

## ABSTRACT

JASROTIA, ASWINI KUMAR SINGH. The Effects of Functional Ingredients and Frozen Storage on Wheat Dough and Bread Quality Studied Using Time-Domain Nuclear Magnetic Resonance, Dynamic Rheology, and Environmental Scanning Electron Microscopy. (Under the direction of Dr. K. P. Sandeep.)

Freezing dough after its preparation and storing it under frozen conditions until it is baked and consumed, is essential for commercial competitiveness, quality, and safety of baked products produced for retail, food service, and in-store bakeries. However, the quality of the resulting bread will not be the same as that produced with fresh dough. Functional ingredients may be added and processing parameters may be modified to produce a bread with quality that is close to that produced from fresh dough. Thus, this study investigated the effect of functional ingredients — gum arabic (GA), calcium stearoyl-2-lactylate (CSL), and diacetyl-tartaric acid ester of monoglycerides (DATEM) — and processing parameters — cooling rates (still air cooling: CR0 and forced air cooling: CR1) and frozen storage time (0, 8, 16, and 26 weeks) on the quality of wheat dough and bread. A full-factorial design of experiments was conducted with two levels for each functional ingredient (eight recipes), two cooling rates, and four frozen storage times. The quality attributes studied for wheat dough were based on the mobility of water, microstructure of frozen dough, and viscoelastic properties of frozen-thawed dough. The quality parameters studied for the bread were baked specific volume (BSV) and parameters that were obtained using textural analysis of the bread crumb and crust.

The mobility of water in frozen-thawed dough was studied using nuclear magnetic resonance (NMR) by obtaining spin-lattice relaxation times ( $T_{11}$  and  $T_{12}$ ) and spin-spin relaxation times ( $T_{21}$  and  $T_{22}$ ) for the frozen-thawed samples. Pulsed field gradient nuclear magnetic resonance (PFG-NMR) was used to determine the self-diffusion coefficient ( $D$ ) of water in the frozen-thawed dough. Environmental scanning electron microscope (ESEM) was used to determine the dough microstructure in frozen state ( $-10$  °C) and at a relative humidity of 100 %. Textural profile analysis (TPA) was performed to determine quality attributes such as cohesiveness, gumminess, and chewiness of the crumb of the bread. Puncture test was used to determine the crust firmness and modulus of elasticity of the crust.

CSL had no significant effect, whereas, GA and DATEM had significant effects on reducing the relaxation time values in frozen-thawed dough. Reduced relaxation time may be attributed to increased binding of water molecules to gluten matrix and starch granules. Longer frozen storage times resulted in low BSV and therefore, deteriorated the quality of the final baked product. DATEM, when added without GA or CSL, resulted in improved BSV with an increase in frozen storage time. DATEM also proved to be a significant ingredient in improving the softness of the crust of the baked product. However, gumminess and chewiness improved only with a specific combination of DATEM, CSL, and GA. A correlation study between the quality parameters of the baked product and quality response variables (at nano- and micro-scales) associated with wheat dough and described using a statistical model, indicated that GA alone accounted for 35.8–55.5 % of variability in the quality attributes (BSV, cohesiveness, chewiness,

gumminess, compressive load at break, and modulus of elasticity of crust) of the baked product.

This study aids the understanding of interaction between emulsifiers/gums and processing parameters (cooling rates and frozen storage time). This may be used to improve the quality of a baked product made from frozen wheat dough. The results present the feasibility of determining an appropriate combination of functional ingredients and processing parameters required for optimizing the quality attributes for baked products made from frozen dough.

The Effects of Functional Ingredients and Frozen Storage on Wheat Dough and Bread  
Quality Studied Using Time-Domain Nuclear Magnetic Resonance,  
Dynamic Rheology, and Environmental  
Scanning Electron Microscopy

by  
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## DEDICATION

*Dedicated to...*

*My Mom, Dad, and Sisters*

*For Their Love and Sacrifices*

*And to My Beautiful Wife*

*For Her Love and Support*

## BIOGRAPHY

Aswini Kumar Singh Jasrotia was born to *Shri.* Santosh and *Smt.* Sudesh Jasrotia on April 30, 1979 in Mumbai, India. He attended Central Schools affiliated with Central Board of Secondary Education in the cities of Jamnagar and Visakhapatnam, India. He graduated from Indian Institute of Technology (IIT), Kharagpur in 2001 with a Bachelor of Technology (Honors) degree in Agricultural and Food Engineering. He was awarded the Institute Silver Medal at IIT, Kharagpur for his first place in academics during undergraduate studies in the department of Agricultural and Food Engineering. After his graduation, he worked with Infosys Technologies Limited, India as a Software Engineer, where he placed first in company's software training program. During 2003 to 2004, Aswini earned Master of Science degree in the Department of Food Science at North Carolina State University under the direction of Dr. Josip Simunovic and Dr. K. P. Sandeep. His research work for the Master of Science program focused on aseptic processing of low-acid multiphase foods. After completion of his MS degree, he worked as a Quality Assurance Manager with Home Bistro Foods Inc., Plattsburgh, NY for one and a half years. Aswini began his Ph.D. program in the fall of 2006 under the direction of Dr. K. P. Sandeep. His Ph.D. research focuses on studying the effects of functional ingredients and frozen storage on quality of wheat dough and bread. He worked as a

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# Chapter 1

## Introduction

### 1.1 Introduction

The production of cereals and baked goods has undergone transformation in the last few decades as a result of consumer demand for diversity of baked products, better product quality and shelf life, healthy ingredients, reduction in industrial labor, and cost of resources (Best 1995). Baked goods are highly perishable and their quality declines rapidly within a few hours of being taken from the oven (Stauffer 1993). One of the challenges faced by the bakery industry is to increase the shelf life (maintaining quality) of baked products after they are taken from the oven. Shelf life improvements for baked products are required due to time involved in transportation — from centralized bakeries (producing freshly baked products) and distribution centers to retail or food service — and due to the time during which baked products remain on the shelf of a retail or food service store before they are consumed. One of the most studied chemical and physical processes that occurs during the aging of bread, leading to deterioration in its quality, is staling. Staling significantly affects the quality of baked products and therefore reduces their shelf life. Staling is associated with increase in firmness of crumb, decrease in crispness of crust, and loss of desired flavors in bread, leading to a

stale flavor (Schiraldi and Fessas 2001). Therefore, minimizing staling (after baking and before the product is consumed) is important for improving the shelf life of baked products. One of the approaches is to reduce the time between baking the product and when it is purchased by the consumers at a retail or food service store. This means manufacturing of dough may take place at a retail or food service store to minimize staling and to produce a product which may be considered ‘freshly baked’.

However, in recent decades, due to high competitiveness in the bakery industry — which is aggressively pursuing cost efficiencies in face of society’s evolving demographic and economic constraints — there has been a centralization of dough manufacturing facilities and retail distribution for dough (Best 1995). In-addition, consumers prefer freshly baked products and seek ethnic, health-promoting, and non-allergenic products (such as gluten-free bread) with a high level of ‘freshness’ (‘freshly baked’) and organoleptic qualities (LeBail and Goff 2008). Therefore, in recent decades, dough has been produced in centralized manufacturing facilities, refrigerated or frozen, and shipped to retail, food service, and in-store bakeries for final baked product manufacturing (Frank 2008). Refrigeration increases the shelf life of dough by facilitating its availability at retail, food service, and in-store bakeries — that may be located far from dough manufacturing facility — for immediate baking needs. Increase in shelf life of refrigerated dough occurs due to delay in the proofing of dough, resulting from inactivation of yeast during refrigeration, and by retarding the growth of spoilage

microorganisms. However, refrigerated dough has a short shelf-life, and is not suitable for long distance transportation and distribution. Increase in the shelf life of dough is critical to the bakery industry due to logistical and economical reasons. Therefore, freezing the dough has been sought out as an alternative. Freezing of dough has been studied in the last two decades to improve the shelf-life of dough and to achieve baked products of quality close to that produced from fresh dough. Freezing of dough has shown a great potential in overcoming the shelf life and quality challenges in the bakery industry and guarantees a steady growth potential (Rosell and Gómez 2007). The production of frozen dough has increased in the last decade due to growth of in-store bakeries and demand for quick and convenient baked products. The frozen dough segment is considered the third largest segment in the baking industry in developed countries and holds a great potential for growth in retail grocery stores and food service due to trends in more meals being prepared outside of home (Schroeder 1999; Asghar and others 2009).

Fermented dough consists of an aqueous continuous medium of gluten and starch containing the dispersed gas phase (Eliasson and Larsson 1993). Frozen and refrigerated dough varies widely in terms of their physiological characteristics and final baked product quality when compared to those for fresh dough. Frozen dough is used in more than 50 % of in-store supermarket bakeries as well as by retail customers. The major shortcoming of frozen dough is the short shelf life of six to eight weeks (Berglund



and others 1991). With extended frozen storage time, the quality of bread made from frozen dough decreases and the proof times of dough increases. This increase in proof time is attributed to weakening of three dimensional protein networks responsible for retention of gas in the dough. Another factor attributed for longer proofing times is the destruction of yeast during freezing, which results in decreased gas production. Freezing and thawing increases the number of disrupted yeast cells in the dough and alters the protein network (Varriano-Marston and others 1980). This directly affects the quality of the baked product (lower baked specific volumes). Water in dough is bound with different components of the dough and understanding this is important in studying the quality of dough during frozen storage.

The minimum ingredient requirements for making a loaf of bread are — flour, yeast, and water. The proportion of the ingredients varies, but typical values are 100:2:65 (ratio of flour: yeast: water). Water is an indispensable and important component of dough and baked products. Water interacts with gluten and starch to form the so-called “bicontinuous network” — the continuous water-containing gluten phase that is inter-penetrated by the continuous “free” water-starch mixture phase. Recently, a lot of focus has been given to developing yeast cells which have high tolerance to freezing and frozen storage. Commercial yeasts show resistance to freezing, but when mixed with flour, they become more sensitive to temperature changes and lose resistance to freezing. This may be attributed to differences in

stability of yeast cells in latent and metabolically active states. Earlier, minimizing yeast activation and delaying the fermentation process before freezing, was considered essential. However, later studies have shown that pre-fermentation before freezing increases the volume of baked bread when freeze tolerant yeasts are used (Rosell and Gómez 2007). Bread-making is affected by the selection of various parameters such as formulation (flour, yeast, and additives — hydrocolloids, emulsifiers, enzymes, and shortenings), processing (cooling rates, frozen storage time, frozen storage temperature), and other ingredients (gluten, egg, sugars, salt, and milk derivatives). Freezing and thawing of dough causes deterioration in the quality of the final baked product. Flour and dough quality are important parameters in determining the quality of a baked product. Wheat varieties with high gluten content and high values of dough deformation energy (measured using alveograph or extensograph) results in better quality of baked products. Therefore, the protein quality and gluten strength are more important than the total amount of proteins present in dough (Rosell and Gómez 2007).

Ultrastructure features of the frozen dough using low-temperature scanning electron microscopy (LT-SEM) have been studied in various research works. It was observed that formation of ice-crystals in non-fermented dough stored for 24 weeks leads to the disruption of gluten matrix, rendering a network separated from starch granules (Berglund and others 1991). The study indicated that water separated into pools, after thawing of frozen dough, resulting in less free water available for

distribution throughout the dough. As frozen storage time increases, a significant increase in proofing time and decrease in loaf volume were observed for all the frozen-thawed dough. Effects of prolonged frozen storage on starch, rheological, and baking characteristics of dough have also been investigated. Rheological studies have shown a decrease in  $G'$  (storage or elastic modulus representing elasticity in dough), due to freezing and thawing, which could be attributed to a loss in polymer cross-bonding. Gelatinization properties of starches isolated from fresh and frozen-thawed dough have been examined using differential scanning calorimetry (DSC). All cultivars in a study (Lu and Grant 1999) showed a significant increase in  $\Delta H$  with increased frozen storage time, indicating migration of water and ice crystallization. Differential thermal analysis (DTA) and DSC have been used to measure un-freezable or bound water in dough. The shortcoming of these methods is that during the heating of samples, some physical and chemical changes such as protein denaturation and starch gelatinization may occur, and thus, the method may not reflect the true binding characteristics of water present in dough (Leung and others 1976; Leung and others 1979).

Water activity ( $a_w$ ) is an important factor in determining the amount of water available for microbial growth and chemical reactions. The measurement of  $a_w$  presumes that the partial vapor pressure above the food material is the same as that of water within the food system. This assumption is true only for very dilute solutions where the diffusion rates of water molecules are high compared to the time scale of

thermodynamic measurements. Since most foods are very complex in terms of their compositions, they are definitely not dilute solutions, and do not present a state of equilibrium during processing and storage. In-addition, when solutes are used to lower  $a_w$ , the response of the system in terms of reaction rates and stability is different. Therefore, both thermodynamics and kinetic factors (mobility) must be taken into account when attempting to explain product stability.

Mobility of water within a food product is an important factor in determining the availability of water which represents the “free” water inside the food. These mobile water molecules easily diffuse within the system and are available for participation in various microbial and chemical reactions within the food material. Since water activity may not represent the true picture of available or free water within a complex food system, a non-invasive method (which can truly represent the picture of free water within a complex food system) is needed. Time-domain nuclear magnetic resonance (TD-NMR) or low-field nuclear magnetic resonance is such a technique which is cost-effective as compared with high-field NMR and involves minimal and non-invasive sample preparation. Due to these properties, TD-NMR holds a great potential in serving as a quality control tool for dough formulation and determination of changes in dough as a result of freezing.

Literature review indicates that there is a substantial difference between the qualities of final baked product made using fresh dough as compared to that made

using frozen-thawed dough. Understanding the effects of dough formulation and processing parameters (such as mixing rate, cooling rate, and frozen storage time) is important in determination of quality attributes (such as baked specific volume or BSV) of the final baked product produced using frozen-thawed dough. Therefore, there is great interest in the bakery industry to understand and quantify the effects of freezing on quality of dough and to improve the quality of baked product produced using frozen-thawed dough. There is a great potential of using a combination of tools and techniques such as rheology, environmental scanning electron microscope (ESEM), and TD-NMR to characterize the micro-structure, physiochemical properties, and redistribution of water in dough during frozen storage and to quantify the quality parameters of the baked product.

However, a comprehensive study on frozen dough involving correlation of dough formulation and processing parameters, rheological properties, microstructure, and NMR relaxometric parameters to the quality parameters of baked products has not been conducted yet. Therefore, this study performed such a comprehensive research work of frozen dough that aids the understanding of dough formulation and processing parameters that were important in achieving quality attributes of baked product, made from frozen-thawed dough, to be close to that made using fresh dough.

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## Chapter 2

### Literature Review

#### 2.1 Bread baking

Bread is probably the oldest “processed” food. Bread making process involves converting wheat flour and other ingredients into a light, aerated, and palatable food. This involves the formation of a cohesive mass of dough, once the flour is hydrated and mixed. The subtle and sometimes complex interplay between raw material composition and processing affects the quality of the baked product in both artisanal and industrial environments. Dough processing generally involves kneading and molding followed by a final baking step. During the kneading step, flour, water, yeast, shortening, salt, and other ingredients are mixed to form dough. In dough, the gluten strands form a biopolymer network in which starch particles and pentosans are dispersed (Bot and de Bruijne 2003).

The cohesive mass, formed after dough processing, also called by bakers as “gluten”, has the ability to trap gases during resting (fermentation and proofing) and baking. This allows the mass to expand, become softer, lighter and more palatable food after the baking process (Cauvain 2001). The ability of proteins present in wheat — to transform wheat flour into glutinous mass by input of mechanical energy during



kneading with water — is limited to wheat and few other cereals seeds (Cauvain 2007). Gluten produced during dough mixing and kneading has the important property of forming elastic network when moistened and worked by mechanical action. This happens by formation of a three-dimensional linkage structure between protein molecules. However, gluten films weaken and then break down under excessive mechanical action if over mixing of dough is conducted. Heating this gluten structure leads to coagulation of gluten and formation of a semi-rigid structure. However, if this gluten has been expanded by gas prior to being heated, then a fairly rigid structure of cellular nature is formed, such as that present inside a loaf of bread. Wheat starch does not form elastic films like gluten. The moistened starch on heating forms a paste and stiffens as it gelatinizes. Therefore, both gluten and starch contribute to the semi-rigid structures during baking of dough. Hard wheat flour or strong flour (high protein content) forms more gluten leading to high stretching strength before tearing, and is therefore chosen to give bread expandability and light density. Soft wheat flour contains less protein, when compared with hard wheat flour, and its gluten film tears more readily, yielding structures that are less chewy and tender. These kinds of flours are preferred for making cakes where more tender and friable structures are desired (Potter and Hotchkiss 1998).

## 2.2 Wheat components and phases in dough

Total lipid content of wheat flour is about 2.5 % w/w. Lipids in wheat flour can be divided into two groups: polar lipids and non-polar lipids. Polar lipids interact with water and form aqueous phases, whereas, non-polar lipids do not form aqueous phases with water (Eliasson and Larsson 1993). Non polar lipids are dominated by triglycerides — mainly occurring in embryo (germ) and as emulsion droplets (spherosomes) in the endosperm of wheat kernel. Polar lipids are dominated by phospholipids and glyco- or galacto-lipids and originate from cell membranes. It has been found that the volume, mixing behavior, and rheological properties of dough at the end of proofing are not greatly influenced by the addition of wheat lipids. However, the loaf volume is influenced by the addition of wheat lipids. Thus, lipids significantly influence the baking of dough. For polar lipids, a minimum loaf volume is achieved quickly and then the loaf volume increases with addition of polar lipids. This can be compared with the increase in the loaf volume with addition of shortenings. With addition of non-polar lipids, the loaf volume decreases. This has been attributed to presence of free fatty acids in non-polar lipids, although, it contains triglycerides.

The protein content in wheat flour varies between 7–20 % w/w (Eliasson and Larsson 1993). It has been shown that increasing protein content of wheat flour increases the loaf volume, thus proving to be an important factor in baking. 90 % of the variation in loaf volume for a single wheat variety was ascribed to the protein content.

However, not only the quantity of protein, but its quality is a significant factor for loaf volume. Wheat proteins exhibit striking viscoelastic properties and gas-holding capacity, which leads to increase in loaf volume, when compared with other cereal proteins. Five classes of proteins can be found in wheat protein: albumins (soluble in water), globulins (soluble in salt solutions), gliadins (soluble in aqueous ethanol), glutenins (soluble, or rather dispersible in dilute acid or alkali), and an insoluble residue. When dough is washed in excess water (or in dilute salt solutions), gluten which is formed as cohesive mass, remains in the dough whereas starch and soluble components are washed away (Eliasson and Larsson 1993). This fraction which remains after dough is washed in water to remove soluble components and starch is a mixture of proteins, lipids, and carbohydrates (pentosans or glycoproteins). Albumins and globulins are referred to as *soluble proteins* and gliadins and glutenins are often described as *gluten proteins* or *wheat storage proteins*.

Starch constitutes the major component of wheat flour varying between 74–90 % on dry basis, depending on the extraction rate. Starch is present as particles in special cells known as amyloplasts (a kind of leucoplast in plants). These starch particles are also known as starch granules and they are the means of storing food in the plants. The size of a starch granule is in the range of 1–30  $\mu\text{m}$  and the size distribution of starch granules is bimodal for wheat, rye, and barley.

Wheat flour has a pale yellow tint after milling. During the aging process the wheat flour changes to white color and improvements in the baking quality is observed. Bleaching agents such as benzoyl peroxide  $[(C_6H_5CO)_2O_2]$ , chlorine, and oxides of nitrogen are added at milling which help in oxidation of carotenoid pigments and reduces conjugated double bonds — thus aiding in decolorizing the wheat flour. Oxidizing agents such as potassium bromate ( $KBrO_3$ ) are added at time of milling which improves the loaf volume, loaf symmetry, and crumb texture of bread. These oxidizing agents also oxidizes sulphhydryl groups (-SH) to disulphide bonds (-S-S-) thereby increasing intermolecular bonding in gluten (Lindsay 1996). Ammonium salts such as  $(NH_4)_2SO_4$  and  $(NH_4)_3PO_4$  are added in wheat flour as a source of nitrogen for yeast growth during dough leavening. Inorganic salts such as  $CaSO_4$  and  $CaHPO_4$  are used to assist in maintaining the desired pH when alkaline water supplies are used during mixing (Lindsay 1996). In a study (Graveland and others 1978), the presence of thiol or sulphhydryl (-SH) groups and disulphide bonds (-S-S-) in wheat flour and dough was tested. It was found that that wheat flour contains 5–7  $\mu\text{mol}$  thiol groups and 11–18  $\mu\text{mol}$  disulphide bonds per gram flour. Jones and others (1974) also conducted a similar study earlier to estimate rheologically important thiol and disulphide groups in dough. Sebesic and others (1981) studied the disulphide-sulphhydryl interchange studies of wheat flour dough. In another study (Watanabe and others 1998) the possibility of protein disulphide isomerase (PDI) duplicating the effect of potassium bromate was

evaluated using dough rheology assessed by fundamental and empirical tests. PDI is an enzyme which has been shown in vitro to catalyze disulphide bond interchange in several “scrambled” proteins. “Scrambled” proteins are prepared by reducing a disulphide bonded protein and allowing it to reoxidize under denaturing conditions, thus causing it to form non-native disulphide bonds. In another research work (Chen and Schofield 1996), the effects of dough mixing and oxidizing improvers on freely reduced and freely oxidized glutathione and protein-glutathione disulphides of wheat flour were studied.

Eliasson and Larsson (1993) described flour dough as a bicontinuous (consisting of two continuous aqueous phases) and phase-separated. The two phases are: water-swelled protein phase (gluten) and the liquid phase (consisting of dispersed starch granules and solubles). The phase separation in the dough is illustrated by the fact that the starch can easily be removed from wheat flour dough by washing, leaving behind a gluten gel. The importance of liquid phase in dough system was illustrated by MacRitchie (1976). Ultracentrifugation studies showed that dough separated into a liquid phase and a solid phase. Conductivity studies showed that the liquid phase (consisting of starch granules and solubles) is continuous and not dispersed.

In a later work (Larsson and Eliasson 1996a), a new model of phase separation for wheat dough, based on ultracentrifugation studies was suggested. In this model, five different phases were proposed: liquid, gel, gluten, starch, and unseparated dough. The

relative amounts of each phase depended on the water content of the dough. In this study, the relationship between phase separation and stress-relaxations behavior for six wheat cultivars at water contents ranging from 38 % w/w to 50 % w/w was studied. Water absorptions for different cultivar of wheat determined using farinograph measurements were attributed to the amount of damaged starch as well as to the protein content. In the same study it was observed that high-damaged starch content cultivar (Prairie) had high water absorption. High water absorption for high protein content cultivar (Sport) was attributed to its higher protein content. The study also observed that the dough containing high water content had a fully developed gluten phase, which allowed remaining water to form a second aqueous phase with starch, thus, resulting in better phase separation when compared with dough containing low water content.

In another study (Larsson and Eliasson 1996b), it was shown that the separation properties of dough into the two aqueous phases (gluten and liquid) depends on the cultivar, mixing time, and additives such as ascorbic acid and lecithin. Increase in mixing time caused an approximate linear increase for incorporation of water into the gluten phase. Adding ascorbic acid to the dough, made using defatted flour, showed an increased separation of the two aqueous phases. Lecithin on the other hand impaired separation which aided in studying the two phases without disturbing the composite system.

To obtain a good product quality after baking, the gluten network should be well developed and hydrated, and the starch particles should be homogeneously distributed. The extent to which this quality can be achieved depends on dough recipe (flour quality and water content) and processing parameters. In particular, kneading temperature, kneader type, energy input, and kneading time are important (Marston and Wannan 1976).

### **2.3 Empirical instruments used to measure quality characteristics of flour and dough**

Empirical techniques (subjective tests) usually depend on the kind of apparatus or instrument used to measure a particular characteristic of a food material. On the other hand, fundamental properties (objective tests) of a food material are independent of the kind of instrument used to measure them. Quality characteristics of flour or dough are measured using various empirical instruments. These instruments are alveograph (also called Chopin extensograph), extensograph, farinograph, and mixograph. Alveograph and extensograph measures stress-strain relationship of dough after it has been mixed. In an alveograph, a bubble is blown into a sheeted piece of dough and the resulting overpressure (P) versus distance at rupture (L) is plotted (graph known as alveogram). This action resembles the inflation action in dough during fermentation and early stages of baking when gases are produced (Sahin and Sumnu 2006). Dough deformation

energy is determined by the area under P versus L curve and is associated with quality of the dough. Similarly, extensograph measures and plots a graph (known as extensogram) between the resistance force to stretching (B) and extensibility (C) of dough. Extensograph measures extension properties of dough in one direction. For use in alveograph or extensograph, a dough sample is prepared using flour, distilled water, and salt in a farinograph, to ensure reproducibility and initial consistency during preparation of dough. After a certain proofing time, the dough is stretched in an extensograph until rupture occurs. Extensogram is used to determine the resistance to extensibility ratio (B/C), energy required for extension, and maximum exerted force. Alveograph and extensograph has been widely used to determine the consistency of flour quality in milling and baking industry (Brabender GmbH & Co. KG 2006b, a). P or B is associated with elastic component and L or C is associated with viscous component of the dough. Protein quality and gluten strength (measured by dough deformation energy using an alveograph) in wheat are more important than the total amount of proteins present in dough in determining the quality characteristics of baked product (Rosell and Gómez 2007).

Farinograph and mixograph are instruments in which a flour and water is filled into a heated measuring mixer, where it is subjected to a defined mechanical stress by rotating mixer blades. In a farinograph, there is a kneading type of mixing using Z-shaped blades that rotate at different speeds in opposite directions. In a mixograph, a



vertical pin, lowered into the dough, rotates in a planetary motion (Sahin and Sumnu 2006). The resistance of dough against the blades or pin — which depends on the dough viscosity — is measured as torque and recorded and plotted as a function of time. This graph is known as farinogram (when produced using a farinograph) or mixogram (when produced using a mixograph). It represents the quality characteristics of flour such as water absorption, development time, dough stability, and degree of softening. Although, alveograph, extensograph, farinograph, and mixograph are user-friendly instruments, the measurements are empirical and these are considered as basic instruments for rheological measurements (Janssen and others 1996). An amylograph is a torsion viscometer used to determine gelatinization characteristics of various starches by recording changes in viscosity of starch as temperature is raised at a constant rate.

## **2.4 Freezing and its effects on quality and safety of foods**

Freezing is one of the oldest methods of food preservation and involves the decrease of temperature to a point somewhere below the temperature where ice crystals forms inside the food. Foods spoil due to bacterial spoilage, oxidation, or enzymatic autolysis. Freezing preserves food by separating water from food and immobilizing it by converting it to ice (Reid 1993; Nesvadba 2009). The separation of water from food during freezing, results in less available water inside the food which increases the

electrolyte concentration leading to a decrease in water activity ( $< 0.7$  in most cases) inside the food. The decreased water activity prolongs the shelf life of frozen food by decreasing the available water for bacterial growth and various reactions (Nesvadba 2009). Freezing also decreases the temperature of food product, which restricts the growth of microorganisms of public health concern.

Freezing and thawing process plays a vital role in determination of the quality of food products in term of its texture, taste and appearance. Some of the important processing operations which include freezing of foods are for food preservation, freeze drying, and freeze concentration. In all of these operations, ice crystal morphology plays an important role in the textural and physical properties of frozen and frozen-thawed foods. For example, smaller ice crystals are preferred in ice creams since large crystals would result in icy mixture. Ice morphology affects the rate of sublimation in freeze drying and the efficiency of ice crystal separation from the concentrated solution in freeze concentration. Stabilizers (hydrocolloids such as locust bean gum, guar gum, carrageenan, carboxymethyl cellulose, sodium alginate, and xanthan gum integrated with emulsifiers) are added in ice cream to inhibit or retard the rate of ice crystal growth or recrystallization during storage. The specific mechanism leading to actions by stabilizers is still under study. However, stabilizer actions are attributed to reduction in the mobility of water, as water is entrapped by emulsifier's entangled network structures in the serum phase (Goff 2004). In recent studies the use of nucleation

agents, antifreeze proteins, ultrasound, and pressure freezing methods, known by the generic name of “freezing assisting techniques” have been proposed to control nucleation and ice morphology.

Three main mechanisms for damage to food during freezing are: 1) mechanical damage to food structure due to ice crystal growth, 2) cross-linking of proteins due to decrease in water availability and increase in electrolyte concentration leading to aggregation and denaturation of actomyosin (in fish and meat), and 3) limited re-absorption of water on thawing. The last two mechanisms are the main cause of the deterioration of quality in frozen foods (Nesvadba 2009). Slower cooling rates results in large ice crystals growth in extracellular locations of plant and animal tissues. Faster cooling rate results in formation of uniform small ice crystals throughout the tissues. The large crystals formation during slow cooling results in food products those are inferior in quality to products frozen using faster cooling rates. In-addition, frozen storage at low and constant subfreezing temperatures is important for quality of the frozen food. Any significant increase of temperature during frozen storage and temperature fluctuations are very detrimental to quality of frozen foods and increases drip loss. Plant tissues are affected by slow freezing due to disruption of metabolic systems, dislocation of enzymes, and cell wall/membrane damage. Damage of cell wall and its cell membrane results in loss of turgor pressure (turgidity) within the cell. The damage results in a permanent transfer of water from intracellular tissues to

extracellular tissues and is irreversible during thawing of foods. Recrystallization — the enlargement of large ice crystals at the expense of smaller ones during frozen storage is one of the most important phenomena leading to loss of quality in foods (Petzold and Aguilera 2009). Dehydrofreezing is a technology to preserve fruits and vegetables by removing part of the water (such as free water) by drying prior to freezing. The partial drying lowers the refrigeration load; lowers transport cost due to reduced weight and volume, and may result in reduced drip loss after thawing. Small foods such as diced vegetable and berries can be frozen as distinct units using individually quick frozen (IQF) technology. IQF is possible when there is no barrier to heat transfer between the food and cooling medium and freezing process is not limited by internal heat transfer due to the small size of the food that is been frozen (Petzold and Aguilera 2009).

Freezing is more complex phenomena in foods — which can be considered very concentrated solutions — when compared with pure water. Three main steps involved in freezing of foods are shown in Figure 2-1.

#### **2.4.1 Supercooling or undercooling**

During freezing, thermodynamics describe the direction and kinetics defines the rate of equilibrium. These two forces compete during supercooling, (below the initial freezing temperature) which is a metastable state of liquid, before nucleation of first crystal can take place. A significant amount of energy barrier is overcome during supercooling,

before the first nucleation occurs. This process known as supercooling or undercooling provides the necessary submicroscopic water aggregates required for nucleation. Pure water (without impurities) can be supercooled to around  $-40\text{ }^{\circ}\text{C}$  where homogeneous ice nucleation takes place. In foods due to heterogeneous ice nucleation, the degree of supercooling is much smaller. Supercooling is one of the mechanisms by which plants and animals cope with sub-zero temperatures or minimize tissue damage that can occur due to ice crystal growth (Nesvadba 2009).

#### **2.4.2 Ice nucleation and ice crystal growth**

Nucleation sites such as cell surfaces acts as nuclei (seeds) in the *heterogeneous nucleation* process in food systems. Such a nucleation site is enhanced if it resembles the structure of water. In pure water, the nucleation sites due to cell surfaces and other impurities are absent and therefore *homogeneous nucleation* takes place due to random fluctuations of water molecules. This happens since water molecules assumes configuration of ice momentarily during these random fluctuations and act as seeds. Nuclei come into existence when critical size at which growth of the nucleus results in reduction in surface energy  $\sigma$  as compared with the increase in Gibbs free energy  $\gamma$  (due to increase in volume). This occurs for a spherical particle when  $\sigma r^2 < (G_v)r^3$  (Nesvadba 2009), where  $r$  is the radius of the nucleus.

### **2.4.3 Recrystallization**

Recrystallization is the phenomena in which the ice crystals undergo relatively unstable changes in size, shape, and number during frozen storage. Due to Oswald ripening, large crystals grown at the expense of smaller ice crystals, reducing the overall surface area of the frozen ice crystals.

## **2.5 Effect of frozen storage on dough quality for baking**

In recent decades, due to high competitiveness in the bakery industry — which is aggressively pursuing for cost efficiencies in face of society's evolving demographic and economic constraints — there has been centralization of retail distribution (Best 1995). This has lead to centralization of dough manufacturing facilities which prepare the dough, freezes it, and ships the frozen dough to the retail, food service, and in-store bakeries for final baked product manufacturing. Frozen bread dough is used in more than 50 % of in-store supermarket bakeries as well as by retail customers (Berglund and others 1991). With centralized manufacturing facilities producing huge amounts of frozen dough, there have been new requirements regarding raw materials, machinery, package and transport to produce a consistent and improved quality product. This has lead to a great interest in studies related to improving quality of frozen dough in last decade (Rosell and Gómez 2007).

The major shortcoming of the frozen dough is the short shelf life of six to eight weeks (Berglund and others 1991). With extended frozen storage, proof times of frozen bread dough increases. Another major concern is the loss of quality that results from mishandling in transportation and storage. The increase in proof time is attributed to the weakening of the three dimensional protein network responsible for gas retention in dough. Another factor attributed for longer proofing times is the destruction of yeast during freezing resulting in decreased gas production. Therefore, freezing and thawing increases the number of disrupted yeast cells in the dough and alters the protein network (Varrianomarston and others 1980).

Weakening of dough during frozen storage and successive freeze-thaw cycles has been attributed to 1) the release of reducing substances from yeast during freezing that may reduce gluten proteins, 2) water redistribution proved by a modification in the water-binding capacity of dough constituents, and 3) a reduction of gluten cross-linking (Ribotta and others 2001). These factors in frozen dough lead to longer fermentation or proofing times, lower loaf volumes in baked product, and undesired changes in textural properties of final baked product.

The quality of a bread product baked from a frozen dough decreases with increasing frozen storage time (Bot 2003). Storage of dough at low temperatures ( $-20^{\circ}\text{C}$ ) has a considerable effect on the final quality of baked bread. The poor quality of baked bread made using dough stored at low temperatures is well reflected in the

lowered baked specific volumes (BSV). When stored at  $-20\text{ }^{\circ}\text{C}$ , water redistributes itself within the dough and alters the properties of gluten and starch. Centrifugation is one of the most direct techniques used to determine the amount of water retained by dough during frozen storage. The amount of water that can be centrifuged increases with the duration of storage (MacRitchie 1976; Räsänen and others 1997a).

