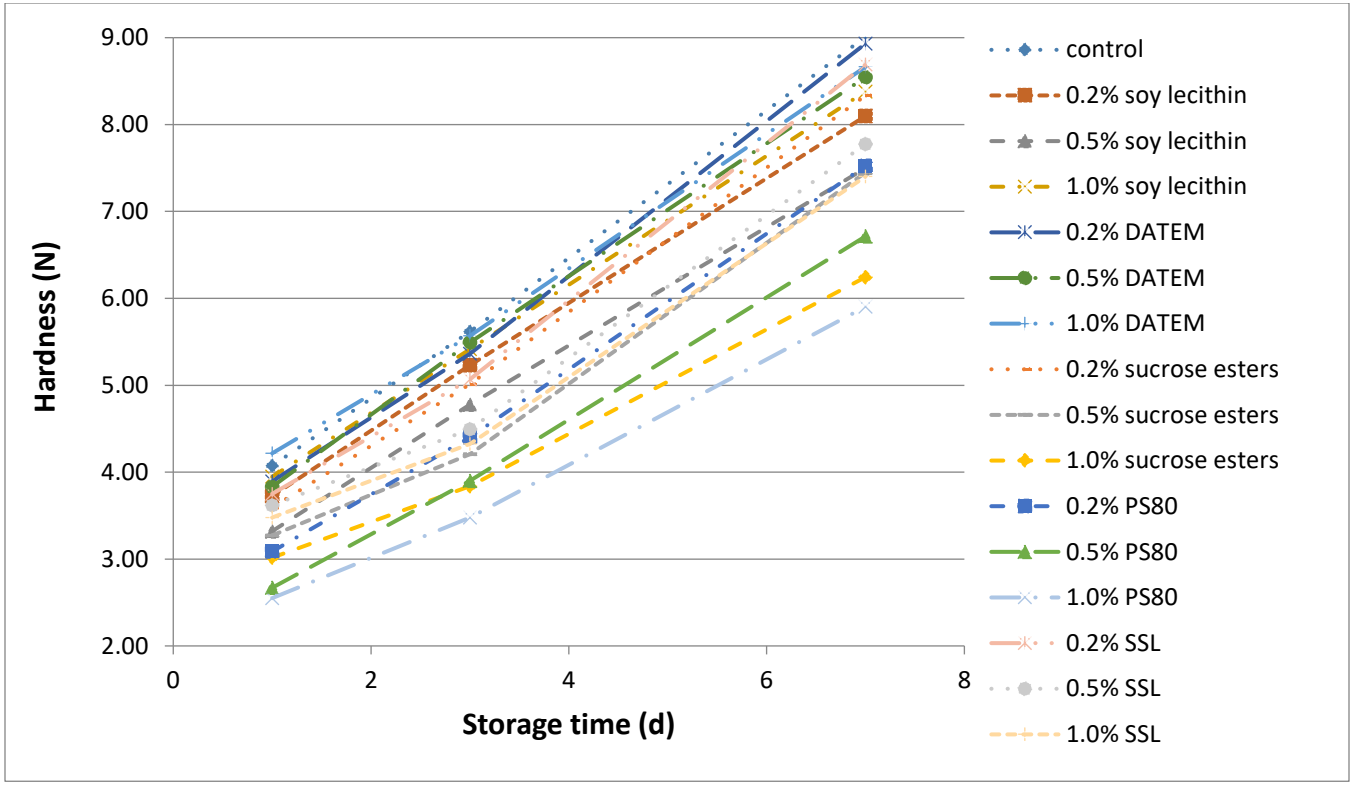


Figure 5.6 Change in crumb hardness over 7 days of storage for whole wheat bread with added emulsifiers

