IMPROVING FROZEN BREAD DOUGH QUALITY THROUGH PROCESSING AND INGREDIENTS

by

JINHEE YI

(Under the Direction of William L. Kerr)

ABSTRACT

The quality of bread made from frozen dough is diminished by changes that occur during the freezing process. The effects of processing conditions and levels of waxy wheat flour (WWF) and water on the quality of frozen dough and bread were investigated. As for processing condition effect, Yeasted bread dough was frozen using four freezing rates, then stored at -10, -20, -30, or -35°C for 30, 60, 90, and 180 days. The properties of the resulting yeasted frozen doughs were measured using nuclear magnetic resonance techniques (NMR), cryo-scanning electron microscopy (SEM), and texture analysis. Specifically, bread staling, a physical property of the bread, was measured using NMR, texture analysis and color analysis. The yeast activity of dough was assessed by measurements of gas production. Our results indicate that dough strength diminishes with time as well as with increasing storage temperature. Cryo-SEM demonstrated that frozen dough stored at -30 and -35°C displayed the least damage to dough structure. NMR studies showed that frozen dough at lower storage temperatures had lower T_2 values (9-10 ms). However, dough stored at higher temperatures displayed higher yeast activity. Baking tests showed that the loaf volume and weight gradually decreased with storage time, and that bread made from dough stored at -20°C resulted in the highest loaf volume. Breads made from dough stored at -30 and -35°C displayed less change in the texture profile during storage as well as less change in T_2 values. As for WWF, the quality of dough and bread quality was investigated. Stickiness of dough increased with higher content of WWF. Dough with higher content of WWF and 60% level of water added were more extensible. NMR studies showed that frozen dough with higher WWF content had lower T_2 values (9-10 ms). Bread made from 15% and 30% WWF had higher specific volume. The results of T_2 value change ratio and firmness showed that bread with higher WWF content had slower rates of staling. Bread made from the combination of 15% or 30% waxy wheat flour and 60% water content had more even distribution of color and higher volume of bread.

INDEX WORDS: Frozen dough, Waxy wheat flour, Freezing rate, Frozen storage temperature and time, Dough quality, Extensibility, Stickiness, Bread staling, Staling rate, Specific volume, Firmness, texture analysis, yeast activity, NMR, Cryo-Scanning Electron Microscopy, Color analysis

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DEDICATION

This dissertation is dedicated

to my family

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INTRODUCTION AND LITERATURE REVIEW

Purpose of the Study

The purpose of this research is to characterize the combination of freezing processing factors such as freezing rate, freezing time and storage time and to improve the quality of frozen bread dough. The use of frozen bread dough has increased in bakeries, supermarkets and restaurants all over the world. Thus, frozen bread dough is used widely in industrial bakeries to facilitate bread production and baking in retail shops. Thus, bread dough freezing allows consumers to get fresh bread at any time of day.

Production of frozen bread dough is basically the same as that of conventional unfrozen dough. Frozen bread dough is made after mixing. The dough is distributed and stored frozen state until it is thawed and baked. The use of frozen dough has advantages such as saving time, space, and equipment costs of retailer. However, there are many limits on the quality of frozen bread dough. The quality characteristics of baked bread from frozen dough are poorer than that of fresh baked bread. Moreover, the physical states changes of frozen dough during frozen storage may contribute to the quality of baked bread (Laaksonen and Roos, 2001). Freezing is based on the physical principles of separation of water from dough as a result of ice crystal formation at sub-zero temperatures. Reid (1994) pointed out that some damage occurs as ice develops and causes migration of water out of cells, or from one region to another. Several studies have reported that the quality of bread made from frozen dough has a smaller volume as compared to bread made from fresh dough.

The quality of bread made from frozen dough is influenced by wheat flour quality, dough improver and dough formulation, and fermentation, as well as processing parameters such as dough mixing time, freezing rate, storage duration, and thawing rate (Inoue and Bushuk, 1991; Neyreneuf and Vanderplaat, 1991; Inoue and Bushuk, 1992; Le Bail et al, 1999a; Maeda and

Morita, 2003). These factors may affect on dough rheology and on yeast activity, which results in poor retention of CO_2 and poor baking performance (Meryman, 1968; Neyreneuf and Vanderplaat, 1991; Le Bail et al, 1999a; Ribotta et al, 2003b).

Different ways to minimize the effect of freezing on doughs are suggested in the literature: find a new yeast strain more resistant to freezing, improve parts of the bread-making process, or use suitable additives and ingredients for frozen doughs. Thus, there have been many studies on improving the quality of frozen dough. However, few studies have been carried out on the combined effect of various treatments on the baking performances of unfrozen and frozen bread doughs.

In this work, I used various treatments to improve the quality of frozen dough and bread. The treatment factors will be processing conditions and partial addition of waxy wheat flour. The analyses used will be dough quality, bread quality, and bread staling.

Two main investigations are:

1. To characterize the combined effect of freezing rate, storage temperature and storage time on frozen bread dough and baked bread compared with unfrozen dough and to investigate the quality of frozen bread dough and of baked bread.
2. To determine the effect of partial addition of waxy wheat flour to improve the quality of frozen bread dough and baked bread and to optimize the proper combination of waxy wheat flour for the quality of unfrozen and frozen dough and the quality of bread.

Literature Review

Bread is an important staple of diet with significant nutritional benefits in the United States and many other countries. However, fresh bread has short shelf life, and numerous chemical and physical alterations, known as staling, occur during its storage period. The texture and flavor of fresh bread deteriorates rapidly, thus, it loses its freshness and crispiness, while crumb firmness and rigidity increase. The pleasant bread flavor disappears, and a stale taste can be felt. These preservation problems have required advanced technology for longer stable dough storage. Freezing is advantageous processing system to preserve the quality of dough and bread. Freezing dough has more benefits than freezing bread. With frozen dough, fresh bread can be produced in restaurant and retail shop (Ribotta et al, 2001; Ribotta et al, 2006).

1.1 Benefits of Frozen Bread Dough

Frozen dough technology began in the 1950s, and much work has been devoted to improving the quality of frozen yeasted dough since then. In 1990, frozen dough was used by more than 50% of in-store supermarket bakeries in the United States (Gelinas et al, 1989; Berglund et al, 1991). Recently frozen dough production has been increasing and the commercial use of frozen doughs has increased significantly in the past 10 years. This recent growing is mostly due to new marketing channels through the growing number of in-store supermarket bakeries and retail shops. Market is requiring benefits of centralized manufacturing. The baking industry has been under pressure to cut costs and improve productivity in the face of a declining labor pool of young, unskilled workers. Meanwhile, total labor costs rose proportionally with increased in-store bakery sales. Retail baking has confronted emerging difficulties. It is lower ingredient cost but higher utility cost and is labor intensive. There are broadening demands for skilled bakers in need of various customers' desire. Availability of young, lower skilled worker who can be trained to service retail baker stores, is declining. However, the number of retail baker unit increased over the same period, which cause increased labor cost more than efficiency of labor. Food service operation must deal with high level of product turn over during very narrow time frame (Kulp et al, 1995).

Frozen bread dough is used widely in industrial bakeries to facilitate bread production and baking in retail shops. The production of cereals and baked goods has been undergoing a continuous transformation promoted by changes in social habits, consumer demands, and the interest of the baked goods producers in saving labor and cost. Necessity of frozen dough in centralized manufacturing is increased. It may be more profitable to rely upon freeze-thaw products. It also makes it possible to offer fresh-baked bakery products to customers. Retail shops may incorporate production efficiencies to drive up the production per labor equivalent and have increased their image value for customer acceptance.

1.2 Dough-Bread Structure Formation - Basic Ingredients and Their Roles

Formulation of frozen dough is usually different from that of unfrozen dough. However the basic formulation and characteristics are essentially the same. The effects of basic dough ingredients on dough and bread processing are discussed below.

Flour - Flour is the most important ingredient in bread making because it modulates the specific characteristics of bread products. It consists of protein, starch and other carbohydrates, ash, fibers, lipids, water and small amount of vitamins, minerals and enzymes. Wheat flour is the most common flour used. Dough quality depends not only on the level but also on the quality of flour protein quality. Because of a process of freezing, storing, and thawing, flour for frozen bread dough should have a high protein content and a great strength (Wolt and D'Appolonia, 1984b). The quality and level of flour protein are affected by the variety and growing environment of the wheat, milling conditions and flour grade (refinement). Yeast leavened frozen bread dough needs high quality wheat flours, high in protein (12-14%). The protein content of most commercial wheat samples varies between 8 and 16%, depending on variety and growing conditions. Hard wheat flour is recommended for frozen bread dough. Hard wheat flour has protein content of 11 – 14% (Pomeranz, 1988; Kamel and Stauffer, 1993; Sahlstrom et al, 2004). Wheat proteins have been traditionally classified into four types according to their solubility: water-soluble albumin, salt soluble globulins, prolamins (gliadins) soluble in 70% ethyl alcohol and glutelin (glutenins) soluble in dilute acids and bases. Glutenin and gliadin are the main protein fractions of the wheat grain. When water is added to wheat endosperm proteins, gliadin and glutenin, gluten proteins are formed (Pomeranz, 1988; Pyler, 1988)).

Waxy wheat flour – Waxy wheat has different starch ratio in comparison with conventional wheat flour. The ratio of amylose to amylopectin differs among starches, but typical ratio of amylose to amylopectin is 25–28 and 72–75%, respectively. However, the starches of some mutant genotypes of barley, amize, rice and wheat, etc. contain either increasing amylopectin content or increasing amylose content. Recently, waxy wheat (amylosefree, glutinous) grains have lower level of amylose content, less than 5%. This different ratio of amylose to amylopectin leads to different starch granule structure, physicochemical properties and the quality of end-use products (Yasui et al, 1996; Seguchi et, 2001; Kim et al, 2003; Hung et al, 2006).

Yeast - *Saccharomyces cerevisiae* is the most common yeast used in bread making. Yeast cells metabolize fermentable sugars under anaerobic conditions producing carbon dioxide, which acts as a leavening agent and expand dough volume to required level. Yeast also supports both the gluten network and aromatic compound production. It gives bread flavor through fermentation process by production of complex chemical compound (Kamel and Stauffer, 1993). Common yeast level in formulation of dough is about 3% on flour basis. An amount of yeast in dough directly affects the rate of gas production. The longer the bulk fermentation time the lower the yeast level should be. As dough temperature increases, the rate of gas production increases (Tuite and Oliver, 1991; Kamel and Stauffer, 1993). There are two types of yeast used in frozen dough. The compressed cake contains approximately 70% moisture so it is highly perishable unless it is refrigerated. Active cells of yeast are available as a compressed cake or in dried form. Active dry yeast is produced by extruding cake yeast in fine strands, which are dried to low moisture content (Charley and Weaver, 1997).

Water - Water is necessary for the formation of dough and is responsible for its fluidity and yeast activity. It is used for the dissolution of salt and sugars and assists the dispersion of yeast cells. It is the medium for food transportation to the yeast through cell membranes. Water is needed for starch and sucrose hydrolysis. It is important for starch gelatinization during baking and contributes to oven spring through vaporization. The water added to the flour activates enzymes, brings about the formation of new bonds between the macromolecules in the flour, and alters the rheological properties of dough. The amount of added water is related to the moisture content and the physicochemical properties of the flour (Gil et al, 1997; Schiraldi and Fessas, 2003).

Sugar – Sugar is basic source of energy, which yeast converts into CO_2 during dough proofing. Sugars are usually used by yeast during the early stages of fermentation. Later more sugars are released by the action of enzymes in the flour and then used for gas production. The concentration of sugar used in dough depends on the type of the product and desired crust characteristics. Sugar is added to provide pleasant flavor and to develop a desired crust color (Sahlstrom et al, 2004). In frozen dough, slightly higher level of sugar is recommended than in unfrozen bread dough. The higher level of sugar in frozen dough gives hygroscopic characteristics, which increases the amount of water absorbed. Therefore, the amount of free water in dough is reduced and yeast damage can be decreased (Kamel and Stauffer, 1993). Sugars also act as antiplasticizers retarding pasting of native starch or function as anti-staling ingredients inhibiting starch recrystallization (Levine and Slade, 1990).

Salt - Salt is considered as a functional ingredient with a functional role. 1.5 – 2.0% of salt is commonly used based on flour basis. Salt has several functional effects on dough. Salt strengthens the gluten, controls the action of yeast and therefore controls the loaf volume. It increases dough stability, firmness, and capacity to retain fermentation gases. Added salts increase gelatinization temperature of starch. However, 1% addition of salts postpones the onset temperature of starch gelatinization of wheat flour by 5 -10˚C (Chinachoti et al, 1991; Kamel and Stauffer, 1993). A small amount of salt in dough improves flavor and favors the action of amylases helping to maintain a supply of maltose as food for the yeast. Salt inhibits the action of flour proteases, which otherwise would depolimerize proteins of the gluten complex. Yeast dough without salt is sticky and difficult to manipulate. In frozen dough products, salt slows the production of carbon dioxide by the yeast delaying their fermentation (Charley and Weaver, 1997).

Lipids **-** Lipids can be used in bread making either in the form of fats or oils and are usually referred to as shortening. They are optional ingredients in bread but can improve dough handling and crumb appearance and contribute to product flavor (Stauffer, 1993). Lipids also improve the keeping quality, softness, and moistness and contribute to bread texture. Both endogenous lipids and added fats are known to play an important role during bread making and staling of bread (Collar et al, 1998). Lipids embedded into the protein matrix are essential as they interact with proteins during dough mixing and contribute to the viscoelastic properties of the gluten network, required for expansion and gas retention during proofing (Demiralp et al, 2000). The incorporation of lipids into bread dough results in a larger final loaf volume (improved oven spring), a less crisp crust and improved keeping quality of the bread (Autio and Laurikainen, 1997).

1.3 Processing Steps and Their Roles in Bread Making

Mixing **-** In initial processing, a mixer is used to blend dough ingredients such as flour, lipid (butter or oil), salt, yeast, and water. The resulting fully mixed bread dough has distinct rheological properties. Dough structure and strength depend predominantly on hydrated wheat flour proteins, generally referred to as gluten. The simple and commonly used dough preparation method is the straight-dough process, in which all ingredients are mixed in a single step. The fully mixed bread dough has distinct rheological properties. Dough structure and strength depend predominantly on hydrated wheat flour proteins, generally referred to as gluten. The unique viscoelastic properties of gluten are developed by interaction of flour proteins during mixing. In frozen dough preparation, the mixing step is especially important, because only during this step gluten formation and modification takes place. The produced dough for freezing must have fully developed gluten and must show optimal rheological properties such as dough extensibility and strength.

Gluten formation in dough preparation - Gluten formation is another factor involved in the freezing injury as well as studying how to improve methods. During dough preparation, the proteins, gliadin and glutenin, are hydrated, then interact with each other to form gluten. Gluten particles transform into a larger cohesive system that exhibits unique rheological properties. In addition to protein interaction, the other components (lipid, starch, non-starch carbohydrates, salts, sugar) also participate in the dough formation. The viscoelastic properties of dough are primarily the result of a continuous protein phase that, in fully developed dough, surrounds the starch granules (Rojas et al, 2002). The rheological behavior of the gluten phase depends on the molecular properties of the interacting components and the type of bonds involved in the polymeric gluten matrix. According to Bushuk and MacRichie (1989), the strength of the polymeric network is attributed to the concentration and strength of cross-links, the molecular weight and molecular weight distribution of the constituent polymers. Inoue and Bushuk (1992) and Inoue et al (1994) postulated that flour type and gluten are both important determinants of the molecular characteristics of flour. The chemical bonds that occur in fully developed flour are covalent and secondary bonds.. The covalent bonds are disulfide bonds, which form inter- and intramolecular crossbonds in the proteins during dough formation by the sulfide-disulfide interchange. The secondary bonds involved are hydrogen, hydrophillic, and ionic bonds and polar interactions (Belton, 1999). Although the secondary linkages are rather weak, their importance cannot be neglected because they are numerous and thus produce a strong association.

The covalent and secondary bonds are disrupted and reformed during freezing of bread dough. The physical chemical reactions that take place during freezing and frozen storage are the results of ice formation and yeast-dough interactions. The ice formation is generally responsible for physical disruption of secondary bonding in the dough and yeast. Loss of the secondary bonding is likely to alter the conformational order of the protein molecules and thus adversely affect their functional properties. The chemical agents that cleave covalent bonds (e.g. disulfide by the reducting action of glutathion) are attributed to the interaction between leached-out compounds from yeast and the surrounding dough protein matrix (Autio and Sinda, 1992). In the production of frozen doughs, these changes are minimized technologically by proper production measures and chemical additives. The optimized production is designed to preserve the dough structure to permit maximum rehydration and restoration of dough structure during thawing. Surfactants complex with gluten proteins to stabilize the gluten structure, and to reform protein disulfides in gluten by the action of added oxidants. Although the physical reactions are not completely reversible, it appears from the actual baking results that, under optimal conditions, most of the dough functionality is preserved by freezing and regained after thawing.

Mixing time - Dough mixing time must be properly controlled, because short mixing generates under-developed structure and long mixing time generates heat and enhanced fermentation. The more fermentation occurs at the dough stage, the more yeast becomes susceptible to freezing injury. Fermentation should be minimized in dough preparation for freezing. Inoue and Bushuk (1992) proposed that high protein flours selected form good wheat varieties are strong and produce frozen dough with good stability. But these types of flour may require longer mixing time to cause more fermentation. Thus, temperature control in dough preparation is very difficult and important.

Molding **-** Dough is divided into pieces of specific weight and is molded to desirable shape after molding. Molding modifies the structure of gas cells as they induce coalescence of small cells into larger ones and contribute as well to the final development of the gluten network (Autio and Laurikainen, 1997)

Freezing **-** Dough pieces are frozen and then stored at the appropriate temperature. Unfrozen doughs are proofed and baked after molding. Doughs for frozen storage are frozen after molding and transferred to a storage freezer. Bread doughs should be frozen rapidly to avoid yeast activation, which is pernicious to storage stability (Gelinas et al, 1995). Blaster freezer and contact freezers are most widely used. Commercial freezing temperatures are usually around -35 to -40°C. During freezing, the dough piece starts to freeze from the outside towards the center. Dough has poor thermal conductivity like other foods compared with metal. Best results are obtained when dough is frozen to a dough core temperature of -10°C, thus ensuring ensure all the free water in the dough freezes. However, It is advantageous not to freeze the entire dough piece solid while blast freezing, due to throughput, minimum energy requirements, and not to subject the exterior of the dough piece to the extreme blast freezer temperatures (-35°C) for prolonged periods, which is detrimental to yeast. After freezing dough pieces are bagged in polyethylene bags and packed in cardboard boxes. The packaged frozen dough pieces are stored freezer at -20˚C usually. The temperature of frozen dough pieces decreases gradually and equilibrates throughout the dough to -20˚C (Singh, 1995; El-Hady et al, 1996; Inoue and Bushuk, 1996).

Packaging **-** Packaging materials and shapes vary according to product specifications. Materials usually applied to frozen bakery products are plastic (films, membranes, etc.) and aluminum (Matz, 1989). In any case, packaging must form an effective, functional barrier to contamination and have sufficient impact and compressive strength to withstand the stresses it is likely to meet. It must perform satisfactorily in storage and transport.

Thawing **-** Frozen dough is defrosted and proofed. This process can be conducted under various time–temperature conditions. Thawing involves the rehydration of the system, mainly of the gluten matrix and yeast cells (Kulp et al, 1995). The process can be completed either at a certain temperature or by stepwise temperature increase, which is more favorable for two reasons. Firstly, during thawing, condensation occurs on the dough surface, as dough is colder than the surrounding air. This results in spotting and blistering of the crust especially when there is a large difference in temperature between the dough surface and the surrounding air. A stepwise increase in temperature minimizes this effect. Secondly, excessively rapid thawing raises the temperature only to the outer regions of the dough, which becomes ready for proofing, while the center of the dough still remains frozen. Retarder–proofer units are used for stepwise thawing and proofing of frozen dough. They allow the temperature of frozen dough pieces to be raised gradually and minimize the temperature differential within them (Kenny et al, 2001b)

Proofing **-** Thawed dough pieces should be proofed before baking, until they obtain desirable volume. The action of yeast attributes to the proofing. Properly proofed dough exhibits optimum rheological properties (optimum balance of extensibility and elasticity) as well as good machinability, and produces bread with desirable volume and crumb characteristics. During proofing, several reactions occur. Alcohol and carbon dioxide are produced. They influence the colloidal nature of the flour proteins and alter the interfacial tension within the dough.

Baking **-** Baking is results in a series of physical, chemical and biochemical changes in bread. The reactions include volume expansion, evaporation of water, formation of a porous structure, denaturation of protein, gelatinization of starch, crust formation and browning reaction, protein cross linking, melting of fat crystals and their incorporation into the surface of air cells, rupture of gas cells and sometimes fragmentation of cell walls (Sablani et al, 2002)

Staling - Bread staling is characterized by many physical and chemical phenomena such as changes in texture, water migration, starch crystallization, and component interactions. Analyses of bread staling have evolved over time, as instrumental technology and knowledge of the subject have progressed. Various analytical techniques are available for monitoring changes at macroscopic, microscopic, and molecular levels.

1.4 Freezing Process: Freezing - Ice Formation

Freezing represents a preservation and maintenance process for food where product temperature is decreased to a point somewhere below the temperature at which ice crystallizes. Freezing crystallizes liquid water into ice, the solid form of water. Lots of changes occur during freezing. In foods, the freezing change is more complex than in pure water. Freezing involves greater structural change and water relocation. The purpose of this process is to reduce the temperature of the product in effort to reduce deterioration reaction rates within the product, thus significantly decreasing the rate of microbial growth, as well as enzymatic and oxidative reactions. In addition, freezing transfers some of the liquid water into ice, leavening less water available to participate in reactions. The quality of frozen foods can be very good, but depends on the freezing process and frozen storage (Reid et al, 1994; Jeremiah, 1996; Kerr, 2004) .

Undercooling – When considering freezing process, thermodynamic and kinetic factors are present. The thermodynamic factors define the positions of equilibrium, and the kinetic factors describe the rate at which these equilbria might be approached. Each dominates the other at a particular point in the freezing process. A significant energy barrier must be surmounted before crystallization at initial freezing point. This energy barrier is demonstrated by withdrawal of sensible heat below 0˚C without a phase change. This process is called undercooling or supercooling, which initiates the formation of submicroscopic water aggregates leading to a suitable interface necessary for a liquid-to-solid transformation. The onset of ice nucleation determines the degree of undercooling (Reid, 1993; Sahagian and Goff, 1996).

***Nucleation* -** Once the solution or melt has been supersaturated, there is a thermodynamic driving force for crystallization. That is, the molecules tend toward the crystalline state to lower the energy level of the system. Liquid-solid transformation takes place. The transformation requires a seed upon which the solid phase can grow. Without this seed, solid phase can not grow because the molecules in liquid phase do not easily arrange into solid configuration. The seed is a cluster of molecules of a sufficient size to sustain growth. These first viable particles, called nuclei, may either be formed from solid particles already present in the system or be generated spontaneously by the supersaturated solution itself. During nucleation, the molecules in the liquid state rearrange and eventually form into a stable cluster which organizes into a crystalline lattice. The ordered arrangement of molecules in the lattice involves a release of latent heat as the phase change occurs. Nucleation also depends on the formulation (types and concentrations of nucleating promoters or inhibitors) and on the processing conditions (heat and mass transfer rates) (Franks, 1985; Reid, 1993; Sahagian and Goff, 1996; Hartel, 2001).

***Recrystallization* –** Ice crystals are relatively unstable and undergo changes in number, size, and shape during frozen storage, known collectively as recrystallization. It is a consequence of the surface energy between the ice and unfrozen phase (Reid, 1983). At constant temperature, Oswald ripening involves the growth of large crystals at the expense of smaller ones and results from the system moving toward reduced surface area. During temperature fluctuation, as temperature increases, small ice crystals tend to melt, while as temperature decreases, the unfrozen water portion does not crystallize again but rather is placed and frozen on the surface of larger ice (Reid, 1983; 1993; Hartel, 2001). Reactions can occur in the solid sate during frozen storage. Recrystallization occurs in pure water as low as -100˚C and it occurs in gels and concentrated aqueous solutions at higher temperatures even in a matter of seconds at temperatures above -50˚C (Mazur and Schmidt, 1968; Mazur, 1970).

In dough, recrystallization may cause separation of water from hydrophilic components and could contribute to the weakening of the three dimensional protein network (Varrianomarston et al, 1980).

1.5 Freezing Process: Freezing Rate and Frozen Food

The freezing rate affects the quality of frozen food. The zone of maximum crystal formation refers to the region between 0ºC and -5ºC where the removal of the heat of fusion takes place. Quick freezing occurs when a food takes less than 30 minutes to pass through the zone of maximum crystal formation. Rapid freezing produces many small ice crystals which cause minimum water movement out of the cell, drip and chemical damage to the cell. Slow freezing produces a few large ice crystals which can lead to mechanical and chemical damage due to partial dehydration of the cells. The food becomes flabby and soft and exuded a lot of drip. Very fast freezing is usually only an advantage in foods consumed raw and some other foods such as frozen vegetables, seafood and poultry (Jeremiah, 1996; Kerr, 2004).

The time a food takes to freeze depends on several factors. Factors affecting freezing rate are (1) shape and size of the food, (2) thermal characteristics of the food (specific heat, thermal conductivity, initial freezing point, food density and moisture content) (3) initial temperature of the food, (4) heat transfer coefficient of the freezing medium (air velocity, degree of contact), (5) characteristics of the packaging. The size and temperature of the freezer and the amount of food placed in the freezer in a single day determines how fast the food will be frozen.

Weight and shape of dough pieces affect freezing rates. For example, a smaller dough piece is frozen more rapidly than a larger dough piece, if the same freezing condition is applied. According, temperature, conveyer speed, or airflow of a freezer should be adjusted for each type of frozen dough product to ensure the appropriate freezing rate. Dough pieces are frozen until the core temperature is below freezing (usually -10˚C to -15˚C). The freezing points of a leaner dough like a French bread is about -5˚C, while that of rich formula doughs like dinner rolls is about -10˚C (Inoue and Bushuk, 1996).

Rapid freezing is generally recommended for foods to minimize the damage due to ice crystallization. Rapid freezing results in smaller ice crystal size. While in freezing dough, rapid freezing seems to have detrimental effects on yeast activity. Although the explanation of the detrimental effect of rapid freezing is not clear, Mazur (1961) postulated that during rapid freezing, intracellular ice crystal, which is lethal to yeast cell membrane, is formed, while during slow freezing, the supercooled intracellular water my be transferred to external ice crystals due to the vapor pressure different. The resulting dehydration may be sufficient to render the small quantity of residual internal water incapable of freezing due to interaction with the solid components of the yeast cell (Mazur, 1961; Mazur and Schmidt, 1968; Mazur, 1970; Hsu et al, 1979a; b; Inoue and Bushuk, 1996)

1.6 Freezing Technology – Freezing Equipment

Freezing of food as a method of preservation and extension of a food product shelf life has been used for long period. Freezing, involves mainly two processes. First, temperature reduction and second, phase transition from liquid to solid. Today many types of freezing equipment exist and recommendations are given for each type of freezer as to the product categories for which it is particularly suited. For most products, more than one freezer can be used. The selection of the technique and freezing rate depends mostly on product requirements and availability or cost. Before a decision made on which freezer should be used, cost-benefit analysis should be taken. However, it is difficult to set a price on everything even though the cost analysis covers the lifetime of freezer. Other factors should be considered, namely: (1) product damage, (2) hygiene, (3) safety, (4) energy recovery, and the freezer as part of processing line. Freezing techniques can be categorized as mechanical (blast, plate, spiral, impingent, immersion, belt or fluidized bed freezers) and cryogenic. The mechanical freezing system became widely accepted and was main commercial methods for long period of time. Cryogenic freezing, which takes palace at temperatures substantially lower than mechanical freezing of foods, is only half as old as mechanical freezing. However, the freezing process has taken successful position in the industry because of the many benefits (Persson and Löndail, 1993; Venetucci, 1995; Ribotta et al, 2001; Roman-Gutierrez et al, 2002; Bhattacharya et al, 2003).

1) Cryogenic freezing equipment

The faster the temperature of a food product is reduced, the smaller the frozen crystal will be and less product damage will occur. Cryogenic freezing produces relatively small ice crystal. Cryogenic freezers differ from mechanical freezers in one fundamental way they are not connected to a refrigeration plant. The heat transfer medium is nitrogen or carbon dioxide.

A cart with trays is used to hold the food; the entire unit is placed into the cabinet; and a predetermined freezer cycle is set. A cryogen such as CO_2 or LN_2 is sprayed into the cabinet and, with the aid of high capacity fans; the temperature of the cabinet quickly drops below the freezing temperature. The food product is subjected to a blizzard condition, which quickly freezes the crust of the product. This is similar to encapsulating the food within a frozen enclosure, thereby reducing moisture loss and keeping the flavor in until the freezing is completed. For lager and continuous production, a 'straight-belt cryogenic freezing tunnel' can be used. This type of freezer is suitable for bread dough, cakes, cookies, pies, rolls, etc. The bakery product can be placed directly on the moving conveyor belt, where it then passes under a cryogenic spray and series of fans.

The 'cryogenic immersion freezer' is available for products that can be dropped directly into a pool of refrigerant (liquid nitrogen, carbon dioxide, Freon). Immersion freezers are good for quick lowering of product temperature, but not adequate for large products requiring substantial heat removal. This design is the fastest way to commercially freeze a food item. It has full advantage of the extremely low -195˚C liquid nitrogen temperature. The immersion time is adjustable, and final freezing can be done in the downstream tunnel, if that is required. It is a method used for berries, diced and sliced fruit, etc. However, it is limited to use in the bakery industry.

2) Mechanical freezer

Mechanical freezing system can be advantageous over expendable gas for freezing applications where cryogen, liquid nitrogen or liquid carbon dioxide is not readily available, for long periods of freezing, and where the time required to ramp down to temperature is not critical. This system also has many benefits. It is a widely accepted, proven system, consists of a simple enclosure, compressor, and evaporative and cooling coils. While there are some disadvantages. Among the disadvantages are its high initial costs, potential high dehydration of food, need for a defrost cycle. Basic refrigeration system is the transformation of a liquid into a gas or vapor, which absorbs heat and cools whatever is around it.

(i) Air-blast freezer

Cold air is blown past the product at high velocity to increase heat transfer. These may be batch or continuous types. In batch-type the product is loaded and removed from the freezer. In continuous type, the product is moved through on trays or conveyer belts. Spiral belt freezers are arranged to allow a long residence time in a smaller space. Air blast freezers are good for high density or large package items (chickens, juice cans). Moisture loss from the product is sometimes a problem. The velocity of the air in a freezer has definite effect on the rate of freezing. Usually high fan velocity produces the most efficient freezing. However, a low fan velocity may be necessary for some food such as bakery products, for which it may be desirable to have a slower freeze to prevent killing of yeast.

(ii) Fluidized-Bed Freezer:

The product is conveyed through the freezer and fluidized by very high velocity air perpendicular to the product movement. The suspension of individual food piece results in very high heat transfer rates and short residence times. Most fruits and vegetables can be frozen in 3-5minutes, Fluidized bed freezers only work with small food pieces (blue berries, straw berries, peas, etc.) Energy requirements to fluidize large items (e.g. chickens) would be prohibitive.

(iii) Contact Freezer – Plate freezer:

Product is brought into direct contact with plate's material at freezing temperature, and is usually pressed between two such plates. Direct contact increases heat transfer (mostly by conduction). The plates are maintained by refrigerant circulating inside. These may be batch or continuous. Less floor space is required, as heat transfer does not require the movement of air. These usually required lower power usage than air blast freezers and only work with products that have fairly flat surfaces. Plate freezers are used mostly in freezing of fish.

1.7. Statement of Problem

The overall quality of bread dough deteriorates gradually during frozen storage (Neyreneuf and Vanderplaat, 1991; Inoue and Bushuk, 1992; Le Bail et al, 1999a; Lu and Grant, 1999; Kenny et al, 2001a; Ribotta et al, 2001). The observed manifestations of loss of baking quality observed are as follows, (1) thawed dough requires longer time to proof to a constant height, (2) proofed loaves show progressively less ovenspring, (3) baked loaves display a flattened top, and (4) the internal structure of the baked bread becomes coarser. Longer prooftimes can, in part, be due to a decline in both viability and activity of yeast; however, the other three symptoms are due to the reduction of gluten cross-linking caused by ice recrystallization, the release of reducing substances from yeast, and by water redistribution provoked by a modification in the water binding capacity of dough constituents

(Varrianomarston et al, 1980; Inoue and Bushuk, 1991; Autio and Sinda, 1992; Ribotta et al, 2001; Ribotta et al, 2003b). Those several phenomena are the main changes to deteriorate the quality of frozen dough.