Frozen storage time and cooling rate effects the ice crystal formation inside dough (Singh 1995; Inoue and Bushuk 1996). Effects of storage conditions and of temperature fluctuations, has been studied on the yeast activity and bread volume (Bail and others 1999). The study indicated the importance of temperature stability during storage on the baking performances of the bread. In another study (Neyreneuf and Delpuech 1993), it was shown that the maximum yeast activity is usually obtained with slow freezing rates. Bail and others (1996) proposed a freezing rate in their study which is supposed to give optimal yeast activity. Even with these studies, there has not been enough work performed on the combined effects of formulation, processing parameters (cooling rate, freezing temperatures, and frozen storage time), and additive ingredients on the baking performance of bread made with wheat flour. The effects on the molecular and microstructural changes that occur during industrial dough processing can be studied using the help of fundamental rheology, and scanning electron microscopy (SEM), differential scanning calorimetry (DSC), dynamic



mechanical thermal analysis (DMTA), and time-domain nuclear magnetic resonance or TD-NMR (Esselink and others 2003a).

## **2.6 Yeast viability, activity, and performance in frozen dough**

Yeast is a vital component in a dough system. Yeast causes the fermentation of sugars present in the dough system during the rise period, and produces the CO<sub>2</sub> necessary for leavening of dough to produce the soft texture found in bread. Yeast is greatly affected by the frozen storage of dough since frozen storage leads to the decrease in the yeast viability, and therefore, stops the fermentation of sugars during frozen storage. Yeast viability is a relative concept. It has been shown that yeast cells may be viable yet not active. The proof time typically increases with the frozen storage time. Methods to detect active yeast in wheat dough based on fluorescence microscopy have been developed (Autio and Mattila-Sandholm 1992). Many studies have been conducted to test the survival of yeast during frozen storage of dough, and many different strains of yeast have been tested for their viability, freeze tolerance, CO<sub>2</sub> production during leavening (gassing power), and freeze-thaw tolerance (Bruinsma and Giesenschlag 1984; Hino and others 1987; Holmes and Hoseney 1987; Autio and Sinda 1992; Gelinas and others 1994; Ribotta and others 2003; Pepe and others 2005). Most of the yeast used commercially and by consumers for home baking is the traditional baker's yeast *Saccharomyces cerevisiae* also known commercially as compressed yeast, "wet yeast", or

“fresh yeast”. The choice of yeast also effect the proofing time of frozen dough. In a study (Wolt and D'Appolonia 1984b) it was observed that proof times for active dry yeast and instant dry yeast were shorter than for compressed yeast, however, as frozen storage time increased the proof times of dry yeast exceeds that of compressed yeast. Active dry yeast is usually rehydrated in 43–46 °C water for 5–15 min and then added to the dough, whereas, instant yeast should be added directly to the dough without rehydration for maximal action (Bruinsma and Giesenschlag 1984).

Freezing and thawing processes decreases the dough resistance to extension measured by the extensograph and this reduction is greater in proofed dough than unyeasted doughs under frozen storage. This is attributed to the release of certain compounds such as glutathione by dead yeast cell during frozen storage which affects the disulphide bonding in dough (Wolt and D'Appolonia 1984a). However other studies using unyeasted dough have shown a decrease in consistency peak measured by farinograph and increase in extensograph extensibility during frozen storage, hence showing that the dough weakness observed upon freezing is not only due to effect of compounds released by dead yeast cell (Wolt and D'Appolonia 1984b; Rosell and Gómez 2007). Baker's yeast show enough freeze resistance, however, its freezing resistance decreases as the environment of the yeast cell is changed due to addition of flour. The yeast when added with flour is less stable to freezing and it might be attributed to the differences in stability of yeast cells in the latent and metabolically

active states. Many freeze tolerant strains of yeast have been studied to improve performance of frozen dough. Some of these strains are from *Saccharomyces* like *Saccharomyces fructuum*, and *Torulasporea delbrueckii* (Takasaki and Karasawa 1992; Hernandez-Lopez and others 2003; Hernandez-Lopez and others 2007; Rosell and Gómez 2007). Also identification of genes in yeast that are responsible for the tolerance of freezing, has opened a new area of research where mutagenesis may produce yeast capable of improved dough performance (Teunissen and others 2002; Panadero and others 2005).

Takasaki and Karasawa (1992) studied the survival of yeast in frozen dough in several stages in the baking process up to 10 weeks of frozen storage. *Torulasporea delbrueckii* was used as a freeze tolerant yeast and *Saccharomyces cerevisiae* as the freeze sensitive yeast. The study found that survival counts were constant in several steps of the bread making process before baking, in both frozen and non-frozen dough prepared using both varieties of yeasts. However, *Torulasporea delbrueckii* showed a decrease in the fermentative ability per yeast cell during longer storage and it had negative effect on the quality of bread for the storage period under three weeks.

Yeast is susceptible to freeze damage with the increase in fermentation time after mixing (Rosell and Gómez 2007). Lower temperatures and short fermentation or resting times after mixing, aid in delaying the start of yeast fermentation. Maintaining final mixing temperatures below 20 °C shows an improvement in gassing power and

overall loaf quality of the baked product. Gluten development is faster in absence of salt during mixing. In addition, simultaneously adding of salt and yeast during mixing increases the risk of osmotic shock to yeast and leads to its cell death. Therefore, the yeast is typically added at the middle of the mixing and the salt near the end of mixing (Wolt and D'Appolonia 1984a; Zounis and others 2002a; Rosell and Gómez 2007).

In a study (Oda and others 1986), many strains from *Saccharomyces* spp. were tested and compared with commercial baker's yeast for their freeze resistance. The study showed that these strains are capable of accumulating higher trehalose content than commercial baker's yeast cell which leads to their lessened freeze injury. This is attributed to the intracellular trehalose which stabilizes membrane lipids under anhydrobiotic conditions and protects many organisms from dehydration. In another study (Meric and others 1995) 4–5 % trehalose content at freezing appeared to be sufficient to prevent yeast from indirect chilling injury during frozen storage. Takano and others (2002) studied fermentation process for frozen dough using freeze sensitive and freeze tolerant yeasts (*Saccharomyces* spp.) and studied grain network structures of baked breads using magnetic resonance imaging (MRI). The study found that prefermentation (fermentation before freezing), punching and remolding, or reheating and molding increased loaf volume by 10–110 % for baked breads made using freeze-tolerant yeast, while loaf volume decreased by 70 % for bread made using freeze sensitive yeast. Wolt and D'Appolonia (1984a) studied the gassing power using a

pressuremeter according to AACC International (1962) method 22-13 and found that both gassing power and baking activity of the yeast decreased during frozen storage. In an another study (Meric and others 1995), gassing analyses was performed using a fermentometer adapted from the volumetric system of Burrows and Harrison (1959), and it was shown to be a reliable indicator of the activity due to cryo-resistance yeast. In another study (Zounis and others 2002a) a fermentograph was used to study the gassing capability of dough during proofing (after the dough was thawed from frozen storage).

It is evident from aforementioned research work that much of the work has been dedicated to studying the freeze tolerance of different yeast strains and there is no evidence to show that the same strain of yeast obtained from a single vendor varies significantly in its fermentation or gassing capabilities when used for studying effects of frozen storage on dough performance. Many studies (Wolt and D'Appolonia 1984a, b; Autio and Sinda 1992; Inoue and others 1995; Räsänen and others 1997a; Räsänen and others 1997b; Stokes and Donald 2000; Zounis and others 2002a; Zounis and others 2002b; Sharadanant and Khan 2003b, a, 2006; Dodić and others 2007) have used fresh commercial baker's yeast also known as compressed/block yeast within one week of its receipt. Many other studies (Holmes and Hosenev 1987; Chen and Schofield 1996; Sahlstrøm and others 1999; Kenny and others 2001; Esselink and others 2003a; Primo-Martin and others 2007; Kim and others 2008b) have used instant/active dry yeast

obtained periodically from single vendor. Therefore, as long as the yeast is obtained periodically from same vendor or used fresh from a sealed packet, there should not be significant variability in yeast leavening activity.

## **2.7 Environmental scanning electron microscope (ESEM) to study dough matrix during frozen storage**

Berglund and others (1991) used low-temperature scanning electron microscope (LT-SEM) to show that in frozen dough less water is associated with both the gluten and starch fractions, and that the water is instead concentrated into large patches of ice crystals. It was observed that formation of ice-crystals in non-fermented doughs stored for 24 weeks, leads to the disruption of gluten matrix rendering a network separated from starch granules. In their study it was indicated that after 24 weeks of frozen storage, water separated into pools causing less free water to be distributed throughout the dough. In other studies (Räsänen and others 1995; Räsänen and others 1997b), the effect of ice crystals on the stability of frozen prefermented dough was studied. It was shown that the pore structure of prefermented dough is extremely sensitive to formation of ice crystals. To prevent physical changes in the pore structure, pre-fermentation time should be shorter, and water content reduced.

In other studies (Esselink and others 2003b, a), the underlying mechanisms of damage caused due to frozen storage at the molecular, microstructural, and

macroscopic level was studied using cryogenic scanning electron microscope (cryo-SEM) and TD-NMR for frozen dough stored at  $-20\text{ }^{\circ}\text{C}$ . Cryo-SEM was used to monitor ice crystal growth at a micro-structural level with increase in frozen storage time. TD-NMR was used to non-invasively study the water mobility of different states of water namely — tightly bound water, water bound to starch surface and gluten matrix, and free water (most mobile) — in thawed dough stored at  $-20\text{ }^{\circ}\text{C}$ . The mobility of water in dough is an important parameter because it relates directly to the quality of the gluten network. It was shown using cryo-SEM that water separates from the starch-gluten matrix to form ice crystals as the frozen storage time increases. This water, on thawing of frozen dough, does not return to its original state. The crystals formed during the frozen storage expand causing serious damage to the gluten-starch structure that may account for the poor baking performance of frozen dough. Microscopy allowed visualization of microstructural heterogeneity of dough including orientation of gluten strands and distribution of starch granules.

Zounis and others (2002b; 2002a) studied the frozen dough structure using LT-SEM. It was shown that LT-SEM proved to be a useful technique in studying the effects of final dough temperature on frozen dough. These studies also suggested that due to lowering of temperature in a LT-SEM the observations were undesirably subjective (due to freezing at very low temperatures) and it would be better to make direct observations of a fractured surface. In a conventional SEM, the sample is imaged under

high vacuum ( $10^{-5}$ - $10^{-7}$  Torr). High vacuum in SEM is required to prevent unacceptable scattering of the primary electron beam (Stokes 2001). Since many biological and food materials contain water, oil, or volatile substances in them, these components will evaporate under high vacuum, thereby changing the specimen's morphology and degrading the vacuum by water vapor since a gaseous environment would deflect the electrons required for imaging. Therefore, the sample to be studied using SEM is required to be dried and coated with a conductive material or cryogenically frozen (as performed in LT-SEM). Drying of sample in SEM is necessary to prevent evaporation of oil, water, or other volatile substance in organic materials under high vacuum conditions present in SEM. SEM requires organic samples to be coated with gold-palladium alloy (2–4 nm thickness) in order to prevent the buildup of negative charge. The coating is required to prevent the buildup of electrical charge on sample surface. Coated specimens give only topographic contrast due to the short escape depths of secondary electrons from metals, and therefore valuable compositional contrast from the underlying specimen is lost (Stokes 2001; Donald 2003b). These requirements make SEM or LT-SEM unsuitable for study of relatively wide range of biological materials in their natural state (Bache and others 2000).

To avoid drying, coating, or cryogen freezing of biological specimens, a variable pressure electron scanning microscope (VP-SEM) or ESEM — where low-pressure and high relative humidity environment is created, instead of vacuum (as in SEM or LT-



SEM) — can be used. VP-SEM or ESEM (Figure 2-2) enables visualization of uncoated, moist, dry, or, oily samples in a gaseous atmosphere in a vacuum range of 1–20 Torr (Stokes and Donald 2000; Roman-Gutierrez and others 2002).

Humidity and temperature in an ESEM can be controlled for imaging frozen dough samples between pressures of 1–20 Torr. This avoids the necessity of drying, coating or cryogenically freezing a frozen dough sample before imaging, as required in SEM or LT-SEM, and thus allows imaging valuable compositional contrast on the specimen surface. Similarly, a low vacuum scanning electron microscope (LV-SEM) works in low pressure ranges of 0–2 Torr and does not require cryogenic freezing of biological samples.

An ESEM or LV-SEM is equipped with a gaseous secondary electron detector (GSED), which can detect electrons in high humid environments and therefore no drying of samples is required. GSED replaces the customary Everhart-Thornley and other solid state backscattered electron detectors used in conventional SEM (Stokes and Donald 2000; Donald 2003b). The GSED relies on the presence of the gas between itself and the sample, as the gas acts as a means of amplifying the signal generated by secondary electrons. Hydrated samples can be stabilized by carefully controlling the temperature and pressure in order to maintain a saturated vapor pressure of water above the sample, in accordance with the saturated vapor pressure curve of water (Stokes and Donald 2000; Waller and others 2008). Typical conditions in an ESEM to

keep a biological sample stable are: 85 % relative humidity, 5–6 Torr, and 5 °C. The sample temperatures in an ESEM are maintained using a special stage incorporating a Peltier chip for cooling (Donald 2003a).

In a study (Bache and Donald 1998) using ESEM, it was shown that at the mesoscopic level, gluten forms into sheets as opposed to fibers, with the network existing on a much shorter-length scale at the molecular level. As is evident from above studies, the most fundamental advantage of ESEM in study of foods is its ability to control the hydration state of the sample thus preventing the dehydration of the food sample. Unlike conventional SEM, ESEM can be used to image both dry and hydrated materials without applying a conductive coating for the material (Stokes and Donald 2000). ESEM has been used to study entirely liquid specimens, soft condensed matter, and delicate samples such as colloids, emulsions, and other complex fluids (Stokes 2001; Thiel and others 2002). In these cases, secondary electron emission processes — only accessible in the ESEM — give rise to the molecular chemistry based contrast mechanism. ESEM is very useful in imaging frozen foods such as ice cream at “storage conditions”, a few degrees below 0 °C, which is not feasible using cryo-SEM (Thiel and others 2002). Maximizing the potential of ESEM requires the development of new techniques and methodologies in order to control the stability of food specimens *in situ*, whilst achieving good quality images and reproducible results (Stokes and others 2002).

## **2.8 Study of frozen water in dough using Differential Scanning Calorimetry (DSC)**

Generally, large ice crystals are formed in slow freezing processes, whereas during rapid freezing, a relatively large number of small ice crystals are formed. Therefore, a rapid freezing rate promotes more uniform crystallization of ice throughout the material, which leads to a higher quality frozen product. However, the freeze-concentrated unfrozen phase often vitrifies (changes to glassy state), and the formation of the maximum amount of ice is controlled by the glass transition of the unfrozen phase. The glass transition exhibits properties of a second-order phase transition, but it occurs over a temperature range. The temperature range over which glass transition occurs depends on a number of factors, such as chain flexibility, molecular weight, plasticizers, etc. The glass transition of freeze-concentrated systems is additionally affected by the amount of plasticizing unfrozen water. The glass transition can be determined using DSC (Laaksonen and Ross 2000).

The physical state and glass transition temperature of frozen dough during frozen storage may affect quality of baked bread. Therefore, it is important to understand phase and state transitions, including glass transition, occurring in dough at sub-zero temperatures. It has been suggested that the glass transition of frozen dough and its components (starch, gluten, gliadins, and glutenin fractions) may affect stability,

as the glass transition may control rates of recrystallization of ice and diffusion-controlled reactions (Laaksonen and Ross 2000).

During frozen storage, major changes in frozen dough — both in gluten structure and in yeast viability are due to ice crystals. The damage is both direct (physical) and consequential (water distribution). A phase diagram of a water-gluten system resulting from DSC experiments (Bot 2003) indicated that part of the water is frozen in the form of ice crystals below the glass transition temperature ( $T_g$ ). The remainder of the water is in the glassy gluten matrix. When the dough is subsequently brought to a temperature  $> T_g$ , the ice crystals melt, but the water from this melted ice crystals is not in the original state. This means that the gluten is slowly dehydrated during storage at low temperatures (below  $T_g$ ) and does not restore to the original hydrated form on subsequent thawing. This dehydration has a negative effect on baking performance since the dehydrated gluten does not hydrate by the water formed due to thawing of ice crystals — that may migrate to the starch phase (Räsänen and others 1998).

In thermodynamic equilibrium, the amount of freezable water depends on the frozen storage temperature only. Lower the storage temperature, the higher the amount of freezable water. In practical situations however, this thermodynamic equilibrium is not attained in concentrated gluten phases within realistic time periods. The non-equilibrium state leads to changes occurring due to enthalpic relaxation. Enthalpic relaxation can be explained as follows: when stored for longer times at low

storage temperatures (below  $T_g$ ) more water migrates from the gluten-glassy state to ice crystals, thereby increasing the concentration of the gluten in the gluten-water glassy system. This leads to an increase in concentration of gluten in the gluten-water glassy state, thereby increasing the  $T_g$  of the gluten-water system. When such a system is thawed, the ice crystals melt only above this new  $T_g$  and the system goes from glass transition curve to melting transition curve along an isotherm. This leads to an enthalpic relaxation peak in the DSC spectrum above the new  $T_g$  (Bot 2003). If the frozen dough is stored well below the  $T_g$ , this slow relaxation process is not seen and the dough is relatively stable during the storage time (Slade and others 1989). Since the commercial frozen storage temperature is approximately  $-18\text{ }^\circ\text{C}$ , which is not too far below the  $T_g$ , such a slow relaxation process can potentially be detrimental to the quality of dough.

## **2.9 Oscillatory or dynamic rheology to evaluate viscoelasticity of dough**

Many food materials exhibit both solid-like (elastic) and liquid-like (viscous) properties and are therefore viscoelastic in nature. A viscoelastic material's properties are time dependent and hence material's properties are function of how long the stress or strain has been employed. Therefore, unsteady state shear measurement of many food materials provide a means of evaluating viscoelasticity. *Oscillatory* rheology is one such

method to measure viscoelasticity of food materials. Oscillatory stress or strain tests are also referred to as “dynamic” tests. The other unsteady shear measurement includes *transient* methods. Oscillatory test are popular for determination of gel strength, starch gelatinization, protein coagulation, dough formation, and shelf-life testing. It is assumed that the stress/strain is constant throughout the sample, sample inertia is negligible, and material behaves as a linear viscoelastic material.

Linear viscoelastic materials properties are dependent on time alone and not on the magnitude of stress or strain applied to the material. Hence there is a linear relationship between stress and strain for all stress rates applied to a linear viscoelastic material. Such behavior is exhibited by many materials at lower stress or strain values. Above a certain stress or strain many materials reflect a non-linear viscoelastic behavior where the stress in a material depends on both shear and shear rate applied to the material. Hence non-linear viscoelastic materials exhibits properties that are not only time dependent but also dependent on the applied stress or strain.

In a controlled stress rheometer, the stress amplitude and frequency of oscillation is controlled and resulting strain is measured. Stress function can be represented by:

$$\sigma = \sigma_0 \sin\omega t \quad (2-1)$$

where  $\sigma_0$  (Pa) is the amplitude of the stress input provided at frequency  $\omega$  (rad/s). The resulting strain or shear ( $\gamma$  — no units) function that is measured by a controlled stress rheometer is:

$$\gamma = \gamma_0 \sin(\omega t + \delta) \quad (2-2)$$

where  $\gamma_0$  is the resulting strain amplitude and phase angle  $\delta$  (rad) is the phase lag between stress and strain function. The ratio of stress amplitude to strain amplitude is represented by complex modulus  $G^*$  (Pa):

$$G^* = \frac{\sigma_0}{\gamma_0} = \sqrt{(G')^2 + (G'')^2} \quad (2-3)$$

$$\tan \delta = \frac{G''}{G'} \quad (2-4)$$

The storage or elastic modulus  $G'$  (Pa) is the in-phase component with strain and the loss or viscous modulus  $G''$  (Pa) is the out of phase component with strain. Therefore we might write:

$$\sigma = G'\gamma + \frac{G''}{\omega} \dot{\gamma} \quad (2-5)$$

or,

$$\sigma = G'\gamma + \eta' \dot{\gamma} \quad (2-6)$$

where  $\eta' = \frac{G''}{\omega}$  is called the dynamic viscosity.  $G'$  represents the elastic nature of the material and  $\eta' \dot{\gamma}$  represents the viscous nature of the material. The linear viscoelastic region (LVR) is important in oscillatory rheology and defines the maximum deformation that can be applied to the sample without destroying the structure. In LVR rheological parameters are not dependent on the stress or strain. To identify this region, stress or strain sweeps followed by frequency sweeps are performed in which storage and loss moduli are plotted against the “sweeping” parameter. Frequency sweep test are used to characterize the mechanical spectra and are performed by varying frequencies over a wide range (0.001 – 100 rad/s, depending of the viscoelastic nature of the material) at a fixed stress or strain value. The data typically indicates material to be one of the three: gel, concentrated solution, or dilute solution. The relationship of the  $G''$  to  $G'$  over the frequency range for these three materials is shown in Figure 2-3.

## **2.10 Oscillatory rheology and dough quality**

Oscillatory rheology is another valuable technique that gives a quantitative measure for the amount of stress in the dough, which is closely related to the quality of the molecular gluten network. The rheological properties of dough are primarily used as quality indicators for the production of many baked products (Asghar and others 2009).



A possible explanation for loss of dough quality during frozen storage is the changes in dough rheology which affects the quality of the baked product (Bot and de Bruijne 2003). During frozen storage, rheological properties of dough changes as water redistributes itself from hydrated gluten to the ice phase. In an earlier study (Lu and Grant 1999), effects of prolonged frozen storage on the starch present in dough along with the rheological and baking properties of frozen dough were studied. In the study, gelatinization properties of starches isolated from fresh and frozen-thawed dough over 16 weeks of frozen storage were examined using DSC. The rheological strength of frozen dough was determined by extensigraph resistance and storage modulus ( $G'$ ). It was shown that these properties vary with the cultivar, but all cultivars showed a significant increase in enthalpy ( $\Delta H$ ) with increased frozen storage time, indicating water migration and ice crystallization.

To study the structural changes that occur within dough in an industrial processing line, a study (Esselink and others 2003a) was designed at industrial scale to map the impact of three processing steps (kneading, extrusion, and molding) on dough structure. It was observed in this study that kneading time affected the homogeneous distribution of the starch particles over the gluten network. Shorter kneading times resulted in inhomogeneous structures, whereas longer kneading times showed more smeared out structures (elongated gluten strands). Extrusion resulted in strong

orientation and sometimes disruption of the gluten strands, whereas, molding had reverse effect on the structure of dough as compared with extrusion.

In other studies (Wolt and D'Appolonia 1984a, b), a significant correlation was found between amylose-amylopectin ratio, proof time, and loaf volume. Amylose-amylopectin ratios were negatively correlated with frozen dough storage time. In another study (Autio and Sinda 1992), gelatinization properties of starch in frozen dough using DSC were studied. It was observed that the onset temperature of starch gelatinization increased after the dough was frozen and later thawed at 4 °C. It was also noted that prolonged storage at 4 °C (> 23 hrs) further increased the onset temperature, which has been attributed to either a delay in the diffusion of water into the starch granules, or to increased growth of ice crystals in the frozen dough. In a study by Miller and Hoseney (1999), dynamic rheological properties of reconstituted flour containing starch, gluten, and various amounts of lyophilized (freeze dried) water-solubles have been conducted. In another study (Newberry and others 2002) conducted to understand the dynamic rheology of yeasted bread dough, it was noted that only a handful studies regarding the dynamic rheological properties of yeasted dough have been conducted.

The negative effects of ice crystals in water-flour mixtures may be controlled by reducing their water content. In a study (Räsänen and others 1997a), the water in water-flour mixture was studied using ultracentrifugation and reduced water content

was seen as smaller amounts of liquid phase with increased dough rigidity ( $G'$  values) during frozen storage. Reduced water content also prevented an increase in the self-diffusion coefficient of water during frozen storage studied using  $^1\text{H}$  NMR techniques.

## **2.11 Time domain nuclear magnetic resonance (TD-NMR) — a historical background**

The physical phenomena of nuclear magnetic resonance (NMR) was put forth in the mid-1930s by the Dutch physicist Cornelis Jacobus Gorter (Gorter 1936), but it was not until 1945 that the American physicists Felix Bloch and Edward Mills Purcell simultaneously discovered NMR (Bloch and others 1946; Purcell and others 1946). Felix Bloch and Edward Mills Purcell were jointly awarded the 1952 Nobel Prize in Physics “for their development of new methods for nuclear magnetic precision measurements and discoveries in connection therewith”. The applications of NMR in chemistry were not appreciated until 1950, when Proctor and Yu (1950) discovered the chemical shift which enabled elucidation of molecular structures in organic compounds. Although originating in physics, it is in chemistry and biological sciences that NMR has provided the greatest applications and possibilities for further research.

In the first continuous wave NMR instruments, spectral resolution was poor. However, with the introduction of signal averaging, sensitivity improved significantly, and by introducing pulsed NMR instruments along with Fourier transformation of the

acquired time-domain relaxation, signal resolution was even further improved. By introducing pulsed NMR the time of acquisition was reduced as an additional advantage due to Fourier transforms. Since the magnetic resonance chemical shift resolution depends on the magnetic field strength, development of instrumentation has progressed towards stronger and stronger magnetic fields. By the introduction of superconducting magnets in the mid-1970s, a leap in magnetic field strength was taken as compared with the electromagnets previously used. This development increased the physical dimensions of the instruments, and with large magnetic stray fields the instruments required a lot of space and were not suited for work outside the laboratory.

In the late 1980s and the beginning of the 1990s application of pulsed field gradients became routine. Besides enabling a large number of specialized pulse experiments in order to fulfill specific demands by researchers, magnetic resonance imaging (MRI) was also made possible, both in the research lab and in hospitals. With the introduction of imaging, NMR really moved into the field of non invasive analysis of large samples, offering completely new prospects for researchers in many fields. Today, it has become an indispensable diagnostic tool in medicine, biological sciences, and life sciences.

Approximately at the same time, when MRI was emerging, a modern superconducting NMR instrument had become very expensive and expert

spectroscopists were required to operate the instruments. It was realized that in many research fields the high spectral resolution offered by these instruments was not always required and development of new small bench-top instruments based on the latest electronics and permanent magnets with a much lower magnetic field strength, started to emerge. These kinds of instruments typically have magnetic field strengths in the range of 0.23–0.70 T that is equivalent to 10–30 MHz angular frequency of precession of the protons. These instruments have particularly found applications in the oil industry for rock core analysis, medical diagnostics, food and cattle feed research. Low-field bench-top instruments have the advantage of being cheaper, lighter, and much less sensitive to changes in environmental and stray fields, as compared to high-field NMR instruments. These qualities of low-field NMR instrument, has aided in applying NMR techniques for on-line quality control measurements in industrial process lines.

Over the years, a number of names have been applied to describe the benchtop NMR instruments such as “low-resolution”, “time-domain”, “wide-line” or “low-field”. Of these only the latter is clearly unambiguous, since a lot of work has been put into producing better magnets and better shimming, thereby producing bench-top instruments capable of showing chemical shift resolution (possessing “high-resolution” and narrow line width capabilities). Furthermore, since high-resolution data may be presented and analyzed in the “time-domain”, this name is considered equally inappropriate by NMR scientists. However, in this dissertation, due to its wide

recognition “time-domain NMR” or TD-NMR will be the term used to describe the techniques associated with 20 MHz instrument used in present study. A great extent of research and development is being carried out on the NMR hardware, continuously offering new possibilities that make both low-field and high-field NMR indispensable tools in research and routine analysis.

## 2.12 Electric and magnetic field

*Electrical field* (**E**) is a property of the space surrounding a stationary charged particle and it exerts force on another charged particle in its vicinity. *Magnetic field* (**B** or **H**) is produced around a moving charge (orbiting electron in an atom), due to changing electric field **E**. Electric and magnetic field are both vector quantities and related by:

$$\mathbf{B} = \frac{(\mathbf{v} \times \mathbf{E})}{c^2} \quad (2-7)$$

where **v** is the velocity of the electric charge in m/s, *c* is the speed of light (299,792,458 m/s), **E** is the electric field measured in N/C or V/m, and **B** is in N·s/(C·m) or Tesla (T). Unlike electric field, magnetic field exerts force **F** only on a moving electric charge, with magnitude and direction given by:

$$\mathbf{F} = q(\mathbf{v} \times \mathbf{B}) \quad (2-8)$$

where *q* is the electric charge that magnetic field is acting upon and is measured in Coulomb (C).

## 2.13 Nuclear spin

Spin properties of nucleons (protons and neutrons) in the nuclei define the overall spin of the nucleus. Nucleons might be thought of as spinning particles. However, spin is not considered as rotation of nucleon and is an *intrinsic* property of a nucleon. When both the atomic number (number of protons) and atomic mass (sum of protons and neutrons) are even, the nucleus is considered as not having a spin. Individual spins of nucleons in such a nucleus cancel out each other. This means only an unpaired nucleon can contribute towards the spin of a nucleus.  $^{12}\text{C}$  and  $^{16}\text{O}$  are examples of nonmagnetic nuclei.  $^{13}\text{C}$  and  $^{17}\text{O}$  each have an unpaired neutron, which contribute towards the spin of their nuclei. If either the atomic number or atomic mass is odd, or both are odd, nucleus is considered to be spinning (possess magnetic properties), and the spin is defined by *spin quantum number* or *intrinsic spin I*. Each unpaired proton or neutron contributes to a spin of  $1/2$  each and has an associated magnetic moment with it. Hence, **I** can take only integer or half-integer values. When **I** = 0, the atom does not possess any spin and is considered to have no magnetic interactions when placed in an external magnetic field (as in case of  $^{12}\text{C}$  and  $^{16}\text{O}$ ).

## 2.14 Magnetic moment

The *intrinsic spin* ( $\mathbf{I}$ ) of a nucleus gives rise to *spin angular momentum*  $\mathbf{p}$ , which points along the spin axis (Seton 2003; Atkins and de Paula 2006):

$$\mathbf{p} = \hbar \mathbf{I} \quad (2-9)$$

where

$$\hbar = \frac{h}{2\pi} \quad (2-10)$$

and  $h$  is Planck's constant with value of a  $6.626 \times 10^{-34}$  J.s or  $4.135 \times 10^{-15}$  eV.s. An external magnetic field forces the vector  $\mathbf{p}$  to approximately orient in a particular direction. Due to the associated electric charge, *intrinsic spin*, and *spin angular momentum* a *magnetic dipole moment*  $\boldsymbol{\mu}$  is associated with such atoms and is given by:

$$\boldsymbol{\mu} = \gamma \mathbf{p} \quad (2-11)$$

where  $\gamma$ , the *gyromagnetic ratio*, is a constant for a given type of nucleus. For protons,

$$\gamma = 26.75 \times 10^7 \text{ (rad/T.s)} \text{ or } \frac{\gamma}{2\pi} = 42.58 \text{ MHz /T .}$$

## 2.15 Interaction of spin with external magnetic field

A magnetic dipole moment causes a nucleus to behave like a tiny magnet. When placed in an external magnetic field  $\mathbf{B}_0$ , a magnetic dipole moment  $\boldsymbol{\mu}$ , interacts with the external magnetic field. The direction of angular momentum arising from the nuclear



spin is quantized (takes certain values), resulting in discrete energy eigen-states. Without an external magnetic field, these states collapse back into one state. This quantization of angular momentum is described by certain values of the *projection quantum number*,  $m_s$ , which can take  $2I + 1$  values from  $-I$  to  $+I$ . For hydrogen,  $I = \frac{1}{2}$ , and hence  $m_s = \pm\frac{1}{2}$ . These two values of  $m_s$  correspond to two energy eigen-states separated by a quantum of energy  $\Delta E$  equal to:

$$\Delta E = \frac{\mu B_0}{I} \quad (2-12)$$

or

$$\Delta E = \gamma \hbar B_0 \quad (2-13)$$

The splitting of the energy into two levels, in presence of a magnetic field, is known as Zeeman splitting (Figure 2-4). Zeeman splitting is a quantum mechanical effect, and without it, NMR and MRI would not exist. At equilibrium, the equivalent population difference which occurs between the two energy eigen-states results in a *Net Magnetization vector* ( $\mathbf{M}_0$ ) in a sample. The transitions from a lower to a higher energy state can be completed by an electromagnetic wave at a frequency  $\omega$  given by:

$$\omega = \frac{\Delta E}{\hbar} = \gamma B_0 \quad (2-14)$$

## 2.16 Boltzmann distribution

Even though a lower energy state is preferred in presence of a external magnetic field,  $\mathbf{B}_0$ , there is only a small excess in number of spins at lower energy state as compared to higher energy state. This occurs because temperature induces thermal motion causing the spins in a lower energy state to transit to a higher energy state. However, an increase in magnetic field increases the number of spins in lower energy state, when compared to the number of spins in a higher energy state (by magnetic transitions) and thus, produces an opposite effect to thermal transitions. The distribution of the populations in lower and higher energy states ( $N_L$  and  $N_U$  respectively) is given by Boltzmann distribution:

$$\frac{N_L\left(+\frac{1}{2}\right)}{N_U\left(-\frac{1}{2}\right)} = \exp\left(\frac{\Delta E}{kT}\right) = \exp\left(\frac{\hbar\omega}{kT}\right) \approx 1 + \left(\frac{\hbar\omega}{kT}\right) \quad (2-15)$$

since,

$$\left(\frac{\hbar\omega}{kT}\right) \ll 1 \quad (2-16)$$

In above equations, k is Boltzmann's constant ( $1.3805 \times 10^{-23}$  J/K), and T is the absolute temperature in Kelvin (K). Therefore the fractional excess of spins in lower energy state is given by:

$$\frac{N_L - N_U}{N_U} \approx \left( \frac{\hbar\omega}{kT} \right) = \left( \frac{\hbar\gamma B_0}{kT} \right) \ll 1 \quad (2-17)$$

At room temperature and with an external magnetic field,  $\mathbf{B}_0$  of 1 T, this fractional excess of protons is  $7 \times 10^{-6}$ . These spins are responsible for the observed phenomena of excitation in spectroscopy or NMR experiments. Since the value of this fractional excess of protons is very small, it can be increased by increasing  $\mathbf{B}_0$  or decreasing temperature (T), thereby increasing the sensitivity of NMR experiments.

## 2.17 Spin packets and net magnetization vector at equilibrium

NMR system is defined at macroscopic levels using the concept of spin packets. Spin packet is the group of spins experiencing same magnetic field strength. The total sum of magnetic fields due to each spin packet can be defined using a summed vector called the *magnetization vector*. The strength or magnitude of this vector is proportional to the spin present in the lower and upper energy levels represented by  $N_L$  and  $N_U$  respectively. *Net magnetizing vector* represented by  $\mathbf{M}_0$ , is the sum of the magnetization vector for all spin packets. In understanding NMR, we focus on  $\mathbf{M}_0$  to describe all phenomenons. As soon as a sample is placed in a magnetic field there is no *net magnetization* ( $\mathbf{M}_0 = 0$ ). This is due to the fact that individual magnetic moments are at random orientations cancelling each other, and therefore, the vector sum over the sample results in no *net magnetization*. After a finite time of placing the sample inside

the magnet, the magnetic moments orient themselves as described by the Boltzmann distribution equation (2-15) to build a steady  $\mathbf{M}_0$ . At this steady state or “equilibrium”,  $\mathbf{M}_0$  is in same direction as the external magnetic field,  $\mathbf{B}_0$ .