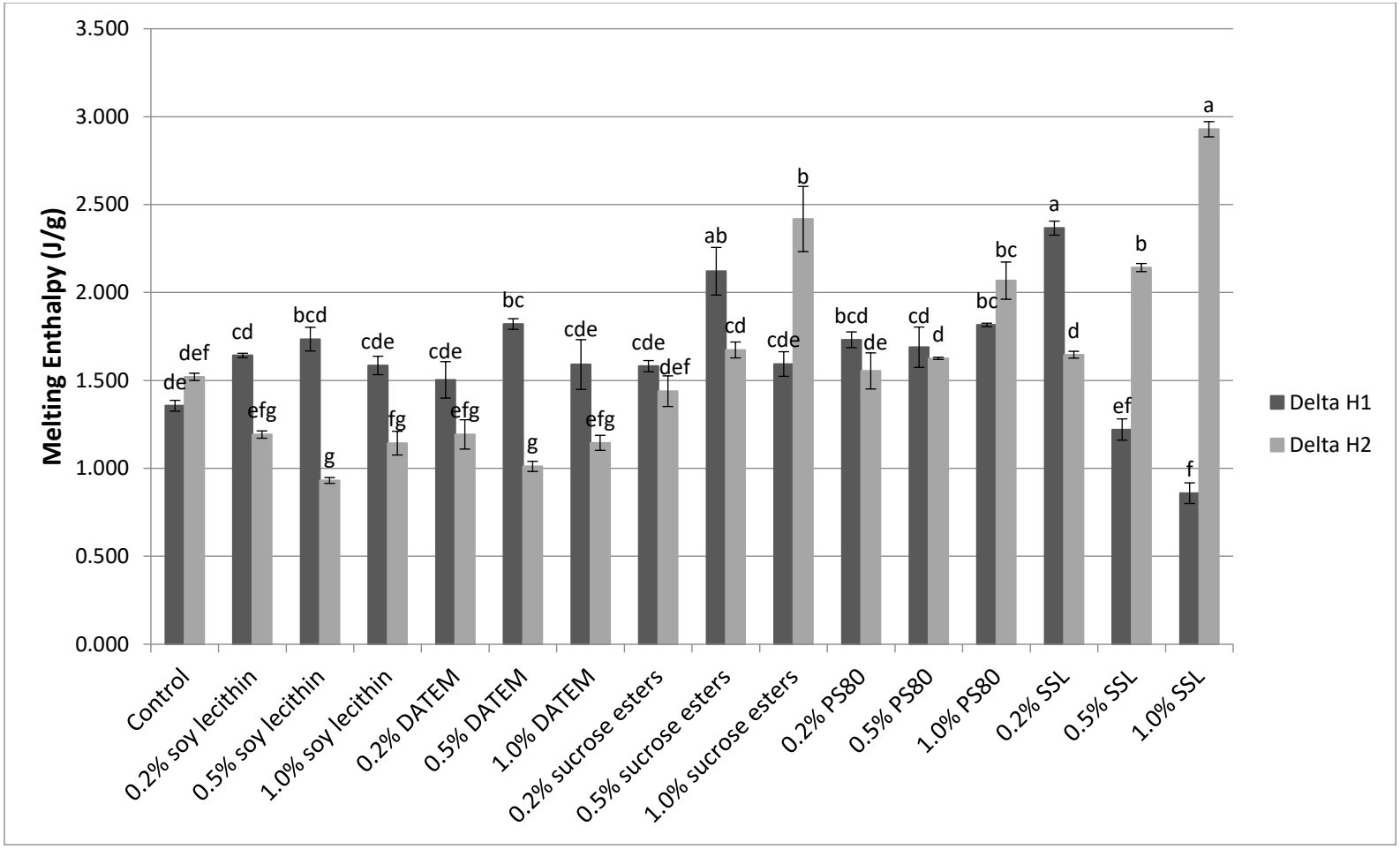


Figure 5.7 Melting enthalpies from DSC thermograms of bread stored for 7 days.
Different letters within the same series are significantly different ($p < 0.05$). Tests were performed in duplicate.