1.7.1 Effects of Ice Formation and Water Redistribution

Freezing is the crystallization of liquid water into ice, the solid form of water. Many changes occur during freezing. Freezing involves greater structural change and water relocation. Even after dough is frozen, changes in the food do not stop. Reaction rates of physical and chemical reactions in frozen dough are reduced as compared to unfrozen dough; however, some reactions are accelerated below the freezing temperature.

Concentration of fermentation acids and crystallization of salts may also lower the pH values. These phenomena can occur in localized dough areas, thus affecting dough stability. The solid ice phase also changes, in that the small ice crystals grow to form large crystals. If salt precipitates, a complex series of pH changes takes place. Undoubtedly, disruption of certain bonds in dough is caused by dehydration and may affect the functionality of doughs.

The temperature fluctuations during frozen storage cause water migration along the temperature gradients. It is evident that the components of the liquid phase in frozen dough are in a metastable state. Changes in the water transfer may lead to moisture loss in some dough components. For example, if the yeast cell membranes are either intact or resist the passage of ice, there is a consequent driving force toward dehydration of the cells by osmotic pressure. Collectively, pH change, osmotic pressure, and water migration caused by freezing affects the quality of frozen dough (Casey and Foy, 1995; Kulp et al, 1995).

1.7.2 Effects of Freezing on Yeast

Lorenz and Kulp (1995) suggested that freezing yeast in a dough system increased the susceptibility to cell damage compared with direct freezing of yeast because the yeast in a dough system was under osmotic pressure and in a state of active fermentation. In addition, cell in active fermentation have a thinner plasma membrane than dormant cells; consequently, they become more susceptible to cell damage. Also, the organic compounds are concentrated by freezing of the aqueous phase, which can cause autolysis of yeast cells (Stauffer, 1993; Casey and Foy, 1995).

In the case of intracellular ice formation, ice formed in cells as a result of freezing likely grows during thawing, especially if warming is slow. Ice formed in cells disrupts cell structure. This sequence of events is believed to be the explanation for yeast cells that exhibit lower survival after slow warming than after rapid warming (Mazur and Schmidt, 1968). Research suggests that the lethal event in rapidly cooled yeast cells is the growth of intracellular ice crystals rather than their initial formation and that the damage observed during warming can occur very rapidly. Mazur (1961; 1968; 1970) speculated that injury occurs because recrystallization exerts sufficient force to rupture plasma membranes of cellular organelles such as mitochondria. Recrystallization of ice crystals has been shown to disrupt protein gels, and cells killed by intracellular damage have been shown to suffer membrane damage and become leaky (Mazur, 1961; Mazur, 1970; Casey and Foy, 1995).

1.7.3 Effects of Freezing on Gluten Network

The covalent and secondary bonds are disrupted and reformed during freezing of bread dough. The physical and chemical reactions that take place during freezing and frozen storage are the results of ice formation and yeast-dough interactions. The ice formation is generally responsible for the physical disruption of bonding in the dough and yeast. Loss of the bonding is likely to alter the conformational order of the protein molecules, thus adversely affecting their functional properties. The chemical agents that cleave covalent bonds (e.g. disulfide by the reducting action of glutathion) are attributed to the interaction between leached-out compounds from yeast and the surrounding dough protein matrix (Autio and Sinda, 1992).

1.7.4 Effects of Freezing on Starch

The reported involvement of starch in frozen dough quality seems to be minor, but it indicates that movement of moisture among the flour and ingredient components does takes place. This observation indicates the presence of a disruption of the association of gluten with starch, which thus may be a part of the dough deterioration process (Kulp, 1995).

1.7.5. Effects of Freezing on Dough Structure

Recently, researchers (Esselink et al, 2003a; Esselink et al, 2003b) used electron microscopy to examine and visualize the breakdown of the reticular pattern of gluten structure, the gluten network of dough subjected to freezing and thawing to assess the potential changes in the rheological properties of frozen dough. Based on these studies, they postulated that changes in the gluten network are partly due to ice recrystallization, which results in an increase in the size of the ice crystals. The ice crystallization process is known to cause a separation of water molecules from the macromolecules to which they are normally attached. The migration of water in dough affects the dough structure (Bache and Donald, 1998; Zheng et al, 2000; Rojas et al, 2002).

In addition, injury to yeast membranes caused by the freezing and thawing process may release certain chemical components of cells, particularly reducing compounds, which can have deleterious effects on dough structures such as the gluten network. However, these researchers had different opinions on the effects of leachates, mainly glutathione, on the gluten network. While these studies (Wolt and D'appolonia, 1984a; b; Inoue and Bushuk, 1991; Casey and Foy, 1995) postulated that leacheates, including glutathione, are certainly involved in the gluten network, the precise manner by which yeast contributes to increased slackening remains the subject of debate.

Starch granules, originally firmly embedded in the gluten network in freshly frozen dough, were observed to become more separated. Less fresh water was associated with either the gluten or starch fractions, concentrating instead into large forms of ice crystals. Gluten strands were also observed to become thinner with time. Collectively these observations, showing changes in the ultrastructure help explain the extended proof times and reduced control.

1.7.6 Effects of Freezing on Proof Time

Proof time for frozen-thawed dough is necessarily longer than that for fresh dough. As mentioned above, this is due to the lower dough temperature at which thawed pieces reach the proof box, and to a certain loss of dough gas retention power and yeast activity caused by the freezing process (Kulp, 1995). The decreased yeast viability and activity are the main causes for the longer proof time. The structure alteration of dough can affect longer proof time. Collectively the ice form, alteration of dough structure, yeast viability and activity affect the proof time of frozen dough.

1.7.8 Effects of Freezing on Oven Spring and Bake Volume

Bread made from frozen dough experiences decreased oven spring and baked volume compared to bread made from fresh dough. This is mainly due to the alteration of frozen dough structure caused by freezing. The alteration of dough texture properties causes reduction of gluten cross-linking by disulfide bonds and less elasticity in bread dough, resulting in diminished gas holding properties associated with frozen dough. Decreased yeast viability and activity result in less gassing power, affecting the oven spring and baked volume. Yeast in frozen dough loses the capacity to produce CO_2, resulting in less gassing power. Release of reducing substances from dead yeast cells during freezing may also reduce gluten protein. Thus, the decreased oven spring and baked volume are caused by alteration of frozen dough structure, yeast viability and activity, and reducing substances.

1.7.9 Effects of Freezing on Bread Staling

Bread made from frozen dough experiences faster bread staling. Freezing process changes the gluten network along with reduced yeast activity and viability. Freezing also affects starch in frozen dough and, therefore, bread staling. Staling is a complex process that involves several physico-chemical changes (Schiraldi and Fessas, 2000). There are different opinions on the role of the gluten network in bread staling. However, since starch is the major constituent in the bread crumb, the physical changes accompanying the retrogradation of starch have been suggested as the main cause of bread staling. When analyzing the aging of the re-baked samples, it was observed that the time of frozen storage produced a progressive increase of the retrogradation temperature range of the amylopectin, while great energy was required for amylopectin melting at longer storage periods, indicating that structural changes of amylopectin were produced during frozen storage. These studies concluded that freezing processes and frozen storage increase bread staling (Barcenas et al, 2003; Barcenas et al, 2004)

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CHAPTER 2

COMBINED EFFECTS OF FREEZING RATE, STORAGE TEMPERATURE, AND STORAGE TIME ON THE PROPERTIES OF FROZEN BREAD DOUGH AND BAKING PERFORMANCE OF FROZEN DOUGH

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ABSTRACT

The quality of yeasted frozen dough and bread made from frozen dough is influenced by processing parameters such as freezing temperature and rate, as well as storage temperature and time. However, few studies have been carried out on the combined effects of these processing factors. Our objective was to compare the effects of freezing temperature and rate as well as storage temperature and time on the quality of frozen dough. Yeasted bread dough was frozen using four freezing rates, then stored at -10, -20, -30, or -35°C for 30, 60, 90, and 180 days. The properties of the resulting yeasted frozen doughs were measured using nuclear magnetic resonance techniques (NMR), cryo-scanning electron microscopy (SEM), and texture analysis. Specifically, the yeast activity of dough was assessed by measurements of gas production. Our results indicate that dough strength diminishes with increased storage time as well as with higher (-10°C) storage temperature. Cryo-SEM demonstrated that frozen dough stored at -30 and -35°C displayed the least damage to dough structure. NMR studies showed that frozen dough at lower storage temperatures had lower T_2 values (9-10 ms) except freezing rate 2. However, dough stored at -20°C displayed the highest yeast activity among samples. Baking tests showed that the loaf volume gradually decreased with storage time, and that bread made from dough stored at -20°C showed the highest loaf volume. Breads produced from -30 and -35°C stored dough displayed less change in the texture profile during storage as well as less change in T_2 values. This research demonstrated that specific combinations of freezing rates and storage conditions contribute to better quality of frozen dough and bread.

Key words: frozen dough; dough stickiness; dough extensibility; NMR; bread volume; bread texture; bread color; bread staling

INTRODUCTION

Bread made from frozen dough has become an increasingly popular alternative to that made directly from unfrozen dough. Frozen dough can be manufactured in large quantities off-site, and then shipped to local restaurants or retail operations. Thus, it can be used to produce freshly baked products while saving on equipment and labor costs. In recent years, the quality of these products has improved owing to advances in technology and formulation, but there is additional room for improvement. Problems associated with frozen dough include long proof time, low volume, poor texture, and variable performance (Kenny et al, 1999). Some of the poorer quality can be attributed to diminished yeast activity, the characteristics of the yeast and their survival after freezing (Hsu et al, 1979a; Wolt and D'Appolonia, 1984b; Hino et al, 1987; Baguena et al, 1991; Takasaki and Karasawa, 1992; Gelinas et al, 1993; El-Hady et al, 1996; Ribotta et al, 2003b). In order to improve performance, frozen dough processors may add extra yeast, use short or no-time dough processing procedures, mix ingredients at relatively low temperatures, or incorporate new strains of freeze-tolerant yeasts (Nemeth et al, 1996).

During freezing, there are also deleterious effects on dough structure. Recent studies (Esselink et al, 2003a; Esselink et al, 2003b) used electron microscopy to examine the breakdown of the reticular gluten network when subjected to freezing and thawing, and related these to changes in the rheological properties of the dough. They postulated that changes in the gluten network are partly due to ice recrystallization during storage, which results in an increase in the size of the ice crystals. The crystallization and recrystallization processes cause separation of water molecules from the macromolecules to which they are normally associated. Migration of water in the dough also affects the dough structure (Bache and Donald, 1998; Zheng et al, 2000; Rojas et al, 2002). Extended frozen storage times have been shown to affect the structure of the gluten protein matrix (Varrianomarston et al, 1980; Berglund et al, 1991), resulting in a weakening of dough strength properties, loss of gas retention properties and deterioration of product quality (Hsu et al, 1979a; Inoue and Bushuk, 1991; Autio and Sinda, 1992; Inoue and Bushuk, 1992; Nemeth et al, 1996)

In addition, injury to yeast membranes caused by freezing and thawing release cellular chemical components that have deleterious effects on the dough structure. However, researchers have different opinions on the effects of specific leachates, mainly reducing compounds such as glutathione, on the gluten network. While studies (Wolt and D'appolonia, 1984a; b; Inoue and Bushuk, 1991; Casey and Foy, 1995) have postulated that leacheates including glutathione affect the gluten network, the precise manner by which yeast injury contributes to increased dough slackening remains the subject of debate. This altered dough structure leads to longer proofing times and less oven spring, resulting in lowered bread quality after baking.

Several techniques are available for examining the quality and changes in dough and bread. Dough ultrastructure and rheological properties are often used to study dough properties. Scanning electron microscopy (SEM) has been useful for studying the extended gluten network, embedded starch and void spaces. More recently, cryo-SEM has allowed direct study of the structure with the ice phase still present (Nicolas et al, 2003). Rheological properties also provide a quantitative measure for the amount of stress in the dough (Bloksma and Bushuk, 1988; Campos et al, 1997), which is related to the quality of the molecular gluten network. Elastic properties of the dough reflect the strength of gluten strands and their degree of crosslinking. Dough adhesiveness is, in some instances, a desirable and necessary property for appropriate function of the baked product (Heddleson et al, 1994). Bread properties are typically assessed by the volume after baking; crumb firmness, cohesiveness or chewiness; colorimetry; and sensory properties (Schober et al, 2005). Nuclear magnetic resonance (NMR) measurements have also proven useful for monitoring the states of water and polymer mobility in dough or bread (Leung and Steinberg, 1979; Seow and Teo, 1996; Chen et al, 1997; Vodovotz et al, 2002; Wang et al, 2004).

Several researchers have studied the effects of storage time or temperature on yeast viability and activity, as well as on dough structure (Mazur and Schmidt, 1968; El-Hady et al, 1996; Havet et al, 2000), but little has done on the influence of freezing rate. Especially in frozen dough preparations, where freezing and sometimes prolonged frozen storage intervene between dough formation and bread baking, several factors still have not been fully investigated (Giannou and Tzia, 2007). In the present study, we examined the combined effects of freezing rate and storage temperature on dough quality and subsequent bread quality. The dough properties were evaluated based on dough extensibility and adhesiveness, and the viability of yeast assayed through measurements of gas production. Both bread volume and firmness were measured in the finished breads. Direct examination of the dough in the frozen state was accomplished using cryogenic scanning electron microscopy (cryo-SEM), allowing visualization of the ultra-structure of gluten-starch association and the state of gluten strands in the network. We also used time-domain NMR to investigate freeze-damage in frozen dough (Ruan et al, 1999; Roman-Gutierrez et al, 2002; Esselink et al, 2003b). These techniques provide a comprehensive understanding of the state of frozen dough made at different freezing rates, and held at various time-temperature storage conditions, as well as its impact on subsequent bread quality.

MATERIALS AND METHODS

Dough Preparation and Freezing

Bread dough was prepared from enriched, bleached hard winter wheat flour (Organic Select Artisan Flour, King Arthur Company, Norwich, Vermont) with 11.5% protein content; Baker's dry yeast (Fleischmann's Yeast, Quebec, Canada); Dominion pure cane sugar (Dixie Crystal, Savannah, GA); non-iodized salt (Morton International, Inc., Chicago, IL); and soybean oil (Wesson Vegetable Oil, ConAgra Foods, Omaha, Nebraska). The basic formulation was (as percent of flour weight): flour (100%), water (60%), sugar (5%), oil (5%), yeast (3%), and salt (2%). The yeast was pre-hydrated with the water, and then all of the dough ingredients were placed in a 6-quart, 575 watt mixer (Kitchen Aid Professional 600 Series, St. Joseph, MI) and mixed for 2 min at speed of 120rpm with a paddle, and for 8 min at 178rmp with a dough hook. Once the dough was formed, it was separated into samples of 50g, and formed into slightly round shapes by hands.

Dough pieces were frozen in circular slabs, approximately 50mm diameter, in a forced-air blast freezer (-35°C, air velocity~10 m/s). As part of the experimental design, four different freezing rates were used (Figure 2.1). Several studies related freezing rate to yeast activity and dough quality. The reduced yeast activity and injury of gluten network caused by freezing are the most cause of declined baking performance of frozen dough. Yeast viability and activity are affected by freezing conditions. Yeast is embedded in dough, resulting in more significant decrease in its viability and activity than direct freezing of compressed yeast. Several researchers reported that slow freezing rate is preferable to preserve yeast activity and higher proofing ability (Mazur and Schmidt, 1968; Neyreneuf and Delpuech, 1993; Le Bail et al, 1999b; Havet et al, 2000).

Freezing rates (*Fr*) were calculated as the ratio of the difference between the initial and the final temperature and the respective duration (International institute of refrigeration, 1986; Havet et al, 2000).

$$Fr = \frac{T_2 - T_1}{t_2 - t_1}$$

In this formula, a beginning criterion (Temperature, T_1 and Time, t_1) and an ending criterion (T_2 and t_2) for freezing must be determined.

- Freezing rate 1- unfrozen dough cooled and frozen at 13.9°C/hr.
- Freezing rate 2- dough frozen at 28.1°C/hr.
- Freezing rate 3- dough frozen at 37.9°C/hr.
- Freezing rate 4- dough frozen at 50.8°C/hr.

After freezing, dough samples were vacuum-packaged in plastic bags and placed at four different frozen storage temperatures, and stored for 30, 60, 90, and 180 days. After reaching to the each desired storage temperature, dough samples were placed in storage freezers at -10, -20, -30, or -35°C.

At each sampling period (30, 60, 90 and 180 days), 3 replications of 16 treatments were withdrawn from the various freezers and placed in a constant temperature chamber (8°C) in order to thaw. Then thawed doughs are placed in a chamber of 36°C and 85% RH for proofing. Optimum thawing-proofing conditions were pre-determined by a pre-test based on time-temperature conditions (Takano et al, 2002). Three replicates of dough sample of 16 treatments were used for dough texture studies, while the three replications of each treatment were baked in a 5-rack gas convection oven (Blodgett BLD-DFG100, Burlington, Vermont) at 180°C for 15 min. After baking, bread samples were allowed to cool for approximately 1hr at prior to most subsequent measurements (Hallen et al, 2004).

Dough Extensibility and Adhesiveness

Extensibility of the dough was measured using a texture analyzer (TA-XT2i, Texture Technologies Corp., Scarsdale, NY) using a modified Kieffer extensibility rig and 5g load cell. Approximately 50 g dough samples were molded into strips approximately 7 mm in diameter and 60mm in length. All samples were left to rest on a grooved plate at 8°C for 20min and 90% RH prior to testing (Anderssen et al, 2004). The dough was pulled at a crosshead speed of 3.3mm/s. The resistance to extension (maximum force) and extensibility (distance to break) were calculated from the force-deformation curves. One advantage of the Kieffer dough extensibility rig is that it uses a micro-extension method involving a very small sample size. It correlates highly with methods such as the extensigraph as indicated by baking performance (Kieffer et al, 1998; Kieffer and Stein, 1999; Suchy et al, 2000; Sharadanant and Khan, 2003a).

Adhesiveness was measured using the texture analyzer with a modified Chen-Hoseney stickiness rig, with conditions as described by Chen and Hoseney (1995) were used. The sample was placed in a cylindrical cell on the base of the texture analyzer, which was then enclosed by a lid with a perforated hole. A small amount of dough was extruded through the hole. The upper cylindrical probe was brought in contact with the exposed dough to adhere to it, and the probe was pulled away from the base at a speed of 1.7mm/s. Both the maximum force and the area under the force-deformation curve required to separate the probe from the test sample were determined as measures of adhesiveness.

Cryo Scanning Electron Microscopy

A Jeol JSM-5410 scanning electron microscope with a CT-500C cryo-unit (Oxford Instruments) was used to investigate the microstructure of frozen bread dough stored for 90 days.

Each frozen dough sample was placed on the cryo-specimen holder, placed in liquid nitrogen (~ -196°C), then transferred to the cryo-unit in the frozen state. Once in the cryo-unit, dough specimens were fractured, sublimated (10 min at -70°C) and sputter coated with gold (4 min at 2 mbar). The prepared dough specimen was transferred to the microscope where it was observed at 15kV and -120°C. Micrographs were taken at 500, 1000 and 2000× magnification.

NMR Measurements of Dough

Proton relaxation measurements were made using a 20MHz Proton (^{1}H) NMR spectrometer (Resonance Instruments, Whitney, UK). Approximately 5g dough samples were taken from the center of the thawed dough. Three replicates were taken from each piece of dough of 16 treatements. Each sample was place in a 10mm diameter glass tube then covered with parafilm. The class tube was placed in a 10°C bath for 20 minutes. After 20 minutes, the glass tubes with dough specimens were placed in 18mm diameter NMR tubes. Transverse (T_2) relaxation curves were developed using the CPMG pulse sequence: 90x-(τ-180y-τ-echo)n (Meiboom and Gill, 1958). Acquisition parameters were set to a 90° pulse of 4.2μs and a recycle delay of 2s. A pulse spacing (τ) of 100μs was chosen to exclude the fast decaying solid-like signal. All measurements were made at 25°C ±1°C. The temperature was controlled by the internal temperature control system in NMR spectrometer. Relaxation curves obtained from CPMG sequences were analyzed using the WinDXP distributed exponential routine (Resonance Instruments, Whitney, UK). This routine calculates a distribution of T_2 terms that best describe the data:

$$g_i = \sum_{j=1}^{m} f_j e^{-t_i/T_{2,j}} \quad (1)$$

where g_i are the values of the exponential distribution at time t_i; f_j are the pre-exponential multipliers, and $T_{2,j}$ are the time constants. In general, there was more than one distributed region, suggesting the existence of various regions of water with similar mobility. In this case, the number of protons in a given region was measured by the integrated signal intensity over the region of the distribution.

Yeast Activity

Yeast activity was measured using AACC method 89-01 (2000) with slight modification. Dough pieces (10g) and 100g water were placed into the reaction vessel, capped, and placed in a water bath at 36 ± 1 °C (Newberry et al, 2002). A hose was connected to the vessel, leading to a volumetric manometer. Gassing power was determined from the volume of carbon dioxide gas trapped after 180 minutes.

Bread Volume

Loaf volume and weight were determined after baking, after cooling for 1 h at ambient temperature (AACC 10-05, 2000). A container was filled with a known volume of rapeseed. The amount of seed displaced when the loaf was introduced was measured in a graduated cylinder, and measures the loaf volume.

Bread Crumb Firmness

The firmness of the bread samples was determined using a texture analyzer (TA-XT2i, Texture Technologies Corp., Scarsdale, NY) with a 36mm radius cylinder probe according to AACC Method 74-10A (2000). Three center slices of each loaf were measured at 25 °C. The compression rate was set at 1.7mm/s. Firmness was the force required to compress the slices (20 mm thick) by 40% strain.

Statistical Analysis

ANOVA was used to analyze data. Three-way mixed design was use for statistical analysis for all data. Fisher's LSD was used to determine significant differences between samples. A *p*-value of less than 0.05 was considered significant.

RESULTS AND DISCUSSION

Dough Extensibility and Adhesiveness

Tables 2.1-2 show the maximum resistance to extension (in g force) and extensibility (in mm) of dough subject to different freezing and storage conditions. For the unfrozen control, maximum resistance was 69.98 g with an extensibility of 84.99 mm. All dough samples subject to freezing had lower resistance and extensibility than the control. Both the maximum resistance to extension and the extensibility decreased with time from 0 to 180 days of frozen storage. For example, maximum resistance ranged from 49.68 to 56.99 g at 30 days, and from 29.17 to 33.68 g at 180 days. Similarly, extensibility ranged from 74.45 to 81.00 mm at 30 days, and from 49.94 to 71.39 mm at 180 days. Storage time had a greater influence on extensibility than temperature. For samples prepared at freezing rate 1 (the slowest rate), and held for 30 days, extensibility varied from 74.45 mm (at -10°C) to 76.84 mm (at -35°C). After 180 days of storage, extensibility ranged from 49.94 mm (at -10°C) to 70.04 mm (at -35°C). Freezing rate also affected the extensibility of frozen dough. Freezing rate 2 produced dough with the highest extensibility, while those from freezing rate 1 (the slowest) had the lowest extensibility. Dough from freezing rate 1 also had lower maximum resistance than those from other freezing rates.

Frozen storage temperature also had some effect on dough extensibility. With lower frozen storage temperature (closer to -35°C), the extensibility increased but there was no significant difference between rates 3 and 4. The interaction between the freezing rate and frozen storage temperature determined extensibility and maximum resistance to extension. For the first 30 days, dough prepared at freezing rate 4 (the fastest freezing) and stored at -35°C had the highest extensibility (81.0 mm); dough prepared at freezing rate 1 (the slowest freezing) and stored at -10°C had the lowest extensibility (74.45 mm). After 180 days, dough prepared at freezing rate 2 and stored at -35°C had the highest extensibility (71.39 mm); those produced at freezing rate 1 and stored at -10°C had the lowest extensibility (49.94 mm).

Decreases in maximum resistance, and in extensibility may indicate deterioration in the gluten network. Extensibility has been attributed to the gluten structure, and particularly to high molecular weight glutenins (Payne and Corfield, 1979; Bushuk and Macritchie, 1989; Belton, 1999; Lindsay and Skerritt, 1999). While disulfide bonds help to maintain the gluten network and provide resistance to stretching, several theories have been developed to explain dough elasticity (MacRitchie and Lafiandra, 1997; Belton, 1999). A polymer science approach suggests that stretching creates additional order in the structure and a subsequent decrease in entropy; a force develops to restore the structure to its original dimensions, and thus maximize entropy. Our results suggest that relatively rapid freezing and low storage temperatures result in the least changes to the gluten structure. Most deleterious were slow freezing rates, low storage temperature (higher frozen storage temperature), and prolonged storage times. It is well known that rapid freezing and low storage temperatures promote the creation of a greater number of small ice crystals, due to enhanced nucleation and crystal growth rates (Kerr et al, 1987). Low freezing rate promotes the growth of relatively large crystals. It has been suggested that ice crystals physically disrupt and damage the gluten network, and dehydrate the gluten so as to cause irreversible structural changes.

Extensibility determines the ability of the dough to extend during gas production by yeast during proofing. Excessively high extensibility results in weak and slack dough, which collapses during the proofing stage or while baking in the oven (Sharadanant and Khan, 2003a). Maximum resistance to extension of the dough measures the ability of the dough to retain gas and subsequently to form springy bread. A very low resistance to extension results in poor gas retention and lower loaf volume. A very high resistance to extension also results in a lower loaf volume because the tough dough is not capable of proofing to an optimum height with the gas produced by the yeast (Sharadanant and Khan, 2003a).

Contrary to our findings, Inoue and Bushuk (1991) reported that extensibility increased with storage time for one week but no clear trend was observed, which is contrary to the results obtained in the present research. However, Sharadanant and Khan (2003) reported that extensibility increased with extended storage time and maximum resistance to extension decreased with storage time.

Table 2.3 shows the adhesiveness of dough samples prepared at different freezing rates, storage temperatures and storage times. Adhesiveness of unfrozen dough was 69.08 g. Adhesiveness of all samples from frozen dough were higher than that of control, indicating more sticky dough. In general, dough adhesiveness increased with storage time. For example, at 30 days adhesiveness values ranged from 74.77 to 80.77 g, while at 180 days values ranged from 93.25 to 115.63 g. Storage temperature was not a significant factor for samples stored at 30 and 60 days, but was for samples stored at 90 and 180 days. At 90 and 180 days, samples stored at lower temperatures were less adhesive.

Freezing rate was also a significant factor for adhesiveness, except for samples stored at 30 days. Samples prepared at freezing rate 2 were less adhesive; those at freezing rate 1 had the highest adhesiveness. In general, adhesiveness values followed a similar dependence as extensibility. Samples stored at -35°C for short times (30 days) were more extensible (with higher force) and less adhesive. Samples stored for long time at low temperature (-10°C) were less extensible (with lower maximum force) and were more adhesive. These results are consistent with extensibility and maximum resistance to extension.

Adhesiveness is defined as the force of attraction acting over a common area between two dissimilar surfaces, in this case the bread dough and the plate of probe from the texture analyzer. Researches to date include study of adhesiveness duto addition of excess water, mixing process, proteolytic enzyme acitivty, and investigation of wheat varieties. As discussed previously, freezing of dough and frozen storage may cause separation of water that is intimately associated with the gluten network resulting from water redistribution due to ice recrystallization. This increased water mobility may be related to disruption of gluten network. This water may be more feely assessable to both surfaces, leading to increased adhesiveness.

Freezing rate 2 also showed less adhesive than other freezing rate. Several researchers reported that dead yeast cell produced reducting substances, leading to deleterious effect on gluten network due to interrupted molecular bonding. The freezing rate results in this work displayed similar dependence as yeast activity. The yeast acitivity showed highest value at freezing rate 2, resulting from higher yeast viability. The higher viable yeast cells might produce less amount of leachets, leading to less injured gluten structure and interaction. The less disrupted structure and network give less water mobility than other rates, leading to lower adhesiveness values.

Microstructure of Frozen Bread Dough

Cryo-SEM was used to visualize the microstructure of frozen dough (in the frozen state) produced at different freezing rates and stored at different temperatures. The frozen dough structure at 90 days showed the typical structure of starch granules embedded in a gluten network (Fig. 2.2-5). All frozen dough samples had voids among the gluten network and starch granules, showing less dense gluten network in those regions. The gluten strands composing the network appeared to be quite damaged after 90 days. Doughs produced at freezing rate 1 and 2 (the slowest rates) were less uniform and had thinner strands. Doughs produced at freezing rates 3 and 4 (more rapid freezing) showed less disrupted structure. Storage temperature had a more notable effect on the gluten network in the dough. With lower storage temperature (closer to -35°C), the dough was less disrupted and had a more uniform gluten network. Regardless of freezing rate, dough with -35 ºC storage temperature showed less disrupted gluten strands in the network than that with -10 ºC storage temperatures.

Microstructural observations help explain the observed rheological properties of dough. As noted, dough stored at higher temperatures and for longer times was less extensible, and more adhesive, than dough stored at low temperatures for shorter times. Lower extensibility can be attributed to the thinner, more disrupted bonding in the gluten. As previously discussed, this disrupted structure is also less able to hold water, and thus more adhesive.

Using SEM, dough microstructure has been investigated by several researchers. Ribotta et al (2004) studied the effect of emulsifiers on frozen bread dough and described the effects of extended frozen storage on the protein matrices. After long storage time, the gluten matrix was found to be quite damaged. Rojas et al. (2000) described dough as a continuous gluten matrix, where starch granule seemed to be scattered among protein network. Discontinuities were observed in the lax matrix surrounding the starch granules under greater magnification. Berglund et al. (1991) found that after 24 weeks frozen storage, the gluten matrix had thinner strands, seemed more disrupted, and was separated from the starch granules. Nicolas et al (2003) observed that during freezing, ice crystals appear to compress the gluten, leading to a significant phase separation between the gluten and ice.

NMR Relaxometry

Pulsed H^1 NMR was used to investigate the relaxation characteristics of dough systems subject to different freezing rate and storage conditions. Table 2.4 shows the transverse relaxation times for the thawed dough samples. The T_2 relaxation time increased somewhat with increased frozen storage time at all storage temperatures. At 30 days, T_2 values ranged from 15.39 to 16.42 ms, while at 180 days T_2 values ranged from 17.62 to 18.71 ms. In most cases, the T_2 times were smallest for samples at freezing rate 2. Frozen storage temperature also had a significant, but small, effect on transverse relaxation times. Samples stored at lower temperature had somewhat shorter T_2 values. For example, at 30 days samples held at -10°C had values ranging from 16.28 to 16.42, while at -35°C values ranged from 15.39 to 15.94 ms.

The amount and state of water play an important role in the preparation and properties of wheat flour dough and their products (Ruan et al, 1999). Ruan et al. (1999) used several methods for presentation and analysis of relaxation time measurement of protons in dough, and suggested there is a continuous distribution of protons having different relaxation times in heterogeneous systems such as dough (Ruan et al, 1999; Esselink et al, 2003b). Time-domain NMR techniques have demonstrated that at high water contents, water is no longer bound in the physical sense.

However, the T_2 relaxation times of this water population are still quite small due to hydrogen exchange with polysaccharide surface hydroxyl groups. In gluten, generally much longer T_2 relaxation times are observed; this has been attributed to a much higher degree of water dynamics (Cherian and Chinachoti, 1996; Esselink et al, 2003b).

In general, longer relaxation times indicate the presence of more mobile water, or at least water that has less restricted rotational freedom. In our studies, T_2 values were greatest at 180 days storage at -10°C, and least at 30 days storage at -35°C. At 180 days at -10°C, we also found the least dough extensibility and maximum dough stickiness, while at 30 days storage at -35°C we found maximum extensibility and the least stickiness. This suggests that changes in T_2 values are most related to changes in the gluten and starch network. As discussed previously, greater changes in the gluten structure and separation of water occur at low storage temperatures and longer storage times. When the dough is thawed, the ice crystals melt but the water is not in the original state. With long frozen storage, ice crystals get bigger and become redistributed through recrystallization. This greater mobility of water once removed from the vicinity of gluten could well explain the increased T_2 values.