## 2.18 Larmor precession for spin

During the NMR or MRI process, energy is put into the system to disturb  $\mathbf{M}_0$  from its equilibrium position (pushing it out of alignment with  $\mathbf{B}_0$ ). Since every single spin precesses,  $\mathbf{M}_0$  also precesses around the direction of  $\mathbf{B}_0$  (Figure 2-5). The precessing net magnetization generates an oscillating magnetic field which is measured by appropriately placed signal detection equipment. Without an external radiofrequency (RF) field,  $\mathbf{M}_0$  is equally distributed in the x-y plane. For a particular magnetic field strength the (resonant) frequency of precession can be calculated via the Larmor equation. The Larmor equation may be derived using classical physics or quantum mechanics. It links the classical and quantum physics descriptions of nuclear magnetic resonance, and is fundamental to NMR and MRI physics. The Larmor equation in classical physics is given by:

$$\omega_0 = \gamma B_0 \quad (2-18)$$

where  $\omega_0$  is the angular frequency of precession of protons in an external magnetic field strength of  $B_0$  and  $\gamma = 2.67 \times 10^8$  rad/(s·T) for  $^1\text{H}$  nucleus. In some texts the right-

hand side of Larmor equation is negative; this simply depends on the direction in which a positive rotation is defined, usually because of the sign of  $\gamma$ . We may also write:

$$\nu_0 = \frac{\omega_0}{2\pi} = \left( \frac{\gamma}{2\pi} \right) B_0 \quad (2-19)$$

where  $\nu_0$  is the Larmor frequency and  $\gamma / 2\pi = 42.56 \text{ MHz/T}$ . For example, the Larmor frequency  $\nu_0$  for a 0.5 T magnet is:

$$0.5 \text{ T} \times 42.56 \text{ MHz/T} = 21.28 \text{ MHz} \quad (2-20)$$

As seen from the Larmor equation (2-18) only a certain amount of energy (electromagnetic wave of certain frequency) can produce transitions in the energy states and hence, this phenomenon is called *resonance* in NMR or MRI. It may be noted that this *resonance* frequency (same as  $\nu_0$ ) is directly proportional to the magnetic field strength  $\mathbf{B}_0$ . When performing a NMR experiment, a RF pulse of short duration (usually lasting about few  $\mu\text{s}$ ), is produced using a RF coil and transmitter to provide a magnetic field  $\mathbf{B}_1$ , such that  $\mathbf{B}_1$  lies in a plane perpendicular to  $\mathbf{B}_0$ . The frequency of this RF pulse must be close or equal to  $\nu_0$  to generate a fluctuating  $\mathbf{B}_1$  field (along x-axis) in order to influence the magnetization vector  $\mathbf{M}_0$ . Even though  $\mathbf{B}_1$  is very small compared to the  $\mathbf{B}_0$ , it may be shown from NMR principles that it easily perturbs  $\mathbf{M}_0$ . This is another way of explaining the *resonance* phenomenon since the selected RF pulse frequency is same as the rate at which the protons precess.  $\mathbf{B}_1$  is held for a certain amount of time that perturbs  $\mathbf{M}_0$  away from  $\mathbf{B}_0$ . When the RF pulse is stopped after  $\mathbf{M}_0$  has flipped by a

certain angle, the magnetic moment  $\mathbf{M}_0$  experience a torque  $\mathbf{L}$  that causes it to spin in precession which attempts to align it along the field  $\mathbf{B}_0$ :

$$\mathbf{L} = \boldsymbol{\mu} \times \mathbf{B}_0 \quad (2-21)$$

After the precession ceases,  $\mathbf{M}_0$  comes back to its equilibrium state, which as described earlier, is reached after a certain time of placing the sample inside the magnet.

## 2.19 Relaxation processes

After the absorption of RF pulse in an NMR experiment,  $\mathbf{M}_0$  perturbs and finally returns to its equilibrium position. This physical phenomenon is termed as “relaxation processes” (Ruan and Chen 1998b, a; Keeler 2005; Atkins and de Paula 2006). Two kinds of relaxation processes are observed: *spin-lattice* (or *longitudinal*) relaxation ( $T_1$ ) and *spin-spin* (or *transverse*) relaxation ( $T_2$ ). The *longitudinal* and *transverse magnetization* decays after the RF pulse is ceased and the recorded signal of magnetization recovery is studied as the relaxation process.  $T_1$  and  $T_2$  are characteristics properties of a sample and are functions of the spin species and the chemical and physical environments surrounding the spins. *Spin-lattice relaxation* is attributed to the interaction of the nuclear spin dipoles with the random fluctuations in magnetic field caused by the motion of the surrounding dipoles in the lattice. Molecular interactions among spin dipoles and inhomogeneity in  $\mathbf{B}_0$ , explains the *spin-spin relaxation* process. Longer  $T_1$  and  $T_2$  values indicate slow relaxation rates of spins.

## **2.20 Advantages and disadvantages of TD-NMR applications in food analysis**

The major advantage of NMR techniques is that it is non-invasive technique in which no sample preparations are required. This feature is particularly important as diagnostic modality in medicine where as compared to X-ray tomography NMR does not use ionizing radiation and does not require contrast agents (Caprihan and Fukushima 1990), although contrast agent are also used in MRI. Another difference is that a typical liquid sample does not attenuate the RF signal required to do the NMR experiments. Since NMR is less likely to suffer from opacity effects, it is very helpful in studying samples involving concentrated solid suspensions where the solids can block the “beam”. Direct measurement of both velocity and particle distributions in concentrated suspension using non-NMR methods is difficult due to high particle-fluid interfaces, which scatter light, sound waves, and particle beams. NMR imaging has proven to be a useful tool to measure fluid velocity and fluid fraction in highly filled suspensions (Altobelli and others 1997). In a research work (Hills 2006), NMR relaxation and diffusion studies were performed on horticulture products to determine ripening, fruit maturity, and their overall quality. <sup>1</sup>H TD-NMR techniques hold potential for use in quality control tools in food, petroleum, and other industries, as it demonstrates a cheap, robust, and rapid method to measure solid fat content, solid/liquid ratios, water-holding characteristics, and other important properties of many materials (Bertram and

Andersen 2006). TD-NMR has also been proven useful in determination of moisture content, pore size distribution, and fluid flow in porous materials (Strange and Webber 1997; Watson and Chang 1997).

Quality control and quality assurance applications with pulsed NMR started about 35 years ago with cooperation of Unilever Research (The Netherlands) and Bruker Physik AG. The collaboration aimed at building a small table-top TD-NMR analyzer for the solid-to-liquid ratio analysis on fat compositions. The analyzer became successful and was widely accepted in the industry. This method is popularly known as solid fat content (SFC) determination and is now recognized as an International Standard Method (Todt and others 2006a).

In the food industry, some major applications of TD-NMR are in determination of the SFC of oils and fats, determination of water and fat content, predicting droplet size distribution, and in determining water mobility in food systems (Padua and others 1991; Li and others 2000; Hinrichs and others 2004; Assifaoui and others 2006; Todt and others 2006b). TD-NMR has also been used in determining the effect of freezing on pasta filata and non-pasta filata Mozzarella cheeses (Kuo and others 2001; Kuo and others 2003). TD-NMR techniques have been successfully applied in characterizing the amount of water in crystallized form in ice cream and in demonstrating the influence of the milk proteins on ice crystal relaxation (Lucas and others 2005). Free induction decay (FID) in  $^1\text{H}$  NMR experiments can be used for studying polymer crystallization by



differentiating NMR signal contributions from crystals, melt-like, and amorphous regions (Hertlein and others 2006). FID acquisition has been shown to be a useful tool for hard/soft ratio or crystallinity measurements, mobile-phase characterization, and online monitoring of crystallization kinetics (Maus and others 2006).

The rehydration capacity of dairy products is a crucial quality criterion for dairy industry. Standard methods to determine quality for dairy products are crude, dedicated to milk powder analysis, and are not suitable for analysis of other dairy powders. The advantage of a newly proposed NMR method in a study (Mariette 2006), for rehydration monitoring is that the measurements are performed under stirring conditions at a controlled temperature. Thus, NMR method provides complete kinetics of the rehydration process and can be used for any composition of a powder.

Even though NMR is a powerful non-invasive method, the technique has its limitations. Relatively few different atomic nuclei have high enough NMR sensitivity to be useful in NMR techniques. However, since proton is abundant in nature and is sensitive to NMR, the technique can be applied in wide range of biological materials. Other limitation is the incompatibility of NMR with nearby ferromagnetic objects, as well as opacity to electrical conductors. Thus, samples containing ferromagnetic particles are ill suited for NMR techniques. However, NMR is appropriate for studying steady state flows or those in which the average fluid behavior changes relatively slowly. Therefore, NMR is ill suited for flow experiments where a property needs to be

measured instantaneously, which can be performed using laser Doppler methods (Caprihan and Fukushima 1990).

## **2.21 TD-NMR — a tool to investigate dough quality**

Water is a very important ingredient in dough as it leads to a bicontinuous phase of the dough system. In this bicontinuous phase the continuous water-containing gluten phase is interpenetrated by the continuous water-starch mixture phase. Water usually constitutes ~40 % w/w of the bread dough and more than 35 % w/w of the baked bread. Water is essential for gluten development, for molecular interactions, and for chemical reactions occurring during the mixing stage of dough production. Water is not evenly distributed among the flour constituents in the dough. Approximately 46 % w/w of water in dough is associated with starch, ~31 % w/w with protein, and ~23 % w/w with pentosans, which is significantly influenced by the amount of protein and damaged starch (Bushuk 1966). Since water interacts with the dough components, its property varies from that of the bulk water. For example, the freezing point of water in a dough system decreases and even after long frozen storage times, not all of the water is frozen (Bushuk and Mehrotra 1977b). Therefore water in the dough is not only distributed among the different components in dough, but is present in different states (Ruan and others 1999).

Differential thermal analysis (DTA) and differential scanning calorimetry (DSC) have been used to measure the bound or un-freezable water in dough (Davies and Webb 1969; Daniels 1975; Bushuk and Mehrotra 1977a, b, c). The bound water content (0.25 g/g sample) in dough was found to be independent of flour protein content, added chemicals, amount of damaged starch, and mixing time. In the study by Bushuk and Mehrotra (1977a) using DTA, it was shown that the binding energy in dough decreased with increasing protein content. This technique does not reveal the true water states or the water-binding characteristics of the dough system as some physical and chemical changes such as protein denaturation and starch gelatinization may occur during heating or cooling of samples. However, when compared to DTA and DSC, pulsed NMR studies require no heating or freezing of the sample and therefore it is a nondestructive technique (Leung and others 1979).

TD-NMR has been applied in various industries such as pharmaceutical, petroleum, and food to study the binding of water molecules in biological systems. NMR can be used to study the spin-lattice relaxation time or longitudinal relaxation time ( $T_1$ ) and spin-spin relaxation or transverse relaxation times ( $T_2$ ) of protons which are characteristic of molecular mobility or the state of water in a dough system (Leung and others 1979; Ruan and others 1999). Due to the low cost of equipment, simple sample preparation, and quick measurement associated with TD-NMR (as compared to high-resolution NMR), it has become a popular quality control investigation tool in academia

and industry. Short  $T_1$  and  $T_2$  indicate that the water is less mobile as compared to its free state, and is present in a bound state in a food system. Staling of bread has also been studied using TD-NMR and it has been shown that the  $T_1$  and  $T_2$  values decreased with increased staling and were independent of the moisture content of the bread (Leung and others 1979). This clearly suggests that relaxation is largely structure-dependent and that physiochemical changes would cause relaxation times to vary. It has been suggested that interpretation of NMR relaxation time measurements in a food system must involve chemical and diffusive exchanges between water protons and biopolymer protons (Hills 2006). Therefore relaxation measurements have been suggested as a useful tool to probe the state of the biopolymers or other species having exchangeable protons. In a study by Haiduc and van Duynhoven (2005), TD-NMR has been used to correlate porous and functional properties of food materials by NMR relaxometry and multivariate analysis. In other studies (Kim-Shin and others 1991; Li and others 1998; Vodovotz and Chinachoti 1998; Baik and Chinachoti 2000; Lin and others 2001), the redistribution, state, and mobility of water has been studied in dough using NMR (by studying relaxation rates or relaxation times), DSC, or DMA.

NMR relaxation times ( $T_1$  and  $T_2$ ), have been used to investigate the state of water in dough (Toledo and others 1968; Leung and others 1979; Ruan and others 1999). Bound water in a system is considered to be the water which persists as liquid phase at low temperatures. Toledo and others (1968) defined bound water as that

which remained liquid at  $-18\text{ }^{\circ}\text{C}$  and was  $0.29 \pm 0.01$  g of water per gram of dry solid for wheat flour dough. This value was independent of the total moisture content of the dough for the same flour with moisture contents greater than 24.6 % w/w. Leung and others (1979) have shown that the NMR relaxation times ( $T_1$  and  $T_2$ ) of bulk water are different from that of bound water in the dough system. Shorter  $T_1$  and  $T_2$  usually mean lower mobility of water in the system.  $T_2$  for bulk water is  $\sim 2$  s, whereas, that for bound water in dough is  $\sim 20$  ms. The shorter  $T_2$  in dough is due to the fact that some water in dough is bound with the flour constituents and therefore, experiences a faster relaxation than bulk water. It was shown that the NMR relaxation curve consists of two components — one with a long  $T_2$  and the other with a short  $T_2$ , corresponding to more mobile and less mobile fractions respectively. The less mobile fraction has a  $T_2$  of  $\sim 20$  ms and accounts for  $\sim 0.62$  g of water per gram of dry solid mass in dough. These values seem to be independent of protein content and mixing time. In an earlier study (Leung and others 1976),  $T_1$  and  $T_2$  measurements were performed for macromolecules such as sodium alginate, pectin, corn starch, casein, and cellulose. It was found that  $T_2$  increased with moisture content for all macromolecules (thus reflecting the increase in mobility) and cellulose showed exceptionally longer  $T_2$  values compared to other materials at the same moisture content. This indicated that  $T_2$  is a characteristic of water binding properties of food materials. Thus, physiochemical changes such as

changes in morphological characteristics and changes in intermolecular forces at microscopic and macroscopic levels causes the relaxation times to change.

## 2.22 Measurement of relaxation times ( $T_1$ and $T_2$ )

Sample  $T_1$  values are most commonly measured using the Inversion Recovery (IR) pulse sequence as shown in Figure 2-6. In an IR pulse sequence, a  $180^\circ$  RF pulse is followed by a  $90^\circ$  RF pulse. The  $180^\circ$  pulse tips the *net magnetization vector*  $\mathbf{M}_z$  to an initial value of  $-\mathbf{M}_0$ . During the period  $\tau$ , between  $180^\circ$  and  $90^\circ$ ,  $\mathbf{M}_z$  gradually recovers to its equilibrium value of  $+\mathbf{M}_0$  as shown in Figure 2-7 (Atkins and de Paula 2006). The rate of recovery depends on the spin-lattice relaxation time of the sample,  $T_1$ , since there is no transverse magnetization component at this stage. A number of repeated  $180^\circ$ - $90^\circ$  experiments — done with different  $\tau$  values and sufficient time (called recycle delay or RD) in between the experiments — to let the magnetization recover towards  $+\mathbf{M}_0$  provides a curve (Figure 2-7) representing equation:

$$M_z(t) = M_0 \left[ 1 - 2 \exp\left(\frac{-t}{T_1}\right) \right] \quad (2-22)$$

$T_2$  is measured using *spin echo* (Atkins and de Paula 2006). The Carr-Purcell-Meiboom-Gill or CPMG pulse sequence is used to measure  $T_2$  which produces the *spin echo*. CPMG is much more tolerant of error in the  $180^\circ$  pulse (due to inhomogeneity of  $90^\circ$  pulse,  $\mathbf{B}_1$ ) and to the loss of precise refocusing due to diffusion when compared to Hahn-echo or

Carr-Purcell (CP) sequences (Ruan and Chen 1998b, a). The envelope in Figure 2-8 of the multiple echo amplitudes reflects the transverse relaxation or decay, from which  $T_2$  can be measured using equation:

$$M_{xy} = M_0 \exp\left(\frac{-t}{T_2}\right) \quad (2-23)$$

### **2.23 Pulsed field gradient NMR (PFG-NMR) to study water diffusion in dough**

Water states in macromolecules solutions and gels can be studied using self-diffusion measurements of water mobility. This can be achieved by means of the PFG-NMR technique. As compared to NMR relaxation techniques the interpretation of PFG-NMR findings are more straightforward. The technique has been used to study water mobility in casein solutions, gels, and cheese, and to study the effect of casein and fat content in model cheese on water diffusion. When diffusion of water is restricted within droplets, the droplet size distribution can be determined — this may be applied to butter to study the distributions of oil and water droplets in it. In-addition, it has been indicated in recent studies that self-diffusion coefficient of water in a freeze-concentrated matrix is a useful parameter for predicting and controlling the recrystallization rate (Hagiwara and others 2006; Petzold and Aguilera 2009). Water

self-diffusion coefficient and staling in white bread has been studied using PFG-NMR by Baik and Chinachoti (2003).

PFG-NMR approach has been applied in determination of fat content in food emulsions like sauces, mayonnaises, margarines, and dairy products. The determination of fat and water content in foods is very important in food industry. However, the difference between the transverse and the longitudinal relaxation times of fat and water is small due to effect of parameters such as temperature, salt concentration, and local environment of the molecules in a sample. Thus common relaxation measurements, such as  $T_1$  and  $T_2$ , alone are not sufficient for establishing good correlation to many sample properties. Combined relaxation experiments where the magnetization at a given time is determined for both the longitudinal and transverse relaxation processes and their analysis conducted using chemometric methods (commonly applied for infrared studies), have been applied to food products containing fat in range of 4–80 % w/w to build simple calibration curves. These techniques have reduced the experimental time required by traditional analytical methods for food analysis (Guthausen and others 2006).

During a NMR experiment, NMR resonance frequency is given by  $\omega$ . If there is a complete homogeneity in the permanent magnetic field,  $\mathbf{B}_0$ , then NMR signal after  $90^\circ$  pulse would decay exponentially with time constant  $T_2$  according to the equation (2-23). In practice, a perfect homogenous field is almost never achieved. Thus, some nuclei



“see” a higher field ( $B_0 + \Delta B$ ) and some lower ( $B_0 - \Delta B$ ). Thus, within the sample, various resonance frequencies ( $\omega$ ,  $\omega + \Delta\omega$ ,  $\omega - \Delta\omega$ ) occur. Therefore, after time  $t$ , phases of nuclei changes from each other due to different precession speeds. Hence, these differences reduces the NMR signal as the signal depends on the vector sum of the magnetic moments of all the rotating nuclei and is maximum when all the individual components are parallel (Bruker Analytik GmbH 2000; Levitt 2001b). If the field distribution is rectangular (i.e. there is a linear field gradient across the sample), then the signal is described to a first approximation by:

$$M_t = M_0 \cdot \exp \frac{\sin(\gamma \cdot \Delta B \cdot t)}{\gamma \cdot \Delta B \cdot t} \quad (2-24)$$

where  $\Delta B$  is the largest deviation of the field from the average value. If individual nuclei do not change their positions, the dephasing of magnetization is reversible due to fixed relationship between the phases. Thus, with a  $180^\circ$  pulse applied after time  $\tau$ , nuclear moments can be refocused and the process is reversible after which they diphas again. This process is referred to as “spin-echo” or “Hahn-echo” (Levitt 2001a). However, due to diffusion of nuclei, the condition that these nuclei do not change their positions in order to ensure that they are in-phase again at time  $2\tau$  is not met. Due to diffusion, the nuclei move in the time between the  $90^\circ$  and  $180^\circ$  pulses and may reach in a position where the field strength is different. Thus, the precession frequency of moved nuclei is changed and the phase relationship is irreversible. After  $2\tau$ , these nuclei are no longer in phase with others and the signal amplitude is therefore reduced. Thus by studying

the relationship between the reduced echo amplitude  $[A(2\tau)]$  and the magnetic field gradient  $G$  (in T/m), we can determine the self-diffusion coefficient of water ( $D$ ) in a system (Bruker Analytik GmbH 2000).

The PFG-NMR techniques are used to study the translational diffusion rate of molecules in solid materials (Stejskal and Tanner 1965; Tanner and Stejskal 1968), which is a modified simple spin-echo experiment. In PFG-NMR,  $G$  is applied after the  $90^\circ$  and  $180^\circ$  pulses in the spin-echo experiment as shown in Figure 2-9. Due to application of  $G$  at time  $t_1$ , a rapid precessional dephasing occurs due to nuclei precessing with different frequencies depending on the position of each nucleus in the sample. Because of diffusion, the nuclei change position during time  $\Delta$ , in between the first and the second application of  $G$ . Therefore,  $180^\circ$  pulse applied at time  $\tau$ , cannot fully refocus the spins. Hence, a second gradient pulse absolutely identical to the first gradient pulse is applied to bring the phases of different nuclei in phase. However, due to diffusion this is not achieved. Thus, the amplitude  $A(2\tau)$  of the echo when compared with amplitude of the echo without application of  $G$  [represented by  $A(0)$ ], gives the measure of the degree of translational diffusional rate of molecules between two RF pulses:

$$\ln \left[ \frac{A(2\tau)}{A(0)} \right] = - \left( \frac{2\tau}{T_2} \right) - \gamma^2 D \delta^2 G^2 \left( \Delta - \frac{1}{3} \delta \right) \quad (2-25)$$

where  $D$  is the translational self-diffusion coefficient in units of  $m^2/s$ . In some cases, the  $\frac{2\tau}{T_2}$  term in the equation (2-25) is negligible compared to the remaining terms (Ruan

and Chen 1998a). Usually, the diffusion coefficient is measured by varying  $\delta$  and/or  $G$

(fixing the other parameters) and plotting  $\ln\left[\frac{A(2\tau)}{A(0)}\right]$  as function of  $\gamma^2\delta^2G^2\left(\Delta - \frac{1}{3}\delta\right)$ ,

thereby obtaining  $D$  from the slope of fitted straight line. The typical values of parameters used in a PFG-NMR experiment are listed in Table 2-1. For  $\Delta \gg \delta$ , the exact diffusion time is  $\Delta$ . When  $\Delta$  is comparable to  $\delta$ , the diffusion time must be considered as

$$\left(\Delta - \frac{1}{3}\delta\right).$$

## **2.24 Functionality of ingredients in dough to improve quality of the baked product**

Bread is a basic food and bread baking is a handicraft with long tradition. However, due to mechanization and large scale production along with an increase in demand for high quality, convenience, and longer shelf-life for bread, a need for functional food additives such as emulsifiers has been created (Stampfli and Nersten 1995). In some studies effects of emulsifiers and enzymes such as diacetyl-tartaric acid ester of monoglycerides (DATEM), sodium stearoyl lactylate (SSL), glyceryl monostearate (GMS), and transglutaminase (TGase) have been studied on the quality of dough products using microscopy, dynamic rheology, baked specific volume measurements, and determination of crumb firmness (Akdogan and others 2006; Xiujin and others

2007; Kim and others 2008b). Effects of gum arabic on dough quality have been studied by Sharadanant and Khan (2003a, 2003b).

Hydrocolloids particles are particles that are dispersed in water with a size  $< 1$   $\mu\text{m}$  and are soluble in water. Water separates out as ice during freezing and the solute concentration of the unfrozen phase in contact with it increases when temperature decreases. After certain concentration of unfrozen phase, no more ice separates due to glass transition temperature coming into effect. There is a maximum freeze concentration of unfrozen phase after which it becomes so viscous that it turns into a glass (since glass transition occurs). This water present in unfrozen phase can be determined by DSC (Laaksonen and Ross 2000).

Effect of various ingredients including dairy products and emulsifiers, has been studied for their effect on increasing the shelf-life and stability of dough to produce a final baked product in similar qualities to that of fresh dough. Wolt and D'Appolonia (1984a, b) studied the influence of SSL and diacetyl tartaric acid on frozen dough stability. These ingredients decreased the effects of frozen storage on rheological properties, but they were ineffective in reducing the dough proofing time. Asghar and others (2009) studied the effects of modified whey protein concentrates (mWPC) using instrumental texture analysis of frozen dough stored for 0, 15, 30, and 60 days. The study showed that the addition of mWPC significantly decreased the values of hardness, cohesiveness, gumminess, and springiness. Ribotta and others (2001) concluded that

frozen dough supplemented with DATEM, gluten, and guar gum resulted in bread with greater volume and more open crumb structure as compared to basic formulation. In a review (Asghar and others 2006a) the functionalities of different additives such as surfactants, hydrocolloids, and gum additives to improve the quality of frozen dough was presented.

Other research works (Asghar and others 2005; Asghar and others 2006b; Asghar and others 2007) studied the rheological and frozen storage time effects of hydrophilic gums on the quality of frozen dough. These rheological studies were performed using empirical techniques such as farinograph and mixograph, and concluded that the use of gum arabic and carboxymethyl cellulose (CMC) at levels of 3 % w/w can improve the overall quality of frozen wheat dough pizza, indicating the potential of hydrocolloids as frozen dough stabilizers. Ribotta and others (2004) studied the effect of emulsifier and guar gum on micro structural, rheological, and baking performance of frozen bread dough. The study concluded that freezing of dough and its frozen storage provoked a decrease in  $G^*$  and  $G'$  values showing a reduction in dough firmness and elasticity. DATEM and guar gum improved volume and texture of bread obtained from non-frozen and frozen-thawed dough. However, neither DATEM nor guar gum could reduce the effects of frozen storage on the dynamic rheological parameters and ultra structure damage. In other studies (Matuda and others 2005; Matuda and others 2006), the effect of vegetable shortening, calcium stearoyl-2-

lactylate (CSL), and polysorbate 80 (PS80 or Tween 80) on unfrozen water and textural properties of frozen French bread dough was studied. It was observed in the study that the value of unfrozen water was 0.30–0.34 g H<sub>2</sub>O/g solids, and additives used during the frozen storage (for up to 56 days) significantly affected the textural properties of frozen French bread dough.

From above literature review, it can be seen that a considerable amount of research work on studying the effects of various ingredients on the quality of frozen dough has been performed. However, there has been minimal research conducted to study the combined effects of various formulation parameters such as water content and emulsifiers (gum arabic, CMC, CSL, DATEM, PS80, and others) with processing parameters such as mixing rates, cooling rates, and frozen storage time on baking performance of wheat flour bread. The effects of combined formulation and processing parameters on the molecular and microstructural changes in dough can be studied using the help of fundamental rheology, ESEM, DSC, DMTA, and TD-NMR (Esselink and others 2003a).

## **2.25 Recent advances in frozen dough research**

In a recent study (Yi and Kerr 2009b), effects of freezing rate (19–69 °C/hr) and frozen storage (for up to 180 days) at four different temperatures (–10, –20, –30, and –35 °C) on dough quality were studied. It was observed using cryo-SEM that dough stored at

–30 and –35 °C, had the least damaged gluten network as well as less change in  $T_2$  values during the frozen storage time. In-addition, the dough strength decreased with longer frozen storage times and higher frozen storage temperature. Also, the loaf volume of bread decreased with longer frozen storage time. In another study (Yi and others 2009), a cultivar of waxy wheat flour (WWF) containing less than 2% amylase was studied. It was observed that with specific combinations of WWF and water, better quality of frozen dough and bread can be produced. NMR studies proved that the  $T_2$  values of the frozen dough with higher WWF were lower as compared to those with lower WWF values. Increasing WWF values resulted in fewer changes to dough properties after frozen storage. However, a higher value of WWF contributed to dough with higher stickiness and bread that was not firm enough. In another research work (Yi and Kerr 2009a), the combined effects of freezing rate, frozen storage temperature, and frozen storage time on dough were studied for up to 180 days of frozen storage time. It was observed that dough stored at lower freezing temperatures had lower loaf volumes. However, dough stored at lower freezing temperatures resulted in breads with lower staling rates. It was concluded that optimization of the freezing process to achieve improved bread quality is unique to each system and is affected by gluten quality, yeast viability, and heat transfer characteristics.

In another NMR study (Lopes-Da-Silva and others 2007), it was suggested that NMR can be used to study the molecular mobility properties, whereas, small-

deformation rheology can be used to study dough properties at supramolecular level. In a research work (Doona and Baik 2007) — that studied relaxation time distributions — the dynamic state and molecular mobility of water in various model systems were investigated using TD-NMR spectroscopy. The study aided in identifying the independent microstructural domains of water present in dough.

In a recent study (Carini and others 2009), relating the effects of shapes (achieved using extrusion, lamination, and lamination under vacuum) on physicochemical properties, it was noticed that the water activity, moisture content, and water mobility were slightly affected by shape of the product. However, cooking loss, tenacity, and extensibility were significantly ( $p$ -value  $< 0.05$ ) affected by shape of the product. In another research work (Serventi and others 2009), conducted to study the effects of formulation parameters on moisture content, water activity, and frozen water content of nutritionally enhanced tortillas (made using carrot, soy, whole meal and their combination) was conducted. It was observed that these ingredients altered the moisture content, water activity, and frozen water content of the tortillas and were dependent on its formulation. In another study (Kim and others 2008a), the effects of mixing and resting on rheology of hard wheat flour dough were studied. It was shown that the stress build up during mixing gradually declined during the 3 hr resting time and the first 30–45 min resting period resulted in structure reformation, probably due to re-polymerization (disulphide bond formation) of the gluten polymer.



In a recent study (Lorenzo and others 2009), the effects of hydrocolloids — hydroxypropyl methylcellulose (HPMC) and mixtures of xanthan/guar and xanthan/HPMC gums — were tested on refrigerated and frozen non-fermented gluten-free dough. ESEM micrographs revealed a continuous matrix formed by hydrocolloid entanglements. It was observed in these micrographs that the starch granules were homogeneously distributed in the dough and acted as inactive fillers. In the study, dynamic oscillatory measurements along with puncture and elongation tests on the unbaked dough were conducted, to study the changes in rheological and textural properties of dough during refrigerated storage. An untrained tasting panel significantly preferred the product made with xanthan/HPMC dough (with a 74 out of 90 score) over the product made with a commercial gluten-free dough.

## **2.26 Full and fractional factorial design of experiments**

A full factorial design with “k” number of factors, and two-levels for each factor involves a  $2^k$  runs. Two-level design is the most popular as it is simple and economical for screening tests and is most useful as a directional tool towards a multilevel response surface design (NIST/SEMATECH 2009). With a full-factorial design, the number of runs increases exponentially as factors rises. Therefore, a fractional factorial design may be chosen if the number of factors increases. In general with two-level designs a fraction  $\frac{1}{2}$ ,  $\frac{1}{4}$ , ..., or  $\frac{1}{p}$  is picked and therefore the design is represented by  $2^{k-1}$ ,  $2^{k-2}$ , ..., or  $2^{k-p}$ .

A well chosen two-level fractional factorial design has the properties of being both balanced and orthogonal. In fractional factorial design, a two-level can be combined with 3 level designs and is known as mixed-level design. Similarly, other mixed-level designs which add 4-level to two-level designs can also be produced in a fractional factorial design (NIST/SEMATECH 2009). In current study a full factorial design of experiments in choose to study all possible interactions among formulation and processing parameters.