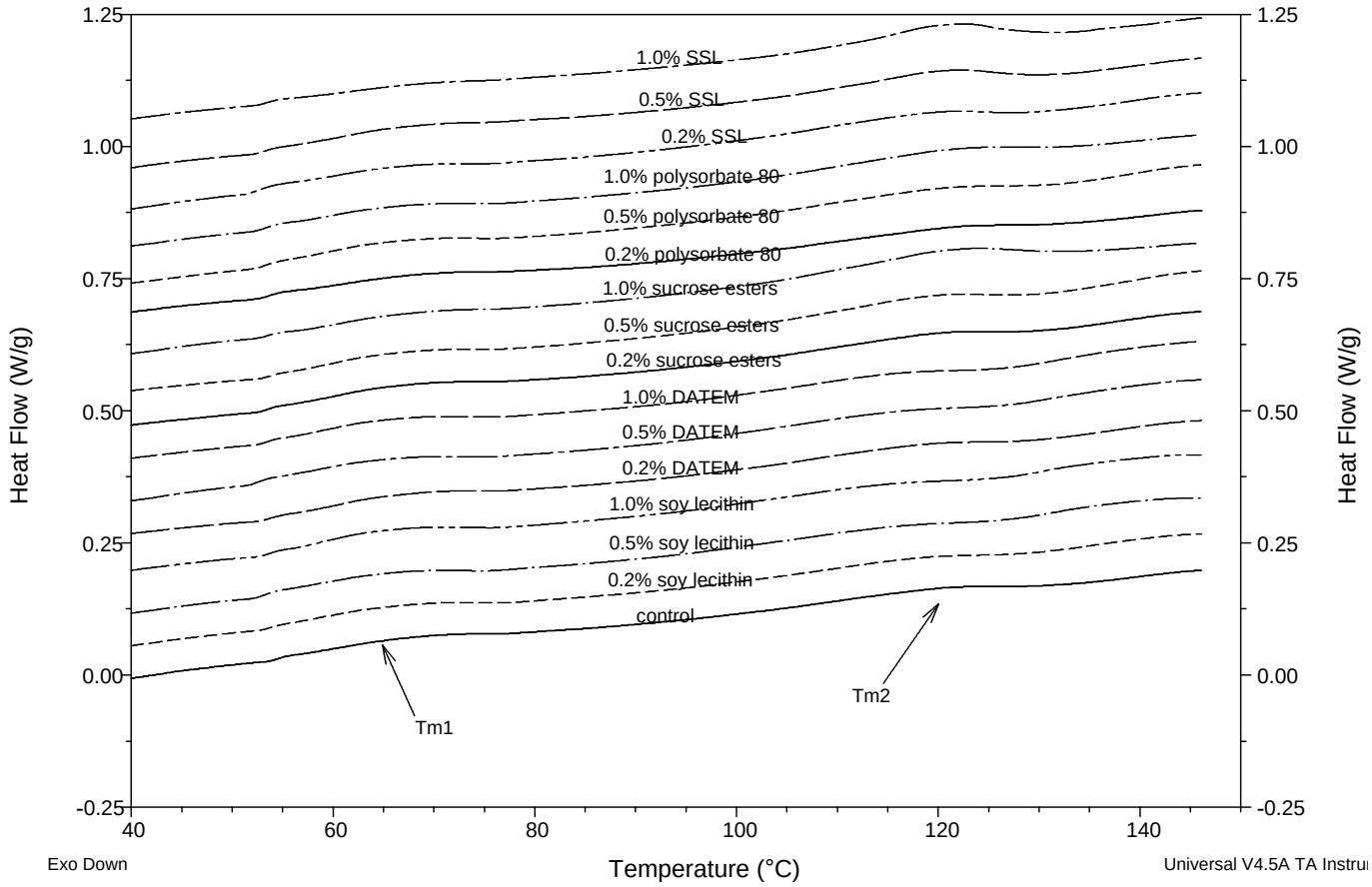


Figure 5.8 DSC thermograms of whole wheat bread with added emulsifiers stored for 7 days

6 Conclusions and recommendations for future work

This work demonstrated the ability of enzymes, hydrocolloids, and emulsifiers to improve the loaf volume and decrease the crumb hardness and staling of whole wheat bread. Of the enzymes, xylanase resulted in the greatest increase in loaf volume and also decreased crumb hardness and staling. Of the five enzymes evaluated, maltogenic α-amylase was the most effective anti-staling agent, and also significantly increased loaf volume. HPMC was determined to be the best hydrocolloid for improving loaf volume of whole wheat bread while maintaining good dough handling properties. CMC was not effective at increasing loaf volume or decreasing hardness or the rate of crumb firming. Of the five emulsifiers tested, soy lecithin and polysorbate 80 increased loaf volume. All emulsifiers decreased the rate of crumb firming, even if they did not improve loaf volume. From these three sets of studies, no clear relationship was found between amylopectin retrogradation and crumb firming. Crumb hardness on Day 1 was not an indicator of crumb hardness on Day 7.

Future studies could examine the combination of different types of enzymes, such as xylanase and amylases, in order to further increase loaf volume while also decreasing staling. Combinations of different classes of improvers could be evaluated using response surface methodology to provide the greatest possible loaf volume. In order to better understand the mechanisms of the effects on volume and crumb firming, future work may examine some of the proposed interactions of the improvers with starch and gluten, and how those influence bread staling. Furthermore, the interactions with the components of wheat bran and germ should be investigated, in order to explain the differences in effects on whole wheat dough and bread compared to reported results in dough and bread made from refined flour.