Yeast Activity and Gas Production

Table 2.5 presents the total gas production for thawed dough slurries after 180 min at 36°C. Yeast activity in frozen dough produced under all freezing and storage conditions was lower than that for an unfrozen control. For example, total gas was 18.5 ml for unfrozen dough as compared to 15.9 ml for frozen dough produced under freezing rate 2 and stored at -20ºC for 30 days. In general, the total gas production decreased with longer storage periods. The highest gas production at 30 days was obtained from frozen dough produced at freezing rate 2 and stored at -20ºC storage temperature (15.9 ml), and the lowest from dough produced at freezing rates 3 and 4 (most rapid freezing) and stored at -30 or -35ºC (9.2-9.5 ml). Dough frozen at rates 1 and 2 (relatively slow freezing) and stored at -10 and -20°C had higher gas production for all frozen dough samples. This same relative gas production was maintained for all storage periods. However, the total gas volume obtained was lower with at longer storage periods at all freezing rates and storage temperatures. The highest gas volume for frozen dough was 14.1 ml at 60 storage days, 10.5 ml at 90 days, and 8.6 ml at 180 days; the lowest one was 7.8 ml at 60 days, 6.8 ml at 90 days, and 5.7ml at 180 days. Greatest volumes were obtained from dough produced at freezing rate 2. In almost all cases, maximum gassing power was observed for dough samples held at -20°C.

It has been observed by several researchers that yeast activity is diminished by freezing and storage (Wolt and D'Appolonia, 1984b; Autio and Sinda, 1992; Neyreneuf and Delpuech, 1993; El-Hady et al, 1996). In addition, yeast may be less viable after freezing (Baguena et al, 1991). The response of yeast to freezing is a complex combination of competing phenomena. Rapid freezing rates produce numerous small ice crystals, both inside and outside of cells. Slow freezing promotes the growth of larger ice crystals outside of the cell, and tends to pull water out of the cell due to the osmotic gradient that forms. For many cellular systems, the latter is more detrimental as the marked changes in ionic strength and pH in the cell lead to protein denaturation, disruption of cell function, and changes in membrane permeability. Yeast, however, can produce osmoregulating substances such as trehalose (Hino et al, 1990; Van Dijck et al, 1995; Majara et al, 1996; Shima et al, 1999) that work to prevent this. In addition, membrane proteins such as aquaporin also help transport water across the yeast cell membrane, and it is thought that this controlled efflux of water during the freezing process reduces intracellular ice crystal formation and resulting cell damage (Tanghe et al, 2002). Rapid freezing may limit the ability of yeast to adapt to freezing stress. In toto, relatively slow freezing rates are optimal for maintaining yeast activity after thawing (Lorenz, 1974; El-Hady et al, 1996; Havet et al, 2000).

Prolonged frozen storage has also been found by others to be detrimental to yeast fermentative activity. This may be related to accumulated damage that occurs with time, and is likely enhanced by recrystallization processes that occur during storage. Fluctuations in temperature have been found to be especially damaging (Berglund et al, 1991; Inoue and Bushuk, 1991; El-Hady et al, 1996). Even partial freeze-thaw cycles allow water to migrate to coalesce with larger ice crystals.

Interestingly, greatest yeast activity was maintained for dough samples stored at -20°C. This temperature is often recommended for storage in industrial settings (Gerdes, 2001, Chilling Developments for Dough). One might expect greater yeast viability at higher storage temperatures.

Bread Volume

Table 2.6 shows the volume of bread made from the various frozen dough treatments. The volume of bread made from unfrozen control dough was 137.8 ml. All breads made from frozen dough had lower volumes. Typically, greatest loaf volumes were attained from dough frozen at rate 2. However, freezing rate was not a strong determinant for loaf volume. For example, after -10°C and 30 days, loaf volume varied only from 111.7 to 113.3 ml, while at -35°C it varied from 91.7 to 94.7 ml. Similarly, for dough stored for 180 days at -10°C loaf volume varied from 86.3 to 90.0 ml. Greatest overall loaf volume (119.0 ml) was seen with bread made from dough frozen at rate 2, and stored at -20°C for 30 days, the same conditions found for greatest yeast activity. Lowest volumes were found with bread made from dough frozen at rates 3 or 4 and stored at -30 or -35°C (83.7 to 84.0 ml at 180 days). There was no significant difference between freezing rate 3 and 4 for all storage times.

Loaf volumes for all breads decreased with increased storage time. For example, at -10°C volumes decreased from 111.7-113.3 ml at -10°C and 30 days to 86.3-90.0 ml after 180 days. At storage temperatures of -30 and -35°C, the effect of storage time was less pronounced. After 180 days, bread volumes from samples frozen at rate 2, and held at -10 or -20°C, were slightly higher than the other treatments, but there were no significant differences amongst volumes for all other samples held for 180 days.

Storage temperature also had a major influence on subsequent loaf volume. In general, samples held at -20°C produced bread with greatest loaf volume, although storage temperature effects interacted with storage time as described above. Again, this seems to reflect, at least in part, the effects of treatment on yeast activity.

Bread Crumb Firmness

Table 2.7 shows crumb firmness for bread made from frozen dough. Measured firmness was 4.03 g for bread made from unfrozen control dough. For frozen dough, crumb firmness increased significantly with frozen storage time of the dough. For example, at -20°C firmness values ranged from 5.99 to 6.88 g at 30 days, and from 7.76 to 8.04 g at 180 days. In general, bread made from dough frozen at rates 1 and 2 was less firm than bread made from dough frozen at rates 3 and 4 for all storage times. The storage temperature also had a significant effect on bread firmness. All dough samples stored at -10 and -20 ºC produced bread that was less firm than other samples for all storage times. At 30 and 60 days, bread made from dough stored at -10°C was less firm than that from dough stored at -20°C.

One usually assumes that a less firm, more tender bread is most desirable for consumers. As noted, firmness of bread from the control was lower than that from frozen dough. Crumb firmness arises from the integrity of the gluten network, the degree of crosslinking, the amount of gas incorporated, and from other structural components of the bread. Ribbotta et al (2001b; 2004) reported similar results to the ones reported in this work with frozen storage temperature. With increase frozen storage time, the firmness increased, which may come from depolymerization of glutenin and higher retrogradation of amylopectin in bread made from frozen dough (Ribotta et al, 2001; 2003a; Ribotta et al, 2004).

CONCLUSION

The relationships between measures takend during this study are decribed in Figure 2.8. This shows a compilation of the contours from response surface fitting. Clearly, storage past 30 days was fairly detrimental to the dough and bread, so Figure 6 shows data for 30 days only. Both volume and firmness depended primarily on storage temperature, and storage temperatures below -20°C were most beneficial. Elasticity and adhesiveness depended on both storage temperature and freezing rate, and generally lower temperatures and higher freezing rates were beneficial. Yeast activity also depended on temperature and freezing rate, but in this case lower temperatures and slower freezing rates were more beneficial. An optimal region for all attributes (shaded in grey) occurs at temperatures between -15 and -20°C and centered around freezing rate 2. Correlations amongst all the data are also instructive (Table 2.8). Extensibility and adhesiveness were negatively correlated (r^2 = -0.856), most likely as these are both related to structural changes of the gluten caused by freezing. Volume was negatively correlated with firmness (r^2 = -0.845). Increased volume leads to lower density, and other things being equal, causes more deformable bread under compression. Yeast activity was somewhat correlated with volume (r^2 = 0.682) and firmness (r^2 = 0.544). Obviously, greater gas production provides the potential for greater volume; however this is mitigated by the diminished and less extensible dough network.

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Table 2.1. Maximum resistance to extension of frozen bread dough (force, g). Values for unfrozen dough 69.98 ± 1.04 g.

Storage Day	Freezing Rate	Storage Temperature			
		-10ºC	-20ºC	-30ºC	-35ºC
30	Rate 1	49.68 ± 1.50	53.28 ± 0.35	54.33 ± 0.74	54.73 ± 0.71
	Rate 2	51.19 ± 1.37	54.01 ± 0.34	54.19 ± 1.27	55.25 ± 0.45
	Rate 3	52.19 ± 1.06	55.60 ± 0.58	56.99 ± 0.66	55.74 ± 0.53
	Rate 4	52.75 ± 1.17	56.05 ± 0.61	56.41 ± 0.68	56.02 ± 0.42
60	Rate 1	47.01 ± 0.76	49.62 ± 1.18	51.77 ± 0.59	51.11 ± 0.70
	Rate 2	47.62 ± 0.68	50.38 ± 0.79	51.86 ± 0.90	52.25 ± 0.65
	Rate 3	48.89 ± 0.19	52.62 ± 0.87	52.65 ± 0.87	51.45 ± 0.17
	Rate 4	48.70 ± 0.27	53.38 ± 0.97	52.74 ± 1.08	52.22 ± 1.03
90	Rate 1	43.78 ± 0.88	45.45 ± 0.88	45.33 ±1.13	45.38 ± 0.70
	Rate 2	44.84 ± 0.69	46.34 ± 0.87	47.50 ± 0.85	47.73 ± 1.12
	Rate 3	45.11 ± 0.51	47.31 ± 0.59	47.73 ± 1.56	47.02 ± 0.32
	Rate 4	45.71 ± 0.38	47.30 ± 0.44	46.64 ± 0.79	47.32 ± 0.31
180	Rate 1	29.17 ± 0.73	30.15 ± 0.75	31.99 ± 0.82	32.01 ± 0.54
	Rate 2	29.54 ± 0.69	33.68 ± 1.04	33.19 ± 0.83	33.06 ± 0.78
	Rate 3	29.49 ± 0.05	32.64 ± 0.71	32.82 ± 0.29	33.12 ± 0.18
	Rate 4	29.71 ± 0.38	32.39 ± 0.55	32.67 ± 1.12	32.13 ± 0.41

Table 2.2. Extensibility of frozen bread dough (distance to peak, mm). Values for unfrozen dough 84.99 ± 2.68 mm.

Storage Day	Freezing Rate	Storage Temperature			
		-10ºC	-20ºC	-30ºC	-35ºC
30	Rate 1	74.45 ± 1.41	76.23 ± 0.48	77.19 ± 0.74	76.84 ± 2.55
	Rate 2	76.12 ± 3.98	78.10 ± 0.59	79.58 ± 0.28	80.92 ± 5.46
	Rate 3	75.86 ± 0.52	77.42 ± 0.51	80.18 ± 0.06	80.99 ± 0.67
	Rate 4	75.22 ± 0.73	77.69 ± 0.87	79.95 ± 0.93	81.00 ± 0.74
60	Rate 1	69.81 ± 1.29	73.37 ± 0.75	75.28 ± 0.51	73.46 ± 0.79
	Rate 2	72.90 ± 0.78	76.09 ± 0.70	77.45 ± 0.20	78.81 ± 0.35
	Rate 3	71.64 ± 1.29	75.23 ± 0.45	76.93 ± 0.53	77.49 ± 1.09
	Rate 4	70.96 ± 0.50	75.30 ± 0.62	77.30 ± 0.73	77.93 ± 0.38
90	Rate 1	63.94 ± 1.76	72.59 ± 0.60	73.94 ± 0.74	74.71 ± 1.17
	Rate 2	67.10 ± 1.37	73.58 ± 0.78	75.76 ± 1.33	75.81 ± 0.35
	Rate 3	66.66 ± 0.71	73.74 ± 0.38	74.93 ± 0.53	75.15 ± 0.23
	Rate 4	65.75 ± 0.08	74.63 ± 1.05	75.63 ± 1.36	75.42 ± 0.50
180	Rate 1	49.94 ± 0.16	67.59 ± 0.41	69.19 ± 0.50	70.04 ± 0.59
	Rate 2	52.43 ± 1.08	69.24 ± 0.31	71.07 ± 0.45	71.39 ± 0.40
	Rate 3	52.00 ± 0.14	68.38 ± 0.63	70.02 ± 0.09	70.30 ± 0.88
	Rate 4	52.42 ± 0.54	69.66 ± 0.45	70.78 ± 0.58	70.48 ± 0.52

Table 2.3 Adhesiveness of frozen bread dough (tension force, g). Values for unfrozen dough 69.08 ± 1.80 g.

Storage Day	Freezing Rate	Storage Temperature			
		-10ºC	-20ºC	-30ºC	-35ºC
30	Rate 1	79.47 ± 2.51	78.03 ± 3.28	79.30 ± 0.59	78.07 ± 0.58
	Rate 2	75.04 ± 4.18	77.20 ± 6.46	78.22 ± 2.60	75.12 ± 1.47
	Rate 3	77.49 ± 1.54	81.00 ± 0.88	80.10 ± 4.71	74.77 ± 0.98
	Rate 4	78.73 ± 0.88	80.77 ± 3.55	79.28 ± 3.55	77.82 ± 2.29
60	Rate 1	82.78 ± 6.73	88.39 ± 0.80	87.70 ± 3.30	83.15 ± 0.49
	Rate 2	77.88 ± 0.59	78.81 ± 0.52	78.16 ± 0.95	81.63 ± 1.12
	Rate 3	82.21 ± 0.27	83.37 ± 1.13	80.30 ± 0.97	82.41 ± 1.14
	Rate 4	82.01 ± 0.43	82.37 ± 1.13	82.00 ± 1.93	80.93 ± 0.73
90	Rate 1	108.45 ± 1.08	97.48 ± 1.48	94.37 ± 3.54	93.15 ± 0.49
	Rate 2	105.86 ± 2.96	91.99 ± 2.79	90.83 ± 0.23	91.63 ± 1.12
	Rate 3	107.84 ± 0.60	92.36 ± 0.52	91.97 ± 0.73	92.41 ± 1.14
	Rate 4	106.24 ± 0.77	92.37 ± 1.06	92.00 ± 1.93	93.43 ± 3.84
180	Rate 1	115.63 ± 0.90	109.60 ± 4.15	97.23 ± 0.96	95.19 ± 1.02
	Rate 2	114.47 ± 1.64	98.83 ± 0.74	93.25 ± 0.90	94.34 ± 0.73
	Rate 3	115.52 ± 0.31	108.69 ± 1.42	94.44 ± 1.54	94.02 ± 1.34
	Rate 4	113.74 ± 3.88	105.32 ± 3.34	94.25 ± 1.20	93.46 ± 0.59

Table 2.4. Transverse relaxation time (T_2 values) of frozen bread dough after thawing at 10°C.

Storage Day	Freezing Rate	Storage Temperature			
		-10ºC	-20ºC	-30ºC	-35ºC
30	Rate 1	16.42	16.32	16.01	15.93
	Rate 2	16.35	16.01	16.35	15.52
	Rate 3	16.28	16.25	16.37	15.66
	Rate 4	16.35	16.45	16.21	15.39
60	Rate 1	16.91	16.98	16.84	16.73
	Rate 2	16.76	16.59	16.43	16.42
	Rate 3	16.82	16.87	16.79	16.80
	Rate 4	16.89	16.93	17.06	16.62
90	Rate 1	17.84	17.96	17.50	17.80
	Rate 2	18.01	17.65	17.85	17.73
	Rate 3	18.20	17.82	17.15	17.60
	Rate 4	17.61	17.67	17.51	17.84
180	Rate 1	18.59	17.56	18.65	18.00
	Rate 2	18.46	18.15	18.31	17.78
	Rate 3	18.71	18.30	18.00	17.62
	Rate 4	18.54	18.03	17.89	17.91

Table 2.5. Yeast Activity (*ml CO_2 gas*) after assigned storage time (Value for unfrozen dough, 24.5 ± 1.27 *ml*)

Storage Day	Freezing Rate	Storage Temperature			
		-10ºC	-20ºC	-30ºC	-35ºC
30	Rate 1	12.6 ± 0.28	14.4 ± 0.92	12.4 ± 0.57	12.2 ± 0.42
	Rate 2	14.0 ± 0.85	15.9 ± 0.64	12.4 ± 0.71	12.2 ± 0.57
	Rate 3	10.2 ± 0.57	10.8 ± 0.57	9.5 ± 1.13	9.2 ± 0.57
	Rate 4	9.8 ± 0.71	10.6 ± 0.35	9.2 ± 0.71	9.4 ± 0.42
60	Rate 1	10.8 ± 0.57	12.6 ± 0.92	10.5 ± 0.71	10.2 ± 0.49
	Rate 2	12.0 ± 0.57	14.1 ± 0.21	10.7 ± 1.06	10.4 ± 0.57
	Rate 3	9.0 ± 0.14	9.4 ± 0.07	8.2 ± 0.71	7.8 ± 0.99
	Rate 4	8.1 ± 0.42	8.6 ± 0.07	8.2 ± 0.42	8.0 ± 0.42
90	Rate 1	8.5 ± 0.14	9.3 ± 1.13	8.1 ± 0.57	7.9 ± 0.1
	Rate 2	9.4 ± 0.42	10.5 ± 0.28	9.3 ± 0.85	9.1 ± 0.71
	Rate 3	7.2 ± 0.42	8.4 ± 0.64	7.1 ± 0.57	6.9 ± 0.49
	Rate 4	6.8 ± 0.85	8.4 ± 0.14	6.9 ± 0.71	6.9 ± 0.57
180	Rate 1	7.2 ± 0.28	7.9 ± 0.85	6.9 ± 0.57	6.8 ± 0.42
	Rate 2	7.8 ± 0.64	8.6 ± 0.42	7.9 ± 0.28	7.7 ± 0.28
	Rate 3	6.1 ± 0.64	6.9 ± 0.42	6.0 ± 0.28	5.8 ± 0.28
	Rate 4	5.7 ± 0.28	6.6 ± 0.85	5.9 ± 0.57	6.0 ± 0.42

Table 2.6. Bread volume (*ml*) made from frozen bread dough. Values for unfrozen dough 137.8 ± 1.15 *ml*.

Storage Day	Freezing Rate	Storage Temperature			
		-10ºC	-20ºC	-30ºC	-35ºC
30	Rate 1	111.7 ± 0.58	115.3 ± 1.53	94.3 ± 2.31	94.0 ± 1.00
	Rate 2	113.3 ± 0.58	119.0 ± 1.00	95.3 ± 0.58	94.7 ± 0.58
	Rate 3	113.3 ± 0.58	113.3 ± 1.53	93.7 ± 0.58	91.7 ± 0.58
	Rate 4	112.7 ± 1.53	113.0 ± 1.00	94.7 ± 0.58	92.0 ± 1.00
60	Rate 1	108.7 ± 1.53	113.3 ± 1.53	92.3 ± 0.58	92.3 ± 1.15
	Rate 2	109.3 ± 0.58	116.3 ± 1.15	93.7 ± 0.58	93.3 ± 0.58
	Rate 3	109.0 ± 1.00	111.7 ± 1.53	91.3 ± 0.58	91.3 ± 0.58
	Rate 4	108.0 ± 1.00	112.7 ± 2.52	91.7 ± 1.15	91.3 ± 1.15
90	Rate 1	104.7 ± 1.53	109.0 ± 1.00	92.0 ± 1.00	90.3 ± 0.95
	Rate 2	107.3 ± 0.58	112.0 ± 1.00	92.7 ± 2.52	90.7 ± 0.58
	Rate 3	101.0 ± 1.00	107.7 ± 0.58	88.7 ± 1.15	88.3 ± 1.15
	Rate 4	102.0 ± 2.65	107.7 ± 1.53	88.7 ± 0.58	88.7 ± 0.58
180	Rate 1	88.3 ± 0.58	88.7 ± 1.15	88.0 ± 2.00	86.0 ± 1.00
	Rate 2	90.0 ± 1.00	91.3 ± 0.58	89.3 ± 2.52	89.0 ± 1.00
	Rate 3	86.3 ± 0.58	87.0 ± 1.00	85.3 ± 0.58	84.0 ± 1.73
	Rate 4	87.7 ± 0.58	87.7 ± 1.53	84.0 ± 1.00	83.7 ± 1.53

Table 2.7. Firmness (N force) of bread made from frozen bread dough. Values for unfrozen dough 4.03 ± 0.53 N.

Storage Day	Freezing Rate	Storage Temperature			
		-10ºC	-20ºC	-30ºC	-35ºC
30	Rate 1	5.00 ± 0.37	6.12 ± 0.22	10.60 ± 0.83	9.0 ± 0.77
	Rate 2	4.56 ± 0.60	5.99 ± 0.56	10.76 ± 0.32	8.9 ± 0.11
	Rate 3	5.78 ± 0.35	6.47 ± 0.11	10.46 ± 1.36	9.3 ± 0.16
	Rate 4	5.91 ± 0.38	6.88 ± 0.33	10.38 ± 0.37	9.5 ± 0.07
60	Rate 1	5.93 ± 0.25	6.80 ± 0.03	11.81 ± 0.54	11.4 ± 1.06
	Rate 2	5.87 ± 0.05	6.64 ± 0.68	12.18 ± 0.34	11.6 ± 0.25
	Rate 3	6.47 ± 0.54	6.90 ± 0.27	12.29 ± 0.28	11.4 ± 0.23
	Rate 4	6.16 ± 0.11	6.95 ± 0.18	11.74 ± 0.39	11.7 ± 0.03
90	Rate 1	8.00 ± 0.33	7.28 ± 0.50	12.26 ± 0.41	12.3 ± 0.38
	Rate 2	7.71 ± 0.11	7.12 ± 0.36	12.36 ± 0.18	11.9 ± 0.18
	Rate 3	9.07 ± 0.55	6.96 ± 0.21	12.04 ± 0.14	11.9 ± 0.09
	Rate 4	8.86 ± 0.25	7.35 ± 0.21	12.40 ± 0.32	12.3 ± 0.51
180	Rate 1	8.78 ± 0.06	8.03 ± 0.38	12.48 ± 0.36	12.9 ± 0.18
	Rate 2	8.46 ± 0.20	8.04 ± 0.29	12.73 ± 0.26	12.7 ± 0.10
	Rate 3	10.18 ± 0.60	7.76 ± 0.21	12.34 ± 0.34	12.6 ± 0.29
	Rate 4	9.73 ± 0.15	8.04 ± 0.21	12.94 ± 0.16	12.8 ± 0.08

Table 2.8. Scatterplot matrix showing significant correlations for dough and bread properties.

	Ext	Adhes	T_2	Volume	Firmness	Yeast
Ext	1	-0.856	0.0568	0.238	0.0406	0.479
Adhes		1	-0.131	-0.408	0.164	-0.657
T_2			1	0.185	-0.179	0.0553
Volume				1	-0.845	0.682
Firmness					1	-0.543
Yeast						1

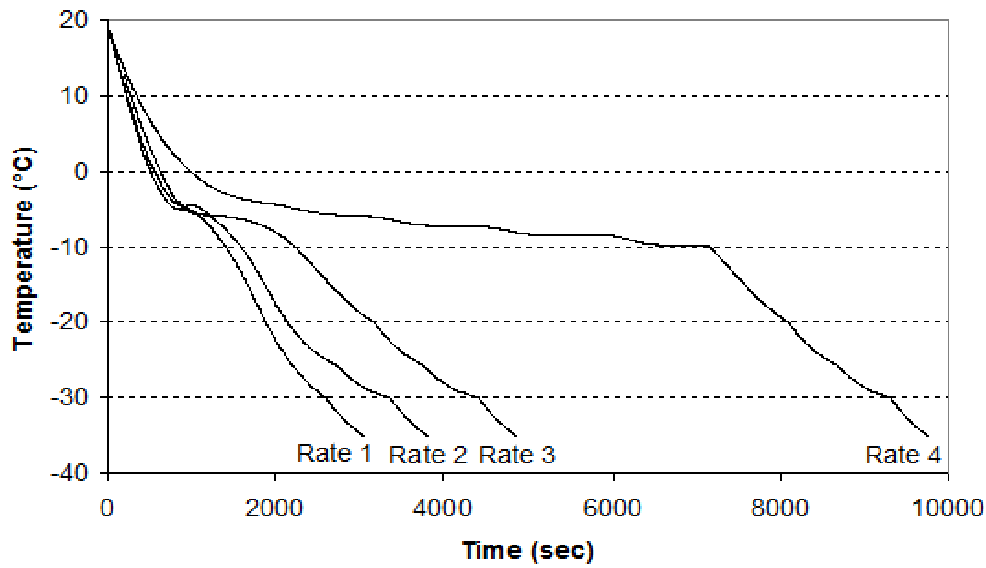

Figure 2.1. Freezing rate protocols for frozen bread doughs. (1) Rate 1- unfrozen dough cooled and frozen at 13.9°C/hr, (2) Rate 2- dough frozen at 28.1°C/hr, (3) Rate 3- dough frozen at 37.9°C/hr, (4) Rate 4- dough frozen at 50.8°C/hr.

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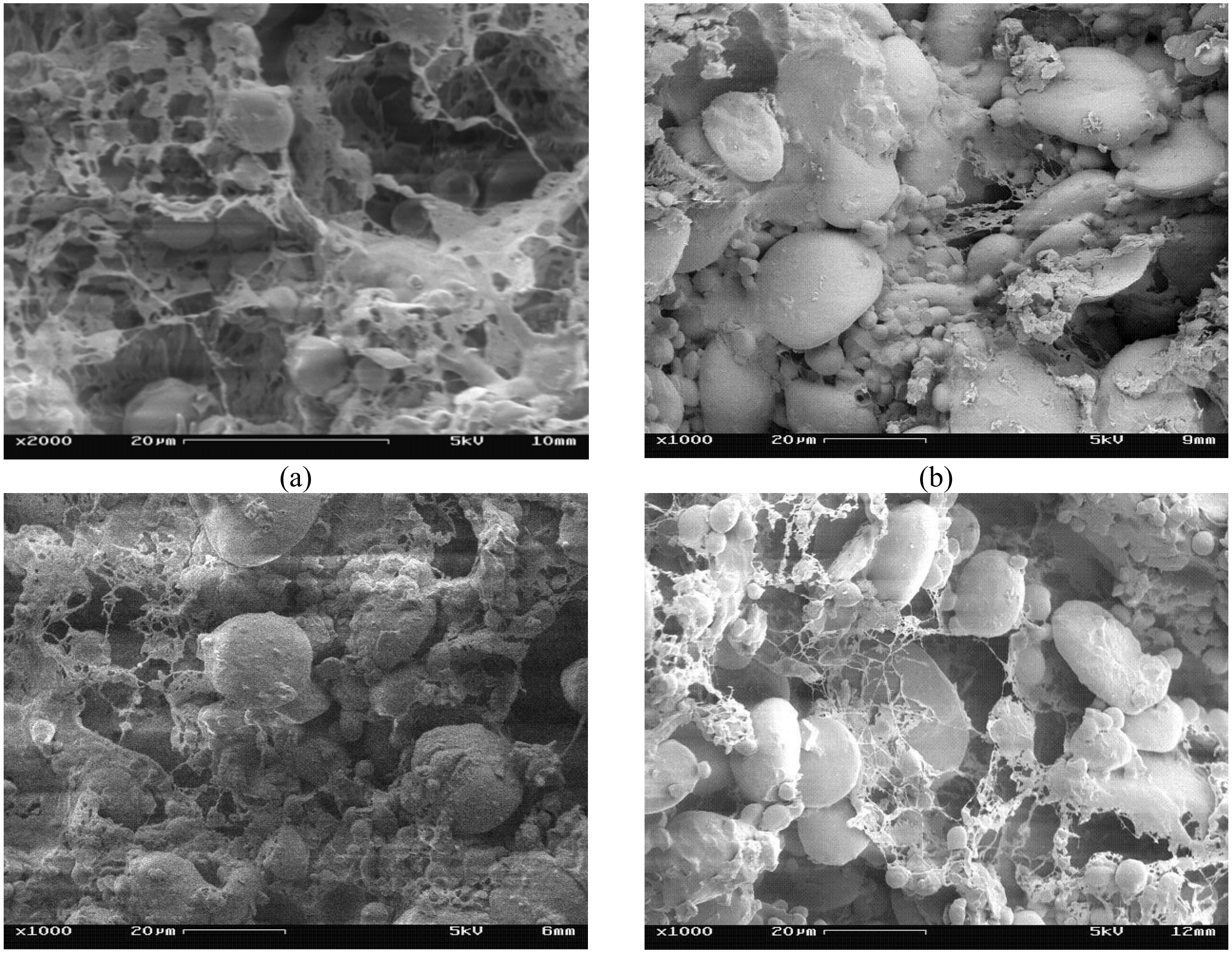


(a) (b) (c) (d)

Figure 2.2. Cryo Scanning Electron Micrograph (Cryo-SEM) of fractured pieces of dough stored for 90 days. Stored at -10 ºC and frozen rate 1(a), rate 2(b), rate 3(c) and rate 4(d)

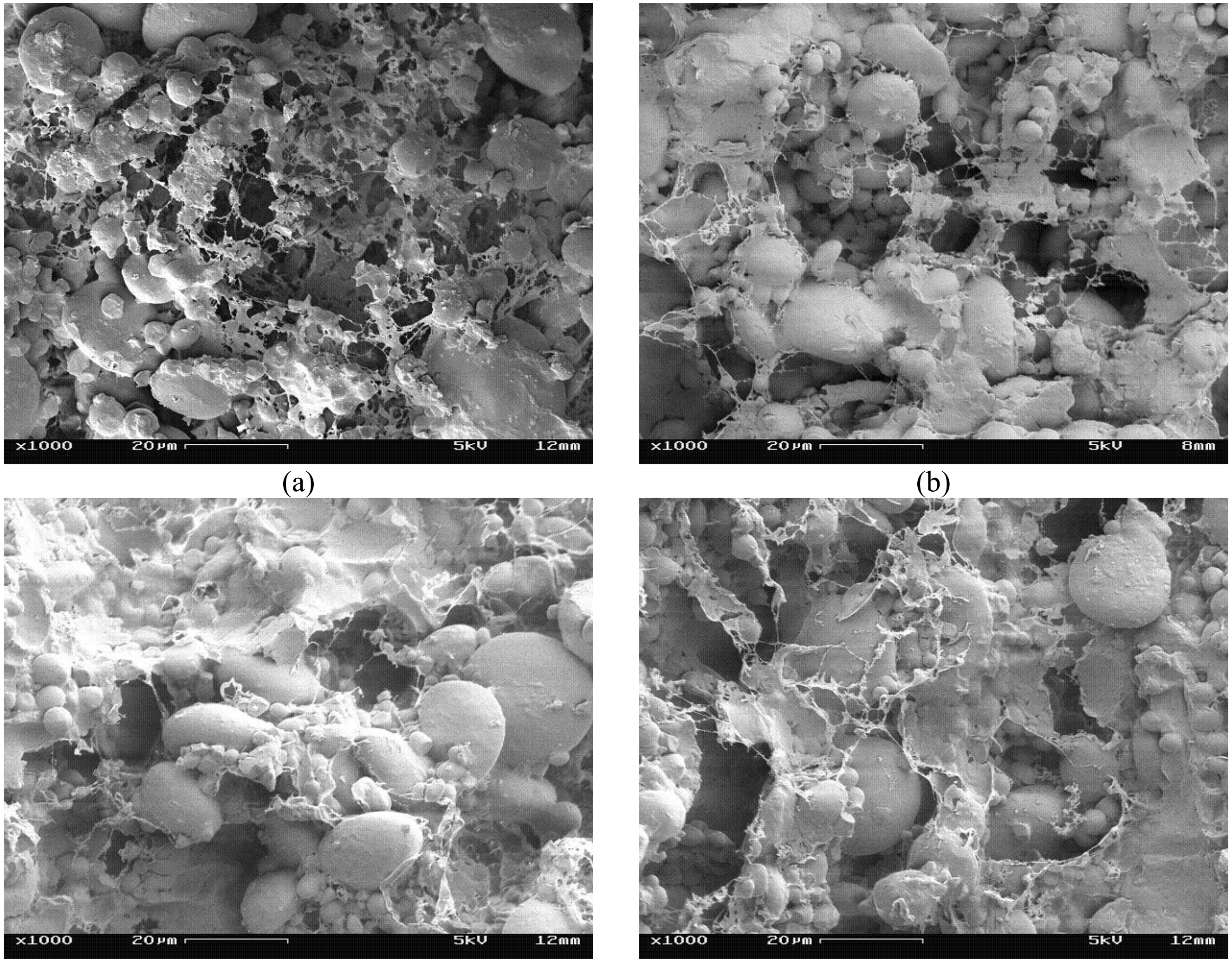
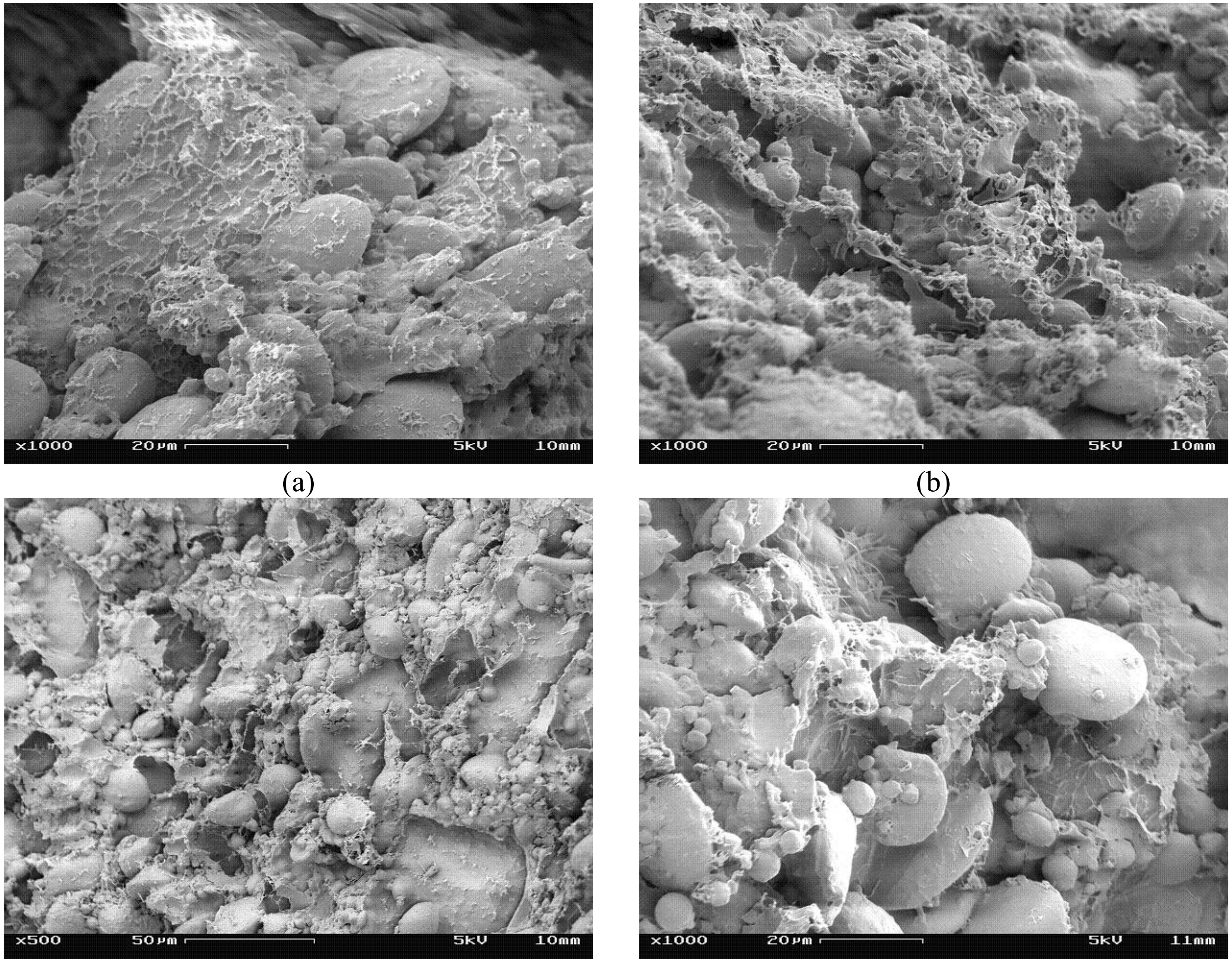