## 2.27 Abbreviations

|          |  |
|----------|--|
| BSV      | Baked specific volume                          |
| cryo-SEM | Cryogenic scanning electron microscope         |
| CMC      | Carboxymethyl cellulose                        |
| CP       | Carr-Purcell                                   |
| CPMG     | Carr-Purcell-Meiboom-Gill                      |
| CR0      | Still air cooling rate                         |
| CR1      | Forced air cooling rate                        |
| CSL      | Calcium stearoyl-2-lactylate                   |
| DATEM    | Diacetyl-tartaric acid ester of monoglycerides |
| DMA      | Dynamic mechanical analysis                    |
| DMTA     | Dynamic mechanical thermal analysis            |
| DSC      | Differential scanning calorimetry              |
| DTA      | Differential thermal analysis                  |
| ESEM     | Environmental scanning electron microscope     |
| FID      | Free induction decay                           |
| FIS      | Free induction signal                          |
| GA       | Gum arabic                                     |
| GMS      | Glyceryl monostearate                          |
| GSED     | Gaseous secondary electron detector            |
| HPMC     | Hydroxypropyl methylcellulose                  |

|         |  |
|---------|--|
| IQF     | Individually quick frozen                        |
| IR      | Inversion recovery                               |
| LF-NMR  | Low-field nuclear magnetic resonance             |
| LT-SEM  | Low-temperature scanning electron microscope     |
| LV-SEM  | Low-vacuum scanning electron microscope          |
| LVR     | Linear viscoelastic region                       |
| mWPC    | Modified whey protein concentrates               |
| MRI     | Magnetic resonance imaging                       |
| NMR     | Nuclear magnetic resonance                       |
| PDI     | Protein disulphide isomerase                     |
| PFG-NMR | Pulsed field gradient nuclear magnetic resonance |
| PS80    | Polysorbate 80 or Tween 80                       |
| RF      | Radio frequency                                  |
| -S-S-   | disulphide bond                                  |
| -SH     | sulfhydryl or thiol group                        |
| SEM     | Scanning electron microscope                     |
| SFC     | Sold fat content                                 |
| SSL     | Sodium stearyl lactylate                         |
| TD-NMR  | Time-domain nuclear magnetic resonance           |
| TGase   | Transglutaminase                                 |
| TPA     | Textural profile analysis                        |
| VP-SEM  | Variable pressure scanning electron microscope   |

|     |                         |
|-----|-------------------------|
| VT  | Variable temperature    |
| w/w | Weight per weight basis |
| WWF | Waxy wheat flour        |

## 2.28 Symbols

|                                 |  |
|---------------------------------|--|
| $\sigma$                        | Surface energy (J/m <sup>2</sup> ) or Stress (Pa)                                |
| $\sigma_0$                      | Amplitude of the stress input (Pa)   |
| $\hbar$                         | Reduced Planck's constant ( $1.054 \times 10^{-34}$ J.s/rad)                     |
| $\eta'$                         | Dynamic viscosity (Pa.s)   |
| $\tau, \delta, \text{ or } t_2$ | Time (s or ms)   |
| $\delta$                        | phase angle or phase lag (rad or °)  |
| $\gamma$                        | Gyromagnetic ratio (rad/T.s) or Strain (no units)                                |
| $\dot{\gamma}$                  | Strain rate (s <sup>-1</sup> )   |
| $\gamma_0$                      | Strain amplitude (no units)  |
| $\mu$                           | Magnetic dipole moment (J/T)   |
| $\omega$                        | Angular, electromagnetic, or resonance frequency (rad/s)                         |
| $\Delta\omega$                  | Change in resonance frequency (rad/s)  |
| $\omega_0$                      | Angular frequency of precession of proton (rad/s)                                |
| $\Delta$                        | Gradient separation time (ms)  |
| $\nu_0$                         | Larmor frequency (MHz)   |
| $\epsilon_0$                    | Strain amplitude (no units)  |
| $a_w$                           | Water activity   |
| $h$                             | Planck's constant ( $6.626 \times 10^{-34}$ J.s or $4.135 \times 10^{-15}$ eV.s) |
| $k$                             | Boltzmann's constant ( $1.3805 \times 10^{-23}$ J/°K)                            |

|  |   |
|--|---|
| $m_s$  | Projection quantum number                               |
| $\mathbf{p}$                                 | Spin angular moment (J.s/rad or eV.s/rad)               |
| $q$  | Electric charge (C)                                     |
| $t_1$  | Pre-gradient time (ms)                                  |
| $\mathbf{v}$                                 | Velocity of the electric charge (m/s)                   |
| $A$  | Amplitude of spin echo (arbitrary unit)                 |
| $B$  | Resistance force to stretching in an extensogram (N)    |
| $\mathbf{B}_0, \mathbf{B}_1$ or $\mathbf{H}$ | Magnetic field (N.s/C.m or T)                           |
| $\Delta B$                                   | Change in magnetic field (N.s/C.m or T)                 |
| $C$  | Extensibility in an extensogram (m)                     |
| $D$  | Translational or self-diffusion coefficient ( $m^2/s$ ) |
| $E^*$  | Complex dynamic mechanical tensile modulus (Pa)         |
| $E'$   | Storage or elastic modulus (Pa)                         |
| $E''$  | Loss or viscous modulus (Pa)                            |
| $\mathbf{E}$                                 | Electric field (N/C or V/m)                             |
| $\Delta E$                                   | Energy difference (J)                                   |
| $\mathbf{F}$                                 | Force (N)   |
| $G$  | Magnetic field gradient (T/m)                           |
| $G_v$  | Gibbs free energy ( $J/m^3$ )                           |
| $G'$   | Storage or elastic modulus (Pa)                         |
| $G''$  | Loss or viscous modulus (Pa)                            |
| $G^*$  | Complex modulus (Pa)                                    |

|  |  |
|--|--|
| $\Delta H$   | Change in enthalpy (J)                             |
| <b>I</b>   | Spin quantum number or intrinsic spin (no units)   |
| <b>L</b>   | Distance at rupture in an alveogram (m)            |
| <b>L</b>   | Torque (N.m or J)                                  |
| <b>M<sub>0</sub>, M<sub>z</sub>, or M<sub>xy</sub></b> | Net magnetization vector (T)                       |
| <b>N<sub>U</sub></b>                                   | Upper energy states population number              |
| <b>N<sub>L</sub></b>                                   | Lower energy states population number              |
| <b>P</b>   | Overpressure in an alveogram (Pa)                  |
| <b>T</b>   | Temperature (°K)                                   |
| <b>T<sub>1</sub></b>                                   | Spin-lattice (or longitudinal) relaxation time (s) |
| <b>T<sub>2</sub></b>                                   | Spin-spin (or transverse) relaxation time (s)      |
| <b>T<sub>g</sub></b>                                   | Glass transition temperature (°C)                  |



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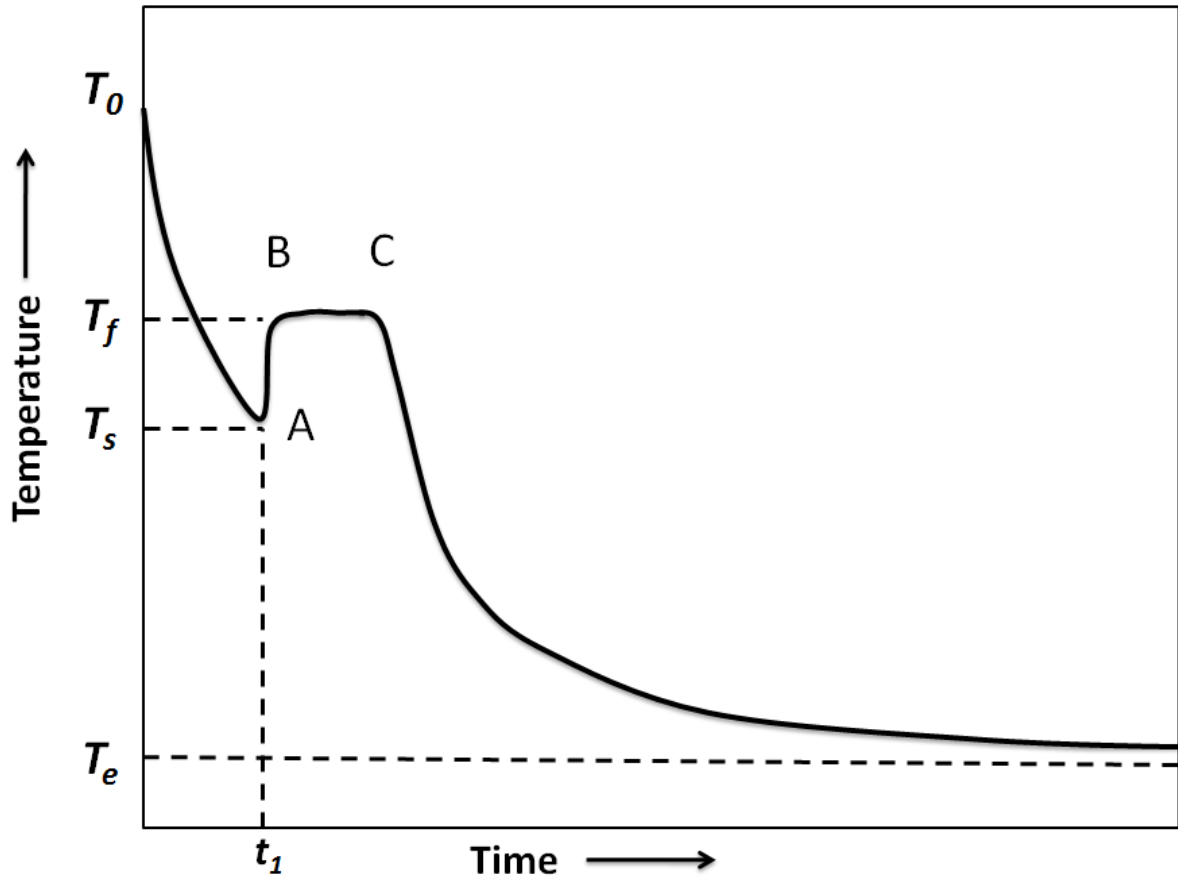
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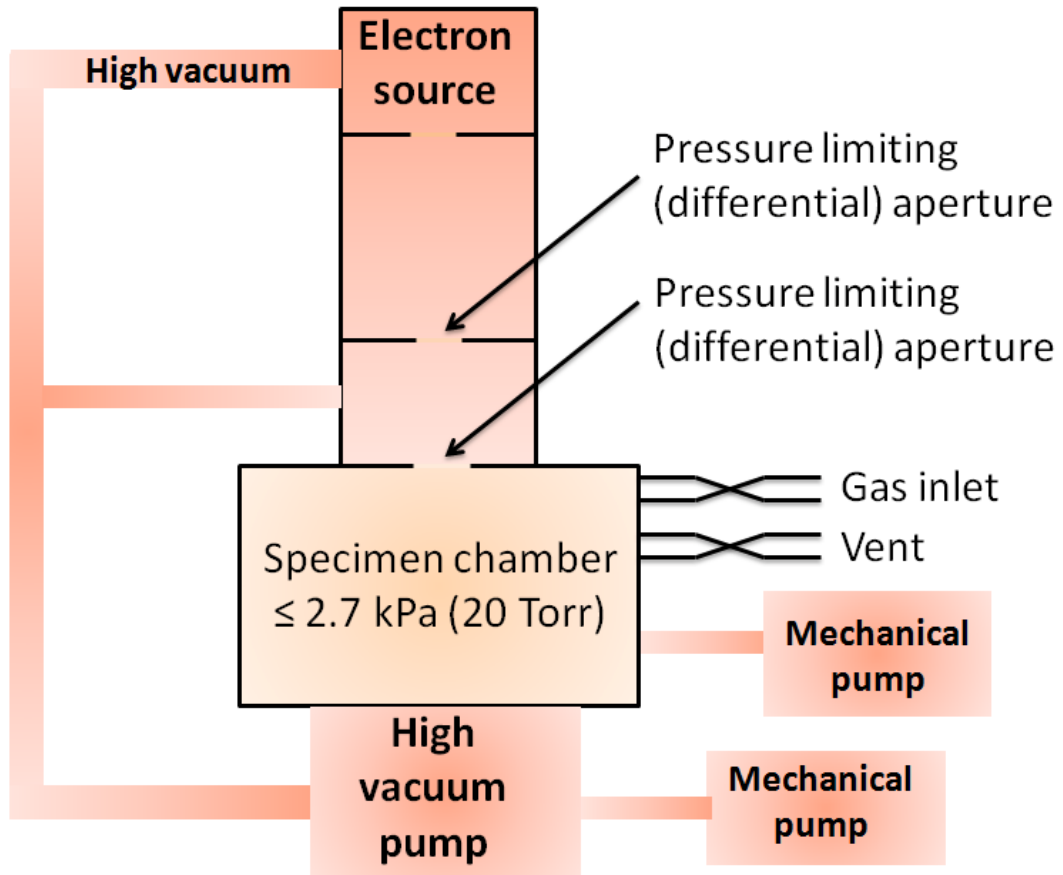
**Table 2-1** Typical values of parameters used for the measurement of water diffusion coefficient in pulse field gradient (PFG) spin-echo experiment

| <b>Time Constant</b> | <b>Description</b>       | <b>Typical value (ms)</b> |
|----------------------|--------------------------|---------------------------|
| $t_1$                | Pre gradient time        | 0.1                       |
| $\delta$             | Gradient on time         | 0.5-3                     |
| $\Delta$             | Gradient separation time | 20                        |
| $\tau$               | Inter p90 time           | 7                         |

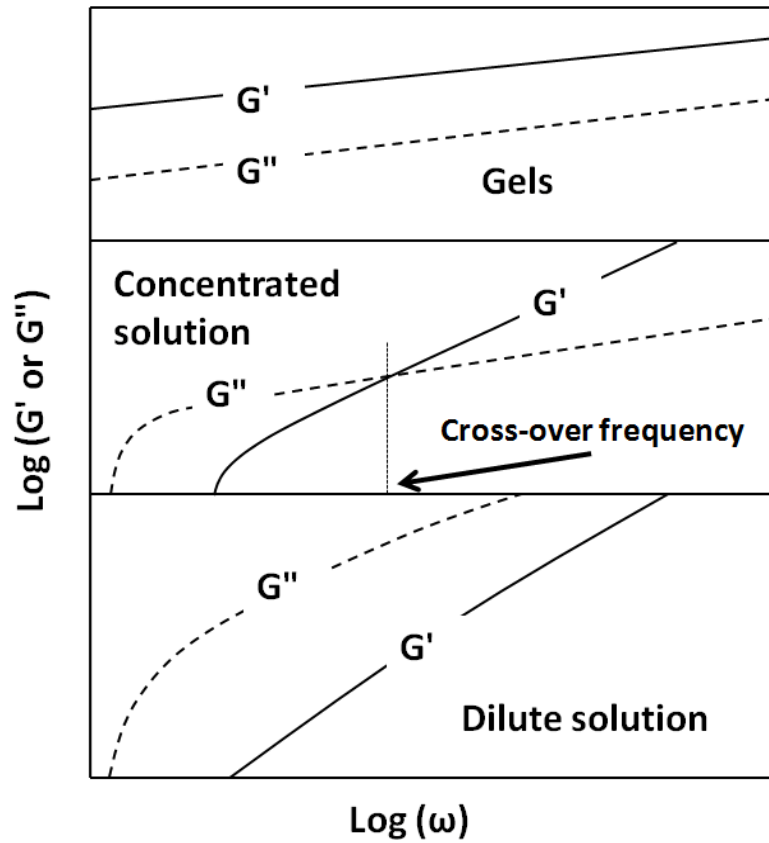


**Figure 2-1** A schematic plot of temperatures in food during freezing, showing the starting temperature,  $T_0$ , the initial freezing temperature,  $T_f$ , the temperature to which the food may supercool,  $T_s$ , the freezing plateau B-C and the equilibrium temperature,  $T_e$ . Adapted from Nesvadba (2009)

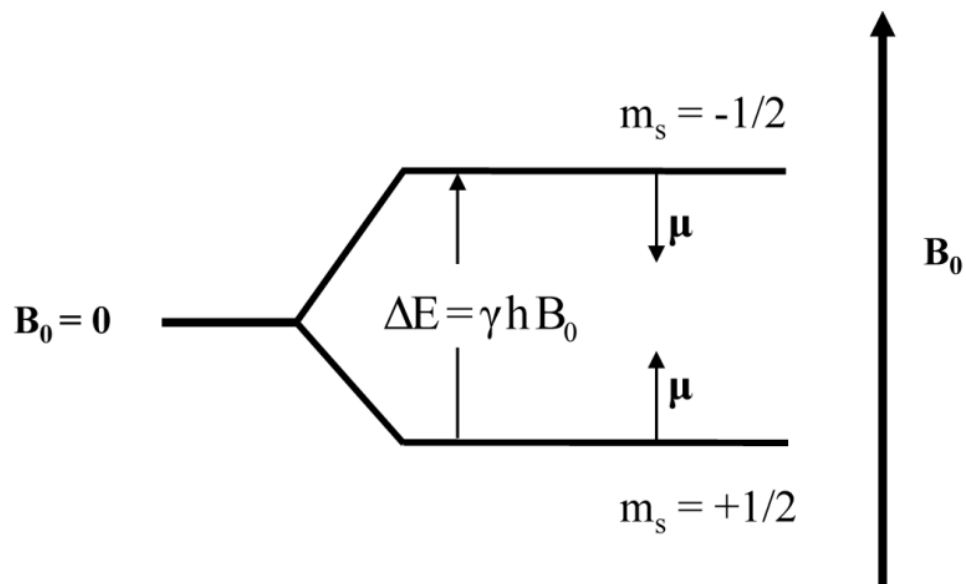




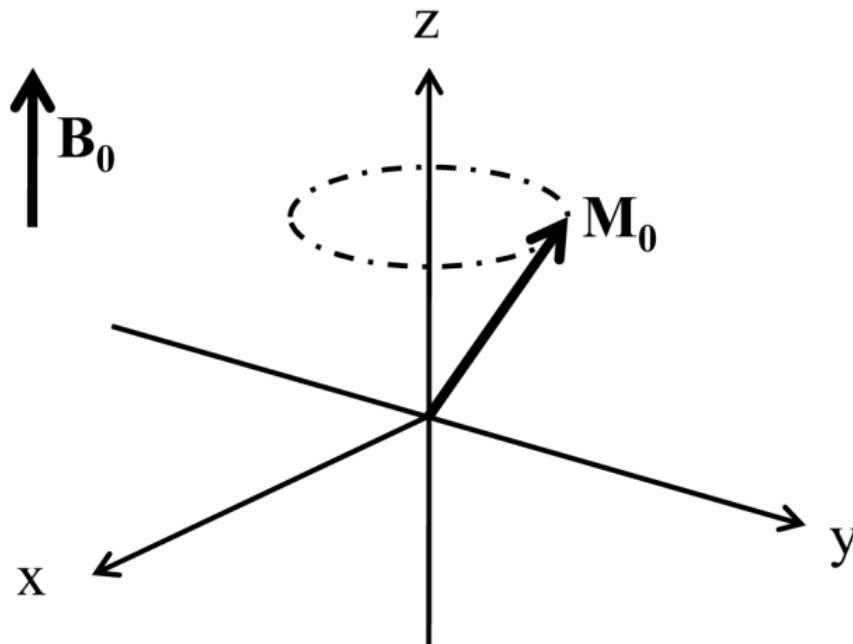
**Figure 2-2** Simplified schematic diagram to show the arrangement of zones and pressure-limiting (differential) apertures of a typical VP-SEM or ESEM. Adapted from Stokes (2008)



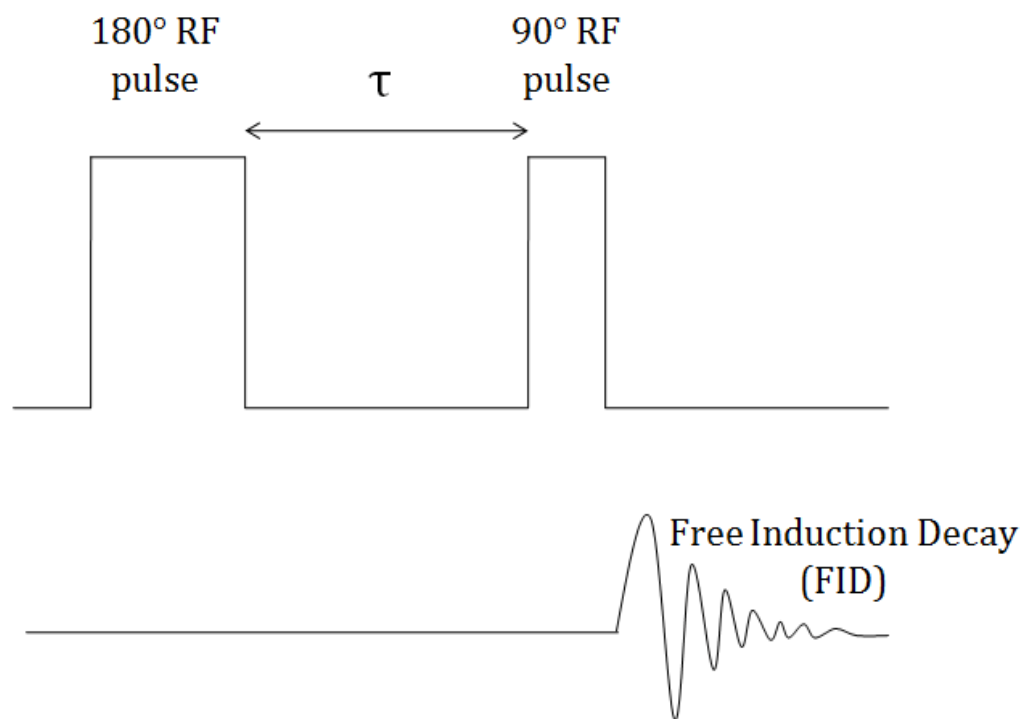
**Figure 2-3** Frequency sweep for gels, concentrated solutions, and dilute solutions.  $G'$  (Pa) is the storage or elastic modulus and  $G''$  (Pa) is the loss or viscous modulus.  $\omega$  is the frequency (may vary between 0.001–100 rad/s) at which measurements are conducted. Adapted from Zhong and Daubert (2007)



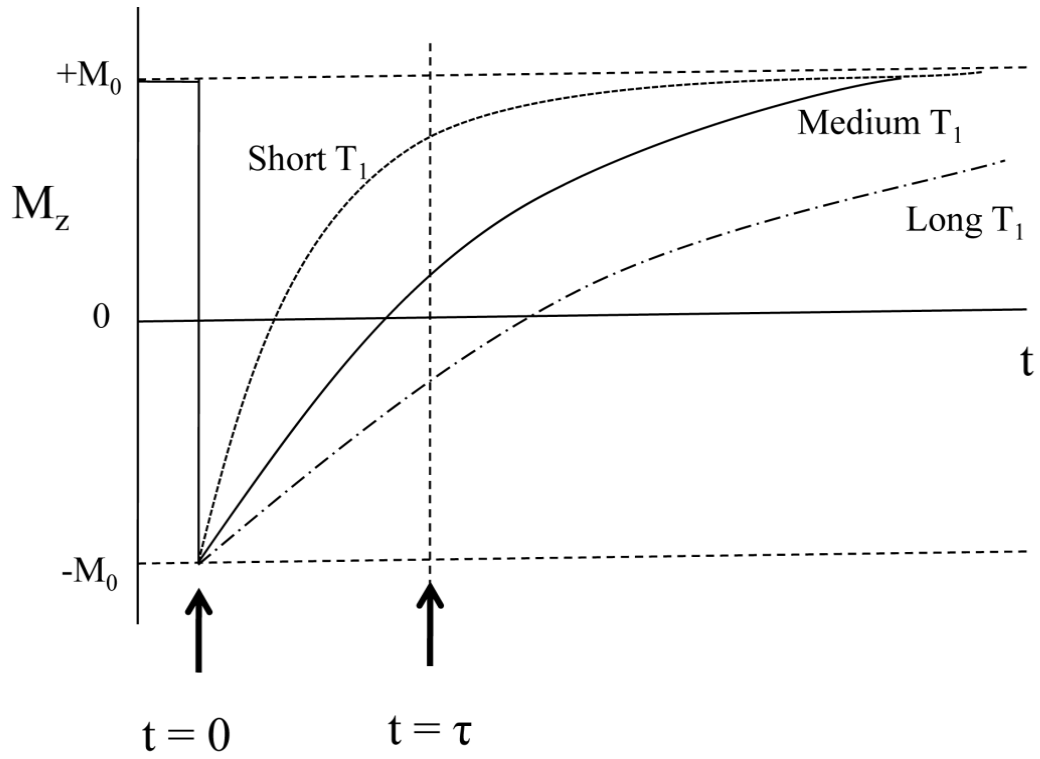
**Figure 2-4** Energy level diagram for  $I = 1/2$  system



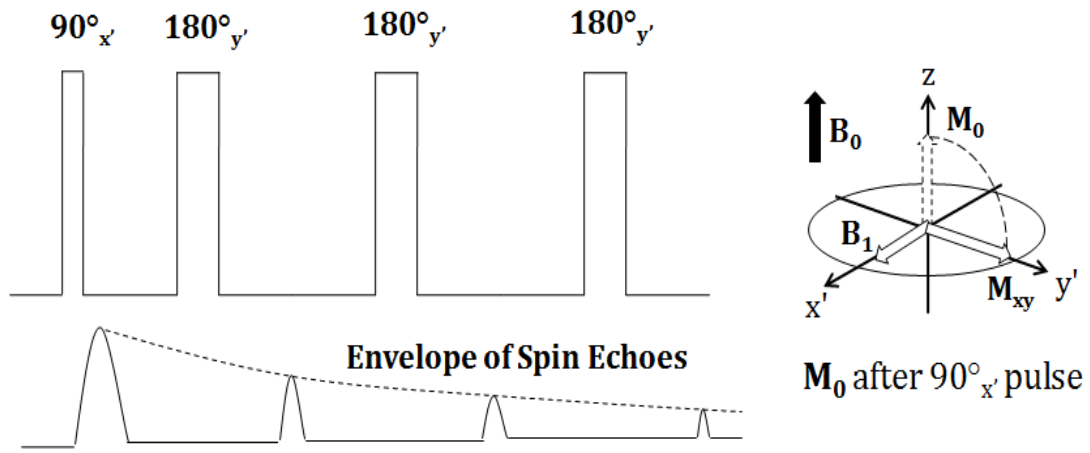
**Figure 2-5** Precession of net magnetization vector  $M_0$  around magnetic field  $B_0$



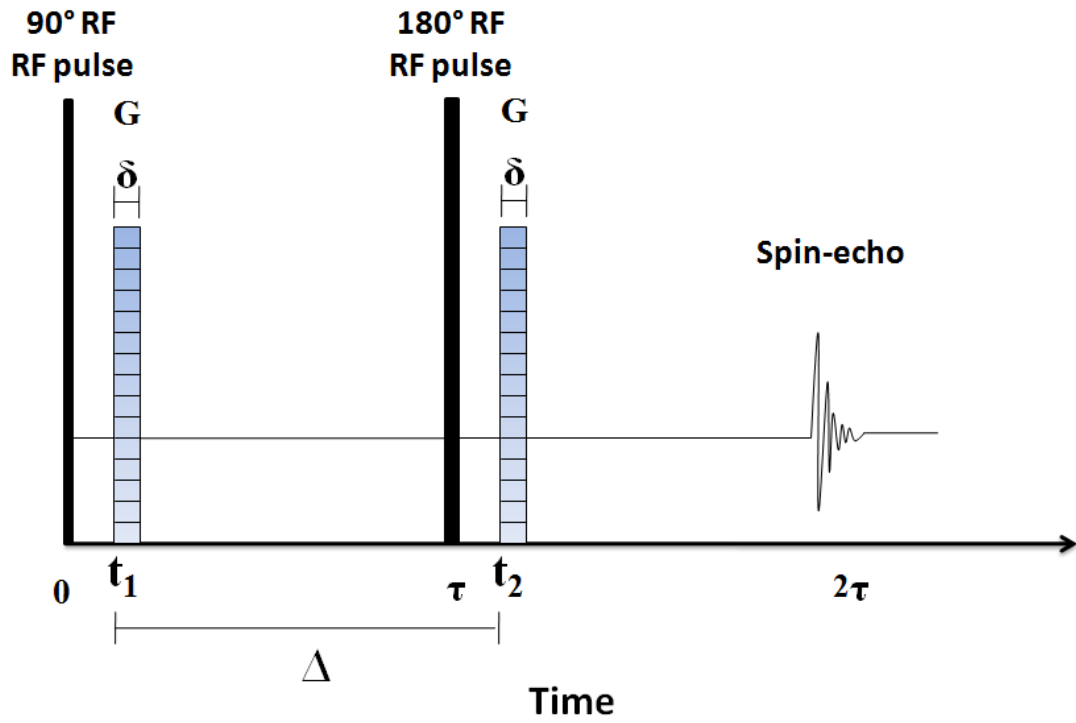
**Figure 2-6** Basic inversion recovery pulse where time  $\tau$  is longer than either of the 180° and 90° pulse (pulse lengths not to scale). Adapted from Seton (2003)



**Figure 2-7**  $T_1$  determination using inversion recovery (IR) pulse sequence for three different kinds of samples. Adapted from Seton (2003)



**Figure 2-8** Multiple echoes generated by the CPMG pulse sequence. Adapted from Seton (2003)



**Figure 2-9** The pulse field gradient NMR (PFG-NMR) spin-echo experiment to determine the self-diffusion coefficient ( $D$ ) of water in dough. Adapted from Ruan and Chen (1998a)



## Chapter 3

### **Project Objectives**

Bread making involves a series of aeration steps in which air bubbles are incorporated during mixing the dough, inflated by carbon dioxide during proofing, and the aerated structure set by baking. There is a substantial difference between the qualities of final baked product made using fresh dough as compared to that made using frozen-thawed dough. From literature, a comprehensive study involving correlation of dough formulation and processing parameters to quality parameters of frozen dough and baked product was never conducted. Therefore, the current research work performed such a comprehensive study which would aid the understanding of the effects of dough formulation and processing on quality of final baked product. This would enable processors to possibly achieve the same quality of bread using fresh and frozen-thawed dough. Techniques such as rheology, TD-NMR, and ESEM will be used to characterize the micro-structure, physiochemical properties, and redistribution of water in the dough during frozen storage. These characteristic properties of the dough depend on the dough formulation and processing parameters such as dough recipe, cooling rate, and frozen storage temperature. The importance of these characteristic properties in the quality attributes of the final baked product will be studied by relating formulation

and processing parameters of dough to quality parameters of a baked product such as BSV, textural properties (TPA and puncture tests using Universal Testing Machine).

The second goal of the current research was to understand and correlate the changes in the macroscopic characteristic properties of fresh and frozen wheat dough, such as, redistribution of water from liquid to solid phase by analyzing NMR relaxometric parameters such as  $T_1$  and  $T_2$  distribution and self-diffusion coefficient of water as a function of formulation and processing parameters.

The third and final goal of the current research was to study the effect of dough ingredients (such as GA, CSL, and DATEM) on quality parameters such as BSV and textural properties of the final baked product. This will aid the understanding of the effect of levels of ingredients on the quality of baked product. Therefore, the main objectives of current research work were as follows:

- 1) Study the effects of formulation and processing parameters such as dough recipe, cooling rate, and frozen storage time on characteristic properties such as the micro-structure, water states, and rheological properties in the dough using ESEM, TD-NMR, and dynamic rheology. These formulation parameters will then be correlated to the BSV and textural properties of the final baked product.
- 2) Correlate the quantitative changes in distribution of water states of frozen-thawed wheat dough by analyzing NMR relaxometric parameters such  $T_2$  distribution and self-diffusion coefficient of water along with the formulation

and processing parameters to the BSV (and other quality parameters of baked product) of final baked product.

- 3) Study the possibility of using functional ingredients (such as GA, CSL, and DATEM) in bringing the final baked product quality (in terms of BSV and other baked product quality parameters) made using frozen-thawed dough close to that made from fresh dough.

## Chapter 4

# **MANUSCRIPT I: Effects of Functional Ingredients, Cooling Rates, and Frozen Storage Time on Quality of Frozen Wheat Dough: Water State, Microstructure, and Dynamic Rheology Studies**

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## 4.1 Abstract

Effects of frozen storage time, cooling rates, and additions of gum arabic (GA) and two emulsifiers — calcium stearoyl-2-lactylate (CSL) and diacetyl-tartaric acid ester of monoglycerides (DATEM) — on the quality of wheat bread dough was studied. Time domain nuclear magnetic resonance (TD-NMR), environmental scanning electron microscope (ESEM), and dynamic rheology (0.002–20 Hz frequency sweep) were used to characterize the redistribution of water, microstructure, and physiochemical properties of dough during frozen storage from 0–26 weeks. The ESEM images were obtained at  $-10\text{ }^{\circ}\text{C}$  and at a relative humidity of 100 % to study the microstructure in frozen dough. A higher cooling rate increased the number of protons and the distribution of  $T_2$  for population 2 (5–30 ms in  $T_2$  distribution). The formulation with addition of only DATEM at 0.6 % w/w showed a significant increase in storage modulus ( $G'$ ) and loss modulus ( $G''$ ) values for the higher cooling rate for the two extreme frequency conditions of 0.002 Hz and 1.62 Hz. This study may aid in understanding and optimizing the important processing and formulation parameters associated with industrial production of frozen dough to obtain baked products with desired quality attributes.

## 4.2 Introduction

Bread is probably the oldest “processed” food. The bread making process involves converting wheat flour, water, yeast, shortening, salt, and other ingredients into a light, aerated and palatable food (Bot and de Bruijne 2003; Cauvain 2001). In recent decades, due to high competitiveness in the bakery industry — which is aggressively pursuing cost efficiencies in face of society’s evolving demographic and economic constraints — there has been a centralization of dough manufacturing facilities and retail distribution (Best 1995). Therefore, dough is produced, frozen, and shipped to retail stores and bakeries for the manufacturing of final baked product (Frank 2008). Frozen dough is used in more than 50 % of in-store supermarket bakeries as well as by retail customers (Berglund et al 1991). A substantial difference exists between the qualities of final baked product made by fresh dough as compared to that by frozen dough. A freshly baked product (made using either fresh or frozen dough) starts to deteriorate soon after it is baked and a substantial difference in the crumb firmness is found within first 24 hrs of baking (Gray and Bemiller 2003; Schiraldi and Fessas 2001). Therefore, freezing the dough aids in prolonging its shelf-life. However, one of the major shortcomings is the 6–8 weeks shelf-life achieved by frozen dough (Berglund et al 1991). The quality of a freshly baked product made using frozen dough decreases with increasing frozen storage time of dough (Bot 2003; Rosell and Gómez 2007). With prolonged frozen storage, water redistributes itself within the dough, which results in a

change in the water binding capacity of dough constituents (MacRitchie 1976; Räsänen et al 1997). Freezing rates, frozen storage time, and frozen storage temperatures also affect the ice crystal growth within the frozen foods (Petzold and Aguilera 2009). Limited re-absorption of water on thawing, resulting in 'drip loss', is another reason for quality changes in frozen foods (Nesvadba 2009). In-addition, there is a loss of quality in baked product made using frozen dough due to inadequate formation of carbon dioxide during proofing since frozen storage adversely affects yeast viability and activity. The aforementioned factors regarding frozen dough leads to longer proofing times, lower loaf volumes, and undesired changes in textural properties of final baked product.

In some studies (Esselink et al 2003a, b), the underlying mechanisms of molecular, microstructural, and macroscopic changes during frozen storage were investigated using cryogenic scanning electron microscope (cryo-SEM) and time domain nuclear magnetic resonance (TD-NMR) for frozen dough stored at  $-20^{\circ}\text{C}$ . TD-NMR was used to non-invasively study the mobility of water in different states namely: rigid-state (primarily in starch particles), bound-state (water bound to the surface of starch and gluten matrix), and free-state (most mobile) in thawed dough. The mobility of water in dough is an important parameter because it relates directly to the quality of the gluten network. Approximately 46 % of water in dough is associated with starch, 31 % with protein, and 23 % with pentosan gum. These values are significantly influenced

by the amount of protein and starch damaged during the milling process (Bushuk 1966). Since water interacts with dough, the thermal and physicochemical properties of water associated with dough vary from that of bulk water. For example, the freezing point of water in a dough system decreases and even after long frozen storage times, not all of the water is frozen (Bushuk and Mehrotra 1977). Therefore, water in the dough is not only distributed among the different components in dough, but is present in different states (Ruan et al 1999). TD-NMR is used to study the spin-lattice relaxation time (also known as the longitudinal relaxation time —  $T_1$ ) and spin-spin relaxation time (also known as the transverse relaxation times —  $T_2$ ) of protons which are characteristic of the molecular mobility or the state of water in a dough system (Leung et al 1979; Ruan et al 1999). In the food industry, some applications of TD-NMR — also known as low-field nuclear magnetic resonance (LF-NMR) — are in determination of the solid fat content (SFC) of oils and fats, determination of water and fat content, droplet size distribution, and mobility of water in food systems (Assifaoui et al 2006a, b; Bertram et al 2002; Doona and Baik 2007; Hinrichs et al 2004; Li et al 2000; Lopes-Da-Silva et al 2007; Padua et al 1991; Todt et al 2006). Staling of bread has also been studied using TD-NMR and it has been shown that the  $T_1$  and  $T_2$  values decreased with increased staling and was independent of the moisture content of the bread (Leung et al 1979). This clearly suggests that relaxation is largely structure-dependent and that physiochemical changes would cause relaxation times to vary, mostly because of the



alteration in the morphology of the food system. The relaxation measurements serve as a useful tool to probe the morphology and the state of the biopolymers or other species having exchangeable protons (Hills 2006). In other studies (Haiduc and van Duynhoven 2005), TD-NMR has been used to correlate porous and functional properties of food materials using multivariate analysis. Water states in macromolecule solutions and gels can be studied using self-diffusion coefficient measurements of water. This can be achieved by means of the pulsed field gradient nuclear magnetic resonance (PFG-NMR) technique. The technique has been used to study mobility of water in different food products such as casein solutions, gels, and cheese (Ghi et al 2002; Godefroy and Callaghan 2003; Kuo et al 2003; Kuo et al 2001; Naji et al 2003). In-addition, it has been used to determine the effects of casein and fat content on diffusion of water in model casein systems (Métais et al 2004).