Appendix A - Statistical analysis code

A.1 SAS: Dunnett's test for multiple comparisons with a single control

```
proc glm data =baking;
class treatment;
model vol = treatment;
means treatment;
means treatment/ dunnett('none/none');
lsmeans treatment / PDIFF=control('none/none');
run;
```

A.2 SAS: Tukey's test for pairwise comparisons

```
proc glm data=TPA plots=none;
class treatment;
model D3Hard=treatment;
means treatment;
lsmeans treatment / stderr pdiff adjust=tukey;
means treatment / tukey lsd lines;
run;
```

A.3 SAS: Simple linear regression analysis for rate of firming

```
proc reg data=control;
model hardness=day;
run;
```

A.4 R code for analyzing volumes of emulsifier treatments

```
############################
 ###           ###
 ### LT: Vol Study Plot ###
 ###           ###
 ############################

 ####################################
 ### Install Any Missing Packages ###
 ####################################

 my.packages <- installed.packages()[,1] # All packages currently installed
 needed.packages <- c("readr","readxl","ggplot2","ggsignif","tidyr") # Those needed for this analysis
 ind.need <- !needed.packages%in%my.packages # Which of the needed are we missing?
 if(sum(ind.need)!=0){install.packages(needed.packages[ind.need])} # Install them

 ################
 ### Packages ###
```

```
 ################

 library(readr) # General package to read in data
 library(readxl) # Specifically reads in excel data
 library(ggplot2) # Package for nicer plots
 library(ggsignif) # Creates the significance indicator level for the plots
 library(tidyr) # Data manipulation package, used to help convert from wide to long data

 ################
 ### Raw Data ###
 ################

 dir <- "C:/Users/marka/Desktop/LT_LVS/Data/" # Where you are storing your data. This is also where the
folder that stores the results will be placed
 dat <- read_excel(paste0(dir,"vol_data.xlsx")) # Loads the Raw Data

 ########################
 ### Data Processing ###
 ########################

 dat$emul <- tolower(sapply(strsplit(dat$`Flour ID`," "),function(x){paste(x[length(x)])},simplify = TRUE)) #
Extract the emuslifier
 dat$level <- sapply(strsplit(dat$`Flour ID`," "),function(x){paste(x[1])},simplify = TRUE) # Extract the level
 dat$level <- ifelse(dat$level=="ww","0%",dat$level) # Convert the control level to something similar to the
other levels
 dat$level[dat$level=="1.0%"] <- "1%" # Consistency issue fix

 colnames(dat) <- c("row.num","emul.level","bake1","bake2","bake3","bake4","bake5","emul","level") #
Change data col names for easier programming

 ########################
 ### Plot Parameters ###
 ########################

 my.plot.title <- "Mean Volume (cc) by Emulsifier and Level" # Title for the plot
 my.x.axis <- "Emulsifier" # Label for the x-axis
 my.y.axis <- "Volume (cc)" # Label for the y-axis
 my.legend.title <- "Level" # Title for the legend

 centered.title <- TRUE # TRUE if you want the plot titled centered, FALSE if you would like the title left-
adjusted
 theme.black.and.white <- FALSE # TRUE if you want the plot to be in the traditional black and white
style, FALSE if you would like the plot to be generated with colors

 plot.width <- 1000 # Plot width in pixels
 plot.height <- round(plot.width*(9/16)) # Plot height in pixels. This calculation is based on the 16:9 aspect
ratio.


##########################################################################################
########################################

##########################################################################################
########################################
 ########################################## NOTHING UNDER HERE SHOULD BE EDITED
##########################################
```

```
#########################################################################################
#####################################

#########################################################################################
#####################################

 ####################################
 ### Convert Wide Data to Long Data ###
 ####################################
 dat.long <- gather(dat,bake,vol,bake1:bake5,factor_key = TRUE) # Convert wide data to long data (each
row represents exactly on volume measurement)

 ##############################
 ### Additional Processing ###
 ##############################
 dat.long <- dat.long[,c("emul","level","bake","vol")] # Only keep these columns
 dat.long <- dat.long[order(dat.long$emul,dat.long$level,dat.long$bake),] # Sort by Emulsifier then level
then bake
 dat.long$emul.level <- factor(paste0(dat.long$emul,"-",dat.long$level)) # Trick to make emul-level groups

 dat <- dat[,c("emul","level","bake1","bake2","bake3","bake4","bake5")] # Only keep these columns