Figure 2.3. Cryo Scanning Electron Micrograph (Cryo-SEM) of fractured pieces of dough stored for 90 days. Stored at -20 ºC and frozen rate 1(a), rate 2(b), rate 3(c) and rate 4(d)



(a) (b) (c) (d)

Figure 2.4. Cryo Scanning Electron Micrograph (Cryo-SEM) of fractured pieces of dough stored for 90 days. Stored at -30 ºC and frozen rate 1(a), rate 2(b), rate 3(c) and rate 4(d)

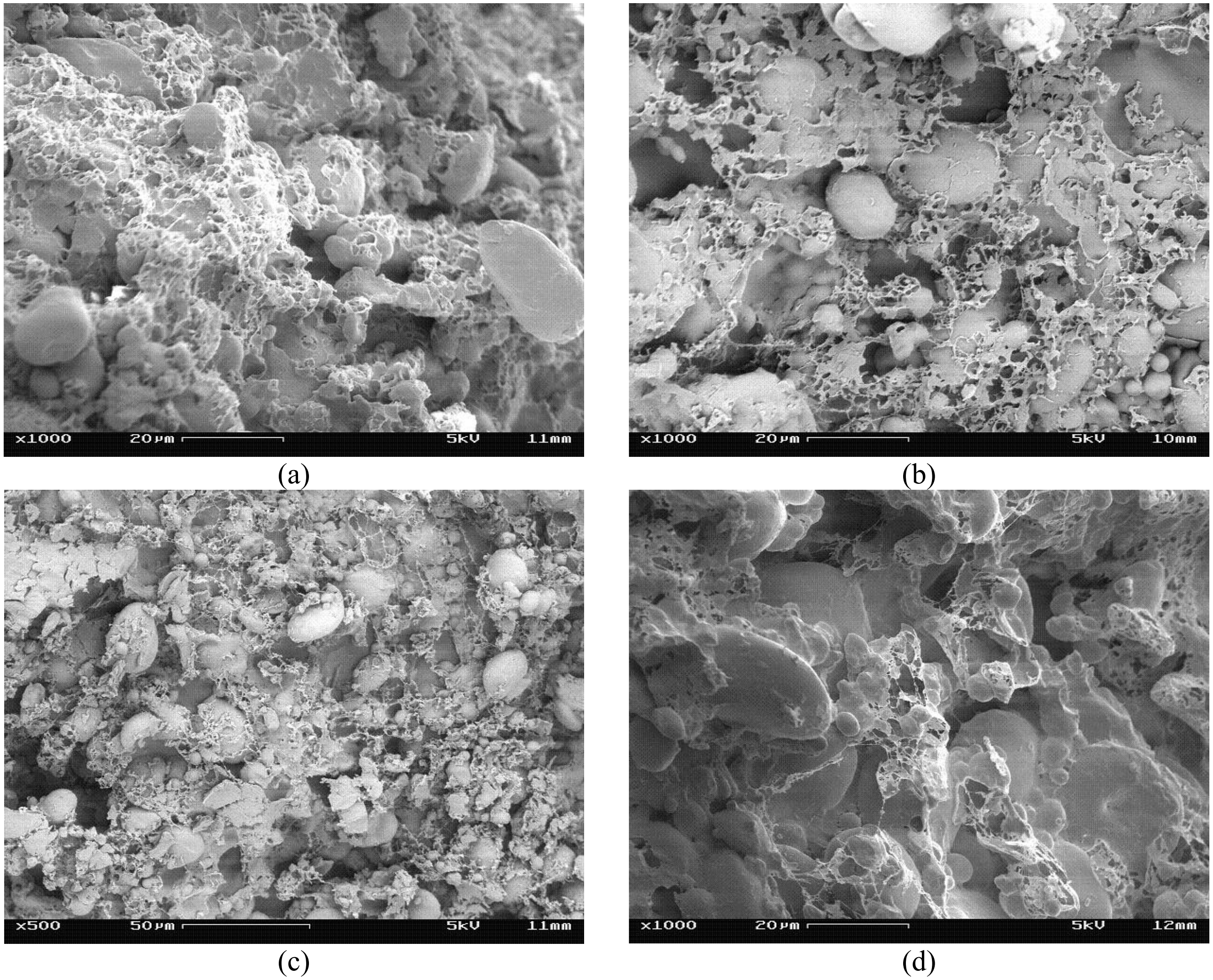


Figure 2.5. Cryo Scanning Electron Micrograph (Cryo-SEM) of fractured pieces of dough stored for 90 days. Stored at -35 ºC and frozen rate 1(a), rate 2(b), rate 3(c) and rate 4(d)

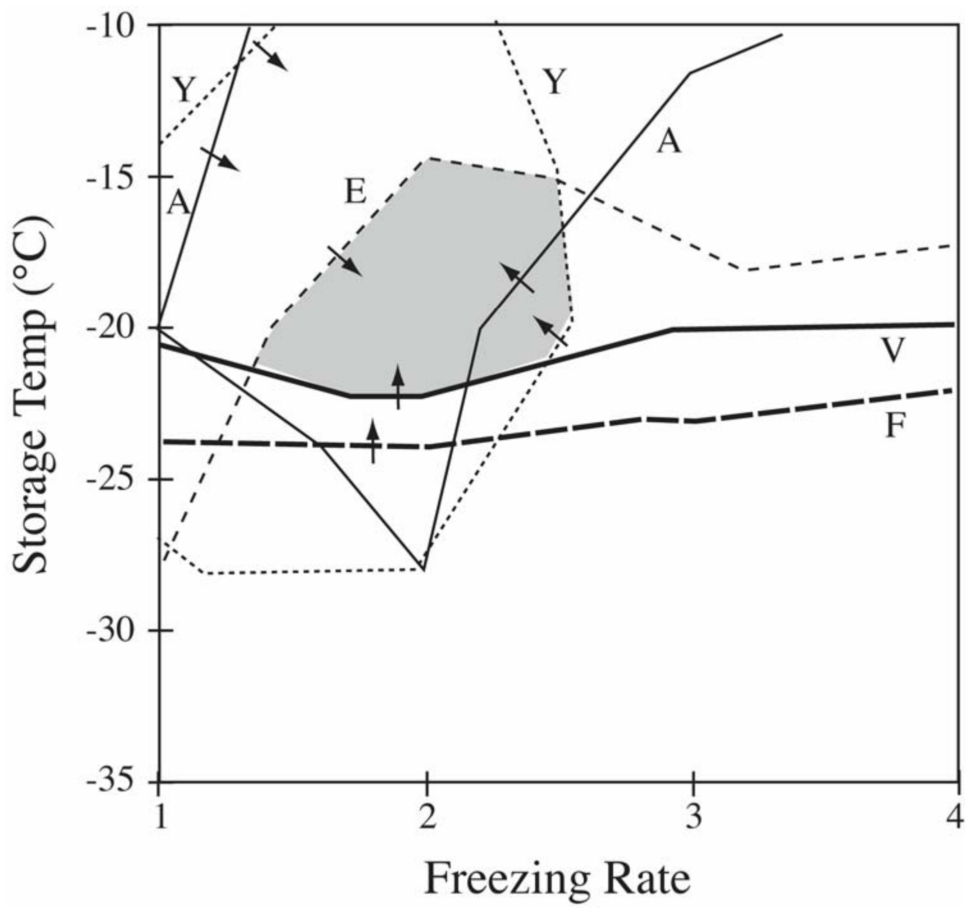


Figure 2.6. Contour plots for elasticity (E), yeast activity (Y), and bread volume (V) at 90% of their maximum value; and adhesiveness (A) and firmness (F) at 90% of their minimum value. Arrows show direction toward more optimal values.

CHAPTER 3

THE EFFECTS OF FREEZING AND STORAGE CONDITIONS ON THE PROPERTIES OF BREAD AND BREAD STALING

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ABSTRACT

The quality factors of bread made from frozen dough is influenced by processing parameters such as freezing temperature and rate, as well as storage temperature and time. However, few studies have been carried out on the combined effects of these processing factors. In this research, the objective was to compare the effects of freezing temperature and rate as well as storage temperature and time on the bread quality factor and bread staling. Yeasted bread dough was frozen using four freezing rates, then stored at -10, -20, -30, or -35°C for 30, 60, 90, and 180 days. The properties of bread made dough under produced those condition were measured with nuclear magnetic resonance techniques (NMR), texture analysis, and color analysis. Specifically, bread staling, a physical property of the bread, was measured using NMR. Our results indicate that longer storage time rendered bread with lower specific volume and darker color. Baking test showed that bread made from dough stored at -20°C and with freezing rate 1 and 2 resulted in the highest specific volume and faster bread staling. The combination of freezing rate 1 and 2 and storage temperature -10 and -20 °C gave bread with higher specific volume and lower firmness owing to higher yeast activity. NMR studies showed that frozen dough at lower storage temperatures had lower T_2 values (9-10 ms) and gave bread with lower bread specific volume and lower bread staling rate. However, dough stored at higher temperatures displayed higher yeast activity, resulting in higher loaf volume. Regardless of bread quality, color seemed to be darker and non-uniform surface color distribution with lower dough quality. Increased understanding of the combined effects of processing conditions is essential for producing better quality of dough and predicting bread quality. This research demonstrated that specific combinations of freezing rates and storage conditions contribute to better bread quality and staling.

Key words: frozen dough; crumb firmness; specific volume; NMR; bread volume; bread color; bread staling

INTRODUCTION

Bread is a very popular and important food product, and has developed as a staple food in many countries due to its significant nutritional, sensorial and textural characteristics (Cauvain and Young, 1999). In the last few decades, alternative or novel methods have developed for the production and preservation of bakery products, including freezing and modified atmosphere technologies (Kulp et al, 1995; Laaksonen and Roos, 2000; Bhattacharya et al, 2003). Conventional breadmaking is time and labor consuming and requires specialized skills, while bread is a food product with a limited shelflife and rapidly degrading sensory characteristics (Kulp et al, 1995; Cauvain, 1998; Giannou and Tzia, 2007). In contrast, frozen dough can be manufactured in a centralized facility, and shipped to stores where bread can be prepared as needed without any serious requirements in space and equipment. Currently, frozen bakery products occupy an increasingly greater portion of the food market (Rouille et al, 2000; Giannou and Tzia, 2007).

Bread quality depends on a variety of factors including texture, flavor, color and appearance of the loaf. Freshly baked bread has a soft, elastic, and moist crumb. Firmness is an often-measured texture property, and increases in firmness are indicative of staling. Firmness is also related to the bread density, which may differ with the type of bread. Typically bread volume is used as a measure of appropriate density, and is a result of the elastic properties of the dough and the activity of the yeast. Flavor is a complex combination of inherent taste and aroma compounds, as well as those that develop from yeast and browning reactions during baking. Expected color also varies with bread variety, but typically consumers expect a well-developed and even distribution of color, but without signs of over-baking. As moisture content, staling, and polymer properties are intimately related to interactions with water, several researchers have used NMR relaxometry to study the state of water in flour dough and bread (Chen et al, 1997; Ruan et al, 1999; Vodovotz et al, 2002). For example, the spin-spin relaxation time (T_2) is a function of the spin species as well as the chemical and physical environments surrounding the spins. Measurements of T_2 and T_2 distributions by proton NMR can be related to various domains of water, the mobility of water, and the interactions of water with food macromolecules (Chen et al, 1997; Hallberg and Chinachoti, 2002; Vodovotz et al, 2002; Wang et al, 2004).

Freezing and prolonged frozen storage may cause deleterious effects on thawed dough and the bread from which it is made, but the factors leading to this have not been fully investigated (Giannou and Tzia, 2007). Part of these changes may be related to alterations of dough structure, while others are related to yeast products or changes in yeast activity or viability. Researchers (Esselink et al, 2003a; Esselink et al, 2003b) have postulated that changes in the gluten network are partly due to ice recrystallization, which results in an increase in the size of the ice crystals. This leads to separation of water from gluten macromolecules, with thinning and reduced pliability of the network (Bache and Donald, 1998; Zheng et al, 2000; Rojas et al, 2002). Others (Wolt and D'appolonia, 1984a; b; Inoue and Bushuk, 1991; Casey and Foy, 1995) have suggested that leacheates from yeast cells, including glutathione, contribute to slackening of the gluten network. The altered dough structure causes decreased oven spring, longer dough proofing time, and diminished bread quality after baking.

Bread made from frozen dough also stales more quickly than that made from fresh dough. Bread staling is frequently attributed to starch retrogradation, which is considered as the main factor responsible for the increase in crumb firmness during storage, although interactions with protein and lipids may also play a role. While studies on extended storage of frozen dough are limited, for part-baked samples longer frozen storage times produced a progressive increase in the retrogradation temperature range of the amylopectin, and greater energy was required for amylopectin melting, indicating that structural changes of amylopectin were produced during frozen storage. In addition, the hardening rate was greater for rebaked samples made from part-balked doughs stored frozen for greater times (Barcenas et al, 2003; Barcenas et al, 2004).

Several researchers have studied the effects of storage temperature on bread quality factors (Hsu et al, 1979b; Le Bail et al, 1999b). Little is known about the combined effects of freezing rate and storage temperature on the quality of bread made from frozen dough. Previous research in this lab has shown that freezing rate, temperature and storage time are all relevant factors to the structure and properties of frozen-thawed dough. In this research, we examined several quality factors of pan-bread prepared from frozen dough produced under different freezing and storage conditions. This included measurements of texture, bread volume, and color distribution. In addition, we measured the activity of yeast during proofing for each of these conditions. Finally, we studied the rates of staling and changes in water using 1H-NMR techniques.

MATERIALS AND METHODS

Dough Preparation and Freezing

Dough was prepared from enriched, bleached hard winter wheat flour (Organic Select Artisan Flour, King Arthur Company, Norwich, Vermont) with 11.5% protein content. Other ingredients (as % flour weight) included: 60% water, 3% Baker's dry yeast (Fleischmann's Yeast, Quebec, Canada); Dominion pure cane sugar (Dixie Crystal, Savannah, GA); non-iodized salt (Morton International, Inc., Chicago, IL); and soybean oil (Wesson Vegetable Oil, ConAgra Foods, Omaha, Nebraska). The yeast was pre-hydrated with the water, and all of the ingredients placed in a 6-quart, 575 watt mixer (KitchenAid Professional 600 Series, St. Joseph, MI) and mixed for 2 min at speed of 120rpm with a paddle, and for 8 min at 178rmp with a dough hook. Once the dough was formed, it was separated into samples of 50g, and formed into slightly round shapes by hands.

Freezing and Storage Conditions

Approximately 50g samples were placed in bread pans to be frozen. Doughs were frozen in circular slabs, approximately 50mm diameter. Freezing was accomplished in walk-in blast freezers located in the Department of Food Science, set to give several rates of freezing. These included:

- Freezing rate 1- unfrozen dough cooled and frozen at 13.9°C/hr.
- Freezing rate 2- dough frozen at 28.1°C/hr.
- Freezing rate 3- dough frozen at 37.9°C/hr.
- Freezing rate 4- dough frozen at 50.8°C/hr.

After freezing, each dough sample was placed into 4 different frozen storage temperatures; -10, -20, -30, and -35 °C. When the core temperature of dough sample reached a desired point, the frozen dough pieces were vacuum-packed and stored for 30, 60, 90, and 180 days.

Bread Preparation

Samples were withdrawn from the freezer after the specified time and were placed in an environmental chamber at 8 °C to thaw. Optimum thawing-proofing conditions were pre-determined by a pre-test based on time-temperature conditions (Takano et al, 2002). Thawed dough was proofed in an environmental stability chamber (Model HEC10R, Hotpack, Warminster, PA) at 36 ºC and 85% relative humidity for 180min. Proofed dough was baked at 180 °C for 15 min (Blodgett DFG-100, Burlington, VT). After baking, bread samples were allowed to cool for approximately 1hr at ambient temperature (Baardseth et al, 2000).

Bread Volume

Loaf volume and weight were determined 1 hr after baking at ambient temperature according to the AACC 10-05 (2000) procedure. Loaf volume was determined by the rapeseed displacement test and specific volume was expressed as the ratio of bread volume to dough weight (Pyler, 1988; Hallen et al, 2004). The loaf was put in a container with known volume (V_C). The container was topped up with rapeseed, the loaf removed and the volume of the rapeseed measured (V_R). Loaf volume (V_L) could then be calculated and recorded according to:

$$V_L\,(ml) = V_C - V_R$$

After measuring volume, the loaf weight (W) was determined on a digital scale. Specific volume (V_S) of bread was calculated as

$$V_s(ml/g) = \frac{V_L}{\mathrm{W}}$$

Bread Crumb Firmness

The firmness of the bread samples was determined using a texture analyzer (TA-XT2i, Texture Technologies Corp., Scarsdale, NY) with a 36mm radius cylinder probe according to AACC Method 74-10A (2000). Three center slices of each loaf were measured at 25 °C. The compression rate was set at 1.7mm/s. Firmness was the force required to compress the slices (20 mm thick) by 40% strain.

Bread Crust Color Measurement

The bread image was acquired with a digital camera (Canon PC1114, Japan) and was used to evaluate the crust color of each baked sample. This method is non-destructive and allows measurement of a larger bread surface area than attained with conventional colorimeters (Purlis and Salvadori, 2007). Images were taken under natural light conditions and the camera was placed perpendicularly to the bread surface at 0.3 m distance. Each image was saved as a jpeg file, at 3072 × 2304 pixel resolution. Images were cropped to keep only the bread sample surface, resulting in circular images with a diameter of 1200 pixels, resulting in a spatial resolution of approximately 125μm/pixel. The digital image was analyzed using the software Image J (http://rsb.info.nih.gov/ij/). The color image was converted to 256 level gray scales. Using bars of known lengths, pixel values were converted into distance units, producing distribution of gray scale. The distribution gives mean and standard deviation of gray scale (Datta et al, 2007).

NMR Relaxometry and Bread Staling

Proton relaxation measurements were made using a 20MHz Proton (^{1}H) NMR spectrometer (Resonance Instruments, Whitney, UK). Approximately 2g bread sample was taken from the center of each loaf, with three replicates were taken from each loaf of bread. Each sample was placed in a 10mm diameter glass tube then covered with parafilm, then placed within a larger 18mm diameter NMR tubes. Transverse (T_2) relaxation curves were developed using the CPMG pulse sequence: 90x-(τ-180y-τ-echo)n (Meiboom and Gill, 1958). Acquisition parameters were set to a 90° pulse of 4.2μs and a recycle delay of 2s. A pulse spacing (τ) of 100μs was chosen to exclude the fast decaying solid-like signal. All measurements were made at 25°C ±1°C, controlled by the internal temperature control system in NMR spectrometer. Relaxation curves obtained from CPMG sequences were analyzed using the WinDXP distributed exponential routine (Resonance Instruments, Whitney, UK). This routine calculates a distribution of T_2 terms that best describe the data:

$$g_i = \sum_{j=1}^{m} f_j e^{-t_i/T_{2,j}}$$

where g_i are the values of the exponential distribution at time t_i; f_j are the pre-exponential multipliers, and $T_{2,j}$ are the time constants. In general, there was more than one distributed region, suggesting the existence of various regions of water with similar mobility. In this case, the number of protons in a given region was measured by the integrated signal intensity over the region of the distribution. Staling that occurred during bread storage was measured using the normalized ratio of the transverse relaxation time (Chen et al, 1997; Vodovotz et al, 2002):

$$\Delta T_2 = \frac{T_{2i} - T_{2t}}{T_{2i}}$$

Where T_{2i} is the initial relaxation time (at day 0) and T_{2t} , the relaxation time measured at each day thereafter.

Statistical Analysis

An ANOVA procedure was used to statistically analyze all data, using a three-way mixed design. Fisher's LSD was used to determine significant differences between samples. A *p*-value of less than 0.05 was considered significant.

RESULTS AND DISCUSSION

Specific Bread Volume

Table 3-1 shows the specific volume of bread made from frozen dough processed at different conditions. The specific volume of bread from unfrozen control dough was 3.14 ml, and all breads made from frozen dough had lower specific volumes than control. The greatest specific volumes were attained from dough frozen at rate 2, but freezing rate did not have a strong influence on volume. For example, at -10°C and 30 days, specific volume varied from 2.58 to 2.71 ml/g, and at -35°C it varied from 1.98 to 2.16 ml/g. After 180 days at -10°C, specific volume varied from 1.98 to 2.07 ml/g. In general, there was no significant difference between freezing rate 3 and 4 for all storage period. Greatest loaf volume (2.73 ml/g) was observed for bread made from dough frozen at rate 2, and stored at -20°C for 30 days. Lowest volumes (1.66 to 1.94 ml/g) were found with bread made from dough frozen stored at -35°C for 180 days.

Specific volumes for all breads decreased with storage time. For example, at -10°C volumes decreased from 2.58-2.71 ml/g at -10°C and 30 days to 1.98-2.07 ml/g after 180 days. At -35°C, volumes ranged from 1.98-2.16 ml/g at 30 days and 1.66-1.94 ml/g after 180 days.

Similar results have been reported by other researchers (Inoue and Bushuk, 1992; Kenny et al, 1999).

Dough storage temperature also had a major influence on bread loaf volume. In most cases, samples stored at -20°C produced bread with the greatest loaf volume, although storage temperature effects interacted with storage time as described above. In most cases, there was no significant difference in specific volume between bread made from frozen dough stored at -30 and -35 ºC for the whole storage period.

Specific volume of bread is an important quality factor as it indicates dough inflating ability and oven spring. For bakery products there is usually an ideal relation between dough weight and loaf volume that yields the most desirable texture and grain (Pyler, 1988; Giannou and Tzia, 2007). That is, specific loaf volume should be not too large or too small because it affects the crumb grain. Too small specific volume gives very compact and closed grain structure and too large loaf volume gives a very open grain structure (Sharadanant and Khan, 2003b).

Bread Crumb Firmness

Table 3-2 shows the firmness of bread made from frozen dough. In comparison, the firmness of bread made from unfrozen dough was 4.03 g, and all bread made from frozen dough had higher degrees of firmness. Overall, minimum bread firmness (4.56 g) was attained from dough at freezing rate 2, and stored at -10°C for 30 days. Maximum firmness (12.9-12.94 g) came from dough stored at -30 or -35°C at 180 days. In general, frozen dough stored at -10 and -20 ºC produced less firm bread than that made from dough stored at -30 and -35 ºC for all storage times and all freezing rates. There was no significant difference in bread firmness for samples made from dough stored at -30 and -35 ºC. At 30 and 60 days, bread made from dough stored at -10°C was less firm than that from dough stored at -20°C. Crumb firmness increased significantly with frozen storage time of the dough. For example, at -20°C firmness values ranged from 5.99 to 6.88 g at 30 days, and from 7.76 to 8.04 g at 180 days.

Freezing rate also had an effect on bread firmness. Dough prepared at freezing rates 1 and 2 gave bread with lower firmness than freezing rates 3 and 4 for storage temperatures of -10 and -20 ºC, and for the whole storage time except for 180 storage days. After 180 days storage, freezing rate 1 gave rise to bread with lower firmness than that from freezing rates 3 or 4. For dough stored at -30 and -35 ºC, there were no differences in bread firmness due to different freezing rates. Philmolsipol (2008) has reported similar results to the ones reported in this work with frozen storage temperature. Bread crumb firmness increased with increasing storage time and that made from frozen dough stored at lower temperature showed less firmness among different treatment of frozen doughs.

Crust Color

The development of color during baking is an important indicator of bread quality. Crust and crumb color of bread samples should be golden brown and creamy white, respectively, and are both expected to be uniform and appealing (Giannou and Tzia, 2007). Photographic images from the bread crust were taken and converted to 256 level gray scales. This provided a scale of overall bread darkness or lightness, with the scale ranging from 0 for black to 255 for white. In addition, this was evaluated in each 125 µm length pixel of the crust, and the distribution of light and dark areas measured by the standard deviation. Table 3-3a-b gives the mean value and the standard deviation of the mean. Storage time was the most influential determinant of crust color, and in general, crusts were darker on average for bread made from dough held at longer storage times. For example, at 30 days and -10°C, values ranged from 79.9 to 82.1, while after 180 days dough storage values ranged from 77.1 to 78.3. There were no differences for bread made from dough held for 60 and 90 days at -10 or -20 ºC. The lightest bread crust (83.11) resulted from dough held 30 days at -35°C; the darkest (70.38) from dough stored for 180 at -35°C. Other researchers (Sharadanant and Khan, 2003b; Giannou and Tzia, 2007) have also found that bread crust is somewhat darker when made from dough subject to extended storage time. The latter reported that storage under freezing conditions causes an increase in crust darkness, but with formation of white spots in the crust surface.

Storage temperature also had significant effect on the color. Bread made from dough stored at -30 and -35 ºC for 30 days was slightly lighter (values between 81.19 and 83.11 at -35°C) than that made from dough stored at -10 and -20 ºC (values between 79.92 and 82.08 at -10°C). However, after 180 days storage, bread made from dough stored at -10 and -20°C was somewhat lighter. Freezing rate also played a role, and in general samples from the freezing rate 2 group were lighter in color, particularly at storage temperatures of -10 and -20°C.

Table 3-3b shows the distribution of dark and light color, with higher numbers indicating greater variation in the surface appearance. In general, storage time was the most dominant factor, and greater variation was observed for bread made from dough stored for longer time at all temperature and freezing rates. Storage temperature also had significant effect on the variation of color distribution. In general, bread made from dough stored at -10 or -20°C showed less color variation than that made from dough stored at -30 or -35°C. In addition, color variation was less for samples prepared at freezing rates 1 or 2. The combination of freezing rate 2 and a storage temperature of -20 ºC resulted in the most even bread crust color, and samples prepared at freezing rate 2 resulted in the lowest variation at all storage times.

Transverse Relaxation Time (T_2)

The spin-spin (transverse) relaxation time (T_2) is a function of the spin species and the chemical and physical environments surrounding the spins. The T_2 value has been used to indicate the state of water in food polymers such as flour dough and bread (Leung et al, 1979; 1983; Cherian and Chinachoti, 1996; Ruan and Chen, 2001; Esselink et al, 2003b). With storage time, bread gets firmer and loses water, and results in lower T_2 values.

Figure 3-1a-d shows the ratio of the measured T_2 of bread over 5 days as compared to the initial value, and was used as a measure of bread staling. Storage time of the frozen dough had the most significant effect on bread staling. With the increase of storage time of frozen dough, the T_2 ratio increased in all cases. In general, the normalized T_2 ratio increased from 0 to 0.25-4 over the 5 days in which the bread was kept.

Storage temperature of the frozen dough was also a critical factor determining the subsequent rate of staling, with lower storage temperature resulting in bread with the highest rates of staling. Dough stored at -10 ºC resulted in bread with the fastest rate of staling. For example, after 180 days storage, the normalized T_2 ratio varied between 0.38 and 0.40 after 5 days for bread made from dough stored at -10°C, and 0.31 and 0.34 for bread made from dough stored at -35°C. Staling rates did not differ for bread made from dough stored at -30 or -35 ºC.

Freezing rate also had some affect on subsequent bread staling. Freezing rate 1 resulted in bread with the fastest staling rate and degree of staling for all storage temperatures. Freezing rate 2 also produced bread with greater, but there was no significant difference in staling rate for bread produced from dough at freezing rates 3 or 4. The combination of dough storage temperatures of -30 and -35 ºC and freezing rate 3 and 4 resulted in bread with the lowest degree of staling, while storage at -10 and -20 ºC and freezing rate 1 resulted in the greatest degree of bread.

DISCUSSION

A decrease in specific loaf volume is typically seen in frozen dough, and we found that frozen storage time was the greatest contributor to diminished load volume. Similar results were reported by (Sharadanant and Khan, 2003b). Berglund et al (1991) reported that with longer frozen storage, the proof time increased, the bread loaf volume decreased, and the bread firmness increased. They speculated that the cause for the long proof times of frozen doughs could be the changes in the ultra-structure of starch and gluten. Frozen storage causes starch damage which may be mainly responsible for increased moisture retention resulting in increased loaf weight and lower loaf volume (Berglund et al, 1991). Damaged starch in flour absorbs more water and produces bread with decreased loaf volume (Farrand, 1972). Lorenz and Kup (1995) reported that the decreased bread volume with frozen storage may be caused from the decreased yeast viability as well as damage to gluten and starch. Previous microstructural studies and rheological measurements from our laboratory confirmed that frozen storage contributes to weakening of the gluten network (Yi, 2008). In addition to changes in the gluten network and damage to starch, frozen storage leads to diminished yeast activity.

Higher frozen storage temperatures rendered bread with higher specific volume. However, dough at higher frozen storage temperature (-10 or -20°C) was found to be stickier and had lower extensibility and maximum resistance to extension, leading to lower oven spring and loaf volume in principle (Yi, 2008). Interestingly, lower frozen storage temperature (-30 or -

35°C) resulted in better dough quality, with greater extensibility and maximum resistance to extension. However, it was concluded that too large a resistance to extension could cause lower oven spring, even though the dough could better retain gas. In addition, it was found that yeast activity was greatest at -10 to -20°C, and the increased production of CO2 contributed to overall greater volume. Freezing rate 2 rendered bread with the highest specific loaf volume. This may be due to the fact that an optimum freezing rate exists at which there is the least damage to yeast cells (Casey and Foy, 1995; Yi, 2008). While fast freezing rates promote better dough quality, they may reduce yeast viability (Yi, 2008). The fact that the combination of freezing rate 2 and storage temperature of -20 ºC led to the greatest bread specific volume suggests that maintaining yeast viability is the most important factor.