Zounis and others (2002a; 2002b) studied frozen dough structure using cryogenic low temperature scanning electron microscope (LT-SEM). It was shown that LT-SEM proved to be a useful tool for studying the effects of final mixing temperature on the structural stability of a frozen dough system. However, these studies also suggest that due to lowering of temperature in a LT-SEM, the observations were undesirably subjective (due to freezing at very low temperatures which alters the temperature, morphology, and surface characteristics of frozen dough), and it would be better to make observations of a fractured frozen dough surface at practical freezing

temperatures (used in industry) and without altering its natural state. To use a conventional scanning electron microscope (SEM), biological samples must be dried, coated, or cryogenically frozen to prepare the sample for observation. The metallic coating on biological specimens gives only topographic contrast — due to the short escape depths of secondary electrons from metals — and therefore, valuable compositional contrast from the underlying specimen is lost (Donald 2003; Stokes 2001). These requirements make SEM or LT-SEM unsuitable for study of relatively wide range of biological materials (Bache et al 2000). To avoid drying, coating, or cryogenic freezing of biological specimens, an environmental scanning electron microscope (ESEM)—where low-pressure and high relative humidity environment is created instead of vacuum (as in SEM or LT-SEM)—can be used (McDonough et al 1996; McDonough and Rooney 1999; Stokes and Donald 2000; Stokes et al 2002). In a study (Bache and Donald 1998) using ESEM, it was shown that at the mesoscopic level (between microscopic and macroscopic levels), gluten forms sheets as opposed to fibers, with the network existing at a much shorter-length scale at the molecular level. In a recent study (Lorenzo et al 2009), micrographs of non-fermented gluten-free dough obtained using an ESEM have shown a continuous matrix formed by hydrocolloid entanglements with starch granules continuously dispersed as inactive fillers.

Oscillatory rheology is a valuable technique that gives a quantitative measure of the amount of stress in the dough and this is related to the viscoelastic properties of the gluten network. Studies relating dough extensibility and resistance to stress are important for evaluating properties of dough since they influence the quality attributes such as baked specific volume (BSV) of the baked product. In a study by Miller and Hosney (1999), dynamic rheological properties of reconstituted flour containing starch, gluten, and various amounts of freeze-dried water-solubles were determined. It was found that both gluten and freeze-dried water-solubles affected the dynamic rheological properties of dough. In-addition, the doughs made from strong flours (high protein content) had lower  $\tan \delta$  values,  $\delta$  being the phase angle, than doughs made with medium or weak flours (low protein content). In an another study (Newberry et al 2002) conducted to understand the dynamic rheology of yeasted bread dough, it was revealed that fermentation had a more complex influence on polymeric dough composition (determined using size-exclusion *high-performance liquid chromatography*) and on its rheological properties that was not observed in previous studies using non-yeasted doughs. In an earlier study (Lu and Grant 1999), effects of prolonged frozen storage on the gelatinization properties of starches and baking quality of yeasted doughs were studied. This study indicated that storage modulus ( $G'$  which is measure of elasticity) decreased for all cultivars except one for the entire 16 weeks of frozen storage. In a recent study (Leray et al 2010) it was observed that rheological

properties of non-yeasted wheat dough were most altered during the first few days whereas rheological properties of non-yeasted gluten-free were most altered only during longer storage times. A similar observation was made in a study (Angioloni et al 2008) where it was shown that the effects of freezing on viscoelastic properties of frozen dough were more concentrated during the first 15 days of freezing.

The effects of various ingredients including dairy products, hydrocolloids (particles less than 1  $\mu\text{m}$  in size dispersed and soluble in water), and emulsifiers has been studied for their effect on increasing the shelf-life and stability of dough to produce a final baked product with similar qualities as that produced using fresh dough have been studied. Wolt and D'Appolonia (1984a, b) studied the influence of emulsifiers—sodium stearoyl lactylate (SSL) and diacetyl tartaric acid—on frozen dough stability. These ingredients decreased the effects of frozen storage on rheological properties, but they were ineffective in reducing the proofing time of dough. Ribotta and others (2001) concluded that frozen dough supplemented with diacetyl-tartaric acid ester of monoglycerides (DATEM), gluten, and guar gum resulted in bread with greater volume and more open crumb structure as compared to that using a basic formulation. In some studies (Asghar et al 2009; Asghar et al 2006; Asghar et al 2007; Lorenzo et al 2009; Selomulyo and Zhou 2007; Wang et al 2006), the functionality of different additives such as surfactants, hydrocolloids, modified whey protein concentrates (mWPC), ascorbic acid, honey, green tea extract, and gum additives to

improve the quality of frozen dough was investigated. It was observed that addition of mWPC significantly decreased the values of hardness, cohesiveness, gumminess, and springiness in baked product. Ribotta et al (2004) studied the effect of DATEM and guar gum on micro structural, rheological, and baking performance of frozen bread dough. It was observed that DATEM and gum guar improved the volume and texture of bread obtained from non-frozen and frozen dough. However, neither DATEM nor gum guar could prevent the detrimental effects of frozen storage on the dynamic rheological parameters and microstructure of dough. In other studies (Matuda et al 2008; Matuda et al 2005; Matuda et al 2006), the effect of vegetable shortening, calcium stearoyl-2-lactylate (CSL), guar gum, xanthan gums, and polysorbate 80 (PS80 or Tween 80) on unfrozen water, proofing, and textural properties of French frozen dough were studied. In these studies, the amount of unfrozen water was 0.30–0.34 g H<sub>2</sub>O/g solids and the additives used during the storage for up to 56 days, significantly affected the textural properties of frozen dough.

Bread is a basic food and bread baking is a handicraft with long tradition. However, due to mechanization, large scale production, and increase in demand for higher quality, convenience, and longer shelf-life for bread, a need for functional food additives such as gums and emulsifiers has been created (Stampfli and Nersten 1995). Gum arabic or GA (a tree exudate) is an amphiphilic compound that improves the water holding capacity of dough. CSL (manufactured by the reaction of stearic acid with lactic

acid and its conversion to a calcium salt) improves the mixing and gas holding capacity of dough. DATEM acts as a dough conditioner to strengthen the gluten and this increases the softness of crumb and volume of bread. In some studies, effects of emulsifiers and enzymes such as DATEM, sodium stearoyl lactylate (SSL), glyceryl monostearate (GMS), and transglutaminase (TGase) on the quality of dough products (using microscopy, dynamic rheology, baked specific volume measurements, and determination of crumb firmness) were investigated (Akdogan et al 2006; Kim et al 2008; Xiujin et al 2007). It was observed that SSL, GMS, and lecithin were most effective at different levels of concentration in determining textural properties of whole wheat tortillas. TGase significantly improved BSV and hardness for bread made using frozen dough but was detrimental to bread made using fresh dough. Effects of hydrophilic gums on quality of frozen dough have been studied by Sharadanant and Khan (2003a, b, 2006). It was observed in these studies that locust bean gum (LBG), GA, and carboxymethyl cellulose (CMC) improved bread characteristics to varying degrees and only  $\kappa$ -carrageenan showed a detrimental effect on frozen dough. It was also observed that frozen dough with LBG and GA showed better retention of the gluten network during frozen storage as compared to frozen dough without LBG or GA. In other studies (Baik and Chinachoti 2000; Kim-Shin et al 1991; Li et al 1998; Lin et al 2001; Vodovotz and Chinachoti 1998), the redistribution, state, and mobility of water in dough have been studied using nuclear magnetic resonance (NMR), by studying relaxation rates or

relaxation times, differential scanning calorimetry (DSC), or dynamic mechanical analysis (DMA). Self-diffusion coefficient of water and staling in white bread was studied using PFG-NMR by Baik and Chinachoti (2003). It was observed that the water in bread crumb had an adverse effect on the amorphous structural components and therefore caused more rapid firming despite less amylopectin recrystallization. It has been indicated in recent studies (Hagiwara et al 2006; Petzold and Aguilera 2009) that self-diffusion coefficient of water in a freeze-concentrated matrix is a useful parameter for predicting and controlling the recrystallization rate.

From the literature, it can be seen that a considerable amount of research work has been conducted separately on studying the effect of various ingredients and processing parameters on the quality of the frozen dough. However, there are relatively few studies devoted to understanding the combined effect of various functional ingredients (such as gums and emulsifiers) and processing parameters on the quality of wheat bread dough. In-addition, only a handful of studies regarding the dynamic rheological properties of yeasted dough has been conducted (Newberry et al 2002) and no studies exist for a frozen storage time of up to 26 weeks. Therefore, the objective of this study was to understand the combined effects of different dough formulations (obtained by varying concentration of GA, CSL, and DATEM) and processing parameters (such as cooling rates and frozen storage time) on quality of frozen dough. Techniques such as TD-NMR (studying relaxation time constants and self-diffusion coefficient of

water), ESEM, and dynamic rheology were used to study the quality of wheat dough by characterizing the redistribution of water, microstructure, and viscoelastic properties of the dough during frozen storage.

### **4.3 Materials and Methods**

Wheat bread dough was prepared using different formulations and processing parameters and was analyzed using rheological, TD-NMR, and ESEM techniques to study the effect of functional ingredients, cooling rates, and frozen storage time on quality of wheat dough. Measurements on three replicate samples were used for all TD-NMR and rheological measurements and means with one standard error were reported in this study.

#### **4.3.1 Materials**

Commercial organic whole wheat flour (The King Arthur Flour Company, Inc., Norwich, VT) with 14.0 % w/w protein content and 1.5 % w/w ash was used to prepare the dough. Active dry yeast (Fleischmann's Yeast, Chesterfield, MO), vegetable oil (ConAgra Foods, Inc., Omaha, NE), and salt were used for all formulations. Active dry yeast was obtained from a single vendor and kept in air-tight containers until use. The hydrocolloid used for the current research work was GA (Colloides Naturels, Inc.,



Bridgewater, NJ). The emulsifiers used were CSL (Caravan Ingredients, Lenexa, KS) and DATEM (Danisco Inc., New Century, KS).

### **4.3.2 Design of experiments**

Design of experiments for the current study involved seven variables used for formulation and processing parameters. A full factorial design was used for the design of experiments involving the seven variables at levels listed in Table 4-1. Treatments obtained by the full factorial design were tested using TD-NMR, small amplitude oscillatory shear (SAOS), and/or ESEM. Figure 4-1 represents a flow diagram showing preparation of dough and the experimental techniques used. “Fresh dough” in this study implies dough samples that were never cooled or kept in frozen storage. Thus, fresh samples for all dough formulations were obtained after mixing followed by 10 minutes of fermentation at  $25 \pm 1$  °C and tested using TD-NMR and SAOS (Figure 4-1). These TD-NMR and SAOS measurements obtained using freshly prepared samples were used as control for comparing TD-NMR and SAOS measurements for rest of the treatments — all obtained by mixing dough, followed by fermentation for 10 mins at  $25 \pm 1$  °C, freezing at the desired cooling rate (CR0: Still air cooling or CR1: Forced air cooling), followed by frozen storage (0, 8, 16, or 26 weeks) and then thawing at 5 °C for 24 hrs. FT0 refers to freshly prepared dough samples that are cooled at the desired cooling rate of CR0 or CR1 until frozen to a stable internal temperature below  $-18$  °C in the freezer

(24 hrs of frozen storage resulted in a stable internal temperature of  $-18\text{ }^{\circ}\text{C}$ ) and then immediately thawed at  $5\text{ }^{\circ}\text{C}$  for 24 hrs. TD-NMR and SAOS measurements were then immediately performed after the thawing. FT0 treatments are referred to as “0 week” treatments. Dough without GA, CSL, or DATEM (Dough 1 in Table 4-2) was used as the control dough for comparison with the rest of the dough treatments. Triplicate measurements were performed for all TD-NMR and SAOS measurements. ESEM images for frozen dough (0, 8, 16, and 26 weeks frozen storage time) were imaged at magnifications of  $300\times$ ,  $1000\times$ ,  $1500\times$ ,  $2500\times$ , and  $5000\times$  to reveal the structure in the range of  $5\text{--}100\text{ }\mu\text{m}$ .

### **4.3.3 Dough formulation**

For all dough formulations, the ratio of wheat flour : yeast : oil : salt was 50:2:1:1. This ratio was calculated from the base recipe: whole wheat flour (4200 g, 61.35% w/w), yeast (168 g, 2.45% w/w), salt (84 g, 1.23% w/w), vegetable shortening (84 g, 1.23% w/w), and water (2310 g, 33.74 % w/w) as used by Zounis and others (2002a). Since we have two levels of two different emulsifiers (CSL and DATEM) and two levels of one hydrocolloid gum (GA), the full factorial design used in this study yielded eight dough formulations (dough 1–8) as presented in Table 4-2.

#### 4.3.4 Dough preparation and processing parameters

As indicated in Table 4-1, two processing variables were studied—cooling rate and frozen storage time. 500 g of dough was prepared for each of the eight treatments of dough formulations. A classic stand mixer (KitchenAid, K45SSWH 4.3L, St. Joseph, MI) with six speed levels (settings available were Stir, 2, 4, 6, 8, and 10) was used to mix the dough ingredients. A moderate mixing rate was achieved using a speed setting of #4 (planetary speed: 135 rpm and beater speed: 442 rpm). The mixer (bowl volume of 4½ quarts) had a dough hook that was used for mixing. Yeast is more susceptible to freeze damage with increase in fermentation/resting time and therefore, minimizing the fermentation time before freezing is important (Rosell and Gómez 2007). Therefore, starter samples were allowed to sit for only 10 min at  $25 \pm 1$  °C to minimize yeast activity before freezing (Dodić et al 2007).

A heavy duty commercial chest freezer (model # FFC13C3AW2, Electrolux Home Products, Inc., Cleveland, OH) with an air temperature of approximately  $-22$  °C was used for cooling dough. CR0 (still air cooling) for dough was achieved by keeping the dough inside the freezer (still air conditions). CR1 (forced air cooling) was achieved using a small table fan (8" high velocity personal fan, Wal-Mart Stores, Inc., Bentonville, AR) kept inside a separated chamber in the freezer. To achieve CR1, samples were kept at the centerline of air flow generated by the fan set at its highest speed. Air speed at the centerline of the fan at its highest speed setting was 0.1 m/s (determined using a digital

anemometer — model # 840002, Datalogging Anemometer Hotwire with CFM Air Flow Meter, Sper Scientific, Scottsdale, AZ). For frozen samples stored for 8, 16, and 26 weeks, the samples were cooled till the core reached  $-18\text{ }^{\circ}\text{C}$  and then immediately placed in a plastic zip lock freezer bag to prevent any moisture exchange between the frozen dough and the surroundings during frozen storage. Temperature at the center of sample was recorded for 20 hrs (enough time to freeze sample cores to  $-18\text{ }^{\circ}\text{C}$ ) to calculate the cooling rates — CR0 and CR1. Temperatures were recorded using a Digi-Sense Scanning Thermocouple Thermometer (Model # 92800-10, Cole-Parmer Instrument Co., Niles, IL) connected to 12" thermocouples (Model # TMQSS-062-12, Quick Disconnect Thermocouples, Omega Engineering, Inc., Stamford, CT). After the dough was stored for desired frozen storage time (0, 8, 16, or 26 weeks) and then thawed at  $5\text{ }^{\circ}\text{C}$  for 24 hrs, TD-NMR ( $T_1$ ,  $T_2$ , and self-diffusion coefficient —  $D$ ) and SAOS measurements were performed.

#### **4.3.5 TD-NMR instrument and cryostat setup**

A bench-top time-domain NMR instrument minispec mq-20 (Bruker Optics, Billerica, MA) operating at 20 MHz with a variable temperature gradient probe was used for all TD-NMR measurements (including determination of self-diffusion coefficient,  $D$ ). The variable temperature gradient probe was set at  $25\text{ }^{\circ}\text{C}$  for fresh samples and at  $5\text{ }^{\circ}\text{C}$  for frozen-thawed samples using a cryostat (Model # MD F32, Julabo USA, Inc., Allentown,

PA). A water and ethylene glycol mixture (to cool the variable temperature probehead) was circulated from the cryostat to the probehead dewar through a flexible Tygon<sup>®</sup> tubing by an inbuilt pump within the cryostat. The tubing was insulated and was long enough to allow the cryostat to be located at least 0.5 m from the magnet to minimize the effect of vibrations caused by the circulator (which can degrade the performance of the NMR instrument). To prevent interference of NMR signal from the protonic circulating fluid, the variable temperature dewar was shielded from the NMR coil. The normal operational temperature range for the variable temperature probehead was  $-5^{\circ}\text{C}$  to  $65^{\circ}\text{C}$ . Temperatures outside this operational range are not recommended since the minispec mq-20 may not be able to regulate the magnet temperature. After the cryostat and magnet temperatures were stabilized, the temperature attained by the cryostat was verified using a digital thermometer (Eutechnics Precision Temperature 4500 Thermometer, Alpha Technics, Irvine, CA) inserted into water kept in a 10 mm diameter tube placed in the NMR probe (placed in between the magnets). The temperature of the magnet in the NMR instrument was stabilized to  $40^{\circ}\text{C}$  before performing calibration checks using the “update settings” program. A “daily check” program using a “daily check sample” was run during each 24 hr period to ensure that the calibration settings remain validated. The  $90^{\circ}$  pulse and  $180^{\circ}$  pulse lengths obtained after calibration checks were  $5.76\ \mu\text{s}$  and  $11.78\ \mu\text{s}$  respectively. The receiver dead time obtained for the NMR probe was  $17.4\ \mu\text{s}$ . NMR profiling was performed to determine

the optimum filling height (1 cm) for maximum NMR visibility of the dough samples in the tubes. 10 mm glass tubes were weighed before and after filling them with dough samples to determine the exact weight of dough in the tube, so that NMR signals used for determining  $T_2$  distribution can be weight normalized. These optimally filled tubes were tightly covered using a Teflon tape and capped to avoid the moisture loss during measurements. A dry bath system (Model # TCON 2000, Duratech, Indianapolis, IN) was used to maintain the temperature of fresh and thawed dough samples in the capped tubes before performing NMR measurements at 25 °C and 5 °C respectively. All measurements were performed within 5 minutes of placing samples within the tubes in order to minimize the amount of time that samples were held in the dry bath.

#### 4.3.6 $T_1$ and $T_2$ measurements and $T_2$ distribution

Inversion recovery (IR) and Carr-Purcell-Meiboom-Gill (CPMG) pulse sequences (Callaghan 1991) were used to acquire  $T_1$  and  $T_2$  data respectively. "CONTIN" program (Provencher 1982a, b) was used to determine  $T_2$  distributions. The IR pulse sequence is shown in Figure 4-2. In an IR pulse sequence, a 180° RF pulse is followed by a 90° RF pulse. The 180° pulse tips the net magnetization ( $\mathbf{M}_z$ ) to an initial value of  $-\mathbf{M}_0$ . During the period  $\tau$ , between the 180° and 90° pulses,  $\mathbf{M}_z$  gradually recovers to its equilibrium value of  $+\mathbf{M}_0$  (Atkins and de Paula 2006; Levitt 2001; Seton 2003). A number of these experiments conducted with different values of  $\tau$  and sufficient time in between the

experiments — called recycle delay or RD (set to  $5T_1$ , where initial value of  $T_1$  is arbitrarily set and later revised after the experiment is conducted, and this process repeated until accurate  $T_1$  value is attained) to let the net magnetization recover to its thermal equilibrium position  $+M_0$  — provides the curve (Figure 4-3) represented by the equation:

$$M_z(t) = M_0 \left[ 1 - 2 \exp\left(\frac{-t}{T_1}\right) \right] \quad (4-1)$$

$T_1$  of a sample depends on the magnetic field strength ( $B_0$ ) of the permanent magnet, the gyromagnetic ratio ( $\gamma$ ), and temperature of the sample. Although not a fundamental property of a material, it is one of the key parameters in understanding mobility of different components (such as oil and water) that may be present in different states in a food system. Longer  $T_1$  values are associated with very mobile water molecules that take a long time to reach their thermal equilibrium state and are relaxing slowly (Ruan and Chen 1998b). The curve generated using an IR pulse sequence (Figure 4-3) to determine  $T_1$  is usually a multi-exponential decay curve and is typically fitted to sums of exponential decay components — representing shorter ( $T_{11}$ ) and longer ( $T_{12}$ ) spin-lattice relaxation times of the sample. Since  $5T_1$  (or  $5T_{12}$ , when  $T_{12}$  can be determined) is used as the RD in a NMR experiment,  $T_1$  or  $T_{12}$  determination is important in a NMR experiment. Therefore, the study involving the longer component of  $T_1$  ( $T_{12}$ ) is of great importance in understanding the most mobile state of protons as well as to determine

the parameters settings for a NMR experiment. In the past, many NMR studies have dealt with crystallization, microencapsulation, rheo-NMR, and determination of polymorphic forms of emulsifiers (Azoury et al 1988; Carini et al 2009; Gabriele et al 2009; Hertlein et al 2006; Plass et al 2001) and very few research works have been conducted to understand the NMR relaxometric parameters obtained using TD-NMR in foods with different emulsifiers and gums or on understanding relaxometric parameters obtained using TD-NMR relating to emulsifiers incorporated in dough products.

CPMG (Figure 4-4) is much more tolerant to error in the  $180^\circ_{y'}$  pulse ( $y'$  is the axis around which the magnetization is rotated by the pulse) when compared to Hahn-echo or Carr-Purcell (CP) sequences (Ruan and Chen 1998a, c; Seton 2003). The error in  $180^\circ_{y'}$  pulse occurs due to inhomogeneity of  $\mathbf{B}_1$ . The envelope (Figure 4-4) generated by the maximum amplitude at multiple echoes  $[M_{xy}(t)]$  at time  $t$  reflects the transverse relaxation decay from which  $T_2$  can be determined by performing a least-square fit to a function of the form:

$$M_{xy}(t) = M_0 \exp\left(\frac{-t}{T_2}\right) \quad (4-2)$$

where  $M_0$  is the maximum transverse relaxation value obtained immediately after the  $90^\circ_{x'}$  pulse. This fit to determine  $T_2$  is usually a multi-exponential decay curve and is typically fitted to sums of exponential decay components — representing shorter ( $T_{21}$ )



and longer ( $T_{22}$ ) spin-spin relaxation times of the sample. Free Induction Decay (FID) program was used to determine the minispec mq-20 gain (in dB) for maximum signal, which was kept the same for all samples so that the comparison of  $T_2$  distributions (using "CONTIN" program) obtained after normalization of the NMR signal becomes relevant (normalization is performed by dividing the signal value by the sample weight). 8 scans for FID program, 4 scans for  $T_1$  program, and 4 scans for  $T_2$  program were used to improve the signal-to-noise ratio. For the program used to determine  $T_1$ , the gain parameter was optimized to get a strong signal with no clip-error. In-addition, for determination of  $T_1$ , the first pulse separation and number of data points for fitting were adjusted for each sample so that the starting negative amplitude value was sufficiently small and the  $T_1$  IR curve flattened when the measurement was completed.  $T_1$  was determined by adjusting RD in the  $T_1$  experiments such that the desired  $T_1$  curve (as mentioned before) is achieved and RD would be long enough to be greater or equal to  $5T_1$ . After performing many preliminary experiments to determine  $T_1$  with different recipes of dough (Table 4-2) at 5 °C and 25 °C (temperatures of the samples at which experiments were performed) it was determined that a RD of 5 s was sufficient to bring back all the net magnetization to its equilibrium. For CPMG experiments, the initial amplitude was close to 80 % of the clip-error value to enable accurate resolving of the different relaxation components ( $T_{21}$  and  $T_{22}$ ) in the dough sample. The 90°-180° pulse separation for CPMG experiments was set to 0.5 ms. Triplicate measurements were

performed on three different samples to report the means and one standard error values.

#### **4.3.7 Self-diffusion coefficient (D) of water in dough measured using Pulse Gradient Unit (PGU)**

The Pulsed Field Gradient Nuclear Magnetic Resonance (PFG-NMR) technique is used to study the transitional diffusion rate of molecules in solid materials. This technique is a modified simple spin-echo experiment (Stejskal and Tanner 1965; Tanner and Stejskal 1968) and is used to determine D (in m<sup>2</sup>/s). In PFG-NMR, a field gradient pulse of strength, G (T/m), is applied using a PGU after the 90° and 180° pulses in the spin-echo experiment as shown in Figure 4-5. D is then determined using the following equation:

$$\ln \left[ \frac{A(G)}{A(0)} \right] = -\gamma^2 G^2 D \delta^2 \left( \Delta - \frac{\delta}{3} \right) \quad (4-3)$$

where A(G) and A(0) are the amplitude of the signal at echo (occurring at 2τ) with and without G respectively. δ, Δ, τ, and γ are the gradient pulse width, gradient pulse separation, 90°-180° pulse separation, and gyromagnetic ratio (of the proton) respectively. A Pulsed Gradient Unit (mq-PGU4, Bruker Optics, Billerica, MA) with probehead gradient coils of 4 T/m gradient strengths was used for determination of D of water in dough. An application program, *Diffusio* (Bruker Analytik GmbH 2000, 2003), included in the minispec mq-20 application pool was used for determination of

D. To determine  $D$ , *Diffusio* used a plot of  $\ln\left[\frac{A(G)}{A(0)}\right]$  versus  $\gamma^2 G^2 \delta^2 \left(\Delta - \frac{\delta}{3}\right)$  — known as the Stejskal-Tanner plot. The benchtop NMR equipment was calibrated according to the method described in section “ $T_1$  and  $T_2$  measurements and  $T_2$  distribution”. Before *Diffusio* can be used,  $G$  must be calibrated for at least three different amplitude settings of PGU using a sample of known diffusion coefficient. Adjustments to the amplitude of the first  $G$  were independently made to bring equality between areas of the two  $G$ s (Figure 4-5) and to achieve an echo precisely at the right position (called “balance”). Distilled water with approximately 1.25 g/L  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  (to shorten the  $T_1$  of distilled water) filled to 1 cm height in a tube (to optimize maximum amplitude using FID) was used as the calibration sample to calibrate  $G$  (Bruker Analytik GmbH 2000). For frozen-thawed samples, the calibration sample was maintained at 5 °C and for fresh samples the calibration sample was maintained at 25 °C.  $G$  was calibrated using the *Diffusio* program and coefficient of determination ( $R^2$ ) value of 0.999 or above was obtained before measurements for  $D$  were performed. All fresh and frozen-thawed samples were prepared in the same manner as described in the section “ $T_1$  and  $T_2$  measurements and  $T_2$  distribution”. 16 scans and 50 % gradient amplitude strength were used for all measurements of  $D$ .  $\delta$ ,  $\Delta$ , and  $\tau$  were set to 0.5 ms, 7.5 ms, and 7.5 ms respectively. The separation between the 90° pulse and first  $G$  was set at 1 ms. Triplicate measurements for  $D$  were performed on three different samples for the same treatment and the means

with one standard error were reported. Replicate measurements obtained during the measurement of relaxation time and  $D$  is an indicator of extent to which exchange might be occurring between the more mobile and less mobile states of water in a dough system during the experimental time frame (Vittadini and Vodovotz 2007).

#### **4.3.8 Imaging of frozen dough using environmental scanning electron microscope (ESEM)**

The ESEM is a variable pressure scanning electron microscope (SEM) that enables visualization of uncoated, moist, dry, or oily samples in a low-pressure gaseous atmosphere at a pressure of 1–20 Torr (Roman-Gutierrez et al 2002; Stokes 2008; Stokes and Donald 2000). Relative humidity and temperature in an ESEM can be controlled for imaging of a biological specimen. The relatively high pressure environment for the sample, as compared to those used in SEM or LT-SEM ( $10^{-5}$ – $10^{-7}$  Torr) eliminates the necessity of fixing, drying, coating or cryogenically freezing the sample before imaging and therefore allows imaging valuable structural features of a biological specimen in its natural hydrated state. A few studies (Donald 2003; Stokes et al 1998; Stokes et al 2002) describe the methodology and the benefits of using ESEM for imaging wet biological samples. These benefits include the capability to image soft matter, complex fluids, and biological specimens without metallic coating and the

capability of achieving a range of temperature, pressure, and relative humidity in the specimen chamber to enable imaging of biological samples in their natural state.

To study the morphology and structural features of frozen dough, an ESEM (FEI Quanta 200 FEG, FEI Company, Hillsboro, OR) equipped with a Peltier stage and a gaseous secondary electron detector (GSED) for *in-situ* studies of samples was used. This ESEM was equipped with a Schottky field emission gun (FEG) for optimal spatial resolution. Very small pieces of frozen dough samples ( $< \frac{1}{2}$  cm size so that they could be kept on the Peltier stage to maintain their frozen state) were fractured using a cold knife and immediately mounted on the pre-cooled Peltier stage (which was cleaned and maintained at  $-10$  °C before mounting the samples). Only the fractured sides of the frozen dough samples were imaged and all frozen dough samples were transferred to the Peltier stage within 5 s of fracturing them to avoid their thawing during the transfer. The Peltier stage was maintained at  $-10$  °C to keep samples frozen during imaging and a 10 mm working distance was used. A relative humidity of 100 % was maintained for the sample environment on the Peltier stage, which resulted in a chamber pressure of 2.16 Torr. These samples were imaged using magnifications of 300  $\times$ , 1000  $\times$ , 1500  $\times$ , 2500  $\times$ , and 5000  $\times$  at an accelerating voltage of 15 kV. This magnification can resolve structural features within a range of 10–100  $\mu\text{m}$ , obtaining clear images for the gluten matrix and starch granules present in frozen dough.

#### **4.3.9 Small amplitude oscillatory shear (SAOS) study for fresh and thawed samples**

SAOS testing was used to determine the viscoelastic properties of dough which are important in determining its quality. For all frozen and then thawed samples, thawing was performed at 5 °C for 24 hrs. SAOS testing was used to determine the dynamic viscoelastic parameters: storage modulus ( $G'$ ), loss modulus ( $G''$ ), complex modulus ( $G^*$ ), and phase angle ( $\delta$ ). SAOS tests were performed at sample temperatures of 5 °C and 25 °C for fresh as well as thawed samples. Dynamic oscillatory measurements were performed on a controlled stress rheometer (StressTech, REOLOGICA® Instruments AB, Lund, Sweden). Sinusoidal stress was applied to a sample and the resulting strain was measured by the instrument to determine the viscoelastic parameters. All measurements for fresh dough samples were conducted using a 20 mm diameter parallel plate attachment with a gap of 2 mm between the plates. The excess dough was trimmed and to prevent drying of sample during the measurement, a thin layer of grease (Super Lube® synthetic multipurpose grease with Syncolon®, Synco Chemical Corporation, Bohemia, NY) was applied at the edges. The desired temperature of 5 °C or 25 °C was achieved for the sample after trimming and coated was performed. To allow further relaxation of stresses generated during sample loading, samples were held at rest for 5 min after the desired temperature was achieved. The time (~15–20 min) during which the desired temperature (5 °C or 25 °C) for the dough sample was

attained after loading it, provided additional time for stresses to relax in the dough before taking measurements.

The linear viscoelastic region (LVR) is important in oscillatory rheology and defines the maximum deformation that can be applied to the sample without destroying its micro-structure. To determine the LVR, a number of stress sweeps at frequencies of 1 Hz and 20 Hz at 5 °C and 25 °C were performed on dough with and without addition of GA, CSL, and DATEM.  $G'$  and  $G''$  vs. stress values from these stress sweeps were plotted to determine the appropriate LVR region for the dough samples. It was determined that 10 Pa (75% of the upper limit of the LVR) at 20 Hz and 25 °C was the appropriate stress at which deformation without destroying the structure of the dough can be achieved. Therefore, a frequency sweep test in the range of 0.002-20 Hz with a 10 Pa stress was applied to the dough samples to obtain  $G'$ ,  $G''$ , and  $\delta$  values. As presented in the study by Ribotta et al (2004), values obtained for  $G'$ ,  $G''$ , and  $\delta$  measured close to 1 Hz (values at 1.62 Hz in this study) were used as the representative values for all the dough samples for comparison purposes. The  $G'$ ,  $G''$ , and  $\delta$  measurements taken at the lower limit of the frequency range (0.002 Hz) enabled studying the rheological behavior of larger macromolecules (such as gluten) present in the dough. Triplicate measurements were performed on three different samples to report the means and one standard error values.

#### **4.3.10 Statistical analysis**

One-way analysis of variance (ANOVA) and generalized linear model (to study main and interaction effects) were used to perform the statistical analysis using the software, JMP® 8.0 (SAS Institute Inc., Cary, NC) at a significance level of 0.05. One-way ANOVA was conducted across eight dough formulations and across nine combinations of cooling rates and frozen storage times. Tukey-Kramer honestly significant difference (HSD) tests were performed for all pair-wise comparisons of means when significant difference existed ( $p$ -value < 0.05) in one-way ANOVA tests. All means reported in this study were obtained using triplicate measurements and the error bars presented in the graphs are one standard error from the mean values.

### **4.4 Results and Discussion**

#### **4.4.1 Cooling rates for dough samples**

Figure 4-6 depicts the temperature data at the center of the dough while cooling at CR0 and CR1 for two dough treatments — with GA, CSL, and DATEM (Dough 2 in Table 4-2) and without GA, CSL, or DATEM (Dough 1 in Table 4-2). The temperature at the center of the dough gradually reached  $-20$  °C and equilibrated throughout the dough. A significant difference ( $\sim 1$  hr) was observed between CR0 and CR1 in the time taken for the center of the samples to reach  $-5$  °C. In-addition, there was a significant difference



(~2 hrs) between CR0 and CR1 in the time required for extraction of latent heat of fusion during the freezing plateau (Figure 4-6). Cooling rates (also referred to, as Freezing rates or Fr) were calculated as the ratio of the difference between the initial and final temperature to the time duration in hrs (Havet et al 2000; International Institute of Refrigeration 1986):

$$Fr = \frac{T_2 - T_1}{t_2 - t_1} \quad (4-4)$$

For calculating Fr,  $T_1$  was the temperature at  $t_1 = 0$  and  $t_2$  was the time when  $T_2$  reached  $-5$  °C. For CR0 or still air cooling, Fr was 6.8 °C/hr and for CR1 or forced air cooling, Fr was 12.1 °C/hr.