 dat <- dat[order(dat$emul,dat$level),] # Sort by Emulsifier then level

 ##############################
 ### Compute Volume Means ###
 ##############################
 dat$mean.vol <- apply(dat[,paste0("bake",1:5)],1,function(x){mean(x,na.rm = TRUE)}) # Compute the
mean volume for each emul-level combination

 ###################################
 ### Tukey-Multiple Comparisons ###
 ###################################
 mod.anova <- aov(data = dat.long,vol~emul) # Fit the ANOVA model
 MC.adj <- TukeyHSD(mod.anova)$`emul` # Perform Tukey Multiple Comparisons
 what.compare <- MC.adj[,1] # What was compared?
 p.val <- unname(MC.adj[,4]) # Adjusted p-values
 star.annotations <- ifelse(p.val>0.05,"",
                        ifelse(p.val>0.01,"*",
                               ifelse(p.val>0.001,"**","***"))) # vvv See below vvv

 #----------------------------#
 #                            #
 # * ==> 0.01 < p.val < 0.05   #
 # ** ==> 0.001 < p.val < 0.01 #
 # *** ==> p.val < 0.001       #
 #                            #
 #----------------------------#

 ######################
 ### Plot Metadata ###
 ######################
 stars.compare <- star.annotations[which(star.annotations!="")] # Which actually have sig?
 compare <- strsplit(names(what.compare[which(star.annotations!="")]),"-") # Which comparisons do we
care about?
```

```
#######################
### Bar Chart Plot ###
#######################

#-------#
# NOTE! #
#-------#

#-------------------------------------------#
# Be sure that you save a copy of this code   #
# somewhere before editing. If you are unsure #
# how ggplot2 syntax works I would recommend  #
# that you do NOT edit the following lines of #
# code.                                       #
#-------------------------------------------#

#-------#
# NOTE! #
#-------#

test.plot <- ggplot(data = dat,aes(x=emul,y=mean.vol))+
  geom_bar(aes(fill=level),stat = "identity",position = "dodge") # Base Bar Chart Plot

for(i in 1:length(stars.compare)){ # For each significant comparison...
  test.plot <- test.plot+geom_signif(comparisons = list(compare[[i]]),annotations = stars.compare[i],
                     tip_length = 0.0,y_position = 750+((i-1)*50)) # ...add on the comparison line...
} # ...fin

test.plot <- test.plot+labs(x=my.x.axis,y=my.y.axis)+ # Change X and Y axis labels
                  ggtitle(label = my.plot.title)+ # Add plot title
                  scale_x_discrete(labels = c("Control","DATEM","Sucrose Esters","Soy
Lecithin","Polysorbate 80","SSL"))+ # Change x-axis group labels
                  scale_fill_discrete(name = my.legend.title) # Change legend title

if(centered.title){test.plot <- test.plot+theme(plot.title = element_text(hjust = 0.5))} # Colored theme with
centered title
if(theme.black.and.white){test.plot <- test.plot+theme_bw()+scale_fill_grey()} # Black and white theme
with left-adjusted title
if(theme.black.and.white & centered.title){test.plot <- test.plot+theme_bw()+theme(plot.title =
element_text(hjust = 0.5))+scale_fill_grey()} # Black and white theme with centered title

#-------------------------------------------------------------#
# Hello future reader of this code (possibly myself),       #
# Do note that I understand the above set of if statements  #
# are order dependent (that is they cannot be rearranged),  #
# I just didn't want to deal the if-else logic this morning, #
# and this does what it is supposed to do.                  #
#                                      - NM                 #
#-------------------------------------------------------------#

setwd(dir) # Put me into the working directory
plot.folder.here <- "BAR_CHART_PLOTS"%in%dir() # Check to see if the bar chart plots directory is
already there
if(!plot.folder.here){dir.create(paste0(dir,"BAR_CHART_PLOTS"))} # If it is not there, then create it
setwd(paste0(dir,"BAR_CHART_PLOTS")) # Go to the plot directory
```

```
 png.titles <-
c("vol_bar_charts_Color_LeftTitle.png","vol_bar_charts_Color_CenterTitle.png","vol_bar_charts_BlackWh
ite_LeftTitle.png","vol_bar_charts_BlackWhite_CenterTitle.png") # Possible plot titles
 png.title.ind <- 2*theme.black.and.white+1*centered.title+1 # Map the possible binary plot parameter
indicators onto indexable integers
 my.png.title <- png.titles[png.title.ind] # Select the correct title

 #-------------------------------------------------#
 # Hello future reader...again,                    #
 # What I just did is the solution to the          #
 # above order dependence issue. Note that this    #
 # solution is not scalable. So be wary of exactly #
 # how many plot parameters you include!           #
 #-------------------------------------------------#

 png(my.png.title,width = plot.width,height = plot.height) # Initalize image file
 test.plot # insert our plot
 dev.off() # close it off. That's it!
```