Firmness increased as a consequence of extended frozen storage and lower storage temperatures, which indicates that the damage to the dough network produced during frozen storage produces effects during the full baking or the posterior cooling that favor firming. Ice crystals damage the protein network, resulting in an altered gluten network that is ultimately responsible for the crumb structure. Yamauchi et al (1999) observed an increase in the crumb hardness of the bread obtained from frozen dough and they ascribed that effect to the degradation of the crumb structure caused by freezing. In fact, Kou and Chinachoti (1991) showed that crumb compression produces mechanical damage and this becomes evident by an increase in the crumb hardness. However, as less damage is incurred at lower storage temperatures, this can only be a partial explanation. Part of the increased firmness may be due to diminished yeast activity found after long frozen storage at low temperatures (Yi, 2008). Reduced activity leads to lower volume and greater density, and thus to greater firmness. Amylose recrystallization also plays an important role in the initial crumb hardness and in the first stages of aging (Kim and

Dappolonia, 1977; Hug-Iten et al, 2003; Barcenas and Rosell, 2006), as the formation of an amylose network contributes to bread hardening. Microscopic studies revealed that during baking there is a phase separation between amylose and amylopectin leading to an accumulation of amylose in the center of the starch granules (Hug-Iten et al, 1999). Conversely, the damage to starch granules produced by the ice recrystallization, which increases with the time of frozen storage, allows leaching of intracellular amylose, increasing the interaction between the intra- and inter-granular amylose and the formation of a network of amylose that brings about an increase in the crumb hardness (Hug-Iten et al, 1999; Barcenas and Rosell, 2006). Freezing rates 1 and 2 (the slowest freezing rates), resulted in bread with lower firmness. As slower freezing rates also contribute to greater yeast activity (Yi, 2008) and higher specific volume, decreased firmness is most likely due to the lower density bread resulting from the more slowly frozen dough. The combination of freezing rate 2 and -20°C gave bread with highest bread volume and lower firmness, and this was found in previous studies to provide dough with greatest yeast activity (Yi, 2008). However, after bread storage for several days, crumb firmness rate was higher (data not shown).

Bread surface color, together with bread texture and flavor, are the main characteristics influencing consumer preference. Hence, the crust color appears as a critical factor in the bread baking process. Bread color comes from thermal reaction including caramelization and non-enzymatic browning reaction (Maillard reaction) during baking, which promote crust flavor and color. The Maillard reaction, that is the non-enzymatic browning reaction of N containing compounds such as proteins or amino acids and reducing sugar produces a variety of reaction compounds, which have a major impact on the flavor profile and color of bread. We found that with increased frozen storage time, the bread color was darker and the color uniformity decreased. This may result from the increase in leached amylose and degraded dextrin which contributes to Maillard browning. Water redistribution and availability could also be partly responsible for the color darkening and non-uniformity. Higher freezing rates and higher storage temperatures (-10 to -20°C) also contributed to color darkening and non-uniformity. As greater preservation of dough structure has been found at storage at -30 to -35°C, lighter and more uniform color most likely result from maintaining the integrity of the dough network, and a lower degradation of amylose and production of dextrins.

Bread staling rate and the degree of staling increased considerably with frozen storage time at all freezing rates and storage temperatures. Bread staling is caused by deterioration of bread structure, texture, and flavor during storage. Firming of the bread and water redistribution are the most important aspects of staling. Bread firming is not a simple phenomenon, but results from loss or redistribution of water, a change in water properties, starch recrystallization, changes in the gluten network, or interactions between gluten protein and starch granules. Some authors state that in addition to starch retrogradation, gluten and lipids also play important roles in bread staling (Schiraldi et al, 1996; Collar et al, 1999; Hallberg and Chinachoti, 2002). Bread firming involves changes in molecular mobility water and dough macromolecules, which can be assessed by nuclear magnetic resonance (NMR) techniques (Kimshin et al, 1991; Martin et al, 1991; Seow and Teo, 1996; Chen et al, 1997; Morgan et al, 1997; Ruan and Chen, 2001). With increasing storage time of frozen dough, the normalized T_2 increased, suggesting that staling was faster and proceeded to a greater extent by the final day of bread storage.

This may also be due to the damaged gluten and starch granule structures incurred during frozen storage, resulting in greater leached amylose. As discussed previously, amylose shows faster and amylopectin shows slower retrogradation. The higher amount of leach amylose intraand interact with amylose and/or amylopectin, resulting in increasing bread firming, faster bread staling. Storage of frozen dough at -10 and -20 °C resulted in greater bread staling. The combination of storage at -10°C and freezing rate 1 (slowest freezing) showed the greatest staling. Those conditions rendered bread with higher volume and lower firmness but showed higher staling rate. As mentioned previously, yeast viability contributed to higher bread volume. However, higher freezing rates and lower storage temperature contribute to better dough quality, and this seems to be the prevailing factor contributing to reduced rates of staling.

CONCLUSION

Based on bread quality in terms of loaf volume, bread volume decreased with increased storage time. Storage temperature and freezing rate 2 also had influences on bread volume. Bread made from dough frozen at freezing rate 2 and stored at -10 and -20°C (low temperature) showed great loaf volume and more tender firmness. However, in the view of bread staling, measurements presented that fast freezing rate (3 and 4) and lower storage temperature (-30 and -35°C) showed slower staling rate and lower staling ratio. To get the better quality of bread, optimum freezing rate (freezing rate 2) for higher yeast activity and higher frozen storage (-10 and -20°C) is proper processing conditions, resulting in bigger proofed and baked volume. However, those freezing rate and storage temperatures for greater volume resulted in higher bread staling in shorter times. On the contrary, fast freezing rate (3 and 4) and lower storage temperature (-30 and -35°C) had lower bread volume and more firm texture caused by lower bread volume. However, those conditions gave slower bread staling.

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Table 3.1. Specific volume (*ml/g*) of bread made from different freezing rates and storage conditions. Values for unfrozen dough; 3.14 ± 0.027 *ml/g*.

Storage Day	Freezing Rate	Storage Temperature			
		-10ºC	-20ºC	-30ºC	-35ºC
30	Rate 1	2.58 ± 0.078	2.65 ± 0.029	2.17 ± 0.060	2.16 ± 0.039
	Rate 2	2.71 ± 0.062	2.73 ± 0.033	2.16 ± 0.079	2.01 ± 0.045
	Rate 3	2.61 ± 0.082	2.64 ± 0.097	2.10 ± 0.050	2.06 ± 0.023
	Rate 4	2.61 ± 0.094	2.58 ± 0.069	2.18 ± 0.013	1.98 ± 0.030
60	Rate 1	2.52 ± 0.070	2.69 ± 0.069	2.14 ± 0.054	2.20 ± 0.194
	Rate 2	2.67 ± 0.052	2.81 ± 0.088	2.12 ± 0.053	1.96 ± 0.035
	Rate 3	2.66 ± 0.068	2.65 ± 0.060	2.08 ± 0.050	2.02 ± 0.022
	Rate 4	2.56 ± 0.063	2.73 ± 0.063	2.12 ± 0.071	1.99 ± 0.049
90	Rate 1	2.43 ± 0.117	2.60 ± 0.037	2.06 ± 0.045	2.16 ± 0.046
	Rate 2	2.54 ± 0.092	2.67 ± 0.069	2.09 ± 0.049	1.88 ± 0.037
	Rate 3	2.13 ± 0.089	2.48 ± 0.042	2.01 ± 0.139	1.91 ± 0.060
	Rate 4	2.38 ± 0.047	2.54 ± 0.021	1.89 ± 0.065	1.95 ± 0.050
180	Rate 1	2.07 ± 0.029	2.10 ± 0.130	1.73 ± 0.037	1.66 ± 0.019
	Rate 2	2.07 ± 0.036	2.13 ± 0.037	1.84 ± 0.049	1.82 ± 0.021
	Rate 3	2.04 ± 0.032	2.10 ± 0.067	2.00 ± 0.116	1.94 ± 0.031
	Rate 4	1.98 ± 0.033	2.06 ± 0.036	1.94 ± 0.017	1.90 ± 0.014

Table 3.2. Firmness (force, N) of bread made from frozen bread dough; Values for unfrozen dough 4.03 ± 0.53 N.

Storage Day	Freezing Rate	Storage Temperature			
		-10ºC	-20ºC	-30ºC	-35ºC
30	Rate 1	5.00 ± 0.37	6.12 ± 0.22	10.60 ± 0.83	9.0 ± 0.77
	Rate 2	4.56 ± 0.60	5.99 ± 0.56	10.76 ± 0.32	8.9 ± 0.11
	Rate 3	5.78 ± 0.35	6.47 ± 0.11	10.46 ± 1.36	9.3 ± 0.16
	Rate 4	5.91 ± 0.38	6.88 ± 0.33	10.38 ± 0.37	9.5 ± 0.07
60	Rate 1	5.93 ± 0.25	6.80 ± 0.03	11.81 ± 0.54	11.4 ± 1.06
	Rate 2	5.87 ± 0.05	6.64 ± 0.68	12.18 ± 0.34	11.6 ± 0.25
	Rate 3	6.47 ± 0.54	6.90 ± 0.27	12.29 ± 0.28	11.4 ± 0.23
	Rate 4	6.16 ± 0.11	6.95 ± 0.18	11.74 ± 0.39	11.7 ± 0.03
90	Rate 1	8.00 ± 0.33	7.28 ± 0.50	12.26 ± 0.41	12.3 ± 0.38
	Rate 2	7.71 ± 0.11	7.12 ± 0.36	12.36 ± 0.18	11.9 ± 0.18
	Rate 3	9.07 ± 0.55	6.96 ± 0.21	12.04 ± 0.14	11.9 ± 0.09
	Rate 4	8.86 ± 0.25	7.35 ± 0.21	12.40 ± 0.32	12.3 ± 0.51
180	Rate 1	8.78 ± 0.06	8.03 ± 0.38	12.48 ± 0.36	12.9 ± 0.18
	Rate 2	8.46 ± 0.20	8.04 ± 0.29	12.73 ± 0.26	12.7 ± 0.10
	Rate 3	10.18 ± 0.60	7.76 ± 0.21	12.34 ± 0.34	12.6 ± 0.29
	Rate 4	9.73 ± 0.15	8.04 ± 0.21	12.94 ± 0.16	12.8 ± 0.08

Table 3.3. Crust color scale of bread made from dough produced under different freezing rates and storage conditions

Storage Day	Freezing Rate	Storage Temperature			
		-10ºC	-20ºC	-30ºC	-35ºC
30	Rate 1	81.75 ± 1.923	81.41 ± 2.267	82.17 ± 2.197	82.46 ± 0.510
	Rate 2	82.08 ± 1.052	82.59 ± 2.610	81.33 ± 2.24	83.11 ± 2.794
	Rate 3	81.37 ± 3.007	80.97 ± 2.731	81.16 ± 1.05	82.27 ± 1.701
	Rate 4	79.92 ± 3.931	80.28 ± 1.163	78.86 ± 2.53	81.19 ± 3.026
60	Rate 1	82.84 ± 2.069	83.53 ± 1.434	81.86 ± 2.235	79.27 ± 1.231
	Rate 2	86.39 ± 0.919	85.15 ± 0.962	80.46 ± 1.254	81.46 ± 3.881
	Rate 3	84.20 ± 2.003	83.63 ± 1.402	80.60 ± 1.481	76.58 ± 2.063
	Rate 4	86.31 ± 1.121	83.30 ± 1.253	79.97 ± 0.620	81.76 ± 1.809
90	Rate 1	81.57 ± 1.695	80.00 ± 2.395	80.06 ± 1.312	80.17 ± 3.246
	Rate 2	82.70 ± 1.595	81.35 ± 1.572	79.47 ± 1.114	79.35 ± 1.398
	Rate 3	82.30 ± 1.948	80.58 ± 1.591	80.40 ± 2.364	80.54 ± 1.404
	Rate 4	82.15 ± 1.735	78.54 ± 1.931	79.68 ± 2.236	79.76 ± 1.969
180	Rate 1	77.15 ± 1.178	78.81 ± 1.636	70.90 ± 0.520	71.65 ± 1.407
	Rate 2	78.29 ± 1.186	80.58 ± 1.807	71.11 ± 2.150	70.38 ± 0.765
	Rate 3	77.71 ± 1.088	78.96 ± 7.700	71.95 ± 1.840	72.27 ± 2.019
	Rate 4	77.89 ± 1.308	79.10 ± 1.523	71.64 ± 2.405	71.54 ± 2.349

Table 3.4. Crust color variation of bread made from dough produced under different freezing rates and storage conditions

Storage Day	Freezing Rate	Storage Temperature			
		-10ºC	-20ºC	-30ºC	-35ºC
30	Rate 1	12.41 ± 0.505	12.73 ± 2.161	14.67 ± 0.787	14.99 ± 0.651
	Rate 2	13.95 ± 0.320	11.55 ± 0.417	15.65 ± 2.523	14.41 ± 1.601
	Rate 3	12.70 ± 1.036	13.29 ± 0.918	13.48 ± 1.421	15.64 ± 2.804
	Rate 4	12.14 ± 1.177	12.43 ± 1.478	12.85 ± 2.694	15.67 ± 0.587
60	Rate 1	13.65 ± 1.697	12.11 ± 2.033	12.20 ± 1.215	14.97 ± 1.154
	Rate 2	12.65 ± 1.697	12.38 ± 2.091	14.68 ± 0.535	14.20 ± 1.997
	Rate 3	13.61 ± 1.044	13.80 ± 1.482	16.77 ± 2.349	14.95 ± 2.078
	Rate 4	13.35 ± 2.230	13.78 ± 1.556	17.44 ± 1.934	14.65 ± 1.248
90	Rate 1	14.90 ± 0.616	16.05 ± 1.596	16.37 ± 1.704	16.29 ± 1.999
	Rate 2	15.84 ± 2.431	15.33 ± 1.000	16.22 ± 0.636	16.15 ± 0.814
	Rate 3	17.78 ± 0.199	16.92 ± 0.972	17.00 ± 2.421	16.67 ± 2.005
	Rate 4	17.23 ± 1.457	17.59 ± 1.728	18.22 ± 0.601	17.46 ± 1.319
180	Rate 1	15.33 ± 0.762	17.09 ± 0.620	17.35 ± 1.009	17.16 ± 1.327
	Rate 2	15.44 ± 2.170	17.00 ± 1.019	17.01 ± 1.798	17.06 ± 2.400
	Rate 3	16.36 ± 0.864	17.65 ± 1.382	17.97 ± 1.471	17.74 ± 1.052
	Rate 4	16.24 ± 1.628	18.12 ± 1.323	17.96 ± 0.843	18.72 ± 2.013

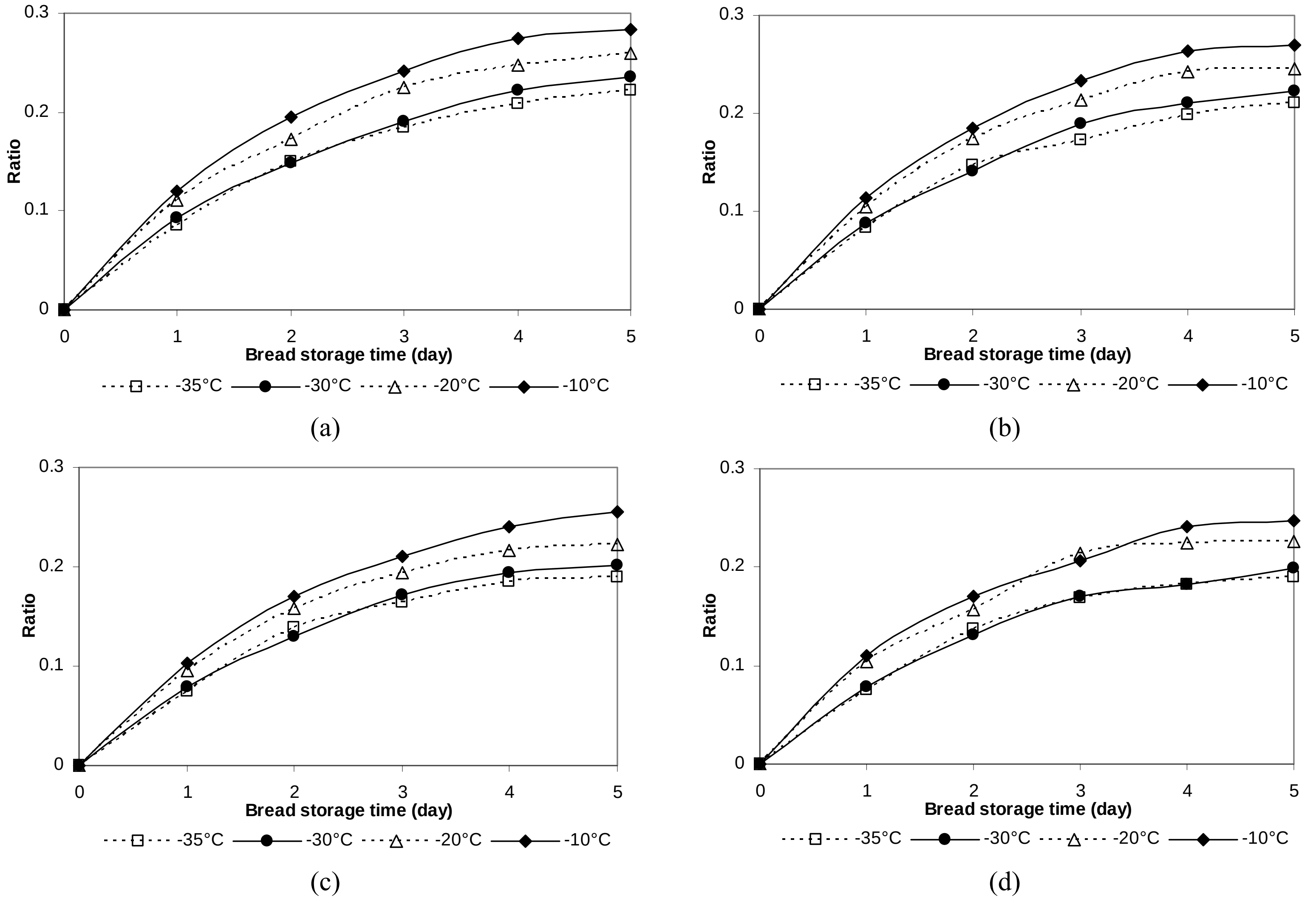

Figure 3.1. The effects of freezing and storage conditions on bread staling measured by transverse relaxation time (T_2) change ratio for 30 day storage time; freezing rate 1(a), rate 2(b), rate 3(c) and rate 4(d).

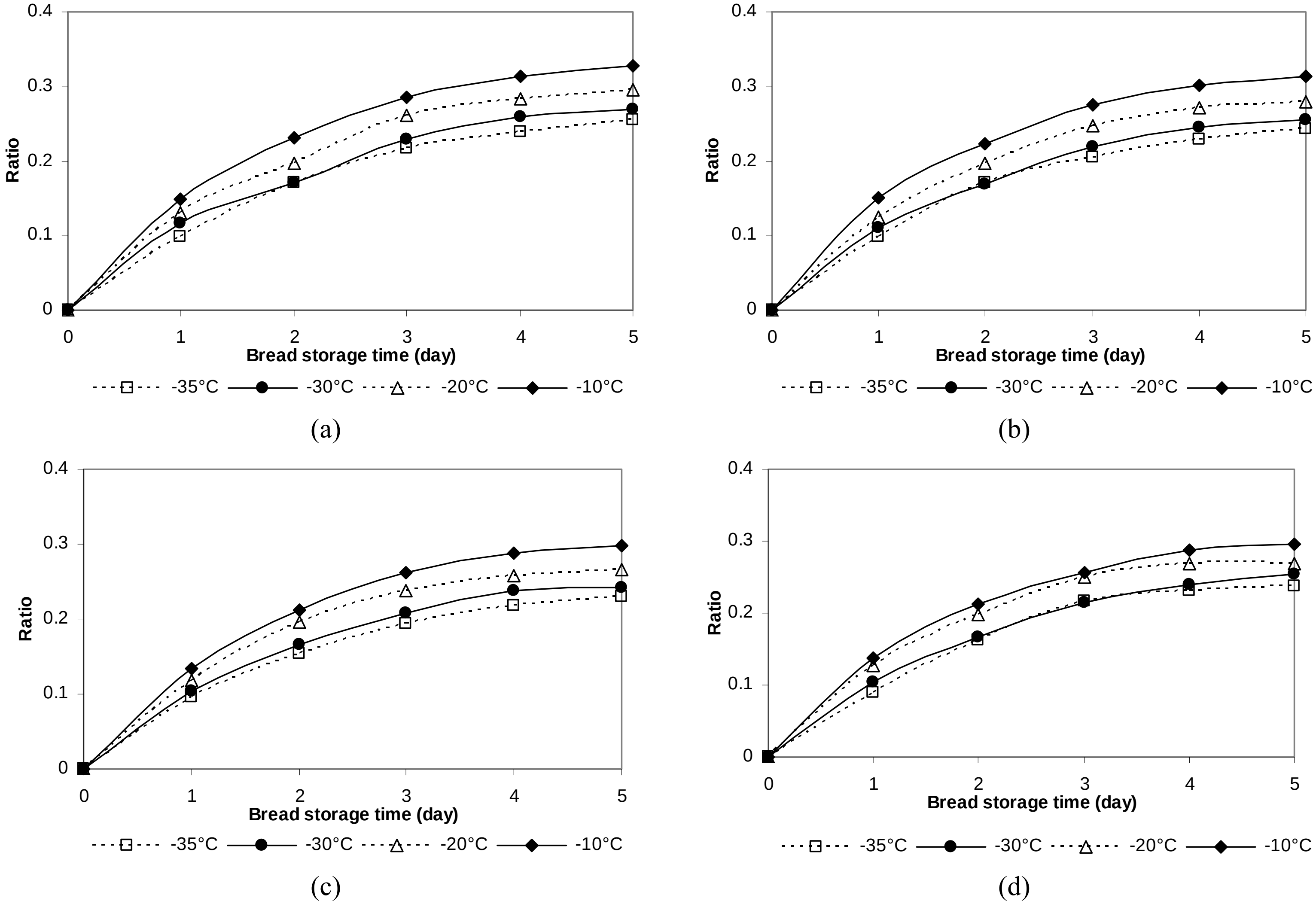

Figure 3.2. The effects of freezing and storage conditions on bread staling measured by transverse relaxation time (T_2) change ratio for 60 day storage time; freezing rate 1(a), rate 2(b), rate 3(c) and rate 4(d).

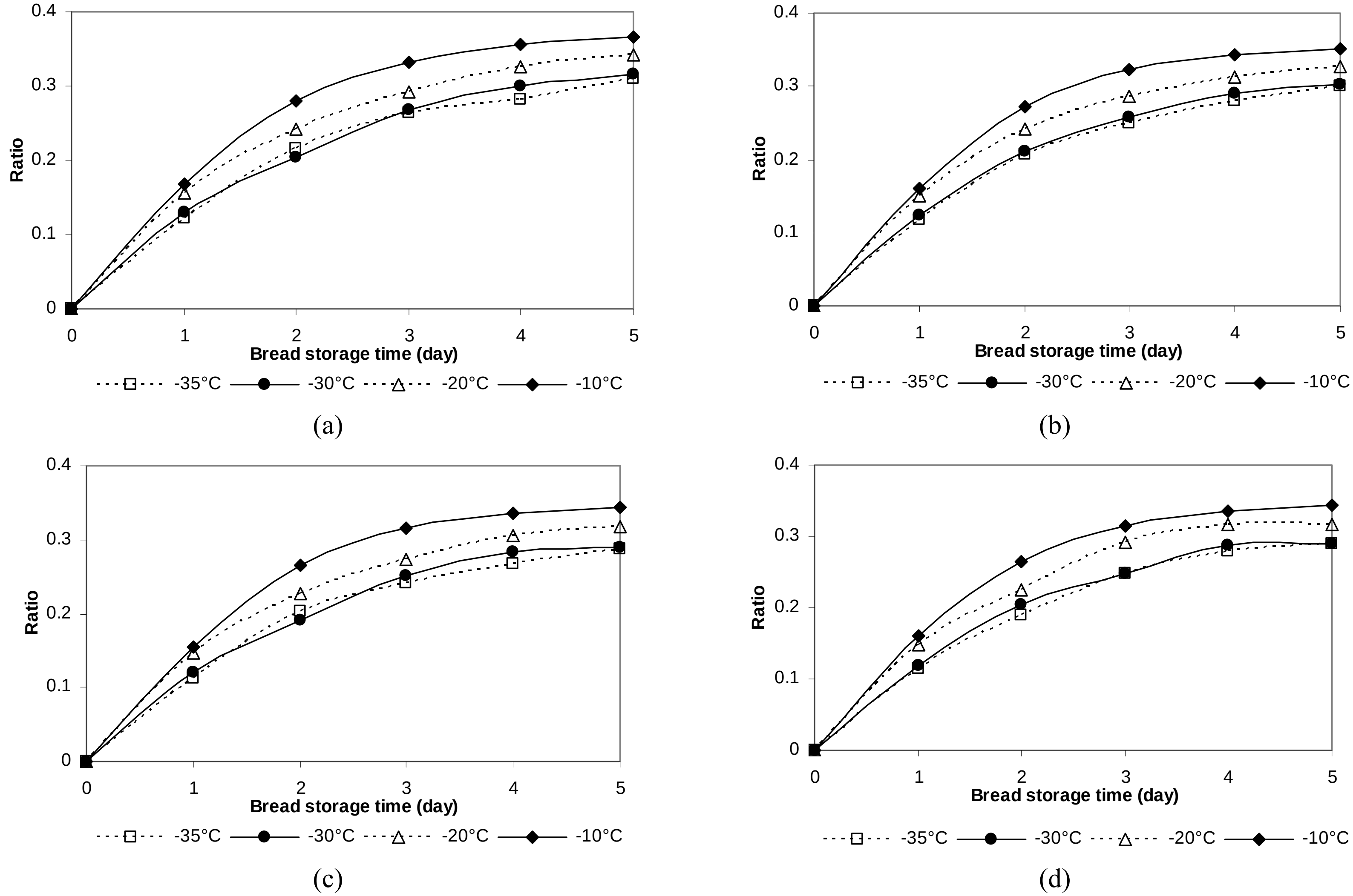

Figure 3.3. The effects of freezing and storage conditions on bread staling measured by transverse relaxation time (T_2) change ratio for 90 day storage time; freezing rate 1(a), rate 2(b), rate 3(c) and rate 4(d).

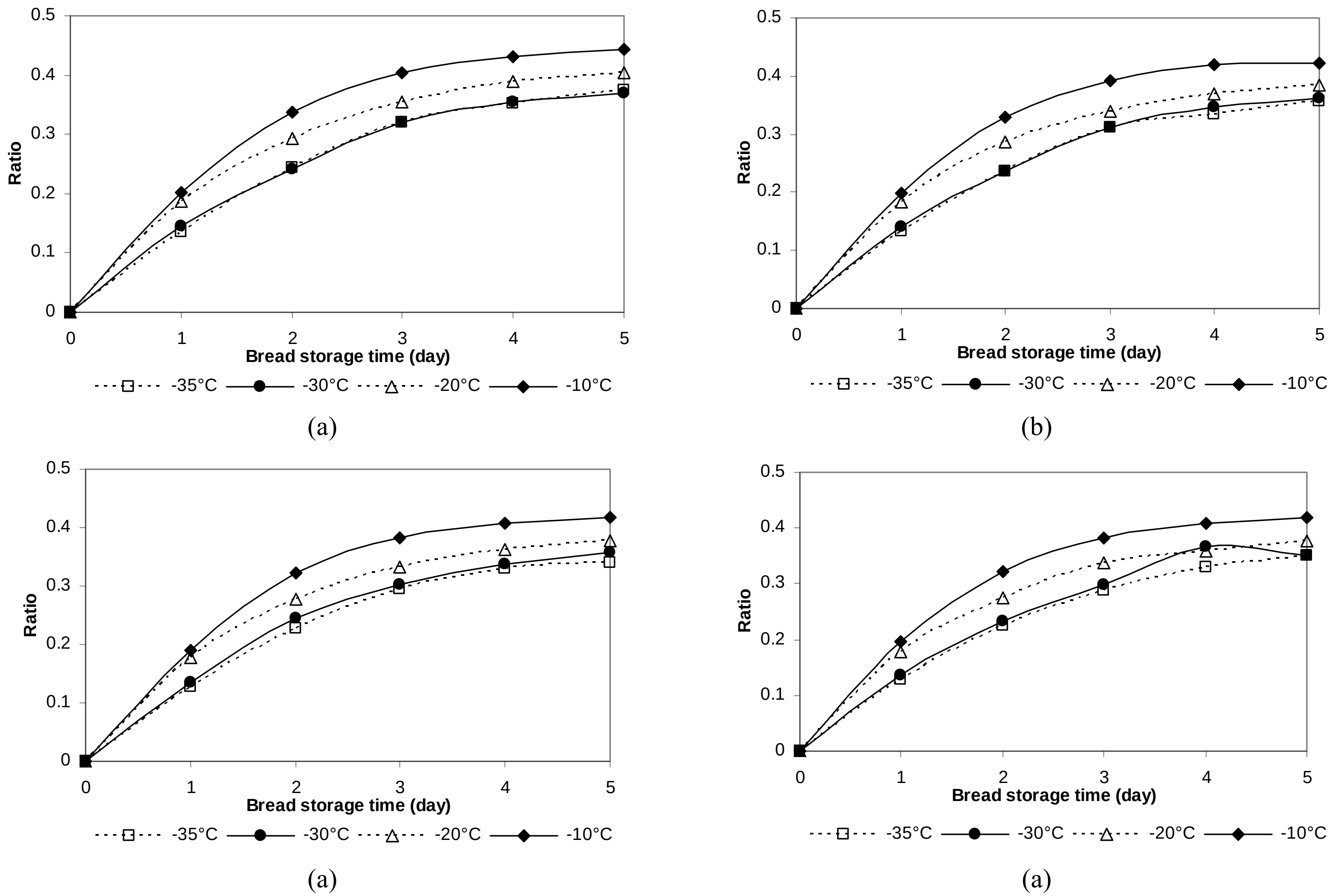

Figure 3.4. The effects of freezing and storage conditions on bread staling measured by transverse relaxation time (T_2) change ratio for 180 day storage time; freezing rate 1(a), rate 2(b), rate 3(c) and rate 4(d).

CHAPTER 4

THE EFFECTS OF WAXY WHEAT FLOUR AND WATER LEVELS ON FROZEN DOUGH AND BREAD PROPERTIES

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ABSTRACT

The quality of bread made from frozen dough is diminished by changes that occur during the freezing process. Recent studies have shown that new varieties of waxy wheat flour (WWF), bred to contain lower levels of amylose, offer unique properties for the production of baked products. In this study, dough properties and baking qualities were investigated by comparing the effects of levels of WWF and water. Stickiness of dough increased with increased content of WWF and water. Dough stickiness increased with higher WWF and water addition. As for freezing and frozen storage, dough of higher WWF and lower water content showed less increased stickiness. Maximum resistance to extension (MRE) decreased with higher WWF and water content. Dough of higher WWF and lower water content showed less decreased extensibility after freezing and frozen storage. Dough with higher content of WWF and water added were more extensible. Nuclear magnetic resonance (NMR) studies showed that frozen dough with higher WWF content had lower transverse relaxation (T_2) time of 9-11ms. As for frozen storage, dough of higher WWF still showed lower T_2. Yeast activity showed that dough WWF 15% showed higher gas production. Bread made from 15% and 30% WWF had higher volume in fresh and frozen dough. Bread firmness decreased with higher amount of WWF and water. This research demonstrated that specific combinations of WWF and water produced a better quality of frozen dough and bread.

Key words: waxy wheat flour; frozen bread dough; stickiness; maximum resistance to extension (MRE); yeast activity; nuclear magnetic resonance (NMR)

INTRODUCTION

Starch is an important component of wheat endosperm (*Triticum aestivum* L.) and the characteristics of the starch is an important factor controlling the texture of bread and other baked goods. Typically, wheat starch contains 70-80% amylopectin and 20-30% amylose. Recently, various techniques such as hybridization, mutagenesis, and somaclonal mutation have produced wheat grains having various ratios of amylopectin and amylose in the starch (Nakamura et al, 1995; KiribuchiOtobe et al, 1997; Kim et al, 2003; Yasui, 2006). Thus, it is possible to produce low-amylose wheat flours. The ratio of amylopectin and amylose contributes to the properties of the flour, and influences the texture, viscosity, and stability of processed foods made from the flour. Research is underway on the use of low amylose or waxy wheat flours in processed foods (Abdel-Aal et al, 2002; Morita et al, 2002a; Kim et al, 2003; Hung et al, 2007a).

Several researchers have reported that waxy wheat starch and WWF have different properties than conventional wheat flour. Waxy wheat starch exhibited higher onset and peak gelatinization temperatures, enthalpy of gelatinization, and degree of crystallization. Products made with waxy wheat starch also showed greater refrigeration and freeze-thaw stabilities than did those from non-waxy starches (Yasui et al, 1996; Hayakawa et al, 1997; Abdel-Aal et al, 2002). Therefore, these new varieties of WWF offer unique properties for the production of baked products.