#### **4.4.2 Effects of emulsifiers, cooling rate, and frozen storage time on relaxation times ( $T_{12}$ , $T_{21}$ , and $T_{22}$ ) of fresh and frozen-thawed dough**

In this study, we compared  $T_{12}$  — the longer component of  $T_1$  — in order to gain an understanding on how fast the relaxation component decays for the most mobile component of water in the dough. Since all  $T_{12}$ ,  $T_{21}$ , and  $T_{22}$  measurements for frozen-thawed samples were performed with a 20 MHz (minispec mq-20) magnet and at a sample temperature of 5 °C, the  $T_1$  results obtained for different frozen-thawed samples were comparable. Figure 4-7 shows the  $T_{12}$  (ms) values for fresh dough at 25 °C and for frozen-thawed dough at 5 °C as a function of time (in weeks) for CR0 and CR1 and for

two different concentration levels of GA, CSL, and DATEM as listed in Table 4-1.  $T_{12}$  values ranged from 56.3–204 ms across all treatments.  $T_{12}$  values declined from 0–8 weeks of frozen storage, increased in the 8–16 week period, and then declined from 16–26 weeks for all treatments. This indicates that the mobility of water in frozen-thawed dough decreased due to freezing after first 8 weeks, which may be attributed to higher number of protons being bound to protein and starch matrix after the initial freezing period of 8 weeks. However, the mobility increased for the 16<sup>th</sup> week and then decreased for 26<sup>th</sup> week and this may be associated with recrystallization of ice, in which ice crystals redistribute themselves and hence affect the association of water with starch and gluten in frozen-thawed dough. The  $T_{12}$  values of fresh dough samples (except Dough 4) at 25 °C were significantly ( $p$ -value < 0.05) higher than those of frozen-thawed samples at 5 °C obtained for both cooling rates — CR0 and CR1. This was expected since the mobility of protons at 25 °C will be higher than their mobility at 5 °C due to increased thermal energy. Since  $T_{12}$  value for Dough 4 was not significantly different between the fresh and frozen-thawed states, it was inferred that the presence of CSL alone in the dough sample might have the effect of decreasing the difference between  $T_{12}$  values of frozen-thawed and fresh dough. This effect was not observed in the other seven formulations (Table 4-2). The average difference between  $T_{12}$  values at 0 week and 26 weeks for both cooling rates was less when DATEM (0.6 % w/w) was present in the dough. This effect due to DATEM may be attributed to its previously

reported dough strengthening effects. At a molecular level, the dough strengthening effects of DATEM possibly corresponds to its capability to bind water molecules tighter to the gluten matrix and starch granules, thereby decreasing the overall mobility of water molecules. In most cases (24 out of 32) across all frozen storage times and different GA, CSL, and DATEM concentrations, there were no significant differences between  $T_{12}$  values at the two cooling rates (still air and forced air cooling) and this might suggest that  $T_{12}$  is not significantly affected by cooling rate.

The spin-spin relaxation time,  $T_2$ , is a better representative of the overall mobility of water molecules in a system as compared to spin-lattice relaxation time,  $T_1$  (or  $T_{12}$ ). Therefore, both the shorter ( $T_{21}$ ) and longer ( $T_{22}$ ) components of  $T_2$  (as presented in the section “ $T_1$  and  $T_2$  measurements and  $T_2$  distribution”) are discussed in this section. Figures 4-8 and 4-9 represent graphs of  $T_{21}$  and  $T_{22}$  respectively, as a function of time, for all treatments studied.  $T_{21}$  values varied approximately from 4.3–10.6 ms and  $T_{22}$  values varied from 19–48.9 ms. The range for  $T_{22}$  values was ~5 times greater than the range for  $T_{21}$  values. This suggests that the higher mobile water molecules were ~5 times more mobile than the lower mobile water molecules. As expected from basic principles of NMR,  $T_{21}$  and  $T_{22}$  values were smaller than  $T_{12}$  values. When all functional ingredients (GA, CSL, and DATEM) were present (Dough 2), the difference between the  $T_{21}$  values for fresh and frozen-thawed dough was significantly less when compared to those for rest of the dough formulations. The average difference

between  $T_{21}$  values at 0 week and 26 weeks for both cooling rates was lesser when DATEM (0.6 % w/w) was present in the dough. This is similar to what was observed with spin-lattice relaxation time ( $T_{12}$ ) and may be attributed to the strengthening effects of DATEM on dough. A very similar overall trend was observed for longer spin-spin relaxation time ( $T_{22}$ ) as was observed for the shorter spin-spin relaxation time ( $T_{21}$ ) across all treatments. In absence of DATEM and GA (Dough 1 and Dough 4), the decrease in  $T_{22}$  was significantly greater in the 0–8 week period when compared to the 8–26 week period for both cooling rates. This decrease of ~28 ms was not observed in any other treatment. This may be attributed to dough strengthening effects of DATEM, which leads to increased binding of water with starch and gluten matrix. In-addition, for both Dough 1 and Dough 4,  $T_{22}$  values for fresh dough were smaller than those for frozen-thawed dough at 0 week, which was not observed for any other treatment. This reduction in mobility of free water (having a  $T_2$  value of ~50 ms), when DATEM or GA were present, might be attributed to greater affinity for water of hydrocolloid gums such as GA and to the water binding capacity of DATEM. Greater affinity for water for GA may be a result of the large number of functional hydroxyl groups available on a hydrocolloid gum resulting in its greater binding capacity to water molecules (due to greater presence of hydrogen bonding). The average difference between  $T_{22}$  values at 0 week and 26 weeks for both cooling rates was less when DATEM (0.6 % w/w) was

present in the dough. This is similar to trends observed for  $T_{12}$  and  $T_{21}$  in the presence of DATEM.

It may be concluded from  $T_{12}$ ,  $T_{21}$ , and  $T_{22}$  trends that CSL had no significant effect whereas GA and DATEM had a significant effect on reducing the relaxation time values between the 0–26 week period. DATEM has been reported to have a significant effect on dough and bread quality attributes due to its dough strengthening effects, resulting in stronger gluten matrix and improved lipid binding properties. These desired properties of DATEM, result in improved dough quality by improving the textural attributes (crumb texture and crust firmness) and by increasing the loaf volume of the baked product (Jacobsberg et al 1976; Sahlstrøm et al 1999; Xiujin et al 2007). As observed in this study, relatively greater effects of DATEM (when compared to CSL or GA) on reducing the mobility of water might suggest reasons for improved dough strengthening properties and better gas retention with DATEM as reported in literature (Jacobsberg et al 1976; Sahlstrøm et al 1999; Xiujin et al 2007).

#### **4.4.3 Effects of emulsifiers, cooling rate, and frozen storage time on distribution of $T_2$ for fresh and frozen-thawed dough**

Two individual exponential decay curves (one each for  $T_{21}$  and  $T_{22}$ ) obtained from a multi-exponential spin-spin relaxation decay does not present all the  $T_2$  values for various mobile states of protons present in a sample. Therefore, to understand the

range and distribution of  $T_2$  as affected by emulsifiers, cooling rates, and frozen storage time, "CONTIN" program (Provencher 1982a, b) was used to obtain  $T_2$  distribution from the  $T_2$  relaxation curve obtained using CPMG pulse sequence. Three distinct populations can be seen in the  $T_2$  distribution for all dough treatments with (Dough 2) and without (Dough 1) GA, CSL, or DATEM as shown in Figures 4-10 through 4-13. The approximate range of  $T_2$  for these populations was: first population from 0.5–5 ms, second population from 5–30 ms, and third population from 30–1000 ms. Each population is represented by a  $T_2$  value (at maximum of the peak and referred to as 'peak  $T_2$ ' value in this study) at which a maximum amplitude of  $T_2$  distribution occurs for that population. Different populations are associated with different environments for protons in the dough which indicates different mobility of protons. The first population corresponds to intra-granular protons inside solid-like components such as starch and proteins where water molecules are tightly associated with the solids (Assifaoui et al 2006a; Ruan et al 1999; Sørland et al 2004). The second population may be attributed to the protons present outside the starch granules and in interaction with starch and emulsifiers/gum of the dough (Assifaoui et al 2006a). The third population may be attributed to loosely bound water or to the apolar protons present in the lipid fraction of dough (Assifaoui et al 2006a). The broader peaks indicate a greater variation in chemical and physical properties of the system and the area under a peak is a measure of the number of protons associated with each population. When compared to fresh

dough (Figures 4-10 and 4-11), a tighter distribution of  $T_2$  occurs for population 1 with increasing time (from 0 to 26 weeks), indicating a more homogenous environment. This indicates a closer association of water molecules in the intra-granular region due to a longer frozen storage time. It is known from literature that ice crystals reorganize to form larger size ice crystals with longer frozen storage times. A similar phenomenon might be attributed to movement of intra-granular protons during longer frozen storage time, during which a closer association of protons with granules is formed, thereby forming a tighter  $T_2$  distribution and a more homogenous environment. In Figure 4-12, a similar effect is seen with addition of GA, CSL, and DATEM at CR0 (still air cooling). However, in Figure 4-13, with addition of GA, CSL, and DATEM at CR1 (forced air cooling), such an effect is not seen, indicating that a faster cooling rate and the presence of emulsifiers and gums affected the intra-granular protons to a larger extent and shifted them to higher mobile phases. This might be attributed to emulsifiers and gums being able to associate with a larger number of protons during smaller size ice crystal formation (providing larger surface area for interaction) at a faster cooling rate than at a slower cooling rate (where larger ice crystals are formed).

The three population peaks of protons present in the dough were deconvoluted to three Gaussian functions using a customized "CONTIN" program (a minispec mq-20 program). The areas under the deconvoluted peaks (referred to as "peak area"), were associated with number of protons in that environment. The standard deviation of the

deconvoluted peaks (referred to as “standard deviation of peak”), provided information regarding the variation in chemical and physical properties (heterogeneity) of the system. Peak  $T_2$ , peak area, and standard deviation of the peak were calculated for the second population. Population 2 was chosen for the study since this population represents the extra-granular protons interacting with starch and emulsifiers/gum within the dough and possibly represents the protons most affected by change in state of water (due to freezing) and by change in ice crystals size (with frozen storage time). The following equations were used to calculate the standard deviation (S) as described by Ruan and Chen (1998b):

$$S = \sqrt{\frac{\sum y_i (x_i - \bar{x})^2}{\sum y_i - 1}} \quad (4-5)$$

$$\bar{x} = \frac{\sum x_i y_i}{\sum y_i} \quad (4-6)$$

where  $x_i$  and  $y_i$  are the  $T_2$  value and its corresponding amplitude respectively under a deconvoluted peak. Peak  $T_2$  values for population 2 in all samples varied in the range of 6.9–18.3 ms (within a  $T_2$  range of ~5–30 ms for population 2). This range of peak  $T_2$  values is similar to results obtained in earlier studies related to dough (Assifaoui et al 2006a; Ruan et al 1999). Peak area and standard deviation of peaks for population 2 in all treatments are presented in Figure 4-15 and Figure 4-16 respectively. For dough with no emulsifiers or gums (Dough 1) cooled at CR0 (still air cooling), there were a



fewer number of protons associated with population 2 at 26<sup>th</sup> week when compared to that for fresh dough. However, the peak T<sub>2</sub> value and homogeneity of population 2 (represented by standard deviation of population 2) was not significantly different for frozen-thawed dough and fresh dough. A similar observation was noted for dough with GA, CSL, and DATEM (Dough 2) cooled at CR0 (still air cooling). Therefore, for dough frozen at CR0, a redistribution of water molecules in frozen-thawed dough (with increasing frozen storage time) resulted in a decreased proton association with starch. For both Dough 1 and Dough 2 cooled at CR1 (forced air cooling), the number of protons associated with population 2 was greater at 26<sup>th</sup> week as compared to that for fresh dough. There was also an increase in the standard deviation (or the heterogeneity) of population 2 at 26<sup>th</sup> week (for frozen dough as compared to that for fresh dough). Therefore, it may be suggested that a faster cooling rate increased the number of protons associated with population 2 and also increased the diversity of population 2. As observed earlier in this study, these results might be attributed to starch and emulsifiers/gums being able to interact with larger number of protons during small ice crystal formation (due to faster cooling) when compared to that at a slower cooling rate (when larger ice crystals are formed).

#### **4.4.4 Self-diffusion coefficient (D) of water for fresh and frozen-thawed dough**

Figure 4-14 presents the self-diffusion coefficient of water ( $D$  in  $\text{m}^2/\text{s}$ ) measured for different levels of frozen storage time, cooling rates, GA, CSL, and DATEM. Without DATEM, there was a decrease in the value of  $D$  from 0 to the 26<sup>th</sup> week. With addition of DATEM (except when all functional ingredients GA, CSL, and DATEM were present), the values increased from 0 to 26<sup>th</sup> week. A significant decrease in  $D$  value was observed at 26<sup>th</sup> week for all dough treatments when compared to that for fresh dough (measured at 25 °C). Since self-diffusion coefficient of water increases with temperature,  $D$  obtained for fresh dough at 25 °C is higher than  $D$  for frozen-thawed dough at 5 °C. When DATEM was added, freezing the dough and storing it under frozen conditions (for any amount of time and then thawing) decreased the overall translational mobility of water present in the dough. The increase in  $D$  associated with addition of DATEM, might be attributed to increased migration of protons in water to higher mobile states as observed from  $T_{21}$  and  $T_{22}$  values. Cooling rate, CSL, or GA did not show any significant effect on  $D$  over 0–26 weeks of frozen storage time.

#### **4.4.5 Frozen dough microstructure studied using ESEM**

Figure 4-17 shows images obtained using ESEM for frozen dough with no emulsifiers and Figure 4-18 shows images obtained using ESEM for frozen dough with all

emulsifiers for different cooling rates and stored for 0, 8, 16, and 26 weeks. Images shown in this study are at 1000× magnification to reveal structural features 40 μm and smaller — which is about the size of starch granules present in wheat flour.

Observation of these images show irregularly shaped particles of different sizes.

Smaller irregular shaped particles with relatively sharper edges represent broken-up endosperm of wheat kernel and lentil-shaped circular particles that are larger than the observed broken-up endosperm particles are the wheat starch granules (Hoseney 1986). The continuous phase of gluten matrix (which includes adhesive proteins) was seen as sheets covering or embedding the starch granules and endosperm particles.

This study used hard wheat dough, which contains relatively higher contents of proteins, damaged starch, and pentosans when compared to soft wheat dough. Due to their much smaller structures, it was not possible to locate pentosan present in the dough (Roman-Gutierrez et al 2002). For dough containing GA, CSL, and DATEM, fully embedded starch granules were observed (Figure 4-18 A-H) as compared to an open structure seen for dough with no functional ingredients (Figure 4-17 A-H), where starch granules are relatively less embedded and more visible in the protein matrix. This less open structure might be attributed to increased strength and elasticity of dough due to dough strengthening effects of emulsifiers (DATEM and CSL), resulting in a closer protein matrix. For dough with no emulsifiers/gum (Figure 4-17), the openness of the structure increases with frozen storage time and was observed to a lesser extent with

increasing frozen storage time in dough with all emulsifiers/gum (Figure 4-18). It was observed in all images, that the protein matrix in dough was in the form of sheets as compared to popularly believed strands or fibrous network. This observation has also been reported in a previous study by Bache and Donald (1998). Thus, at the mesoscopic level, gluten forms into sheets and network formation might exist only at much shorter length scales (at the molecular level). All frozen dough images obtained using ESEM, revealed a non-porous compact structure.

#### **4.4.6 Small amplitude oscillatory shear (SAOS) study of fresh and frozen-thawed dough**

The dynamic rheological properties  $G'$  (in Pa),  $G''$  (in Pa), and phase angle  $\delta$  (in degree) measured as a function of oscillation frequency (at 5 °C and 25 °C) for fresh dough made without and with emulsifiers/gum are shown in Figure 4-19 and Figure 4-20 respectively. For both 5 °C and 25 °C, and with or without emulsifiers/gum, the dough behaved like a gel-type material with  $G'$  greater than  $G''$  in the frequency range of 0.002–20 Hz. Similar observations have been noted in earlier studies (Ribotta et al 2004; Van Bockstaele et al 2008).  $G'$  and  $G''$  values at any frequency were higher at 5 °C than at 25 °C, indicating that lowering the temperature increased the elastic and viscous components of dough. This may be attributed to lower mobility of water molecules at lower temperatures, resulting in greater elastic and viscous nature of dough. The

decrease in  $G'$  and  $G''$  values with increase in temperature, does not fit with the rubber elasticity model in which elasticity increases with increase in temperature. However, the increase in elasticity with increase in temperature holds true only if the proportionality constant (pertaining to elastic element) is a constant value in the rubber elasticity model. In case of frozen-thawed dough, the elastic element may not be a constant value and may decrease relatively faster as compared to the increase in temperature. This decrease in elastic element may be attributed to hydrogen bonds present in frozen-thawed dough — since the strength of hydrogen bonds decreases with the increase in temperature. The phase angle ( $\delta$ ) at low frequencies (0.002–1 Hz) for doughs made with and without emulsifiers/gum was greater at 25 °C when compared to that at 5 °C, indicating a relatively higher viscous nature of dough at greater temperatures. This indicates that larger gluten macromolecules are more elastic at lower temperatures. This might be attributed to a large hydrodynamic radius and less mobility of water at lower temperatures (Fennema 1996). However,  $\delta$  was lower for 25 °C when compared to that at 5 °C (for doughs with and without emulsifiers/gum) at frequencies of 1-20 Hz. This might be attributed to relatively greater elasticity of dough at higher temperatures due to increased mobility, resulting in increased association of water with the gluten network. From Figure 4-19 and Figure 4-20, it was noted that there is a significant shift in  $\delta$  at 25 °C for the sample with emulsifiers/gum as compared to the one without any emulsifier or gum. However, there is no significant

shift in  $\delta$  at 5 °C. The increased viscous component of dough at higher temperatures in the presence of emulsifiers clearly indicates an increase in overall mobility of dough. This may be attributed to emulsifiers/gum being able to associate with a larger number of protons as compared to the dough without emulsifiers/gum.

In order to study the combination of extreme conditions of temperature and frequency, the effects of frozen storage, cooling rate, and emulsifiers were analyzed at 0.002 Hz and 5 °C (Figure 4-21 and Figure 4-22) and at 1.62 Hz and 25 °C (Figure 4-23 and Figure 4-24). Values at 1.62 Hz in this study were used as the representative values for all the dough samples for comparison purposes (Ribotta et al 2004). The trends observed at 0.002 Hz and 5 °C were consistent with trends observed at 1.62 Hz and 25 °C. However, the values of  $G'$  and  $G''$  at 0.002 Hz and 5 °C were significantly lower (in range of 5000-15000 Pa) when compared to  $G'$  and  $G''$  at 1.62 Hz and 25 °C (in range of 10000-40000 Pa). The  $G'$  and  $G''$  values for a particular dough treatment at CR1 (forced air cooling) was either higher or equal to the corresponding values observed at CR0 (still air cooling). The lower elastic modulus values at a slower cooling rate (CR0) might be attributed to the greater damage to gluten matrix due to formation of larger ice crystals as compared to smaller ice crystals formed at a faster cooling rate (CR1). The formulation with addition of only DATEM at 0.6 % w/w, showed a significant increase in  $G'$  and  $G''$  values for a higher cooling rate for the two extreme conditions studied. Thus, it may be concluded that with addition of DATEM alone and with a higher cooling

rate for dough, there may be improved effects seen in rheological properties of frozen-thawed dough.

Wolt and D'Appolonia (1984a) observed that a decrease in extensibility occurs with increasing frozen storage time. This decrease in extensibility was attributed to an overall deterioration in the gluten network occurring due to depolymerization of glutenin aggregates during frozen storage (Ribotta et al 2001). Inoue and Bushuk (1991) studied the extensibility of dough using a modified extensigraph procedure and observed no significant differences in rheological properties during short term frozen storage. Redistribution of water during frozen storage leads to enlargement of ice crystals, resulting in rupture of the gluten matrix and starch in the dough. This leads to a weaker gluten network which results in a decrease in gas holding capacity of the dough during proofing (Ribotta et al 2001). Another reason attributed to weakening of the gluten network is the release of glutathione from yeast cells (Wolt and D'Appolonia 1984a), which breaks the disulphide bonds in gluten during the frozen storage.

## **4.5 Conclusions**

The combined effects of GA, CSL, and DATEM and processing parameters such as frozen storage time and cooling rates on the quality of frozen dough was studied. The average difference between  $T_{12}$  values at 0 week and 26 weeks for both cooling rates was less when DATEM (0.6 % w/w) was present in the dough. This may be attributed to

previously reported dough strengthening effects of DATEM. When DATEM was absent, the presence of GA decreased the  $T_{21}$  values at 0 week and  $T_{21}$  values significantly decreased from 0 to 26<sup>th</sup> week. In the presence of DATEM (0.6 % w/w), no significant differences were observed in  $T_{21}$  values at 0 week and 26<sup>th</sup> week. This observation was similar for the spin-lattice relaxation time constant ( $T_{12}$ ).  $T_{22}$  values between fresh and 0 week frozen-thawed dough were significantly different when DATEM was added. It may be concluded from  $T_{12}$ ,  $T_{21}$ , and  $T_{22}$  trends that CSL had an insignificant effect on reducing the relaxation time values, whereas, GA and DATEM had a significant effect in reducing the relaxation time values. The reduction in mobility of free water when DATEM or GA was added might be attributed to GA's large affinity for water and to the good water binding capacity of DATEM. This suggests the capacity of GA and DATEM in retaining moisture in the starch and gluten matrix (after freeze-thaw) and in their possible combined use to improve the quality of baked product made using frozen-thawed dough. A faster cooling rate increased the number of protons and heterogeneity associated with the environment for population 2 ( $T_2$  values of 5–30 ms). This might be attributed to starch and emulsifiers/gums interaction with larger number of protons during small ice crystal formation at CR1 when compared to that at CR0. Hence, cooling dough at higher rates may retain greater amount of water in dough after freeze-thaw and therefore, may improve the quality of baked product. Presence of DATEM may be attributed to an increased strength in the dough as it might be binding water molecules



tighter to the gluten matrix and starch granules, thereby decreasing the mobility of water molecules. It was observed in all images obtained using ESEM, that the protein matrix in dough was present in form of sheets as compared to popularly believed strands or fibrous network. Thus, at a mesoscopic level, gluten forms into sheets and network formation might exist only at much shorter length scales (molecular level). Formulations with addition of DATEM at 0.6 % w/w resulted in a significant increase in G' and G'' values for a higher cooling rate at the two extreme conditions (of temperature and frequency) studied. Thus, it was concluded that addition of DATEM alone and cooling the dough at a higher cooling rate, can result in improved rheological properties of frozen dough and could improve the overall quality of frozen dough. This study aids the understanding of effects of important processing and formulation parameters on quality of frozen dough. This knowledge can assist in optimizing these important parameters associated with industrial manufacturing of frozen dough to produce baked products with desired quality attributes.

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**Table 4-1** Levels of formulation and processing parameters for design of experiments

| Variables  | Levels | Values of different levels <sup>a</sup>  |
|--|--------|--|
| Water content  | 1      | W1: 33.74 %  |
| Mixing rate  | 1      | M1 (setting: #4 on mixer i.e. planetary speed: 135 rpm & beater speed: 442 rpm)            |
| Cooling rate   | 3      | Fresh (no cooling or frozen storage), CR0: still air cooling, CR1: forced air cooling      |
| Frozen storage time                                    | 4      | FT0: 0 week (for cooling and immediate thawing), FT1: 8 week, FT2: 16 weeks, FT3: 26 weeks |
| Gum Arabic (GA)  | 2      | GA0: 0%, GA1: 3%   |
| Calcium stearoyl-2-lactylate (CSL)                     | 2      | CSL0: 0%, CSL1: 0.5%   |
| Diacetyl-tartaric acid ester of monoglycerides (DATEM) | 2      | DATEM0: 0%, DATEM1: 0.6%   |

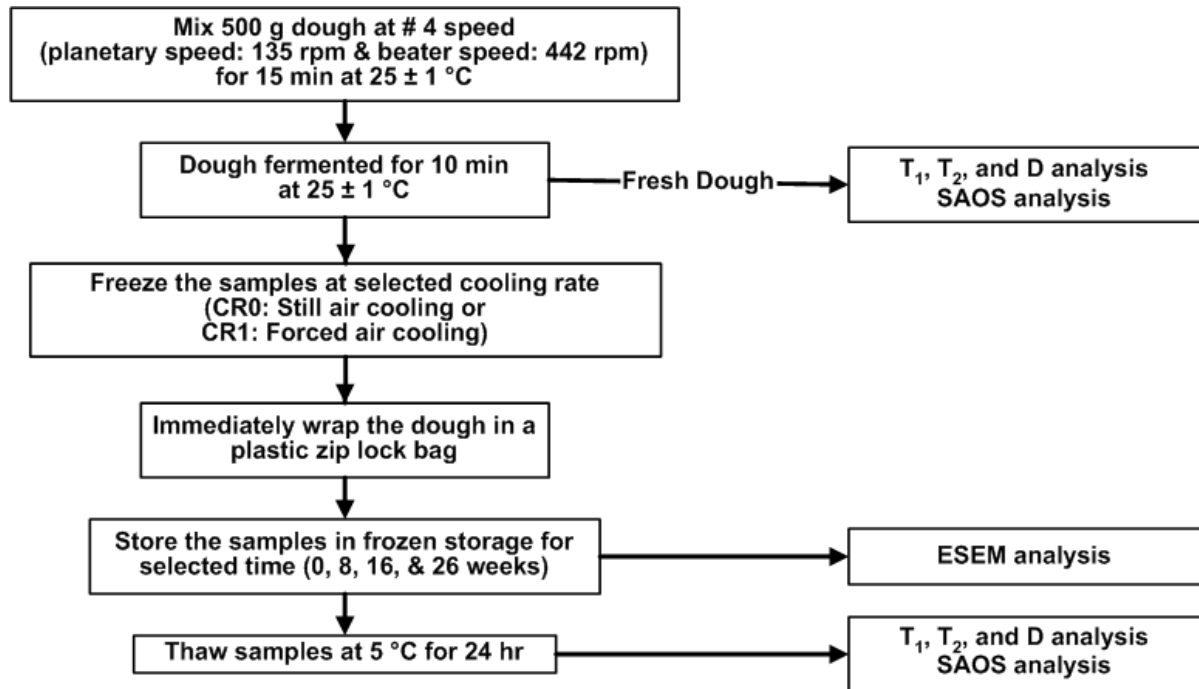
<sup>a</sup> All the % values are in w/w basis

**Table 4-2** Eight dough formulations obtained using full factorial design of experiments using variables described in Table 4-1. All ingredients values in each formulation are in % w/w basis <sup>a</sup>

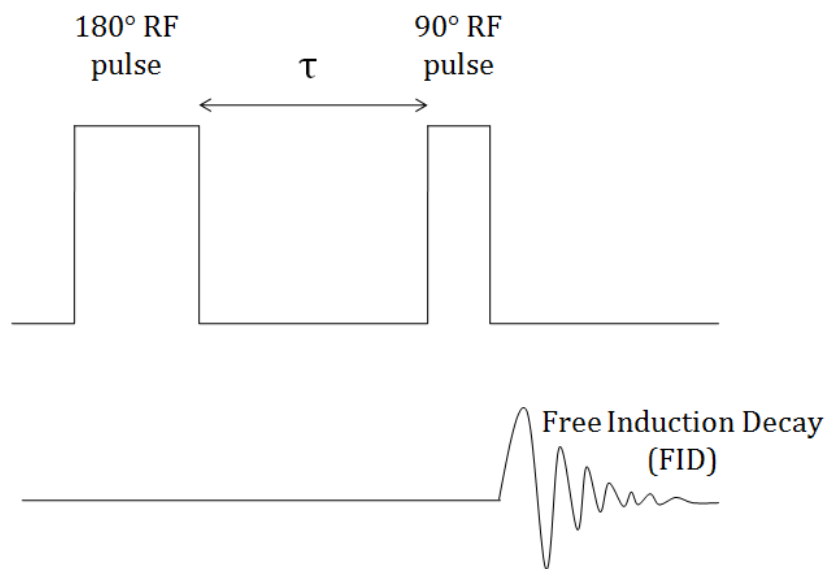
| <b>Formulation <sup>b</sup></b> | <b>Water</b> | <b>Flour</b> | <b>Yeast</b> | <b>GA</b> | <b>CSL</b> | <b>DATEM</b> | <b>Oil</b> | <b>Salt</b> |
|---------------------------------|--------------|--------------|--------------|-----------|------------|--------------|------------|-------------|
| <i>Dough 1</i>                  | 34.25        | 62.26        | 1.00         | 0.00      | 0.00       | 0.00         | 1.25       | 1.25        |
| Dough 2                         | 34.25        | 58.32        | 1.00         | 3.00      | 0.50       | 0.60         | 1.17       | 1.17        |
| Dough 3                         | 34.25        | 59.38        | 1.00         | 3.00      | 0.00       | 0.00         | 1.19       | 1.19        |
| Dough 4                         | 34.25        | 61.78        | 1.00         | 0.00      | 0.50       | 0.00         | 1.24       | 1.24        |
| Dough 5                         | 34.25        | 61.68        | 1.00         | 0.00      | 0.00       | 0.60         | 1.23       | 1.23        |
| Dough 6                         | 34.25        | 58.89        | 1.00         | 3.00      | 0.50       | 0.00         | 1.18       | 1.18        |
| Dough 7                         | 34.25        | 61.20        | 1.00         | 0.00      | 0.50       | 0.60         | 1.22       | 1.22        |
| Dough 8                         | 34.25        | 58.80        | 1.00         | 3.00      | 0.00       | 0.60         | 1.18       | 1.18        |

<sup>a</sup> For all recipes, the ratio of wheat flour: yeast: oil: salt was 50:2:1:1 (Zounis et al 2002a)

<sup>b</sup> *Dough 1* has no emulsifiers or gum in it

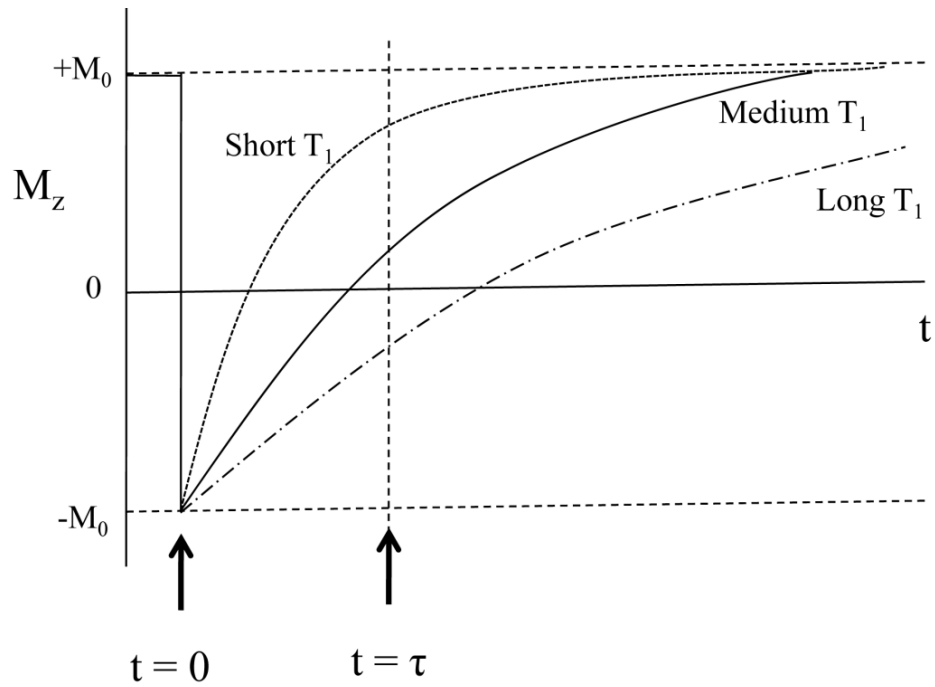


**Figure 4-1** Flow diagram for dough preparation and experimental tests involved studying the quality parameters of fresh and frozen dough

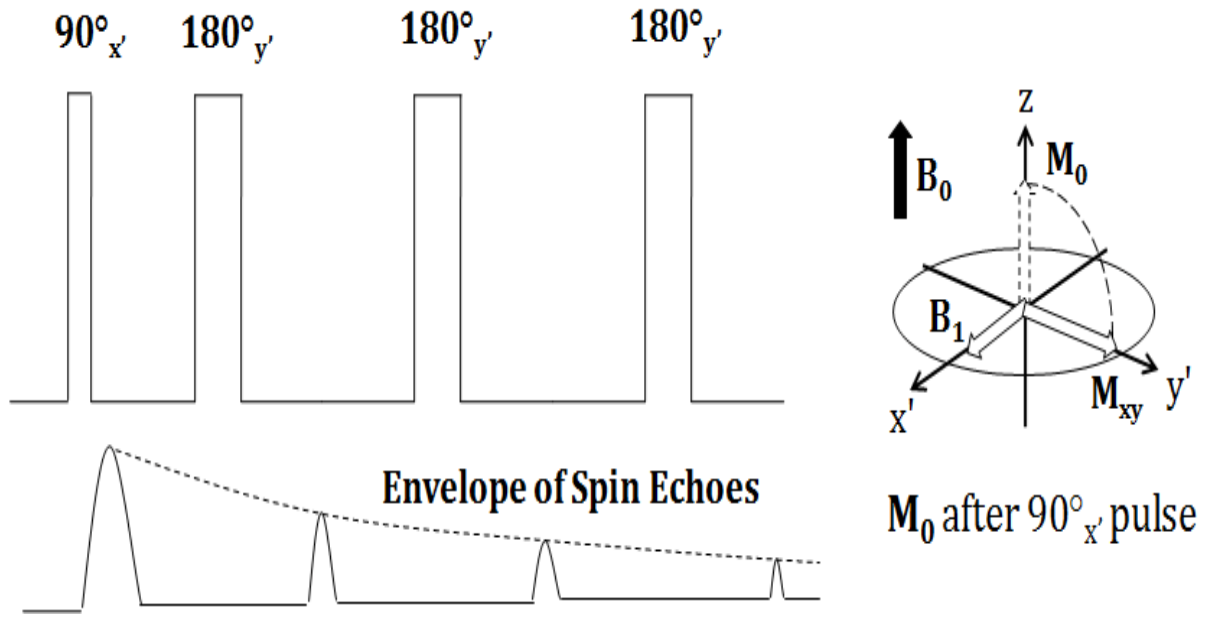


**Figure 4-2** Basic inversion recovery pulse where time,  $\tau$ , is longer than both 180° and 90° pulse. Time lengths are not to scale. Adapted from Seton (2003)