Some studies have examined the properties of bread made from whole waxy wheat flour. WWF bread has been shown to have higher protein and dietary fiber, but mixing stability was decreased, the dough was less extensible, bread volume was reduced, and color was different than that for breads made with conventional wheat flour (Hung et al, 2006). However, these problems are ameliorated by partial substitution with WWF, and bread with softer crumb and better nutrition can be made. There is also evidence that bread containing a portion of WWF has a greater shelf-life due to reduced rates of staling (Bhattacharya et al, 2002).

The opportunity for the use of WWF flours for frozen dough has not been investigated. Frozen dough is an important product in current food industries, due to its extensive use in retail outlets, restaurants and in-store bakeries. Its use allows dough to be produced in large quantities at a central location, saving on energy and labor costs, then transported to local establishments for final baking. The quality of frozen dough, and the bread from which it is made, may be less than that made from unfrozen dough. Structural changes to the gluten network occur during freezing and storage, and the activity of yeast is often decreased (Neyreneuf and Vanderplaat, 1991; El-Hady et al, 1996; Ribotta et al, 2001; Ribotta et al, 2004). The former seems to be related to freezing out and migration of water during freezing, the latter to injury to yeast cells from ice and freeze concentration of solutes. In practice, deleterious effects may be lessened by the addition of dough strengtheners, higher protein, more yeast, or changing processing conditions to limit initial yeast activity. As the interaction of water with amylopectin differs somewhat from that with amylose, it is worth investigating if WWF dough can better withstand the hardships of freezing.

The purpose of this research was to investigate the effects of WWF and water levels on dough quality and baking performance in fresh and frozen dough. In particular, we focused on the dough properties and baking qualities after freezing. Dough properties were assessed by physical measurements of stickiness and extensibility, water binding properties, and yeast activity. Bread properties were assessed by measurements of bread volume and crumb firmness.

MATERIALS AND METHODS

Materials

To examine the relationship between the ratio of waxy wheat and conventional wheat flour on dough quality, normal amylopectin content and non-waxy wheat flour was used in various proportions. In addition, as the waxy wheat flour may absorb different amounts of water in order to form acceptable dough, several levels of water were also examined. The conventional wheat flour used was all-purpose winter red wheat (Martha White Foods Inc., Jackson, TN). Protein content was 9.59%, as determined by the Dumas method (AACC 46-30) using an FP-2000 combustion unit with a thermal conductivity detector (LECO Corp, St, Joseph, MI). Waxy wheat flour used was Shinmi Waxy wheat flour (Shinmi chalmil). The genetic background is the cross: Kanto107/Bai-Huo/3/Bai-Huo/Kanto107//A92-3327/Kanto107. Protein content was 9.65%. Amylose content was 1.2% as determined by the iodine color method (Hung and Morita, 2005; Hung et al, 2007b). The available waxy wheat flour at the time of this study had protein content somewhat lower than the 11-12% typically used for breadmaking. Thus, vital wheat gluten (74.88% protein; King Arthur Flour Company, Inc., White River Junction, VT) was used to adjust the protein content of the flour mixes to 12%. Other ingredients used for dough preparation included (as percent of flour weight): 3% Baker's dry yeast (Fleischmann's Yeast, Chesterfield, MO), 5% sucrose (Pure Cane Sugar, Dixie Crystal, Savannah, GA), 2% non-iodized salt (Morton International, Inc., Chicago, IL), and 5% Crisco vegetable oil (J.M. Smucker Co., Orrville, OH).

Dough Preparation and Baking

Waxy wheat flour (15%, 30%, and 45% by weight of total flour) was blended with the conventional wheat flour. Different levels of water (55%, 60%, and 65% based on the weight of total flour) were used in dough preparation. The yeast was pre-hydrated with the water and all of the dough ingredients were placed in a blender and mixed. The mixing times for best dough development were different depending on water content. Dough with 55% water was mixed for 2 min at 90 rpm and for 9 min at 128 rpm; dough with 60% water was mixed for 8 min at 128 rpm; and dough at 65% water was mixed for 11 min at 128 rpm. Once the dough was formed, it was separated into samples of 50g, and shaped into round balls. Samples were placed in bread pans and frozen in circular slabs, approximately 50mm diameter. The freezer was a built-in blaster freezer (h=35 W/m^2K).

Samples were frozen at approximately 24.6°C/hr and frozen dough samples were stored at -20°C for 1 and 90 days. After the storage period, samples were thawed in a constant-temperature chamber at 8°C until they reached the chamber temperature. Three samples were used for texture studies, while the rest were baked in a convection oven at 180°C for 15 min (DFG-100, Blodgett, Burlington, VT). After baking, bread samples were allowed to cool for approximately 1hr at ambient temperature. Firmness, volume and weight were measured 15 min after baking and cooling (Hallen et al, 2004).

Dough Properties

Stickiness. Stickiness was measured using the TA-XT2i equipped with a modified Chen-Hoseney dough stickiness rig (Chen and Hoseney, 1995) were used. Small pieces of the dough were extruded through a series of small holes on the top of the plate to a height of 1 mm. A one-inch cylinder probe was brought to the surface, then pulled away from the lower rig at a speed of 1.7 mm/s. The stickiness was determined from the peak tensile force.

MRE & Extensibility. Maximum resistance to extension (MRE) and extensibility of dough were measured using a texture analyzer (TA-XT2i, Texture Technologies Corp., Scarsdale, NY) equipped with a modified Kieffer extensibility rig and a 5 Kg load cell. Dough samples were molded on dough form into a strip approximately 7 mm in diameter with 60mm in length. Dough samples were formed and rested at 8°C for 20 min and 90% RH before testing (Anderssen et al, 2004). Dough strips were placed on the rig and ends pulled apart at a speed of 3.3 mm/s. Maximum resistance to extension (MRE) and extensibility were taken as the maximum force and distance incurred at fracture.

NMR Relaxometry. H^1-NMR relaxation measurements of dough were made using a 20MHz Proton (^{1}H) NMR spectrometer (Resonance Instruments, Whitney, UK). Approximately 5g dough sample were taken from the thawed dough center. Three replicates were taken from each piece of dough. Each sample was place in a 10 mm diameter glass tube then covered with parafilm. The glass tube was placed at 10°C bath for 20 minutes. After 20 minutes, the glass tubes with the dough specimens were placed in 18 mm diameter NMR tubes. Transverse (T_2) relaxation curves were developed using the CPMG pulse sequence: 90x-(τ-180y-τ-echo)n (Meiboom and Gill, 1958). Acquisition parameters were set to a 90° pulse of 4.2μs and a recycle delay of 2s. An interpulse spacing (τ) of 500μs was chosen. All measurements were made at 25°C ±1°C. Relaxation curves obtained from the CPMG sequences were analyzed using the WinDXP distributed exponential routine (Resonance Instruments, Whitney, UK). This routine calculates a distribution of T_2 terms that best describe the data:

$$g_i = \sum_{j=1}^{m} f_j e^{-t_i/T_{2,j}}$$

where g_i are the values of the exponential distribution at time t_i; f_j are the pre-exponential multipliers, and $T_{2,j}$ are the time constants. In general, there was more than one distributed region, suggesting the existence of various regions of water with similar mobility. In this case, the number of protons in a given region was measured by the integrated signal intensity over the region of the distribution.

Yeast Activity. Yeast activity was measured using AACC method 89-01 (2000) with slight modification. Dough pieces (10 g, based on dough of 60% water content) were mixed with 100g of water, placed in a reaction vessel (Newberry et al, 2002), and the container placed in a controlled temperature bath at 36 ±1°C. A hose was connected to the top of the container, and directed to a volumetric manometer. Gassing power was determined from the volume of CO_2 gas collected over 180 mins. 1°C and 100g water was placed into the device

Bread Volume

Bread loaf volume and weight were determined according to the AACC 10-05 (2000) procedure. A container was filled to a level surface with a measured volume of rapeseed. Subsequently, the bread loaf was introduced and the volume of rapeseed displaced measured in a graduated cylinder (Pyler, 1988).

Statistical Analysis

The effects of WWF and water content on dough properties, yeast activity, and bread volume and firmness were analyzed by two-way analysis of variance at a level of significance of 0.05. Mean values were compared using Fisher's LSD method with significance level of 0.05. SAS software was used for data analysis (SAS institute, Inc, 2006).

RESULTS AND DISCUSSION

Effects of WWF and Water Content on Dough Properties

Stickiness. Table 4.1 shows the stickiness of fresh (control) dough and frozen/thawed dough with different levels of waxy wheat flour and water. Both WWF and water content had significant effects on dough stickiness. With increasing water content, dough stickiness increased in both fresh and frozen dough. For unfrozen dough, stickiness increased from 76.86 to 83.55 g with no added WWF, and from 85.98 to 98.14 g for dough substituted with 45% WWF. With greater levels of WWF, the dough stickiness increased for all different water contents and storage times. Other researchers have also found that stickiness increases in dough prepared with WWF (Morita et al, 2002a; Hung et al, 2007a).

Stickiness also increased with frozen storage time, although 1 day storage did not seem to make dough samples much stickier. Storage for 90 days, however, rendered dough with much higher stickiness for all water contents and WWF ratios. For example, stickiness values increased from a range of 76.86 - 83.55 g to 104.12 - 116.22 g for 0% WWF samples stored for 90 days. Likewise, for samples containing 45% WWF, stickiness increased from a range of 85.98 - 98.14 g to 108.02 - 122.14 g over 90 days frozen storage. Increased stickiness with frozen storage has previously been observed for dough prepared from conventional wheat flour (Yi and Kerr, 2008). Microstructural and NMR studies indicate that this corresponds to deterioration of the gluten network, leaving some of the water less closely associated with the gluten strands.

Interestingly, the change in stickiness during frozen storage was less for samples with less water or greater WWF content. For example, stickiness of 0% WWF dough with 55% water increased by 32.0% during 90 days storage, while stickiness for samples with 65% water increased by 39.1%. For samples with 45% WWF and 55% water, stickiness increased by 25.6%, while samples with 65% water increased by 24.5%. During dough freezing, as water transforms into ice, water movement leads to weakening of the dough structure. It has been suggested that the addition of waxy wheat flour suppresses water migration, presumably as water is more tightly held by the amylopectin starch (Abdel-Aal et al, 2002; Bhattacharya et al, 2002; Morita et al, 2002b). Thus, waxy starch may decrease the amount of water that migrates away from the gluten-starch matrix, and limit structural changes to that network.

Stickiness (or adhesiveness) is an important textural property of wheat dough (Armero and Collar, 1997). Overly sticky dough does not form well, and may adhere to machine surfaces. In addition, if excessive stickiness carries over to the bread, it may diminish consumer acceptance, resulting in bread that is too chewy, adheres to the mouth, or seems to be underbaked.

MRE & Extensibility. Table 4.2a shows the maximum resistance to extension (MRE) for dough made with various percentages of WWF. The MRE decreased with higher water content in the unfrozen fresh dough, ranging from 53.97 to 58.66 g at 55% moisture, and 17.54 to 28.15 g at 65% moisture. MRE of fresh dough with WWF decreased with higher levels of WWF, ranging from 58.66 to 53.97 g (at 55% water), 40.02 to 34.16 g (at 60% moisture), and 28.15 to 17.54 g (at 65% moisture). Dough having lower levels of water and WWF had higher MRE values, and vice versa. Morita et al (2002b) reported that the gluten of normal wheat flour was not extensible, as the dough was rigid and starch granules were mostly buried in the gluten matrix. The gluten of WWF, however, was not as evenly dispersed and did not cover the starch granules as uniformly or entirely as in normal wheat flour.

After frozen storage, the MRE of all dough samples was reduced. However, the percent change in MRE during storage was less for samples containing higher levels of WWF, as well as for those containing less water. Table 2a also shows the normalized change in MRE. So, for example, MRE values of 0% WWF samples changed by 19, 29 and 46% for dough with 55, 60, and 65% moisture, respectively. In comparison, MRE values of 45% WWF dough changed by only 14, 20 and 38%. Again, this may be related to the ability of waxy starch to better hold water in the vicinity of the gluten matrix. Abdel-Aal et al (2002) reported that the total water separation from waxy starch gels after one week of refrigeration or one freeze-thaw cycle was lower than from nonwaxy starch gel. Other researchers (Abdel-Aal et al, 2002; Morita et al, 2002b; Hung et al, 2007b) have also found that waxy wheat starch has greater water holding power and higher swelling power than conventional wheat starch. Sharadanant and Khan (2003) reported that maximum resistance to extension decreased with storage time.

The extensibility of fresh and frozen dough was also dependent on levels of water and WWF content (Table 4.2b). In general, treatments that resulted in lower MRE resulted in greater extensibility. That is, dough samples that could be pulled farther apart before breaking also developed less force during the stretch. Both are indicators that the viscoelastic dough has a diminished elastic component, which in dough is attributed to the crosslinked gluten fibrils. Extensibility increased with higher water content in unfrozen fresh dough, with values of 57.69, 76.59 and 87.43 mm in 0% WWF dough at 55%, 60% and 65% moisture, respectively. Extensibility of fresh dough increased with higher levels of WWF, reaching values of 61.7, 84.45 and 98.45 mm in 45% WWF dough at 55, 60 and 65% moisture. There was also an interactive effect of moisture and WWF levels. Dough having higher levels of water and WWF showed greater extensibility, while those having lower levels of water and WWF had less extensibility.

During extended frozen storage, extensibility of all dough samples was reduced. It should be noted that over storage both MRE and extensibility decreased. In contrast, at higher levels of WWF or water in the unfrozen dough, extensibility increased while MRE decreased. Extensibility has been attributed to the gluten structure, and particularly to high molecular weight glutenins (Payne and Corfield, 1979; Bushuk and Macritchie, 1989; Belton, 1999; Lindsay and Skerritt, 1999). While disulfide bonds help to maintain the gluten network and provide resistance to stretching, several theories have been developed to explain dough elasticity (MacRitchie and Lafiandra, 1997; Belton, 1999). A polymer science approach suggests that stretching creates additional order in the structure and a subsequent decrease in entropy; a force develops to restore the structure to its original dimensions, and thus maximize entropy. As before, the percent change in extensibility with storage depended on the water level and WWF content. Samples with lower moisture or greater fraction of WWF showed less change in extensibility with frozen storage. For example, samples with 0% WWF stored for 90 days had extensibility values of 14.6, 15.9 and 19.7% smaller than control for samples at 55, 60 and 65% moisture, respectively. For samples with 45% WWF, extensibility decreased by 9.4, 12.5, and 14.5% than control for samples at 55, 60 and 65% moisture, respectively.

Adel-Aal et al (2002) reported that waxy wheat showed greater refrigeration and freeze-thaw stability than did nonwaxy starch, and noted that the extensibility of WWF dough did not change as much during frozen storage. Other researchers (Abdel-Aal et al, 2002; Morita et al, 2002b; Hung et al, 2007b) reported that waxy wheat starch (~ < 5%) has more water holding power than other normal starch. With higher level of WWF, extensibility decreased, resulting from retaining more water due to WWF starch.

Relaxometry of Dough. Dough with higher levels of WWF had lower T_2 values for all water contents and frozen storage time (Table 4.3). For unfrozen dough, T_2 values ranged from 10.76-12.01 ms for 0% WWF dough to 9.98-10.96 ms for 45% WWF dough. This is consistent with findings that waxy wheat starch is better able to bind or immobilize water. For example, Kovacs et al (1997) and Sasaki et al (2000) reported that there was negative relation between amylose content and water uptake or swelling power, indicating that water in dough with higher WWF content is incorporated more strongly than dough with lower WWF. T_2 values are primarily associated with water in the samples, and water with lower rotational freedom will result in lower T_2 values (Ruan et al, 1999). T_2 values increased, albeit slightly, with increased water content for all different WWF contents. T_2 relaxation time would be expected to increase with the addition of water. Extra water is still intimately incorporated in the gluten-starch matrix due to hydrogen exchange with polysaccharide surface hydroxyl groups, but has higher mobility (Cherian and Chinachoti, 1996; Esselink et al, 2003b).

T_2 relaxation times also increased with frozen storage time. For 1 day freezing and storage, T_2 relaxation times increased only slightly. For examples, values for 0% WWF dough increased from 10.76-12.01 ms to 11.74-13.27 ms after 1 day, while those for 45% WWF dough increased from 9.98-10.96 to 10.73-11.89 ms. Longer frozen storage resulted on even greater increase in T_2 values. At 90 days, values for 0% WWF dough had increased to 15.75-18.36 ms, while that for 45% WWF dough had increased to 12.86-13.37 ms. As discussed previously, a major part of the water is bound to starch-gluten matrix and is affected by dough freezing conditions and storage time. Consistent with changes we observed in stickiness and extensibility, and as observed through microstructural studies, frozen storage leads to migration of water away from the matrix, and weakening and thinning of the gluten fibrils. This increase in more mobile water leads to higher average values of T_2. Interestingly, changes in T_2 during storage were less for dough with higher levels of WWF. At 0% WWF, T_2 values increased between 46.3 and 52.9% during 90 days storage. At 45% WWF, they increased by 28.8 to 40.2%. This may reflect the ability of high waxy starch to better hold onto water, even during frozen storage. This water redistribution and dehydration have a negative effect on dough and baking performance, but this may be lessened by the addition of WWF. For freezing and frozen storage, Abdel-Aal (2002) reported that WWF showed higher water absorption ability and WWF starch showed greater stability during refrigeration and freezing-thaw cycle, as compared to convention wheat flour and starch.

Yeast Activity and Gas Production. Table 4.4 shows the total gas production during 180 min for unfrozen dough, and dough thawed after frozen storage. Yeast activity decreased with increasing levels of WWF in the fresh dough. Gas volumes for 0% WWF dough were 21.60, 22.03 and 22.47 ml (at 55, 60 and 65% moisture), while for 45% WWF dough were 16.10, 16.33 and 16.93 ml. However, gas volumes of 15% WWF dough were highest. This may reflect a lower level of fermentable sugars, or total carbohydrate, in the waxy wheat flour. It is somewhat contradictory to findings by some other researchers. For example, Åkerberg et al (1998) showed that high amylose flours have more resistant starch, and lead to a lower glycemic index in consumed bread. Water levels did affect yeast activity in fresh or stored dough, but it was not determinant for yeast activity in dough with higher WWF. For example, for 90 days, reduced gas volume ranged from 47 - 53% with 0% WWF to 39 – 41% with 45% WWF. During frozen storage, dough stored 1 day had slight lower yeast activity than control. After 90 days frozen storage, the activity was lower in all corresponding samples. At 0 days, CO_2 volumes ranged from 16.10 to 22.47, while at 90 days they ranged from 9.40 to 11.40 ml. While dough having higher levels of WWF substitution showed lower yeast activity, the percent change in yeast activity was less than those with lower levels. For example, samples with 0% WWF 47, 50 and 53% lower gas volumes (at 55, 60 and 65% moisture) at 90 days, while those with 45% WWF had 39, 41 and 41% lower volumes. It may result from more restricted moisture redistribution by higher amylopectin content with higher water holding stability.

Effects of WWF and Water Content on Bread Properties

Bread Volume. Bread volume (Table 4.5) decreased only a slight amount with higher levels of WWF. Values from unfrozen dough ranged from 126.8-125 ml for 0% WWF bread to 125.6-124.6 ml for 45% WWF bread. These results agree with other researchers. Waxy wheat flour substitution was reported to decrease bread volume and give a porous crumb structure and glutinous texture (Abdel-Aal et al, 2002; Morita et al, 2002a; Park and Baik, 2007). Hung et al (2006) reported that bread volume decreased with increasing WWF levels. However, in this research, the volume of 15% WWF bread showed higher volume than other WWF levels flour for all moisture levels. Bread volume levels do not coincide with yeast activity levels. That is, breads from dough with highest yeast activity did not necessarily produce bread with the greatest volume. This reflects the fact that a particular bread volume results from both yeast gassing power and the ability of the gluten matrix to stretch and retain gas. A very high extensibility indicates weak and slack dough, which collapses during the proofing stage or while baking in the oven. In other research, bread of higher WWF content collapsed after baking and did not supported well crumb structure during slicing (Park and Baik, 2007). MRE of the dough relates to the ability of the dough to retain gas and give springy bread. A very low MRE results in poor gas retention and a lower loaf volume. A very high MRE also results in a lower loaf volume because the tough dough is not capable of proofing to an optimum height with the gas produced by the yeast. In our studies, dough with greater WWF levels had greater extensibility and lower MRE. Thus, at 15-30% WWF one has somewhat greater extensibility, and yeast activity similar to or greater than that of 0% WWF dough.

The water level also influenced bread volume. Bread with 60% water had higher volume than those with 55% and 65% water, at all WWF levels and storage times. Bread made from dough with 55% water showed greater volume than that from dough with 65% water. Again, this may be related to the MRE and extensibility of dough. Dough with 55% water showed stronger MRE and lower extensibility. Dough with 65% water showed lower MRE and greater extensibility.

Freezing and frozen storage also affected bread volume. All stored frozen dough produced bread with lower volume than bread from unfrozen dough. For example, bread volume from 0% dough decreased from 125.0-131.4 ml to 112.9-120.0 ml over 90 days. For 15% WWF dough, values decreased from 127.3-134.4 ml to 115.6-123.5 ml. In general, bread with higher WWF levels had a slightly less percent change in volume after frozen dough storage. For 0% WWF dough, bread volumes decreased by 8.0-9.0%, while those for 45% WWF dough decreased by 6.8-8.6%.

Bread Firmness. As seen in Table 4.6, bread firmness decreased with higher levels of WWF. For bread made from unfrozen dough, firmness ranged from 608.8-758.9 g for bread made from 0% WWF dough, and from 235.8-561.1 g for bread made from 45% WWF dough. These results agree well with other studies (Abdel-Aal et al, 2002; Morita et al, 2002a; Park and Baik, 2007) that found that waxy wheat flour substitution decreased bread firmness. During baking, dough with higher WWF content showed lower oven spring. This may result from the state of starch in the waxy wheat flour. Starch composition in WWF is mostly amylopectin (amylose < 5%). Amylose is acknowledged to retrograde immediately after baking and provides some support for crumb structure. Recall that dough with higher levels of WWF also showed increased extensibility and lower MRE, but greatest bread volumes were found at 15% WWF. This lower firmness may be related to the state of gluten as well as the bread pore structure. Using SEM, Morita et al (2002a) found that bread from normal wheat flour had more pore structures than those from WWF bread. They also observed normal wheat flour bread had more uniform pore structures, and a thinner gluten sheets that were more evenly covered with starch granules. In contrast, the WWF bread had thicker, sheet-like strands and a crumb that was broken by rougher and more inconsistent size gas cells.

Water content also had a significant effect on bread firmness. With higher water levels in the dough, the resultant bread was less firm. Again, firmness values for 0% WWF bread were 758.9, 611.7 and 608.8 g (at 55, 60 and 65% moisture, respectively), and for 45% WWF bread were 561.1, 248.0 and 235.8 g. Recall that higher moisture reduced the MRE of the stretched dough. One might hypothesize that this would lead to lower resistance to compression in the finished bread. That is, the set gluten network also shows decreased resistance to strain. In addition, water serves as a plasticizer in food polymer systems, increasing the free volume for molecular motions. This increased mobility would be expected to reduce the viscous component of the bread, and thus influence the measured resistance to compression.

Freezing and frozen storage increased breadcrumb firmness. For 0% WWF bread, values ranged from 608.8-758.9 g for bread made from unfrozen dough, and increased to 701.9-883.9 g for bread made from frozen dough after 90 days. For 45% WWF bread, values increased from

235.8-561.1 g to 292.6 - 621.0 g. As with other properties, the percent change in firmness of bread made from frozen stored dough was less for samples with higher levels of WWF. For example, for 90 day frozen storage, the percent increase for 0% WWF bread was 17, 23 and 33% (for 55, 60 and 65% water dough), while for 45% WWF bread the percent increase was11, 18, and 28%.

CONCLUSIONS

The quality of dough and resulting bread texture are determining factors for both processing and consumer response. Dough with higher WWF levels had lower MRE and increased extensibility, while dough with less water had higher MRE and lower extensibility. Dough with greater WWF and water content were soft, viscous, and had a more glutinous texture. Our results also showed that bread with 15% WWF had the largest bread volume, and that with 60% water had the largest volume.

With higher WWF content, bread quality was known to decrease. However, this research showed that proper combination could improve dough and bread quality. For frozen storage, dough of higher water content showed more damaged characteristics, indicating lower water content is desirable for freezing and frozen storage. But there is proper water content for higher bread volume and quality. 60% water content showed the highest bread volume. WWF content contributed to reduced damage to dough quality for freezing and frozen storage, resulting in better bread quality. So for frozen dough stability and bread quality, desirable WWF and water content should be selected.

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Table 4.1. Stickiness (g, force to positive peak) of dough with different levels of waxy wheat flour addition and water content and normalized ratio of stickiness change (R_{St})** of dough for unfrozen and long-term frozen storage

Frozen Storage Day	Water Content (%)	Waxy Wheat Flour (%)							
		0		15		30		45	
		Stickiness	R_{St}	Stickiness	R_{St}	Stickiness	R_{St}	Stickiness	R_{St}
0	55	76.86 ± 1.00	-***	80.26 ± 1.05	-	83.63 ± 0.61	-	85.98 ± 0.60	-
	60	79.63 ± 0.72	-	83.55 ± 0.83	-	87.83 ± 0.68	-	91.90 ± 1.58	-
	65	83.55 ± 0.72	-	88.19 ± 0.50	-	92.73 ± 0.81	-	98.14 ± 1.12	-
1	55	81.19 ± 0.82	0.056	83.93 ± 0.80	0.046	85.89 ± 1.11	0.027	88.11 ± 0.79	0.025
	60	85.00 ± 1.25	0.067	88.02 ± 0.99	0.046	91.35 ± 0.98	0.040	94.50 ± 1.51	0.028
	65	90.28 ± 1.81	0.081	94.19 ± 0.50	0.068	97.93 ± 1.06	0.056	102.30 ± 0.79	0.042
90	55	104.12 ± 1.15	0.355	105.39 ± 0.88	0.313	106.77 ± 0.37	0.277	108.02 ± 0.52	0.256
	60	109.72 ± 0.92	0.378	111.98 ± 1.17	0.331	113.44 ± 1.17	0.292	115.75 ± 1.78	0.259
	65	116.22 ± 1.79	0.391	117.39 ± 0.77	0.331	119.77 ± 0.77	0.292	122.14 ± 2.89	0.245

* Stickiness: force to positive peak, g force

** R_{St}: Stickiness change ratio: normalized ratio of stickiness change of dough with different levels of waxy wheat flour addition and water content for unfrozen and long-term frozen storage, $(S_t - S_0)/S_0$.

S_t: Stickiness after 1, and 90 day frozen storage.

S_0: Stickiness of unfrozen fresh dough

*** Not applicable: it is initial day of dough made fresh

Table 4.2a. Maximum resistance to extension (MRE)[*] of dough with different levels of waxy wheat flour addition and water content and normalized ratio of MRE change (R_{MRE})[**] of dough for unfrozen and long-term frozen storage

Frozen Storage Day	Water Content (%)	Waxy Wheat Flour (%)							
		0		15		30		45	
		MRE	R_{MRE}	MRE	R_{MRE}	MRE	R_{MRE}	MRE	R_{MRE}
0	55	58.66 ± 3.36	-[***]	56.27 ± 2.00	-	56.33 ± 0.90	-	53.97 ± 0.61	-
	60	40.02 ± 0.72	-	38.05 ± 1.38	-	36.20 ± 2.10	-	34.16 ± 1.73	-
	65	28.15 ± 2.98	-	24.28 ± 1.03	-	20.73 ± 1.42	-	17.54 ± 0.91	-
1	55	55.77 ± 1.55	0.05	54.03 ± 2.03	0.04	54.00 ± 0.52	0.04	52.47 ± 0.61	0.03
	60	35.94 ± 0.48	0.10	35.20 ± 1.72	0.07	34.00 ± 2.28	0.06	32.71 ± 0.84	0.04
	65	23.39 ± 2.52	0.17	21.59 ± 0.67	0.11	18.70 ± 1.09	0.10	15.95 ± 0.78	0.09
90	55	47.61 ± 2.16	0.19	46.89 ± 2.52	0.17	47.70 ± 1.27	0.15	46.52 ± 1.11	0.14
	60	28.37 ± 1.32	0.29	27.86 ± 1.72	0.27	28.09 ± 2.25	0.22	27.27 ± 0.82	0.20
	65	15.23 ± 2.06	0.46	14.26 ± 0.83	0.41	13.02 ± 0.52	0.37	10.95 ± 1.16	0.38

[*] Maximum resistance to extension (MRE): force to peak, g force

[**] R_{MRE}: MRE change ratio: normalized ratio of MRE change of dough with different levels of waxy wheat flour addition and water content for unfrozen and long-term frozen storage, $(M_t - M_0)/M_0$.

M_t: MRE after 1, and 90 day frozen storage.

M_0: MRE of unfrozen fresh dough

[***] Not applicable: it is initial day of dough made fresh

Table 4.2b. Extensibility (Ext.)* of dough with different levels of waxy wheat flour addition and water content and normalized ratio of change ($R_{Ext.}$)** of dough for unfrozen and long-term frozen storage

Frozen Storage Day	Water Content (%)	Waxy Wheat Flour (%)							
		0		15		30		45	
		Ext. (*mm*)	R_{Ext}	Ext. (*mm*)	R_{Ext}	Ext. (*mm*)	R_{Ext}	Ext. (*mm*)	R_{Ext}
0	55	57.69 ± 2.03	-***	58.98 ± 0.94	-	59.93 ± 1.47	-	61.72 ± 2.88	-
	60	76.59 ± 3.08	-	78.68 ± 1.21	-	81.36 ± 1.86	-	85.45 ± 2.35	-
	65	87.43 ± 3.01	-	89.90 ± 1.59	-	92.75 ± 2.34	-	98.45 ± 4.10	-
1	55	55.02 ± 1.58	0.046	56.34 ± 0.94	0.045	57.72 ± 1.47	0.037	59.83 ± 2.88	0.031
	60	72.47 ± 2.05	0.054	75.00 ± 1.26	0.047	77.74 ± 1.90	0.044	83.32 ± 2.07	0.037
	65	82.70 ± 2.25	0.054	85.90 ± 2.32	0.044	88.42 ± 2.43	0.047	94.45 ± 1.47	0.041
90	55	49.23 ± 0.92	0.146	51.49 ± 2.79	0.127	53.14 ± 0.59	0.113	55.94 ± 2.33	0.094
	60	64.44 ± 2.37	0.159	67.50 ± 1.09	0.142	71.06 ± 2.45	0.127	74.78 ± 2.62	0.125
	65	70.23 ± 3.07	0.197	75.09 ± 1.73	0.165	77.14 ± 2.58	0.168	84.15 ± 2.84	0.145

* Extensibility of dough (Ext.): Distance to peak, *mm*

** $R_{Ext.}$: Extensibility change ratio: normalized ratio of Extensibility change of dough with different levels of waxy wheat flour addition and water content for unfrozen and long-term frozen storage, $(E_t - E_0)/E_0$.

E_t: Ext. after 1, and 90 day frozen storage.

E_0: Ext. of unfrozen fresh dough

*** Not applicable: it is initial day of dough made fresh

Table 4.3. Transverse relaxation time (T_2)* of dough with different levels of waxy wheat flour addition and water content and normalized ratio of T_2 value change (R_{T2})** of dough for unfrozen and long-term frozen storage

Frozen Storage Day	Water Content (%)	Waxy Wheat Flour (%)							
		0		15		30		45	
		T_2	R_{T2}	T_2	R_{T2}	T_2	R_{T2}	T_2	R_{T2}
0	55	10.764 ± 0.002	-***	10.373 ± 0.002	-	10.207 ± 0.005	-	9.983 ± 0.086	-
	60	11.263 ± 0.079	-	10.997 ± 0.138	-	10.694 ± 0.100	-	10.460 ± 0.077	-
	65	12.013 ± 0.082	-	11.351 ± 0.069	-	11.148 ± 0.101	-	10.961 ± 0.075	-
1	55	11.738 ± 0.052	0.090	11.251 ± 0.060	0.085	11.035 ± 0.083	0.081	10.734 ± 0.022	0.075
	60	12.350 ± 0.075	0.097	11.979 ± 0.067	0.089	11.611 ± 0.085	0.086	11.295 ± 0.106	0.080
	65	13.274 ± 0.069	0.105	12.433 ± 0.069	0.095	12.157 ± 0.009	0.090	11.888 ± 0.008	0.085
90	55	15.746 ± 0.059	0.463	14.240 ± 0.040	0.373	13.722 ± 0.061	0.344	12.864 ± 0.056	0.288
	60	16.913 ±0.049	0.502	15.904 ± 0.009	0.446	15.337 ± 0.054	0.434	14.209 ± 0.067	0.358
	65	18.363 ± 0.101	0.529	16.630 ± 0.142	0.465	16.190 ± 0.110	0.452	15.369 ± 0.078	0.402

* Transverse relaxation time of dough (T_2): *ms*

** R_{T2}: T_2 value change ratio: normalized ratio of T2 value change of dough with different levels of waxy wheat flour addition and water content for unfrozen and long-term frozen storage, $(T_{2,t} – T_{2,0})/T_{0,t}$.