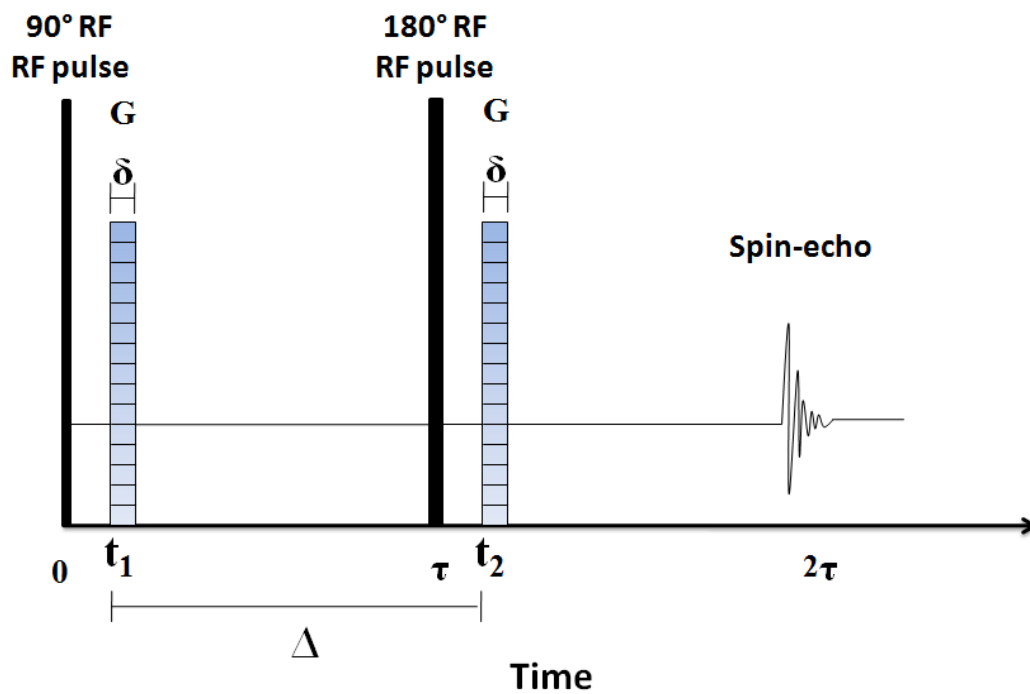




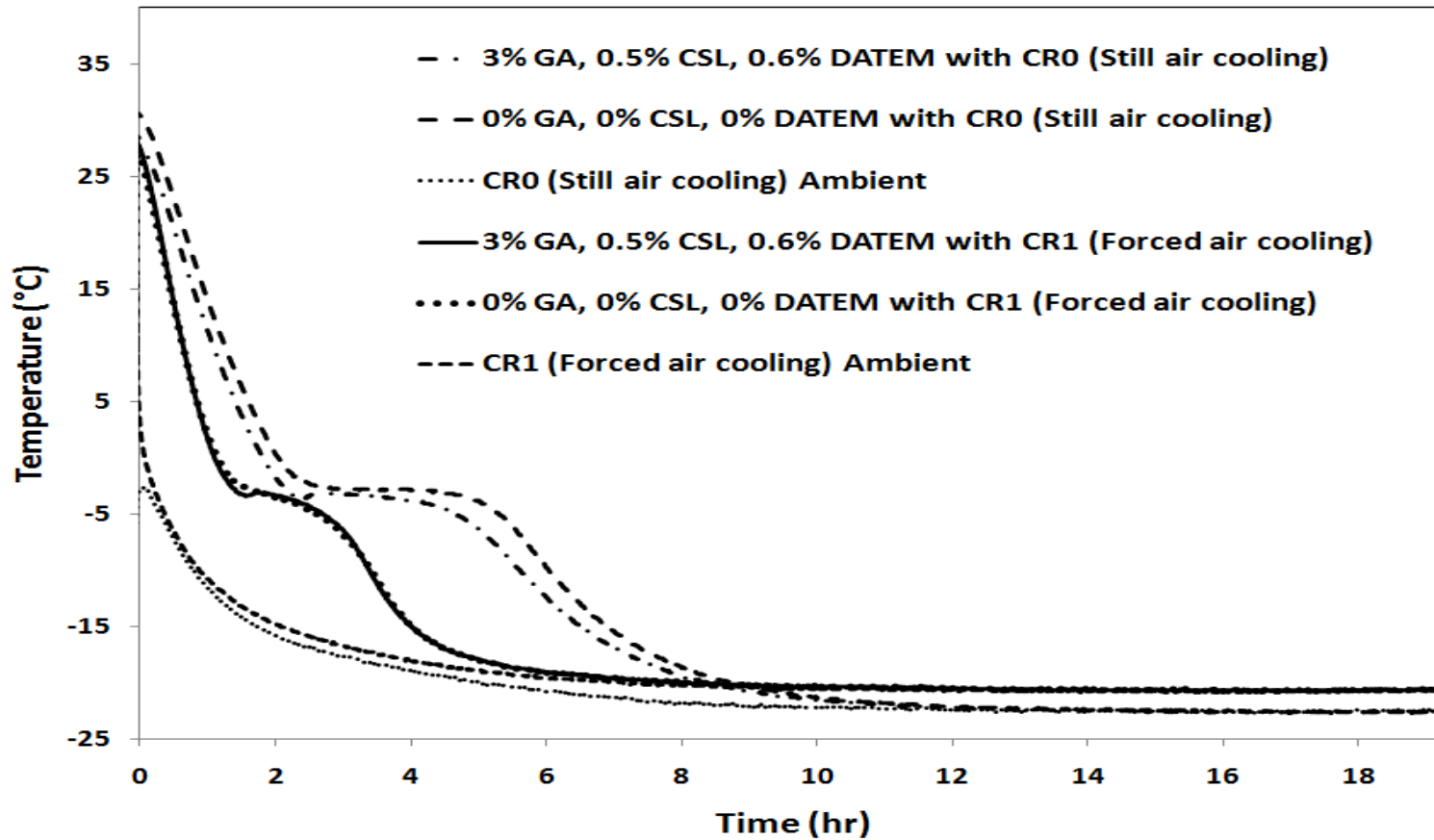
**Figure 4-3** Determination of  $T_1$  using inversion recovery sequence for three different kinds of samples. Adapted from Seton (2003)



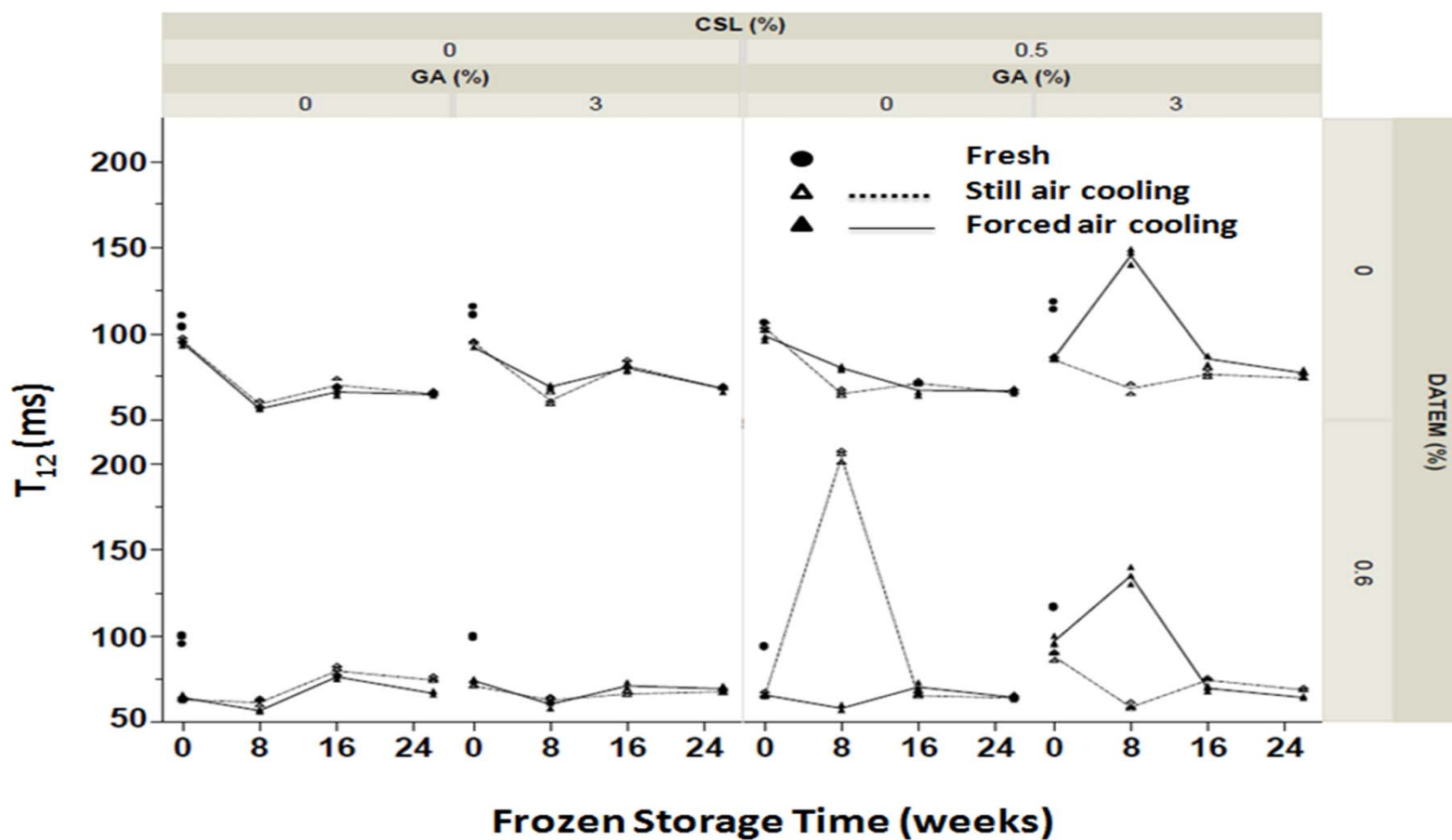
**Figure 4-4** Multiple echoes generated by the CPMG pulse sequence to determine  $T_2$ . Odd-numbered echoes are skipped to avoid accumulation of  $180^\circ_{y'}$  pulse error. Adapted from Seton (2003)



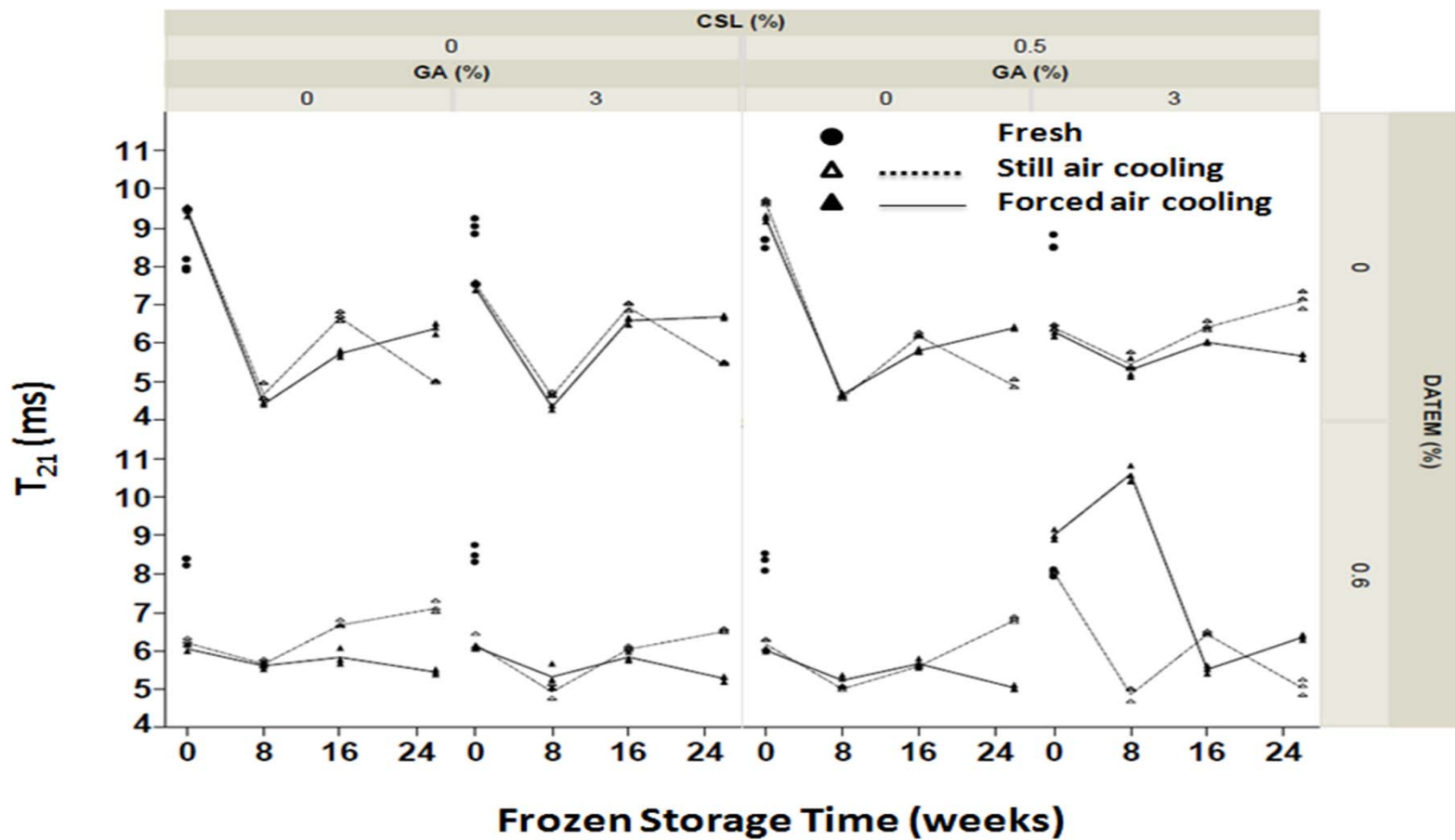
**Figure 4-5** The pulse field gradient (PFG) spin-echo experiment to determine the self-diffusion coefficient of water ( $D$ ) in dough. Adapted from Ruan and Chen (1998a)



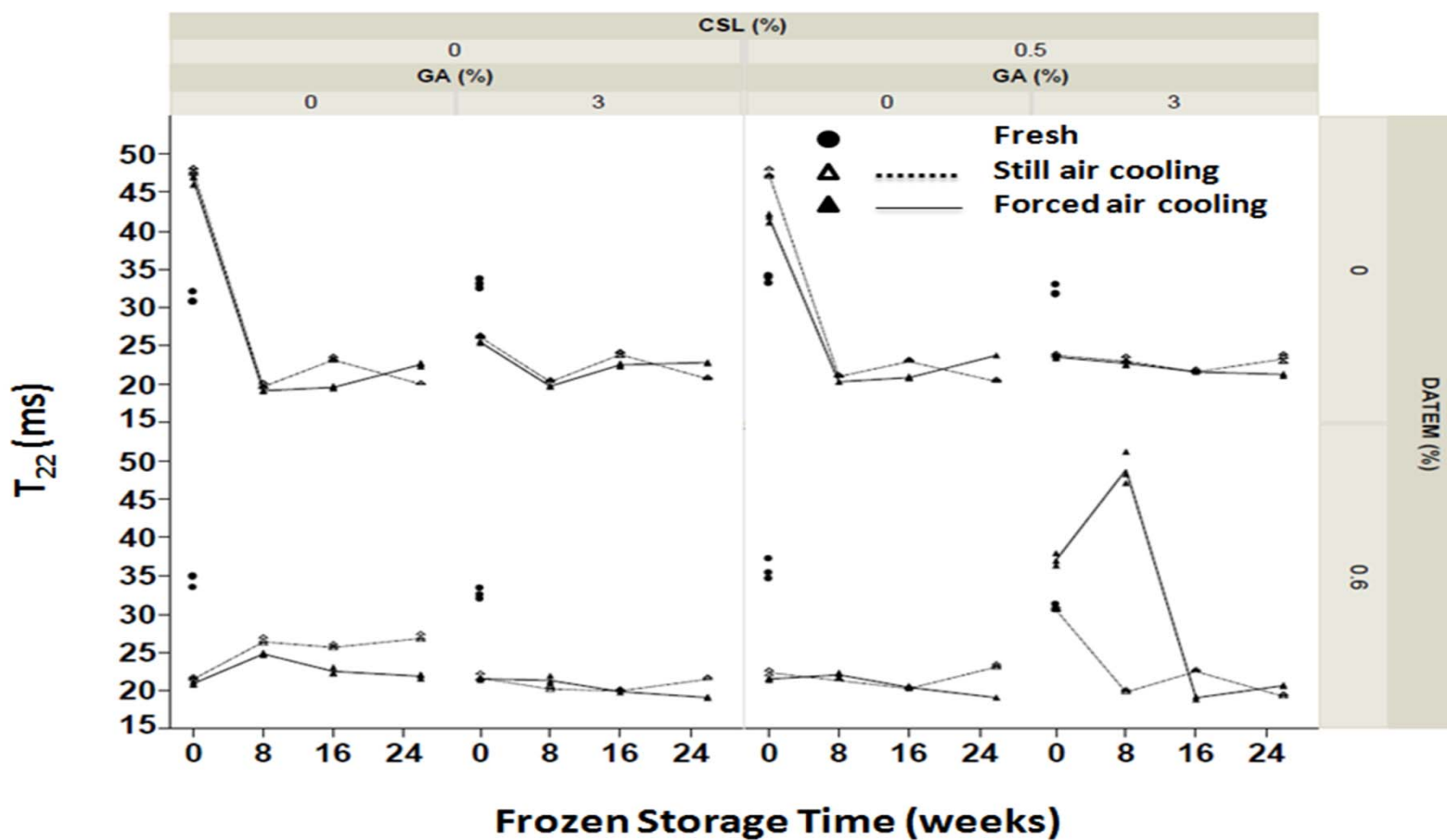
**Figure 4-6** Temperature distribution for cooling of doughs with and without emulsifiers/gum to freezing temperatures below  $-20\text{ }^{\circ}\text{C}$  at cooling rates of CR0 (still air cooling) and CR1 (forced air cooling). GA, CSL, and DATEM stands for Gum Arabic, Calcium stearoyl-2-lactylate, and Diacetyl-tartaric acid ester of monoglycerides respectively; all % are in w/w basis



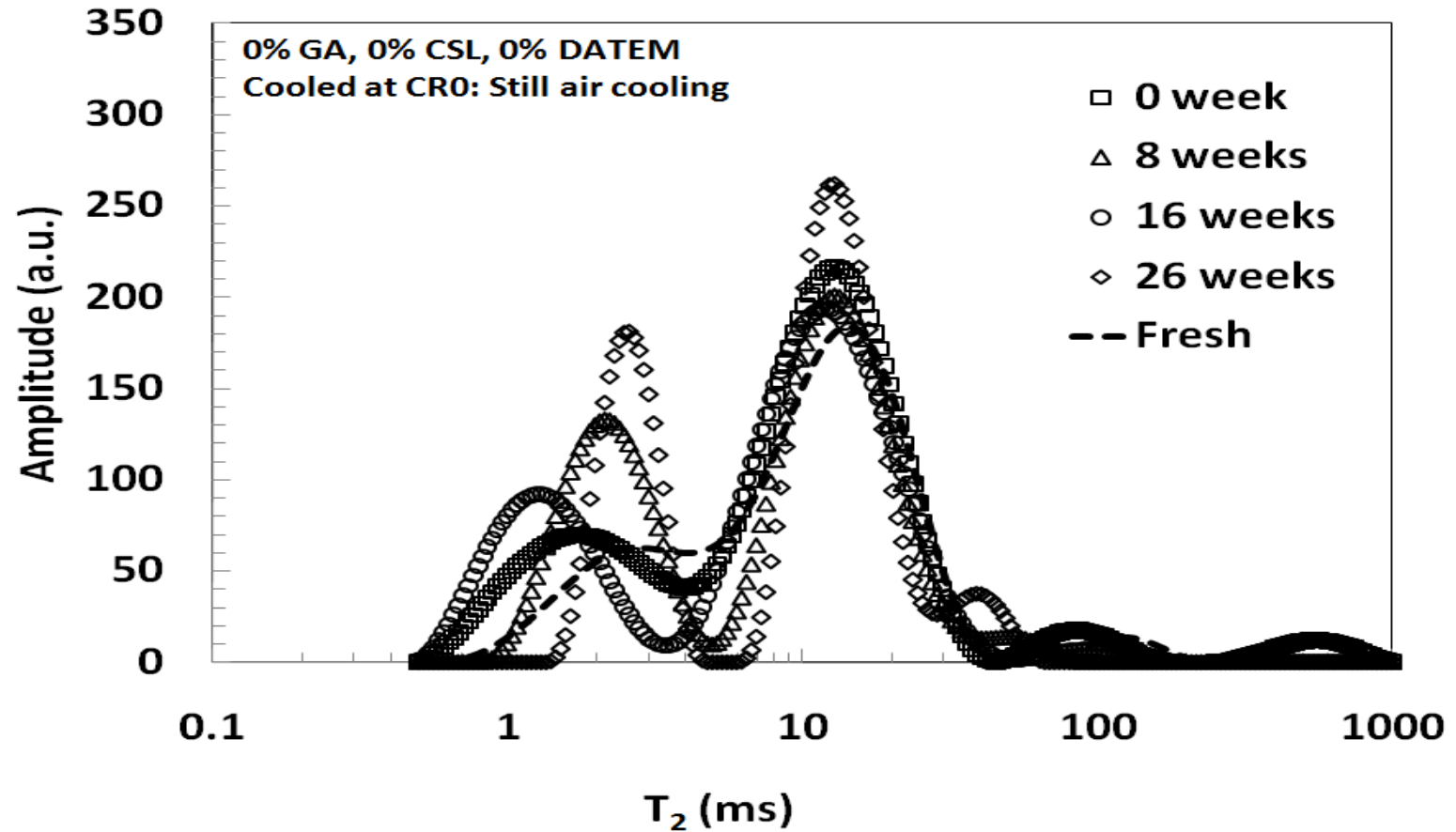
**Figure 4-7** Proton spin-lattice relaxation time constant ( $T_{12}$ ) of fresh dough at 25 °C (fresh samples represented by ● at 0 week) and for frozen-thawed to 5 °C samples, as a function of frozen storage time. GA, CSL, and DATEM stands for Gum Arabic, Calcium stearoyl-2-lactylate, and Diacetyl-tartaric acid ester of monoglycerides respectively; all % are in w/w basis



**Figure 4-8** Proton spin-spin relaxation time constant ( $T_{21}$ ) of fresh dough at 25 °C (fresh samples represented by ● at 0 week) and for frozen and then thawed to 5 °C samples, as a function of frozen storage time. GA, CSL, and DATEM stands for Gum Arabic, Calcium stearoyl-2-lactylate, and Diacetyl-tartaric acid ester of monoglycerides respectively; all % are in w/w basis

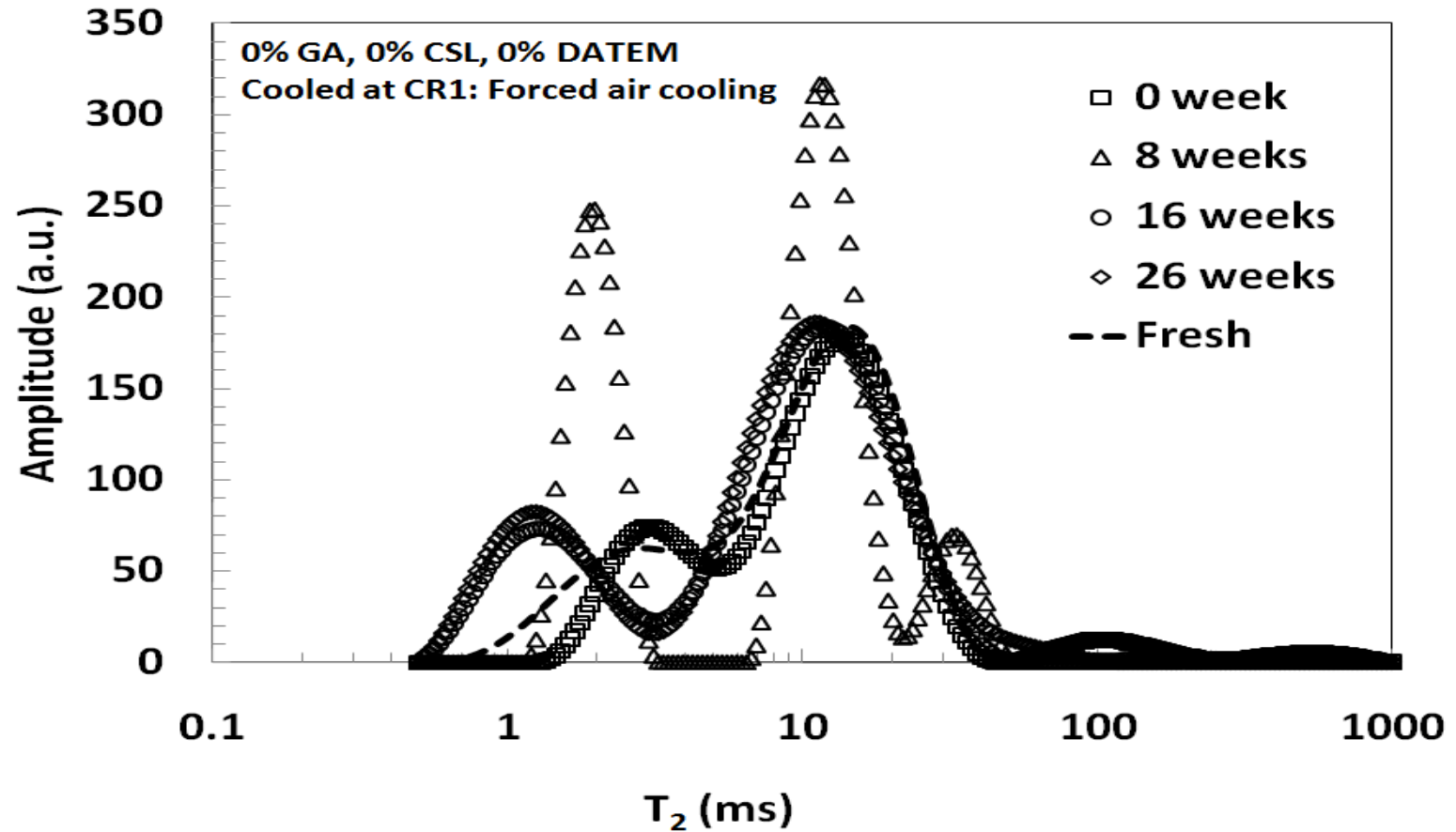


**Figure 4-9** Proton spin-spin relaxation time constant ( $T_{22}$ ) of fresh dough at 25 °C (fresh samples represented by ● at 0 week) and for frozen and then thawed to 5 °C samples, as a function of frozen storage time. GA, CSL, and DATEM stands for Gum Arabic, Calcium stearoyl-2-lactylate, and Diacetyl-tartaric acid ester of monoglycerides respectively; all % are in w/w basis

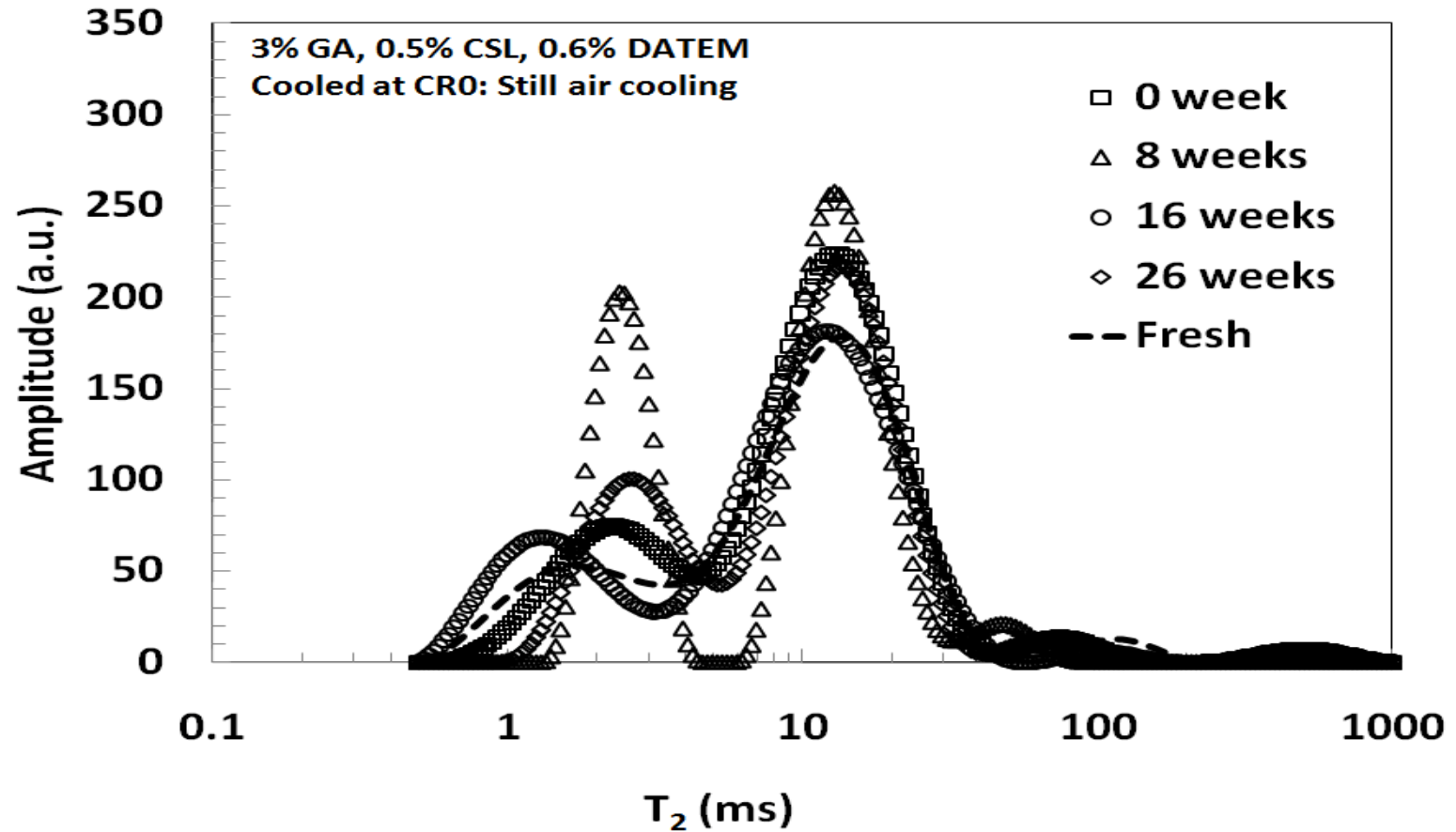


**Figure 4-10** Distribution of proton spin-spin relaxation time constant ( $T_2$ ) of dough for 0% GA, 0% CSL, 0% DATEM formulation at CR0: still air cooling. GA, CSL, and DATEM stands for Gum Arabic, Calcium stearoyl-2-lactylate, and Diacetyl-tartaric acid ester of monoglycerides respectively; all % are in w/w basis

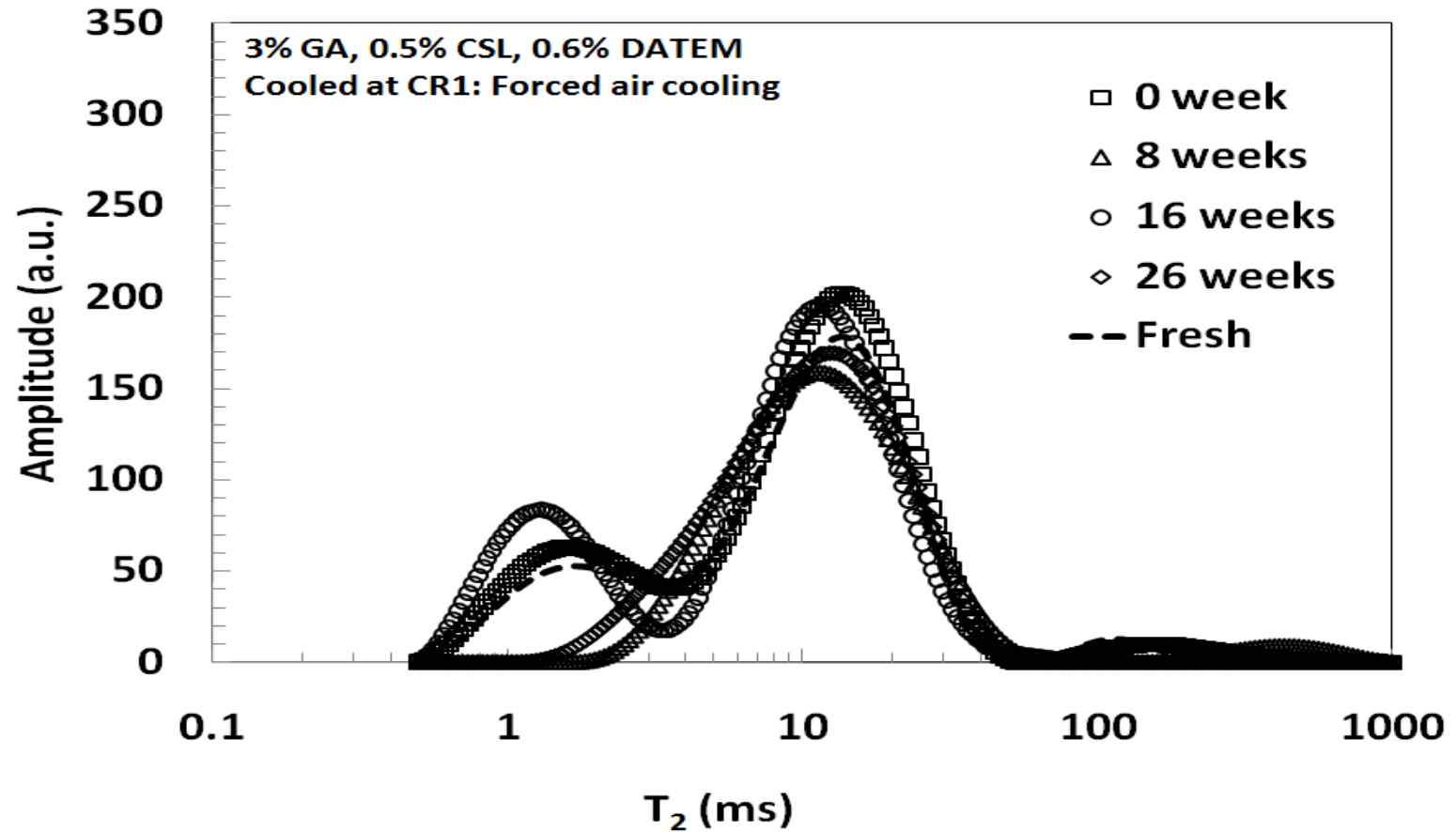




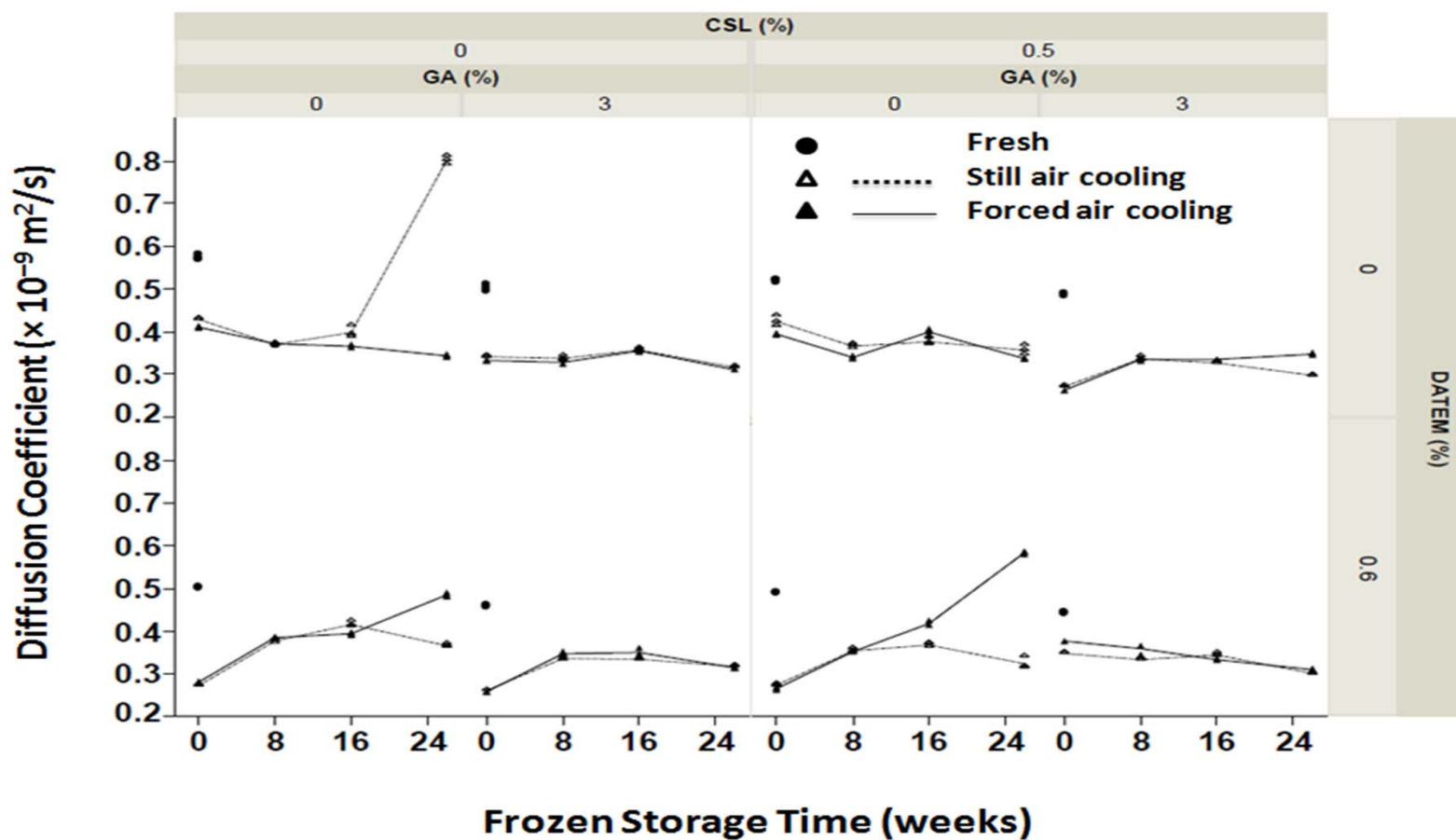
**Figure 4-11** Distribution of proton spin-spin relaxation time constant ( $T_2$ ) of dough for 0% GA, 0% CSL, 0% DATEM formulation at CR1: forced air cooling. GA, CSL, and DATEM stands for Gum Arabic, Calcium stearoyl-2-lactylate, and Diacetyl-tartaric acid ester of monoglycerides respectively; all % are in w/w basis