$T_{2,0}$: T_2 value of unfrozen fresh dough.

$T_{2,t}$: T_2 value after 1 and 90 day frozen storage.

*** Not applicable: it is initial day of dough made fresh.

Table 4.4. Yeast activity[*] of fresh dough with different levels of water and waxy wheat flour

Frozen Storage Day	Water Content (%)	Waxy Wheat Flour (%)							
		0		15		30		45	
		Volume (*ml*)	R_Y[**]	Volume (*ml*)	R_Y	Volume (*ml*)	R_Y	Volume (*ml*)	R_Y
0	55	21.60 ± 0.70	-[***]	22.90 ± 1.14	-	19.30 ± 0.72	-	16.10 ± 0.36	-
	60	22.03 ± 0.78	-	23.10 ± 1.60	-	19.13 ± 1.63	-	16.33 ± 0.65	-
	65	22.47 ± 0.51	-	23.03 ± 0.32	-	18.90 ± 0.92	-	16.93 ± 0.96	-
1	55	18.40 ± 0.98	0.15	19.80 ± 1.22	0.14	16.60 ± 0.70	0.14	14.50 ± 0.66	0.10
	60	18.40 ± 0.72	0.16	19.13 ± 1.07	0.17	16.70 ± 0.72	0.13	14.57 ± 0.83	0.11
	65	17.93 ± 1.60	0.20	18.30 ± 1.28	0.21	15.50 ± 0.72	0.18	13.93 ± 1.11	0.18
90	55	11.40 ± 1.15	0.47	12.80 ± 1.44	0.44	10.47 ± 0.67	0.46	9.90 ± 0.26	0.39
	60	11.07 ± 0.95	0.50	12.87 ± 0.95	0.44	10.27 ± 0.85	0.46	9.63 ± 1.15	0.41
	65	10.60 ± 0.92	0.53	13.10 ± 1.11	0.43	10.33 ± 1.03	0.45	9.40 ± 0.50	0.41

[*] Yeast activity was expressed with gas volume produce, *ml*

[**] R_Y: Yeast activity change ratio: normalized ratio of yeast activity change of dough with different levels of waxy wheat flour addition and water content for unfrozen and long-term frozen storage, $(Y_0 – Y_t)/Y_0$.

Y_0: Yeast activity of unfrozen fresh dough.

Y_t: Yeast activity of dough after 1 and 90 day frozen storage.

[***] Not applicable: it is initial day of dough made fresh.

Table 4.5. Bread volume (ml)* made from dough with different levels of waxy wheat flour and water content and normalized ratio of bread value change (R_{Br})** of dough for unfrozen and long-term frozen storage

Frozen Storage Day	Water Content (%)	Waxy Wheat Flour (%)							
		0		15		30		45	
		Volume (*ml*)	R_{Br}	Volume (*ml*)	R_{Br}	Volume (*ml*)	R_{Br}	Volume (*ml*)	R_{Br}
0	55	126.8 ± 0.40	-***	129.4 ± 0.99	-	127.6 ± 0.56	-	125.6 ± 0.70	-
	60	131.4 ± 0.67	-	134.4 ± 0.81	-	131.8 ± 0.95	-	129.8 ± 0.85	-
	65	125.0 ± 0.81	-	127.3 ± 0.60	-	125.5 ± 0.59	-	124.6 ± 1.67	-
1	55	125.0 ± 0.23	0.014	127.7 ± 2.13	0.013	126.3 ± 2.30	0.010	124.8 ± 2.00	0.006
	60	129.0 ± 1.36	0.018	132.5 ± 1.23	0.014	130.3 ± 1.20	0.011	128.6 ± 3.15	0.009
	65	122.1 ± 2.31	0.023	124.8 ± 1.23	0.020	123.4 ± 2.61	0.017	122.9 ± 2.15	0.014
90	55	116.6 ± 1.96	0.080	119.7 ± 1.26	0.075	118.4 ± 1.50	0.072	117.1 ± 1.50	0.068
	60	120.0 ± 0.16	0.087	123.5 ± 2.53	0.081	121.3 ± 0.94	0.080	120.1 ± 3.00	0.075
	65	112.9 ± 2.15	0.097	115.6 ± 2.13	0.092	114.3 ± 0.70	0.089	113.9 ± 2.50	0.086

* Bread volume was measured by rapeseed methods, *ml*

** R_{Br}: Bread volume change ratio: normalized ratio of volume change of bread made from dough with different levels of waxy wheat flour addition and water content for unfrozen and long-term frozen storage, $(V_{Br,0} – V_{Br,t})/V_{Br,0}$.

$V_{Br,0}$: Bread volume of unfrozen fresh dough.

$V_{Br,t}$: Bread volume after 1 and 90 day frozen storage.

*** Not applicable: it is initial day of dough made fresh.

Table 4.6. Firmness* of bread crumb made from dough with different levels of waxy wheat flour and water content and normalized ratio of bread value change (R_{Fr})** of dough for unfrozen and long-term frozen storage

Frozen Storage Day	Water Content (%)	Waxy Wheat Flour (%)							
		0		15		30		45	
		Firmness (g)	R_{Fr}	Firmness (g)	R_{Fr}	Firmness (g)	R_{Fr}	Firmness (g)	R_{Fr}
0	55	758.9 ± 6.83	-***	697.0 ± 8.30	-	644.4 ± 15.06	-	561.1 ± 7.57	-
	60	611.7 ± 8.57	-	543.3 ± 19.26	-	414.7 ± 13.75	-	248.0 ± 7.17	-
	65	608.8 ± 9.95	-	469.7 ± 14.71	-	380.0 ± 12.09	-	235.8 ± 10.92	-
1	55	783.0 ± 10.35	0.032	712.2 ± 7.93	0.022	664.5 ± 15.29	0.031	573.7 ± 9.45	0.023
	60	677.9 ± 11.23	0.111	600.4 ± 15.92	0.105	457.6 ± 11.29	0.104	270.7 ± 9.04	0.092
	65	608.8 ± 9.03	0.137	528.3 ± 16.23	0.125	423.6 ± 18.40	0.115	263.4 ± 14.38	0.117
90	55	883.9 ± 11.92	0.165	801.3 ± 8.91	0.150	725.8 ± 14.75	0.126	621.0 ± 10.09	0.107
	60	753.4 ± 14.47	0.232	662.7 ± 17.71	0.228	507.3 ± 13.26	0.223	292.6 ± 18.47	0.180
	65	710.9 ± 10.28	0.328	613.1 ± 14.92	0.305	499.8 ± 15.52	0.316	301.2 ± 14.25	0.277

* Bread firmness was measured by compression force, *g*

** R_{Fr}: Bread firmness change ratio: normalized ratio of firmness change of bread made from dough with different levels of waxy wheat flour addition and water content for unfrozen and long-term frozen storage, $(F_0 – F_t)/F_0$.

F_0: Bread volume of unfrozen fresh dough.

F_t: Bread volume after 1 and 90 day frozen storage.

*** Not applicable: it is initial day of dough made fresh.

CHAPTER 5

THE EFFECTS OF WAXY WHEAT FLOUR AND WATER LEVELS ON THE BREAD PROPERTIES AND BREAD STALING

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ABSTRACT

The quality of bread made from frozen dough is diminished and staling rate is increased by changes that occur during the freezing process. Recent studies have shown that new varieties of waxy wheat flour (WWF), bred to contain higher levels of amylopectin, offer unique properties for the production of baked products. In this study, bread qualities and staling were investigated by comparing the effects of levels of WWF and water after freezing and long-term (90day) frozen storage. The specific volume was the highest with 15% WWF substitution and 60% water content in bread of unfrozen and frozen dough. With higher level of WWF and lower water content, bread staling rate decreased. After freezing and long-term frozen storage, bread of higher WWF and lower water content showed slower staling rate. Color intensity increased with higher level of WWF and lower water content, and it got darker after freezing. Color variation was big with higher WWF. However, after long term frozen storage, the color variation of bread crust increased with higher WWF and water content. Nuclear magnetic resonance (NMR) studies showed that bread with higher WWF and water content had higher transverse relaxation (T_2) time of 9-11ms. For bread staling, bread with higher WWF and lower water content showed less reduced T_2 values, meaning that the bread had retarded bread staling. After long-term frozen storage, bread of higher WWF still showed lowered T_2 change on bread staling. This research demonstrated that specific combinations of WWF and water produced a better quality of bread after dough freezing.

* Key words: waxy wheat flour (WWF); specific volume; firmness; bread staling; color analysis; nuclear magnetic resonance (NMR)

INTRODUCTION

Bread is an important food product in many countries due to its significant nutritional, sensorial and textural characteristics (Cauvain and Young, 1999). During the last decade, changes in the way food products are manufactured, distributed and retailed, led to the development of alternative or novel methods for the production and preservation of bakery products such as freezing or MAP technology (Kulp et al, 1995; Laaksonen and Roos, 2000; Bhattacharya et al, 2003). Frozen bakery products can be prepared in-store when needed without major requirements for space and equipment. The quality of bread made from frozen dough may not be the same as that from unfrozen dough. Freezing may change the gluten structure and yeast activity, leading to changes in important quality factors including loaf volume, color and appearance, flavor, and texture characteristics such as firmness (Autio and Sinda, 1992; Bhattacharya et al, 2003; Sharadanant and Khan, 2003b; Giannou and Tzia, 2007).

Bread made from frozen dough also experiences faster rates of staling. Crumb firmness increases, crispness of bread crust decreases, and the bread loaf losses its flavor with a concomitant loss in bread quality. As starch is the major constituent in the bread crumb, the physical changes accompanying the retrogradation of starch have been suggested as the main cause of bread staling. Staling is a complex process, however, that involves several physico-chemical changes (Schiraldi and Fessas, 2000) including starch retrogradation as well as changes in gluten, lipids, and, starch-gluten interactions (Schiraldi et al, 1996; Gerrard et al, 1997; Collar et al, 1999; Gerrard et al, 2001; Hallberg and Chinachoti, 2002). It is well known that freezing causes structural changes the gluten network and a reduction in yeast activity and viability (Yi, 2008). Freezing also affects starch in frozen dough and, therefore, all these factors should influence staling in bread made from frozen dough. It has been shown that the time of frozen storage produced a progressive increase of the retrogradation temperature range of the amylopectin, while greater energy was required for amylopectin melting at longer storage periods, indicating that structural changes of amylopectin were produced during frozen storage (Barcenas et al, 2003; Barcenas et al, 2004). These studies concluded that freezing and frozen storage increase bread staling.

Water also plays an important role in bread staling. During storage, water may migrate and become redistributed in the bread structure. Hardening of the crumb is related to moisture redistribution. The moisture migrates from crumb to crust over time. Gluten undergoes a first order transformation resulting in the release of water from gluten and absorption of this water by retrograding starch (Kay and Willhoft, 1972; Leung et al, 1983; He and Hoseney, 1990; Baik and Chinachoti, 2000).

One approach to limiting staling in baked products is to incorporate modified starches, particularly those that contain greater amounts of amylopectin. Alternately, enzymes such as α-amylase may be incorporated to break down amylose in such a way as to reduce retrogradation, while providing more dextrins for yeast activity. An interesting new advancement is waxy wheat flour (WWF), an amylose-free variety which has been developed through classical breeding and genetics (Graybosch et al, 2003). Many uses have been suggested for WWF, including as a source of blending flour to improve shelf life stability, processing quality, or palatability of baked and sheeted wheat products (Lee et al, 2001). Hung et al (2007a) produced bread from WWF that had higher protein and dietary fiber, but showed better quality was attained with partial substitution of conventional flour wit 10-50% WWF. Bhattacharya et al (2002) showed that bread with 10-30% WWF had decreased staling during 5 days storage as compared to control samples. It is also known that amylopectin and amylose have differing propensities to hold water during freezing and thawing. To date, no studies have documented the use of WWF to produce frozen dough to be used for bread making.

In this research, we studied several quality characteristics of bread made from frozen bread dough having different waxy wheat flour and water content. This included frozen dough stored for up to 90 days. Quality factors included loaf volume, crust color and color distribution, crumb firmness and rates of staling.

MATERIAL AND METHODS

Materials

To explore the relationship between ratio of waxy wheat to conventional flour and dough quality, blends were made from normal amylopectin and non-waxy wheat flour. The conventional flour was a winter red wheat variety (Martha White Foods Inc All-Purpose, Jackson, TN), with a protein content is 9.59% as determined by Dumas combustion method (AACC 46-30) using the FP-2000 nitrogen analyzer (Leco, St. Joseph, M). Waxy wheat flour used was Shinmi Waxy wheat flour (Shinmi chalmil) with a protein content of 9.65%, and amylose content of 1.2% as determined by the iodine color method.. The genetic background is the cross: Kanto107/Bai-Huo/3/Bai-Huo/Kanto107//A92-3327/Kanto107. Vital gluten (74.8% protein, King Arthur Flour Company Inc., White River Junction, VT) was used to adjust the flour mixes to 12.5%, a more suitable for bread making. Other ingredients used for dough preparation were dry yeast (3%, flour basis, Fleischmann's Yeast, Chesterfield, MO), commercially available sugar (5%, flour basis, Dixie Crystal, USA),non-iodized salt (2%, flour basis, Morton International Inc., Chicago IL), and Crisco voy oil (5%, flour basis, J. M. Smucker Co., Orrville, OH).

Dough Preparation and Baking

Waxy wheat flour was blended with conventional wheat flour at levels of 15%, 30%, and 45% of total flour weight. Yeast was pre-hydrated with the water and all of the dough ingredients were placed in a blender and mixed together. Enough water was added to give 55%, 60%, and 65% water in the final dough. The mixing times for best dough development were different depending on water content. Dough at 55% water was mixed for 2 min at a speed of 90 rpm and for 9 min at 128 rpm, 8 min at 128 rpm for 60% water, and 11min at 128rpm for 65%. After the dough was formed, it was separated into 50g samples, and shaped by hand into round balls before placing in bread pans.

The dough samples were frozen in circular slabs, approximately 50mm diameter in a built-in blast freezer in the department (Eliatt-Williams, Indianapolis). The freezing time was approximately 1 hour at -20°C and frozen dough samples were stored at -20°C for 90 days. After storage for 0, 1 and 90 days, samples were thawed in an environmental stability chamber (HotPack, Warminster, PA) at 8°C. The thawed dough was then placed at 36°C and 85% RH to proof. Proofed samples were baked in a convection oven at 180°C for 15 min (Blodgett, Burlington VT). After baking, bread samples were allowed to cool for approximately 1hr at ambient temperature prior to subsequent tests (Hallen et al, 2004).

Bread Volume

Loaf volume and weight were determined according to the AACC 10-05 (2000) procedure. Loaf volume was determined by the rapeseed displacement test and specific volume was expressed as the ratio of bread volume to dough weight (Pyler, 1988; Hallen et al, 2004). The loaf was placed in a container with known volume (V_C). The container was topped with rapeseed, the loaf removed and the volume of the rapeseed noted (V_R). Loaf volume (V_L) was calculated as:

$$V_L\ (ml) = V_C - V_R$$

After measuring volume, the loaf weight (W) was determined on a digital scale. Specific volume (V_S) of bread was calculated as

$$Vs(ml/g) = \frac{V_L}{\mathrm{W}}$$

Crumb Firmness

The firmness of the bread samples was determined using a texture analyzer (TA-XT2i, Texture Technologies Corp., Scarsdale, NY) equipped with a 36 mm cylinder probe attached to a 5Kg load cell (AACC method 74-10A, 2000). Three center slices of each loaf were measured at 25 °C. The compression rate was set at 1.7 mm/s, and firmness determined as the force required to compress the slices (20 mm thick) by 40% strain.

Bread Crust Color

A digitized image was used to evaluate the crust color of each sample. The bread image was acquired by digital camera (Canon PC1114, Japan) (Purlis and Salvadori, 2007). Images of the bread surface were taken under natural light conditions with the camera placed perpendicularly to the surface at 0.3 m distance. Each image was saved as a jpeg file, at 3072 × 2304 pixel resolution (approximately 125 μm resolution). Images were cropped to keep only the bread sample surface, resulting in circular images with a 1200 pixel diameter. The digital image was analyzed using Image J software (http://rsb.info.nih.gov/ij/). The color image was converted to 256 level gray scales, representing degrees of darkness. Using bars of known lengths, pixel values are converted into distance units, producing distribution of gray scale. The distribution gives the mean and standard deviation of gray scale (Datta et al, 2007).

NMR Measurements

Proton relaxation measurements were made using a 20MHz Proton (^{1}H) NMR spectrometer (Resonance Instruments, Whitney, UK). An approximately 2g bread sample was taken from the center of each bread loaf. Three replicates were taken from each loaf of bread. Each sample was placed in a 10mm diameter glass tube then covered with parafilm. The glass tubes with bread specimens were then placed in 18mm diameter NMR tubes. Transverse (T_2)

relaxation curves were developed using the CPMG pulse sequence: 90x-(τ-180y-τ-echo)n (Meiboom and Gill, 1958). Acquisition parameters were set to a 90° pulse of 4.2μs and a recycle delay of 2s. A pulse spacing (τ) of 100μs was chosen to exclude the fast decaying solid-like signal. All measurements were made at 25°C ±1°C. Relaxation curves obtained from CPMG sequences were analyzed using the WinDXP distributed exponential routine (Resonance Instruments, Whitney, UK). This routine calculates a distribution of T_2 terms that best describe the data:

$$g_i = \sum_{j=1}^{m} f_j e^{-t_i/T_{2,j}}$$

where g_i are the values of the exponential distribution at time t_i; f_j are the pre-exponential multipliers, and $T_{2,j}$ are the time constants. In general, there was more than one distributed region, suggesting the existence of various regions of water with similar mobility. In this case, the number of protons in a given region was measured by the integrated signal intensity over the region of the distribution.

Statistical Analysis

The effects of WWF content and water content on bread volume, firmness, and crust color were analyzed by two-way analysis of variance at a level of significance of 0.05. Mean values were compared using Fisher's LSD method with significance level of 0.05. SAS software was used for data analysis (SAS institute, Inc, 2006).

RESULTS AND DISCUSSION

Bread Specific Volume

Table 5.1 shows the specific volume of bread formed at different levels of WWF and water. The results also show effects of long term frozen storage over 90 days. Without frozen storage, bread made from 15% WWF showed the highest volume (3.05, 3.18 and 3.01 ml/g at 55, 60 and 65% water, respectively), but volume was decreased at higher levels of WWF at all moisture levels. Bread made from 60% water dough had higher specific volume than did bread made from 55% and 65% water dough.

Loaf volume was also measured after 1 day and 90 days of frozen storage. Freezing decreased the loaf volume for all levels of. For example, for 15% WWF bread volumes ranged from 2.95-3.14 ml/g after 1 day storage and 2.76-2.93 ml/g after 90 days storage. Although loaf volume was decreased, greatest volumes were always attained from the 15% WWF treatments, and with dough at 60% moisture. Loaf volume for all breads decreased with long term storage. For example, for 45% WWF bread, specific volumes decreased from 2.87-3.04 ml/g for unfrozen dough to 2.71-2.86 ml/g after 90 days of frozen storage. Table 5.1 also shows the relative change in bread volume (RSV) as compared to bread made from unfrozen dough. In general, thre was less change in bread volume for samples with higher moisture content and greater levels of WWF. For example, at 0% WWF, the specific volume of bread from unfrozen dough was 2.98 ml/g for 55% water and 2.95g for 65% water dough. After 90 days, this decreased to 2.78 ml/g for 55% and 2.64g for 65% water dough. This represented a change of 6.9% for the 55% water dough and 9.0% for the 65% water dough. As noted, bread with higher WWF content showed less change in volume subsequent to frozen storage.

The specific volume of bread is a characteristic quality parameter and is related to the ability of the dough to inflate and develop oven spring. For every bakery product there is usually an ideal relation between dough weight and loaf volume that yields the most desirable texture and grain (Pyler, 1988; Giannou and Tzia, 2007). That is, specific loaf volume should be not too large or too small because it affects the crumb grain. Too small of a specific volume gives a very compact and closed grain structure and too large of a loaf volume gives a very open grain structure (Sharadanant and Khan, 2003b).

Bread Crumb Firmness

Table 5.2 shows crumb firmness for bread made from unfrozen and frozen dough having different levels of WWF and water. For bread from unfrozen dough, maximum firmness (758.9 g) was attained from dough with 0% WWF and 55% water. Minimum firmness (235.8 g) was attained for dough with 45% WWF and 65% water. Firmness decreased with increased waxy wheat flour content for all breads. For example, firmness values ranged from 608.8 to 758.9g for 0% WWF and 235.8 to 561.1 g for 45% WWF substitution. With respect to water content, bread firmness decreased with increased water content of the dough. For example, at 15% WWF substitution, bread firmness of 55% water content was 697.0 g and decreased to 469.7 g for 65% water content.

Bread firmness increased for all different WWF and water levels of dough after freezing and frozen storage. At 0 days, firmness ranged from 235.8 to 758.9 g while after 1 day frozen storage, values ranged from 263.4 to 783.0 g. For 15% WWF and 60% water, values increased from 543.3 g at day 0 to 600.4 g after 1 day frozen storage. After long-term storage (90 days), the firmness increased even further, with values ranging from 292.6 to 883.9 g. At 15% WWF, firmness values ranged from 469.7 to 697.0 g at day 0 to 613.1-801.3 g after 90 days frozen storage.

The relative change in firmness (R_F) due to frozen storage is also shown in Table 5.2. Bread made from dough with greater water content had a more change in bread volume after frozen storage. Bread with higher levels of WWF showed less change in volume due to frozen storage. For example, for 0% WWF dough volumes increased by 16.5, 23.2 and 32.8% for moisture levels of 55, 60 and 65%, respectively. For 45% WWF dough, bread firmness increased by 10.7, 18.0 and 27.7 % after 90 days storage.

One usually assumes that a less firm, more tender bread is most desirable for consumers, although this depends on the bread variety. Crumb firmness arises from the integrity of the gluten network, the degree of cross linking, the amount of gas incorporated, and from other structural components of the bread. Giannou et al (2007) have reported similar results to the ones reported in this work with frozen storage temperature. They reported that bread crumb firmness increased after freezing and had increased with prolonged frozen storage. Phimolsiripol et al (2008) also reported bread firmness change related to frozen storage and fluctuation temperature. They showed that the lower temperature of storage gives more tender characteristics to bread made from frozen dough.

Crust Color

The development of color during baking is an important indicator of bread quality. Crust and crumb color of bread samples should be golden brown and creamy white, respectively, and are both expected to be uniform and appealing (Giannou and Tzia, 2007). In this research, the color image was converted to 256 level gray scales, with 0 corresponding to pure black and 255 to white. Table 5.3 presents the mean value and the standard deviation of the mean. As seen in Table 5.3, the crust became darker with increased WWF content and decreased water content. Values ranged from 108.8-122.4 for 0% WWF to 90.7-104.2 for 45% WWF (at 0 days). Also at day 0, values ranged from 90.7-108.8 for samples at 55% moisture and from 104.2-122,4 for samples at 65% moisture. (Hung et al, 2007a) also found that increased levels of WWF resulted in darker bread color.

Frozen storage also had a significant effect on the crust color. In general, values decreased with frozen storage time, indicating a darker crust color. For dough stored at longer time, samples with higher WWF levels were still darker. However, the relative change in darkeness as compared to bread from unfrozen dough was less with greater WWF content. For example, values for bread made from 0%WWF/60% moisture dough decreased by 2.6% after 90 days frozen storage, while those for bread made from 45% WWF/60% moisture increased by 1.89%.

Table 5.4 shows the color distribution of each image. The variation in darkness increased with both WWF level and moisture content, indicating a bread with a less even distribution of color. In addition, bread color variation increased with long term frozen storage. Giannou and Tzia (2007) also reported that storage under freezing conditions causes darker, less even crust color, which they attributed to the formation of white spots in the crust surface. As with volume and firmness values, we found that the percent change in darkness after frozen storage was less for samples with higher levels of WWF.

Bread Staling

As bread stales, the texture of crumb changes from soft and spongy texture to one that is firm. Hence, firmness is one of the most commonly used methods to determine the degree of bread staleness, which has been shown to correlate with bread staling as measured by consumer actability (Axford et al, 1968; Gray and Bemiller, 2003). Changes in bread firmness during 5 days storage after baking are shown in Figure 5.1-3. Regardless of treatment group, the firmness of all breads increased most rapidly during the first two days.

Bread with greater levels of WWF showed slower staling rates. For example, for bread made from unfrozen dough with 0%WWF/60% moisture, firmness increased from 616.7 to 1551.1 g over 5 days. For bread made from 45% WWF/60% moisture dough, firmness increased from 248.0 to 830.8 g over 5 days. The percent change in firmness was also less with greater proportions of WWF in the bread.

Bread made from dough with higher water content showed lower overall firmness after 5 days storage, but had a lesser relative change in firmness over time as compared with bread of lower water content. For example, at 15% WWF/65% water, firmness increased from 469.7 g at day 0 to 1276.5 g (a 172% increase), while that for 15% WWF/60% water increased from 343.3 g to 1336.7 g (a 289% increase).

Freezing and frozen storage also increased bread staling rates. One day after freezing, all treatments had increased initial bread firmness and increased staling rate. As seen by comparing Figures 5.1 and 5.2, initial firmness was not so different between breads made from unfrozen and frozen dough, but after 5 days storage, firmness values were higher. After frozen storage for 1 day, bread with higher WWF showed less increased firmness and slower firming rates. Long-term frozen storage (for 90 days) of dough also significantly increased bread staling. As seen in

Figure 5.1 and 5.3, after 90 days storage, frozen dough gave bread with higher firmness compared with unfrozen dough. The initial bread firmness of long term frozen dough was higher than that of unfrozen dough, and final firmness increased even higher. For example, at 15% WWF/60% water content, firmness of bread from unfrozen dough increased from 543.3 g to 1336.7 g (a 146% increase). However, after the 15% WWF/60% moisture samples were stored frozen for 90 days, firmness increased from 662.7 g to 1740.4 g (a 163% increase). With respect to WWF content, bread staling with higher WWF bread increased more slowly than that with lower WWF levels. For example, for 0% WWF/60% moisture dough, bread firmness increased from 735.4 g 1972.4 g over 5 days, while for 45% WWF/60% moisture dough it increased from 326.3 g to 1107.8 g. In general, higher WWF and lower water content retarded staling in bread made from unfrozen and frozen dough.

The results indicate that WWF has some effect on retarding bread staling, which agrees with other researchers. Bhattcharya et al (2002) reported that addition of WWF resulted in lower firmness values during bread storage. Biladeris (1992) stated that starch retrogradation was a biphasic phenomenon consisting of early complexion of amylose followed by a slow and gradual recrystallization of amylopectin. Therefore, reducing the amylose content could reduce bread staling. Hug-Iten et al (1999) postulated that reorganization of intragranualar amylose fractions enhances the rigidity of the starch granule during bread staling. Our research results also suggest that reducing the fraction of amylose in bread reduces staling. It is hypothesized that highly branched amylopectin polymers bind water more strongly, resulting in reduced moisture migration that encourages bread staling. In addition, upin freezing and frozen storage, dough with higher levels of amylopectin may have stronger association with water, resulting in a softer crumb and retarded bread staling. Higher water availability better plasticizes the starch network giving a softer texture, but migration of water away from the network results in faster staling.

NMR Relaxometry and Bread Staling

Pulsed H^1 NMR was used to investigate the relaxation characteristics of bread systems subject to different WWF and water content after freezing and frozen storage. Figure 5.4 - 6 shows the transverse relaxation times for bread samples during storage of bread samples for 5 days. The T_2 relaxation time decreased over storage time for all treatments. Bread with higher WWF and water content showed higher initial T_2, but the change in T_2 during storage were different. Bread with higher WWF showed less decrease in T_2 and always had higher final values than bread with lower WWF content after 5 days. As for freezing and frozen storage, bread with higher WWF showed higher T_2 values and higher T_2 values were maintained during 5 days storage. However, bread with higher water content showed higher initial T_2 values but showed a greater decrease in T_2 during storage. These results indicate that bread with higher WWF content holds more water and showed more retarded bread staling, while bread with higher water content showed higher mobilility moisture but faster bread staling than bread with lower water content.

Many researchers have used relaxation times of NMR to indicate the state of water in food polymers such as bread (Leung and Steinberg, 1979; Seow and Teo, 1996; Chen et al, 1997; Vodovotz et al, 2002; Wang et al, 2004). The spin-spin (transverse) relaxation time by T_2 is a function of the spin species and the chemical a physical environments surrounding the spins. In other words, the relaxation time constants are a fundamental property of the chemical and physical environment. During bread storage, drying out of moisture does not explain staling, but accelerate reactions leading to staling. The moisture relationships with bread crumb are one of several important considerations in bread staling. Schiraldi and Fessas (2000) concluded that the overall picture of the crumb could be described as interpenetrated gels separated by aqueous interphases which contain most of the low molecular weight solutes. This water is rather mobile and can facilitate mutual displacement of the incompatible gel phases, thus behaving as a plasticizer, and can enhance the crumb to crust migration of moisture. As staling progresses, moisture is incorporated in the reorganized starch structure, resulting in lower mobility. These phenomena lead to lower T_2 values.

With higher WWF, we observed lower staling rate and higher T_2 values. As mentioned, the high ratio of highly branched amylopectin in WWF binds water more strongly, resulting in slower retrogradation than found for amylose, and an overall reduced rate of bread staling. Thus, these results suggest that on freezing and frozen storage, dough with greater amylopectin content has stronger association with water than dough of higher amount of amylose, resulting in higher T_2 values and lower staling rate.

CONCLUSIONS

Addition of WWF to commercial flour at 15, 30, and 45% affected bread quality, resulting in highest volume in 15% WWF substitution. Bread with higher WWF levels gave softer texture and retarded bread staling. On freezing and frozen storage, bread with 15% WWF also gave the highest bread volume and reduced staling rate. Water level is a critical factor in forming a good dough, and bread made from dough with 60% water had the highest bread volume for unfrozen and frozen-stored dough. Bread with 60% water also showed reduced bread staling than bread with 65% water, and had a softer texture than bread from 55% water dough even after freezing and long-term frozen storage. These results suggest that 15% WWF and 60% water content is an optimal condition for better bread quality after freezing and storage. For frozen storage, addition of WWF is a possible way to reduce the effects of freezing damage on bread quality.

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Table 5.1. Specific volume* of bread made from different freezing rates and storage conditions

Frozen Storage Day	Water Content (%)	Waxy Wheat Flour (%)							
		0		15		30		45	
		Specific volume	R_{SV}**	Specific volume	R_{SV}	Specific volume	R_{SV}	Specific volume	R_{SV}
0	55	2.98 ± 0.010	-***	3.05 ± 0.023	-	3.01 ± 0.013	-	2.95 ± 0.017	-
	60	3.11 ± 0.016	-	3.18 ± 0.019	-	3.14 ± 0.023	-	3.04 ± 0.020	-
	65	2.95 ± 0.019	-	3.01 ± 0.014	-	2.94 ± 0.014	-	2.87 ± 0.038	-
1	55	2.94 ± 0.005	0.014	3.01 ± 0.050	0.013	3.01 ± 0.055	0.000	2.95 ± 0.047	0.000
	60	3.04 ± 0.032	0.023	3.14 ± 0.029	0.014	3.10 ± 0.029	0.014	3.04 ± 0.074	0.000
	65	2.90 ± 0.055	0.017	2.95 ± 0.029	0.020	2.90 ± 0.061	0.016	2.87 ± 0.051	0.011
90	55	2.78 ± 0.047	0.069	2.85 ± 0.030	0.065	2.83 ± 0.036	0.063	2.77 ± 0.059	0.055
	60	2.84 ± 0.051	0.089	2.93 ± 0.060	0.082	2.89 ± 0.022	0.080	2.86 ± 0.071	0.059
	65	2.64 ± 0.050	0.090	2.76 ± 0.051	0.083	2.71 ± 0.017	0.081	2.71 ± 0.059	0.056

* Specific volume of bread was expressed as, *ml/g*. After measuring the loaf volume and weight, the specific volume was calculated.
** R_{SV}: Specific volume ratio: ratio of specific volume change of bread made dough after freezing and frozen storage, $(SV_0 - SV_t)/SV_0$.
SV_t: Specific volume after 1, and 90 day frozen storage.
SV_0: Specific volume of unfrozen fresh dough
*** Not applicable: it is initial day of bread baked fresh

Table 5.2. Firmness* of bread crumb made from dough with different levels of waxy wheat flour and water content and Normalized ratio of bread value change (R_{Fr})** of dough for unfrozen and long-term frozen storage

Frozen Storage Day	Water Content (%)	Waxy Wheat Flour (%)							
		0		15		30		45	
		Firmness (g)	R_{Fr}	Firmness (g)	R_{Fr}	Firmness (g)	R_{Fr}	Firmness (g)	R_{Fr}
0	55	758.9 ± 6.83	-***	697.0 ± 8.30	-	644.4 ± 15.06	-	561.1 ± 7.57	-
	60	611.7 ± 8.57	-	543.3 ± 19.26	-	414.7 ± 13.75	-	248.0 ± 7.17	-
	65	608.8 ± 9.95	-	469.7 ± 14.71	-	380.0 ± 12.09	-	235.8 ± 10.92	-
1	55	783.0 ± 10.35	0.032	712.2 ± 7.93	0.022	664.5 ± 15.29	0.031	573.7 ± 9.45	0.023
	60	677.9 ± 11.23	0.111	600.4 ± 15.92	0.105	457.6 ± 11.29	0.104	270.7 ± 9.04	0.092
	65	608.8 ± 9.03	0.137	528.3 ± 16.23	0.125	423.6 ± 18.40	0.115	263.4 ± 14.38	0.117
90	55	883.9 ± 11.92	0.165	801.3 ± 8.91	0.150	725.8 ± 14.75	0.126	621.0 ± 10.09	0.107
	60	753.4 ± 14.47	0.232	662.7 ± 17.71	0.228	507.3 ± 13.26	0.223	292.6 ± 18.47	0.180
	65	710.9 ± 10.28	0.328	613.1 ± 14.92	0.305	499.8 ± 15.52	0.316	301.2 ± 14.25	0.277

* Bread firmness was measured by compression force, *g*

** R_{Fr}: Bread firmness ratio: ratio of firmness change of bread made from dough after freezing and frozen storage storage, $(F_t - F_0)/F_0$.

F_0: Bread volume of unfrozen fresh dough.

F_t: Bread volume after 1 and 90 day frozen storage.

*** Not applicable: it is initial day of dough made fresh.

Table 5.3. Crust color scale* of bread made from dough produced under different freezing rates and storage conditions

Water Content (%)	Waxy Wheat Flour (%)							
	0		15		30		45	
	Color Scale	R_{COL}**	Color Scale	R_{COL}	Color Scale	R_{COL}	Color Scale	R_{COL}
55	108.8 ± 0.41	-***	98.4 ± 0.76	-	93.5 ± 0.58	-	90.7 ± 0.13	-
60	114.5 ± 0.57	-	103.2 ± 0.35	-	96.5 ± 0.34	-	93.7 ± 0.45	-
65	122.4 ± 1.48	-	114.0 ± 0.05	-	108.6 ± 1.27	-	104.2 ± 1.12	-
55	107.7 ± 0.46	0.0096	97.5 ± 0.79	0.0097	94.5 ± 1.44	-0.0099	89.8 ± 0.94	0.0098
60	113.5 ± 0.86	0.0093	102.2 ± 0.71	0.0095	97.2 ± 1.71	-0.0077	92.8 ± 0.69	0.0097
65	121.3 ± 1.56	0.0091	112.9 ± 0.77	0.0093	107.6 ± 1.20	0.0094	103.2 ± 0.16	0.0096
55	105.0 ± 1.24	0.0350	95.4 ± 0.84	0.0310	90.9 ± 0.43	0.0285	88.2 ± 0.65	0.0272
60	110.5 ± 1.22	0.0263	99.9 ± 1.55	0.0227	93.6 ± 1.65	0.0365	91.0 ± 0.46	0.0189
65	117.8 ± 2.18	0.0290	110.0 ± 1.49	0.0261	105.0 ± 1.85	0.0240	100.8 ± 0.26	0.0231

* Crust color scale is an average of three means of gray scale histogram of bread image.

** R_{COL}: Color intensity ratio: ratio of color intensity change of bread made from dough after freezing and frozen storage, $(Col_0 – Col_t)/Col_0$.

Col_0: Color intensity of unfrozen fresh dough.

Col_t: Color intensity after 1 and 90 day frozen storage.

*** Not applicable: it is initial day of dough made fresh.

Table 5.4. Crust color variation* of bread made from dough produced under different freezing rates and storage conditions

Water Content (%)	Waxy Wheat Flour (%)							
	0		15		30		45	
	Volume (*ml*)	R_{Var}**	Volume (*ml*)	R_{Var}	Volume (*ml*)	R_{Var}	Volume (*ml*)	R_{Var}
55	10.74 ± 0.75	-***	11.25 ± 1.09	-	12.39 ± 0.72	-	15.90 ± 0.82	-
60	10.83 ± 0.78	-	11.39 ± 0.36	-	13.34 ± 0.75	-	16.50 ± 0.60	-
65	11.38 ± 0.55	-	12.98 ± 0.59	-	14.19 ± 0.92	-	17.39 ± 1.43	-
55	10.85 ± 0.87	0.010	11.35 ± 0.45	0.009	12.99 ± 0.86	0.004	16.07 ± 0.93	0.011
60	10.96 ± 0.90	0.012	11.50 ± 1.26	0.010	13.47 ± 1.21	0.010	16.65 ± 0.84	0.009
65	11.57 ± 0.39	0.016	13.15 ± 0.47	0.013	14.27 ± 1.15	0.006	17.57 ± 1.22	0.010
55	14.73 ± 0.56	0.372	13.72 ± 0.74	0.220	14.93 ± 1.01	0.154	17.23 ± 0.75	0.084
60	15.42 ± 1.35	0.424	15.69 ± 1.03	0.378	17.39 ± 0.66	0.304	18.94 ± 0.63	0.148
65	16.89 ± 0.65	0.484	18.22 ± 0.72	0.403	18.74 ± 1.05	0.320	20.59 ± 1.50	0.184

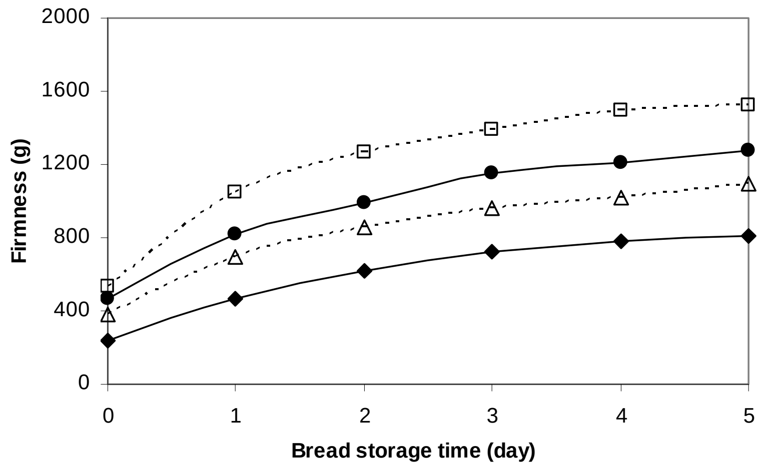
* Crust color variation is mean of the degree of histogram distribution of color analyzed. (scale ranges 0 black to 255 white)

** R_{Var}: Color variation ratio: ratio of color variation change of bread made from dough after freezing and frozen storage, $(Var_0 – Var_t)/Var_0$.

Var_0: Color variation of unfrozen fresh dough.

Var_t: Color variation after 1 and 90 day frozen storage.

*** Not applicable: it is initial day of dough made fresh.



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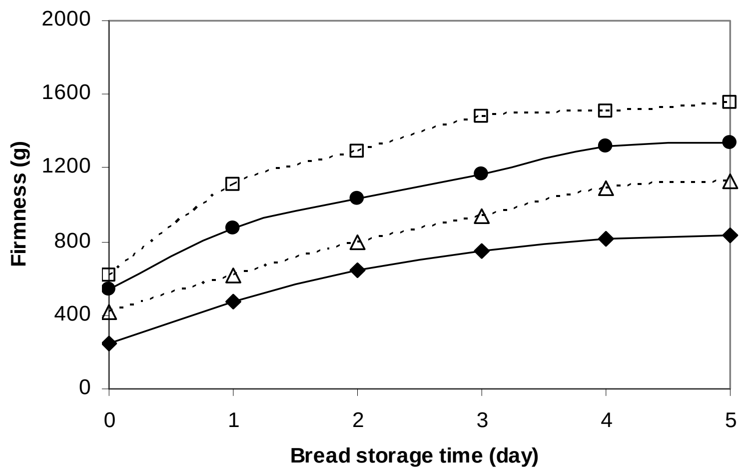

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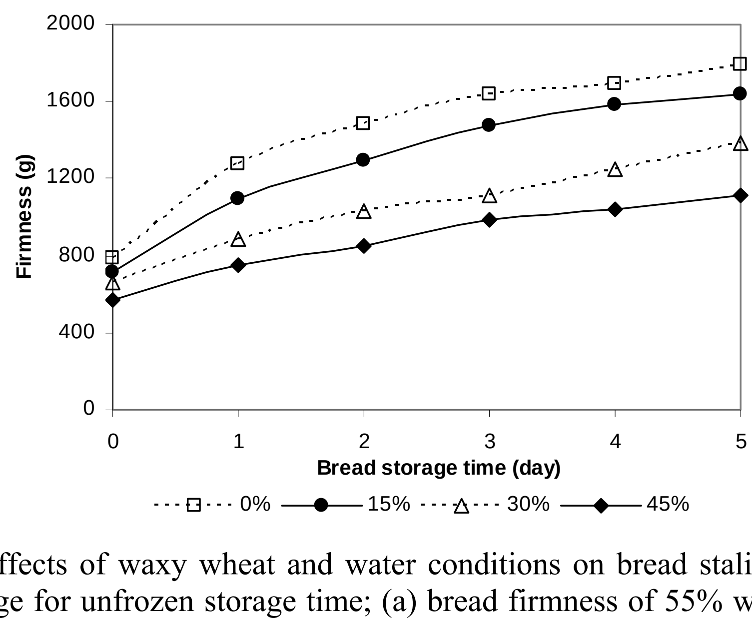
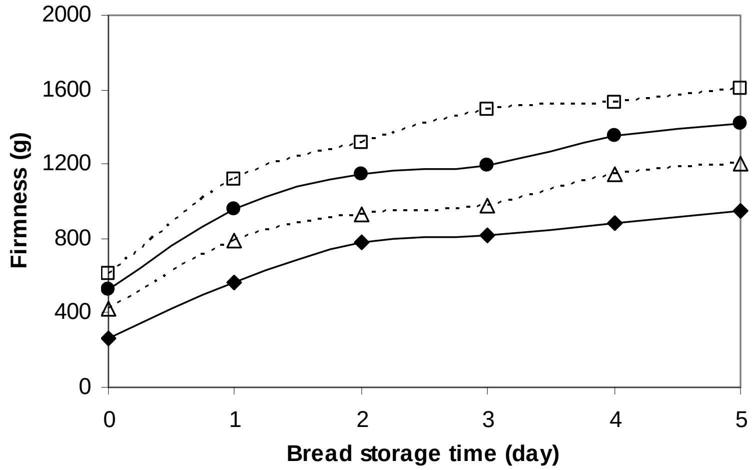


(c)

Figure 5.1. The effects of waxy wheat and water conditions on bread staling measured by firmness (g) change for unfrozen storage time; (a) bread firmness of 55% water content, (b) bread firmness of 60% water content, and (c) bread firmness of 65% water content.



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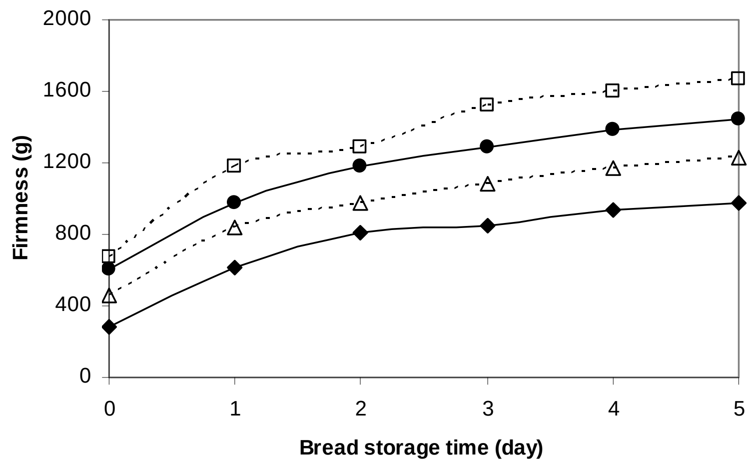

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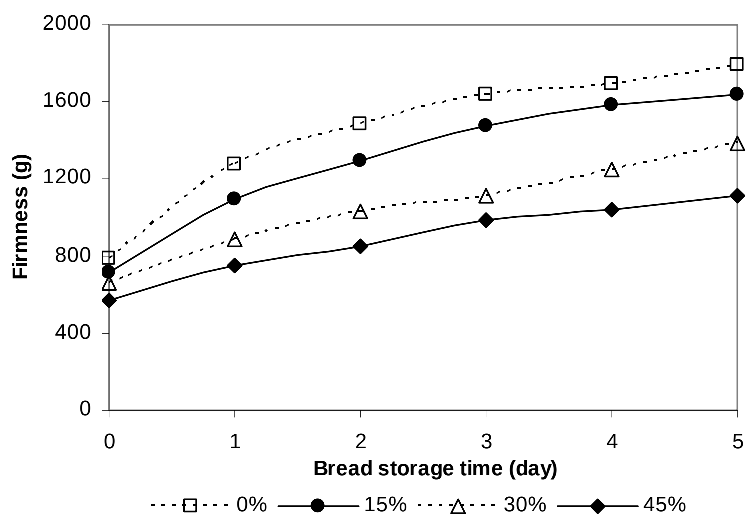
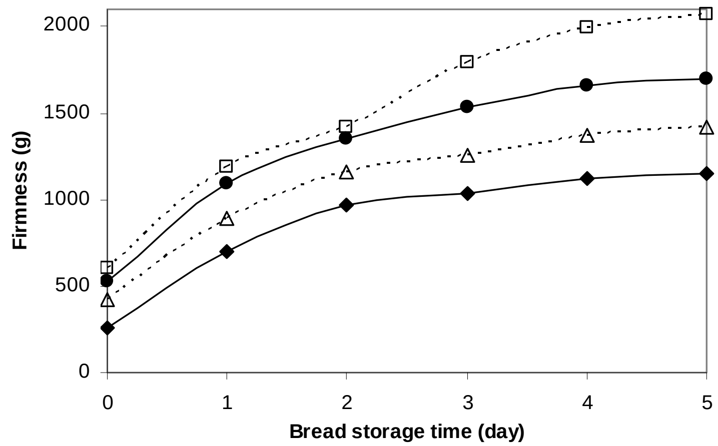


(c)

Figure 5.2. The effects of waxy wheat and water conditions on bread staling measured by firmness (g) change for 1 day frozen storage time;(a) bread firmness of 55% water content, (b) bread firmness of 60% water content, and (c) bread firmness of 65% water content.



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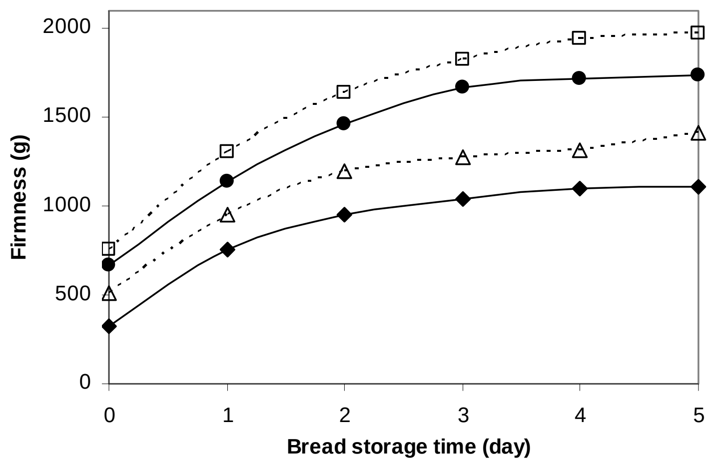

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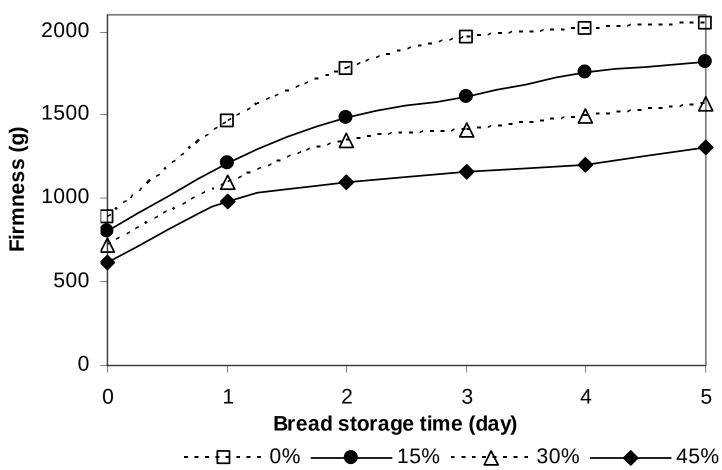
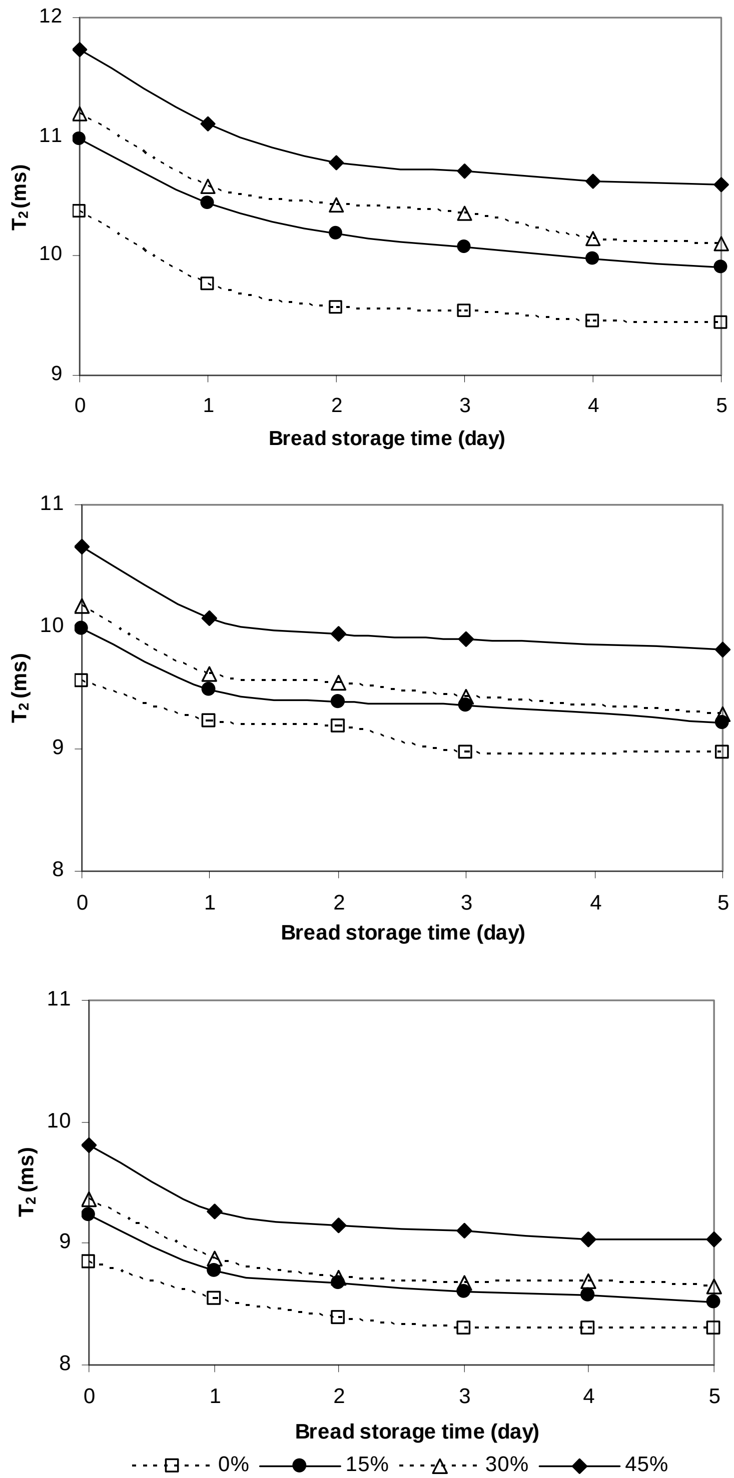


(c)

Figure 5.3. The effects of waxy wheat and water conditions on bread staling measured by firmness (g) change for 90 day frozen storage time;(a) bread firmness of 55% water content, (b) bread firmness of 60% water content, and (c) bread firmness of 65% water content.



(a)

(b)

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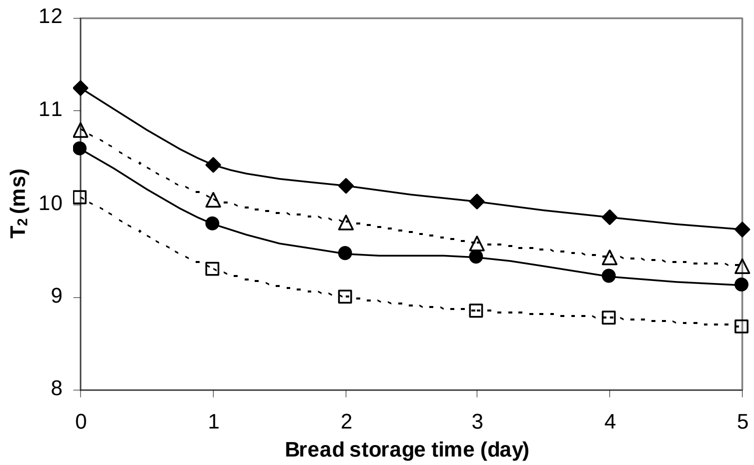
Figure 5.4. Water mobility on bread staling measured by Transverse relaxation time (T_2) for bread of unfrozen fresh dough ; (a) T_2 of 65% water content, (b) T_2 of 60% water content, and (c) T_2 of 55% water content.



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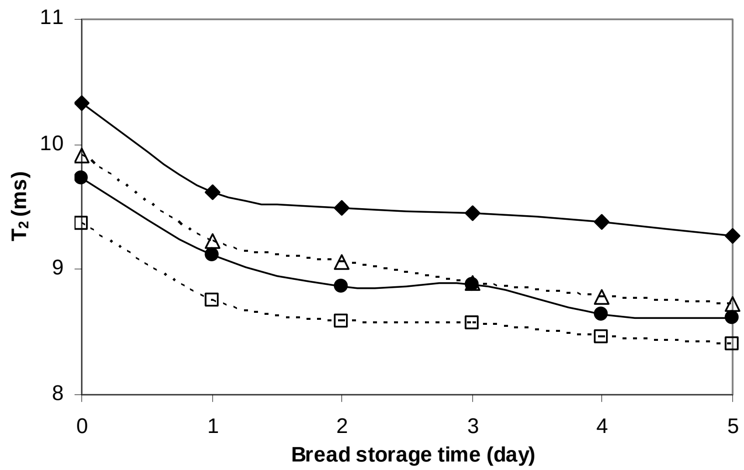

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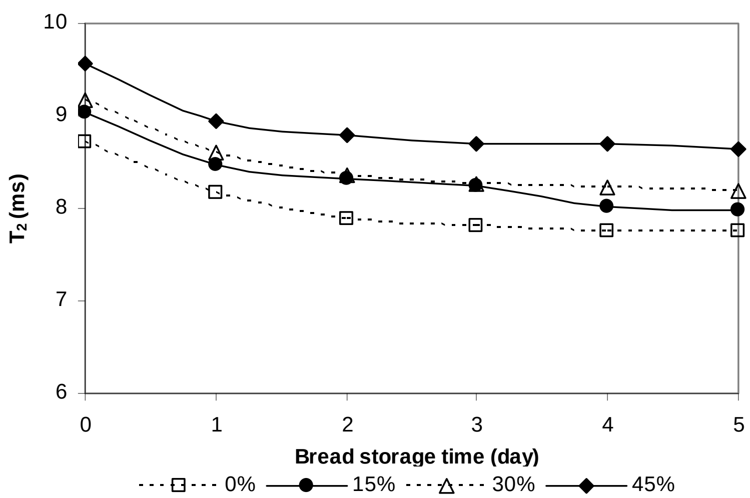
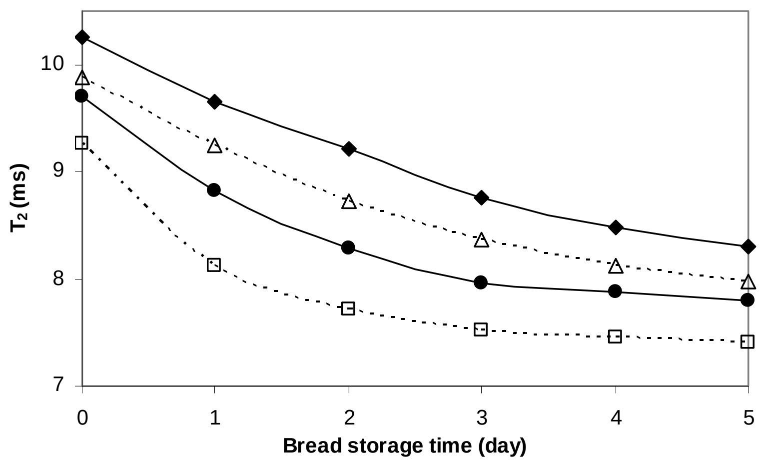
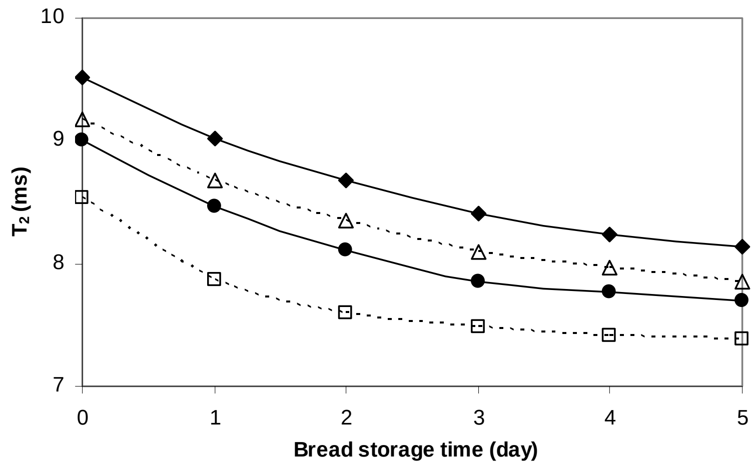


(c)

Figure 5.5. Water mobility on bread staling measured by Transverse relaxation time (T_2) for bread of 1 day frozen storage dough ; (a) T_2 of 65% water content, (b) T_2 of 60% water content, and (c) T_2 of 55% water content.



(a)



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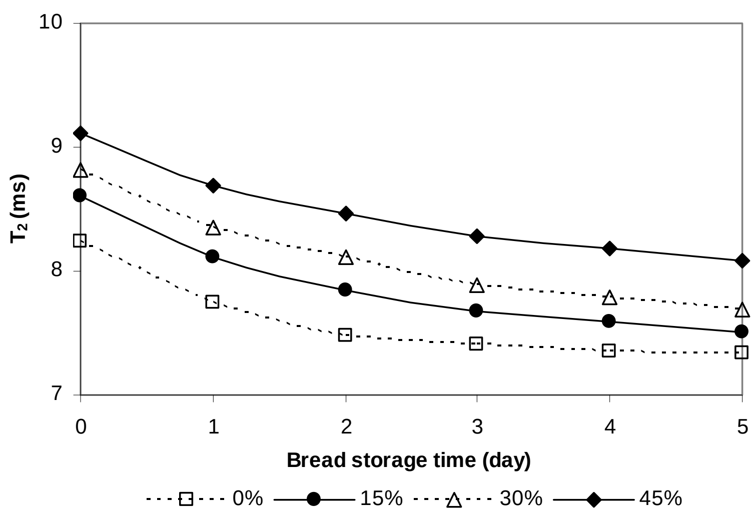

(c)

Figure 5.6. Water mobility on bread staling measured by Transverse relaxation time (T_2) for bread of 90 day frozen storage dough ; (a) T_2 of 65% water content, (b) T_2 of 60% water content, and (c) T_2 of 55% water content.

CHAPTER 6

SUMMARY AND CONCLUSION

The purpose of this research was to characterize the effects of processing conditions and waxy wheat flour on dough and bread to improve the quality of frozen bread dough and bread. First, freezing processing factors such as freezing rate, frozen storage temperature and storage time were investigated to understand its combined effects on dough quality and bread quality and to optimize better processing conditions by measuring dough quality, yeast activity, and bread quality and staling. Secondly, partial addition of waxy what flour (WWF) was investigated to understand its combined effects with water content on fresh and frozen dough. WWF was substituted with common flour and water was used at the ratio of 55, 60, and 65% on flour basis. The combined effects of WWF and water content investigated to characterize the combination of different WWF and water content, and to optimize the better combination through the measurements of dough quality, yeast activity, and bread quality.

The quality of yeasted frozen dough and bread made from frozen dough was influenced by processing parameters. Yeasted bread dough was frozen using four different freezing rates, then stored at -10, -20, -30, or -35°C for 30, 60, 90, and 180 days. The properties of the resulting yeasted frozen doughs were measured using nuclear magnetic resonance techniques (NMR), cryo-scanning electron microscopy (SEM), and texture analysis. Specifically, bread staling, a physical property of the bread, was measured using NMR, texture analysis and color analysis. The yeast activity of dough was assessed by measurements of gas production. Our results indicate that dough strength diminishes with time as well as with increasing storage temperature. Cryo-SEM demonstrated that frozen dough stored at -30 and -35°C displayed the least damage to dough structure. NMR studies showed that frozen dough at lower storage temperatures had lower T2 values (9-10 ms). However, dough frozen at freezing rate 2 and stored at higher temperatures (-20°C) displayed higher yeast activity. Baking tests showed that the loaf volume and weight gradually decreased with storage time, and that bread made from dough stored at -20°C resulted in the highest loaf volume. Breads produced from -30 and -35°C stored dough displayed less change in the texture profile during storage as well as less change in T_2 values. This research demonstrated that for better quality of dough and slower bread staling, fast freezing rate and lower storage temperature (-30, and -35°C) showed better quality of dough and slower bread staling. However, slower freezing (freezing rate 2) and higher frozen storage temperature (-20°C) showed higher loaf volume and more tender texture.

The quality of bread made from frozen dough is diminished by changes that occur during the freezing process. Recent studies have shown that new varieties of waxy wheat flour (WWF), bred to contain higher levels of amylopectin, offer unique properties for the production of baked products. In this study, the effects of levels of WWF and water on the quality of frozen dough and bread were investigated. Yeasted bread dough was made using four levels of WWF (0, 15, 30, and 45%) and three levels of water (55, 60, and 65%) and stored frozen for up to 90 days. Doughs with different treatments were frozen at the freezing rate of 0.37 °C/min and store at -20°C. Unfrozen and frozen dough stored for 1 day and 90 days (long-term) were measured to investigate the quality of dough and bread. The properties of the resulting yeasted frozen doughs were measured using nuclear magnetic resonance techniques (NMR), and texture analysis. The yeast activity of dough was assessed by measurements of gas production. Physical property of the bread was measured using NMR, texture analysis, loaf volume and weight, and curst color analysis. Changes in moisture distribution and polymer mobility were measured by NMR.

Stickiness of dough increased with higher content of WWF. Dough with higher content of WWF and 60% level of water added showed were more extensible. NMR studies showed that frozen dough with higher WWF content had lower T2 values (9-10 ms). Bread made from 15%

and 30% WWF had higher specific volume. The results of T_2 value change ratio and firmness showed that bread with WWF content had decreased rates of staling. Dough and bread made from the combination of 15% or 30% waxy wheat flour and 60% water content had more even distribution of color. This research demonstrated that WWF substitution showed better dough quality and bread in fresh dough. In freezing and frozen storage, WWF substitution showed better conserved quality of dough and bread quality from freezing damage. With 15% addition of WWF, it had highest bread volume and slower bread staling than bread made from dough of 0% WWF. This means specific level of WWF addition may produces higher volume, good color distribution, and reduced staling.