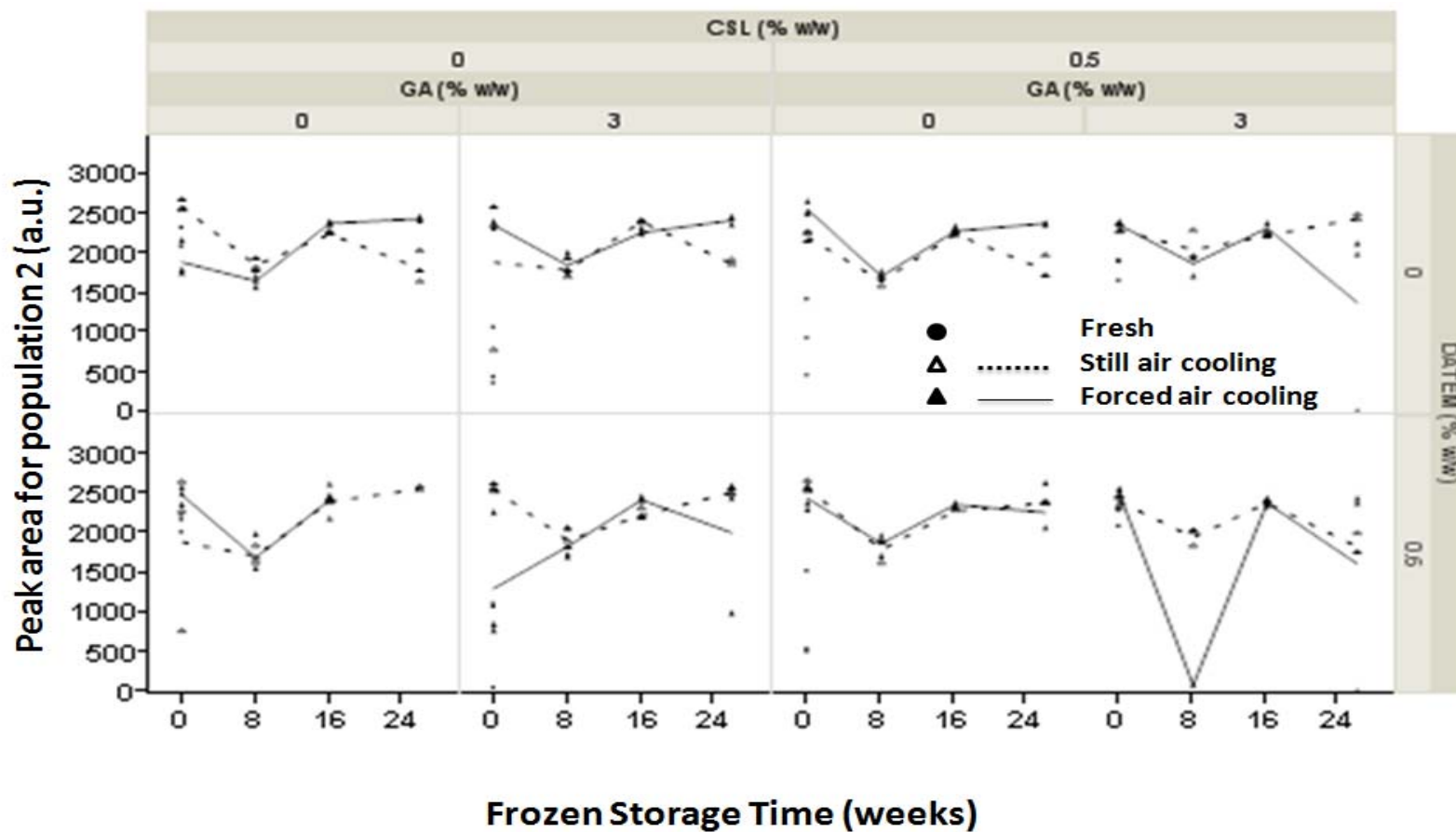
**Figure 4-12** Distribution of proton spin-spin relaxation time constant ( $T_2$ ) of dough for 3% GA, 0.5% CSL, 0.6% DATEM formulation at CR0: still air cooling. GA, CSL, and DATEM stands for Gum Arabic, Calcium stearoyl-2-lactylate, and Diacetyl-tartaric acid ester of monoglycerides respectively; all % are in w/w basis



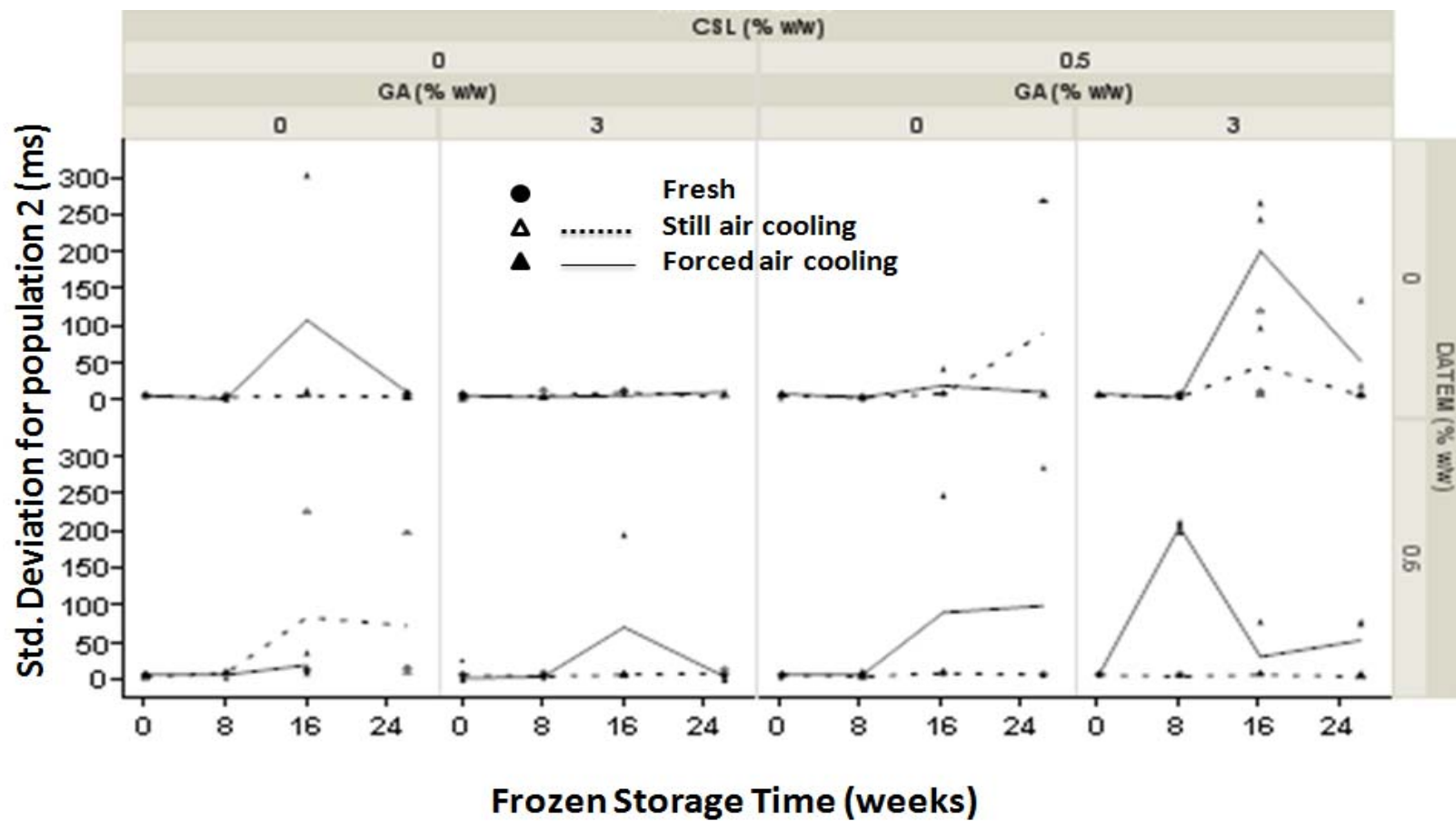
**Figure 4-13** Distribution of proton spin-spin relaxation time constant ( $T_2$ ) of dough for 3% GA, 0.5% CSL, 0.6% DATEM formulation at CR1: forced air cooling. GA, CSL, and DATEM stands for Gum Arabic, Calcium stearoyl-2-lactylate, and Diacetyl-tartaric acid ester of monoglycerides respectively; all % are in w/w basis



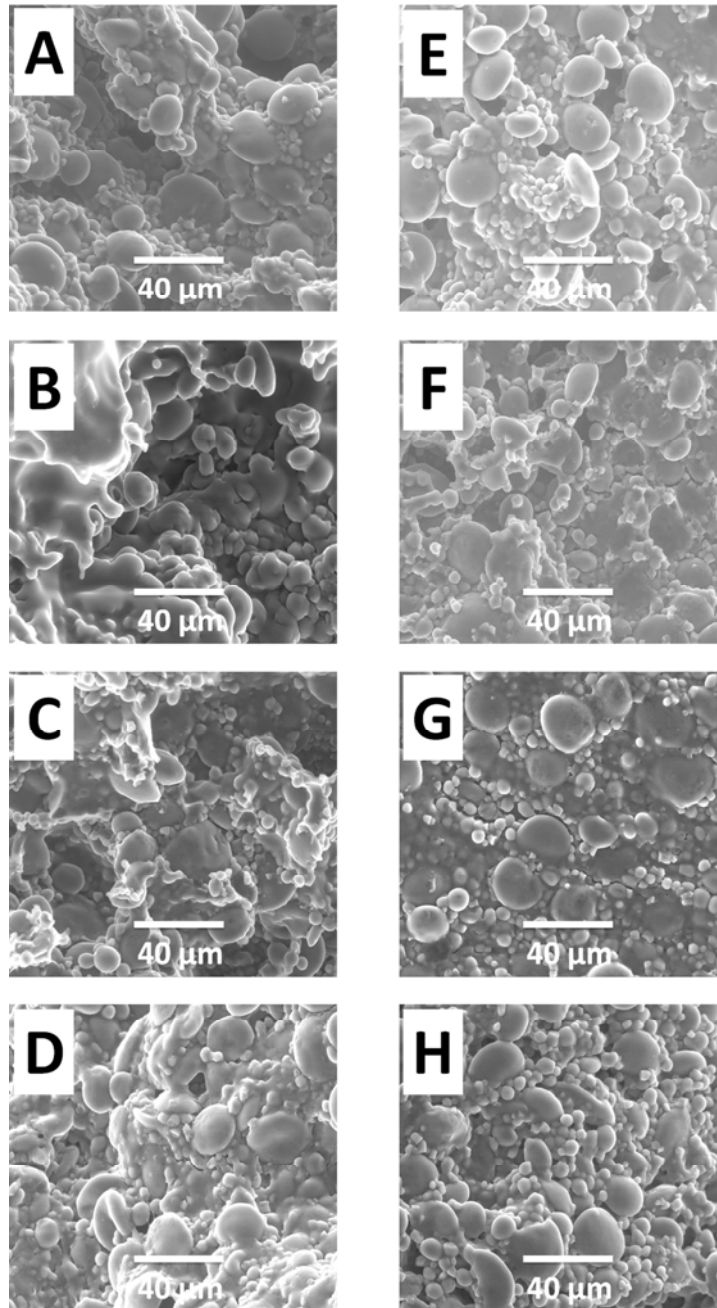
**Figure 4-14** Self-diffusion coefficient ( $D$  in  $m^2/s$ ) of water in fresh dough at  $25\text{ }^\circ\text{C}$  (fresh samples represented by ● at 0 week) and for frozen-thawed samples, as a function of frozen storage time. GA, CSL, and DATEM stands for Gum Arabic, Calcium stearoyl-2-lactylate, and Diacetyl-tartaric acid ester of monoglycerides respectively; all % are in w/w basis



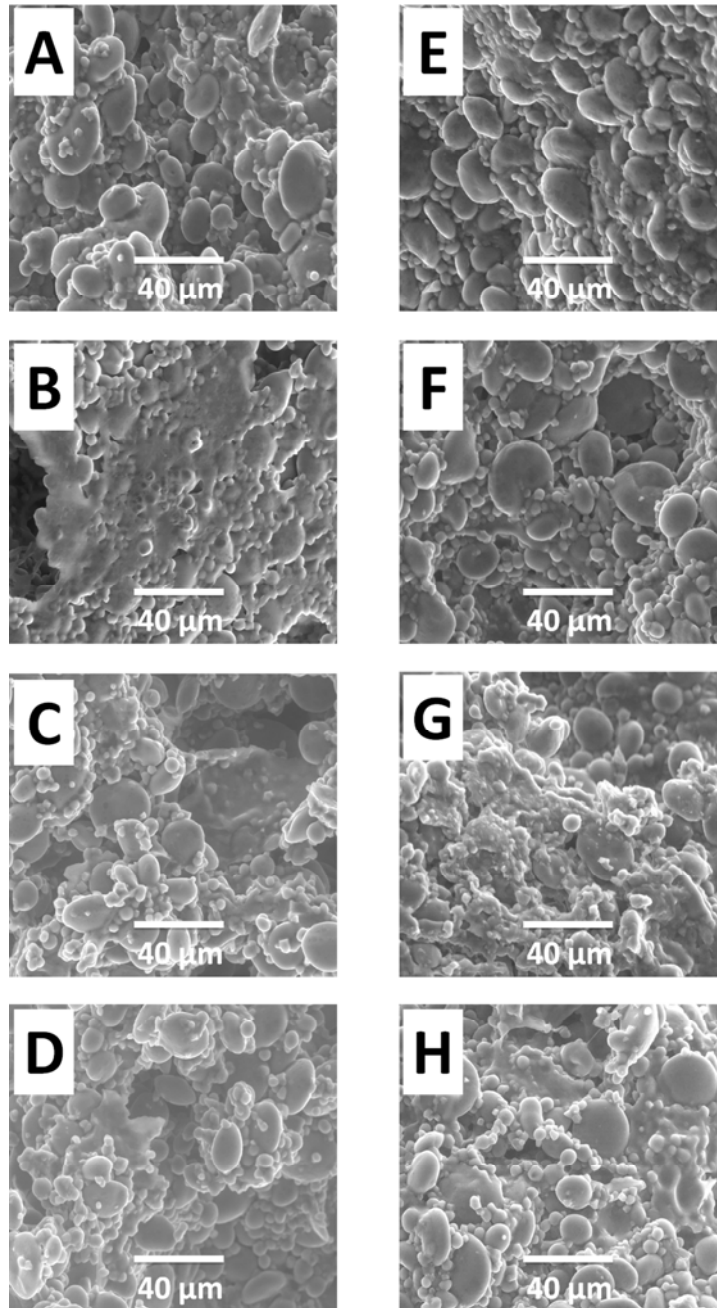
**Figure 4-15** Peak area for population 2 of protons in fresh dough at 25 °C (fresh samples represented by ● at 0 week) and for frozen-thawed samples, as a function of frozen storage time. GA, CSL, and DATEM stands for Gum Arabic, Calcium stearoyl-2-lactylate, and Diacetyl-tartaric acid ester of monoglycerides respectively; all % are in w/w basis



**Figure 4-16** Peak standard deviation for population 2 of protons in fresh dough at 25 °C (fresh samples represented by ● at 0 week) and for frozen-thawed samples, as a function of frozen storage time. GA, CSL, and DATEM stands for Gum Arabic, Calcium stearoyl-2-lactylate, and Diacetyl-tartaric acid ester of monoglycerides respectively; all % are in w/w basis

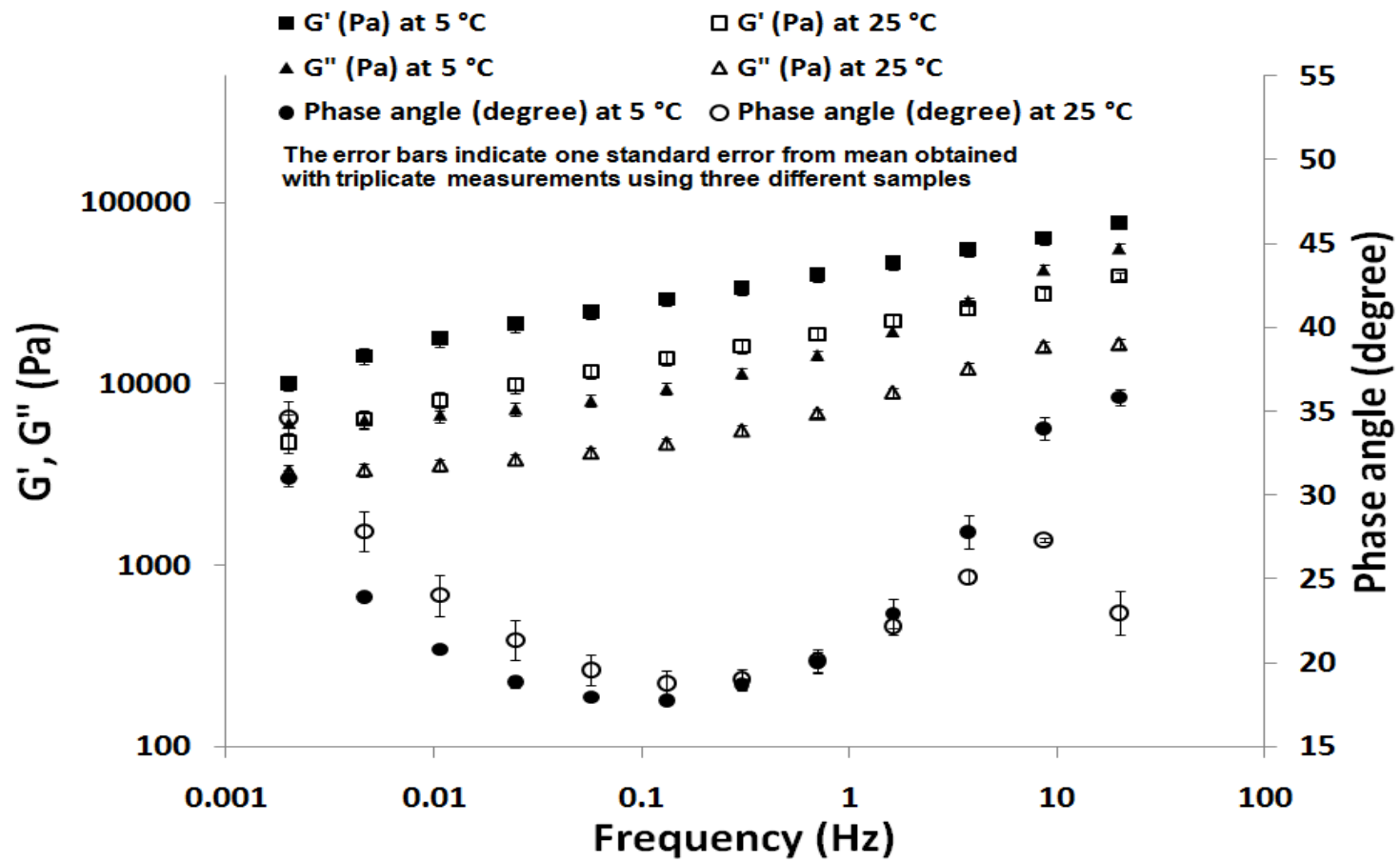


**Figure 4-17** ESEM micrographs of frozen dough ( $-10\text{ }^{\circ}\text{C}$ ) with no emulsifiers/gum. A, B, C, and D represents micrographs for 0, 8, 16, and 26 weeks frozen storage time respectively at CR0 or still air cooling. E, F, G, and H represent micrographs for 0, 8, 16, and 26 weeks frozen storage time respectively at CR1 or forced air cooling

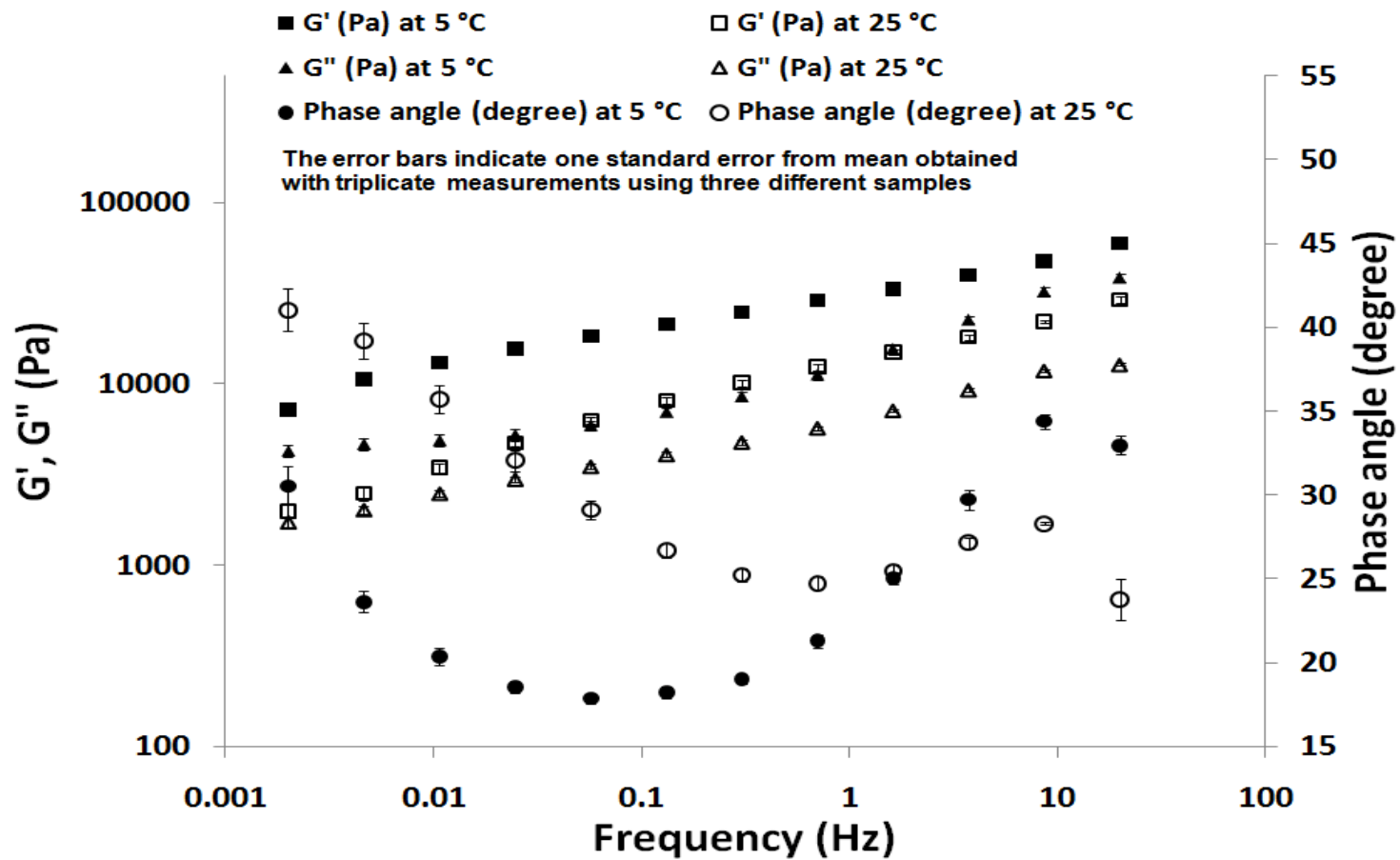


**Figure 4-18** ESEM micrographs of frozen dough ( $-10\text{ }^{\circ}\text{C}$ ) made using with 3% GA, 0.5% CSL, 0.6% DATEM. GA: Gum Arabic, CSL: Calcium stearoyl-2-lactylate, and DATEM: Diacetyl-tartaric acid ester of monoglycerides. A, B, C, and D represents micrographs for 0, 8, 16, and 26 weeks frozen storage time respectively at CR0 or still air cooling. E, F, G, and H represent micrographs for 0, 8, 16, and 26 weeks frozen storage time respectively at CR1 or forced air cooling

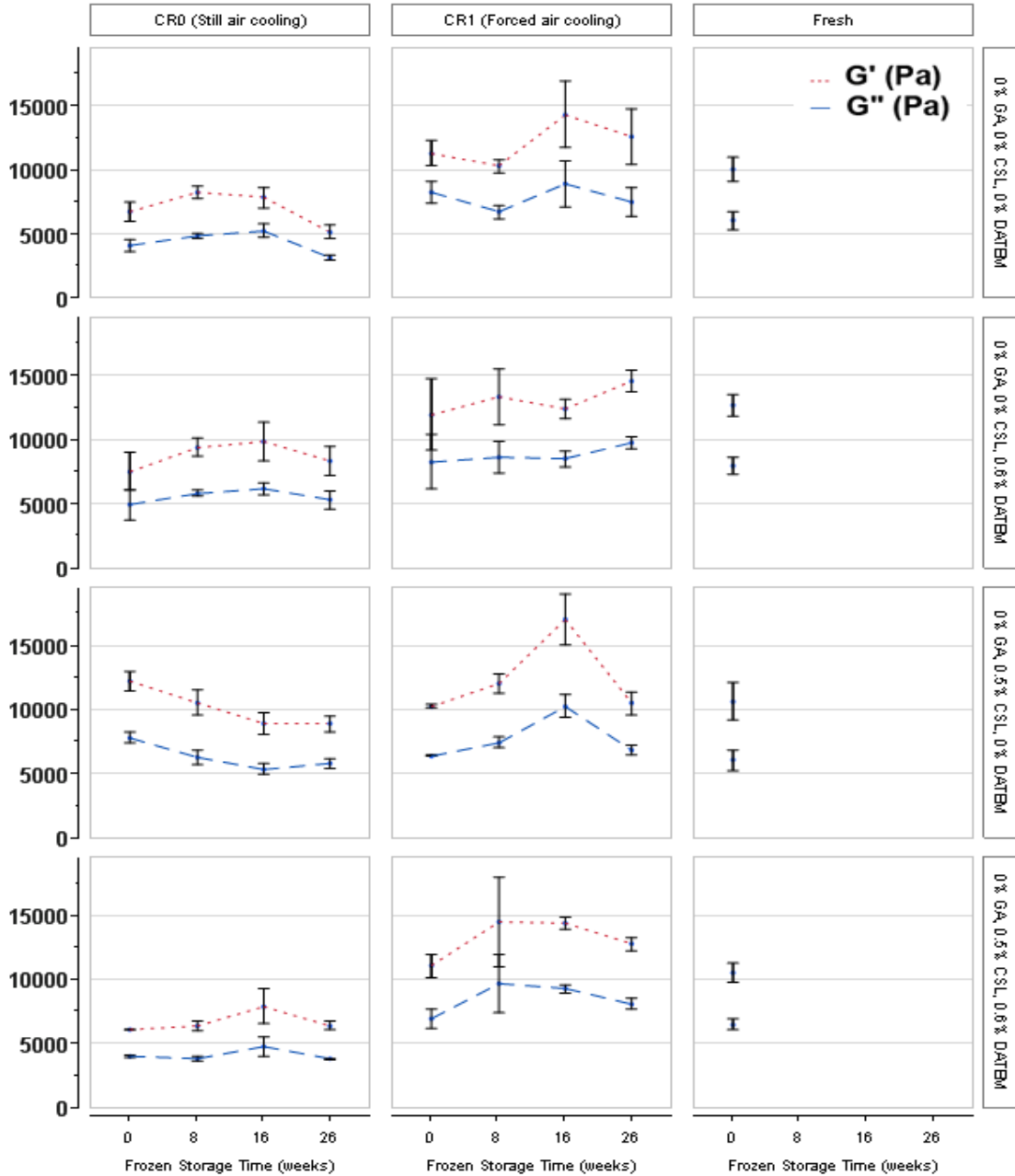




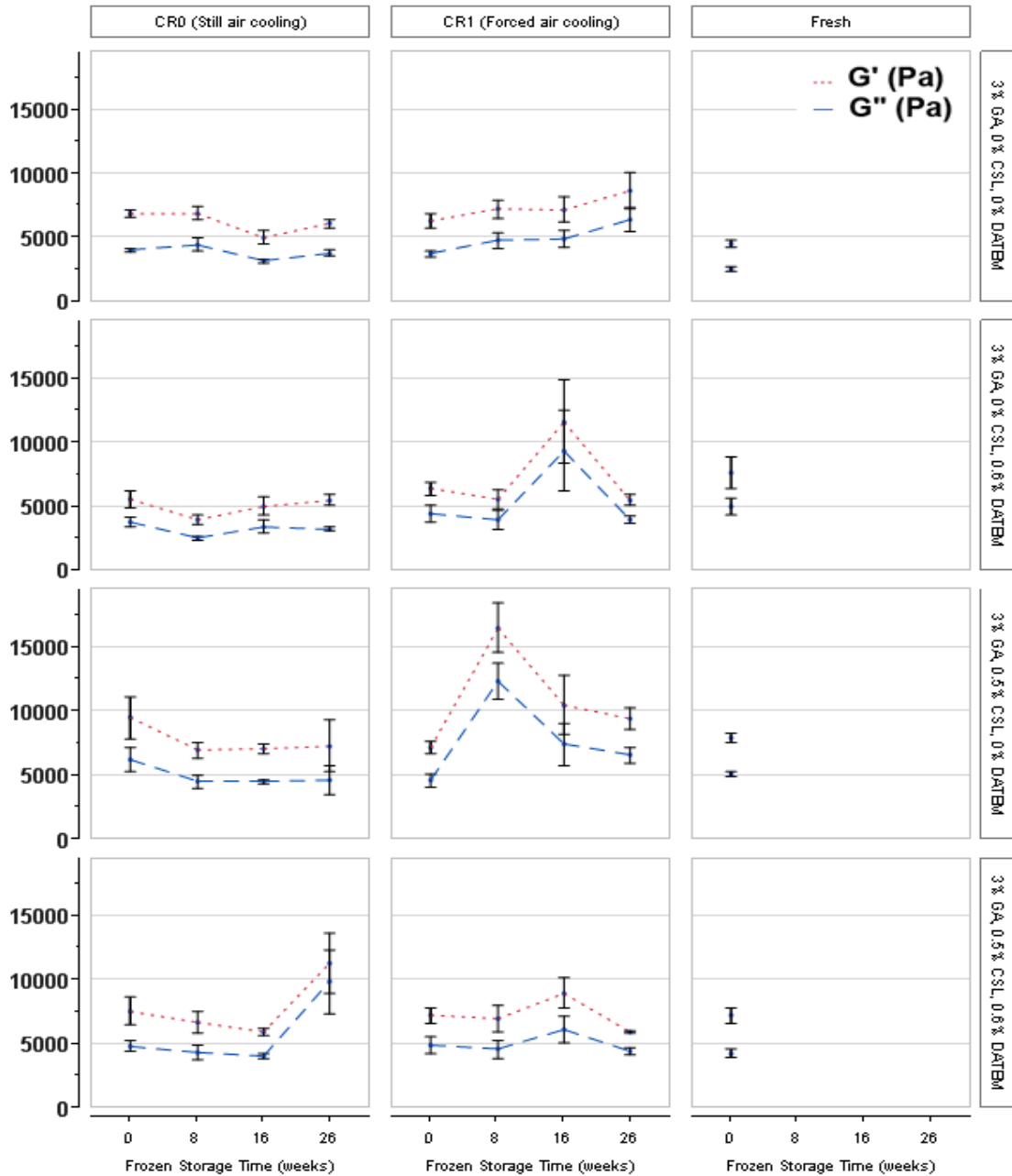
**Figure 4-19** Storage modulus ( $G'$ ), loss modulus ( $G''$ ), and phase angle ( $\delta$ ) versus frequency for fresh dough made without any emulsifiers or gums at 5 °C and 25 °C



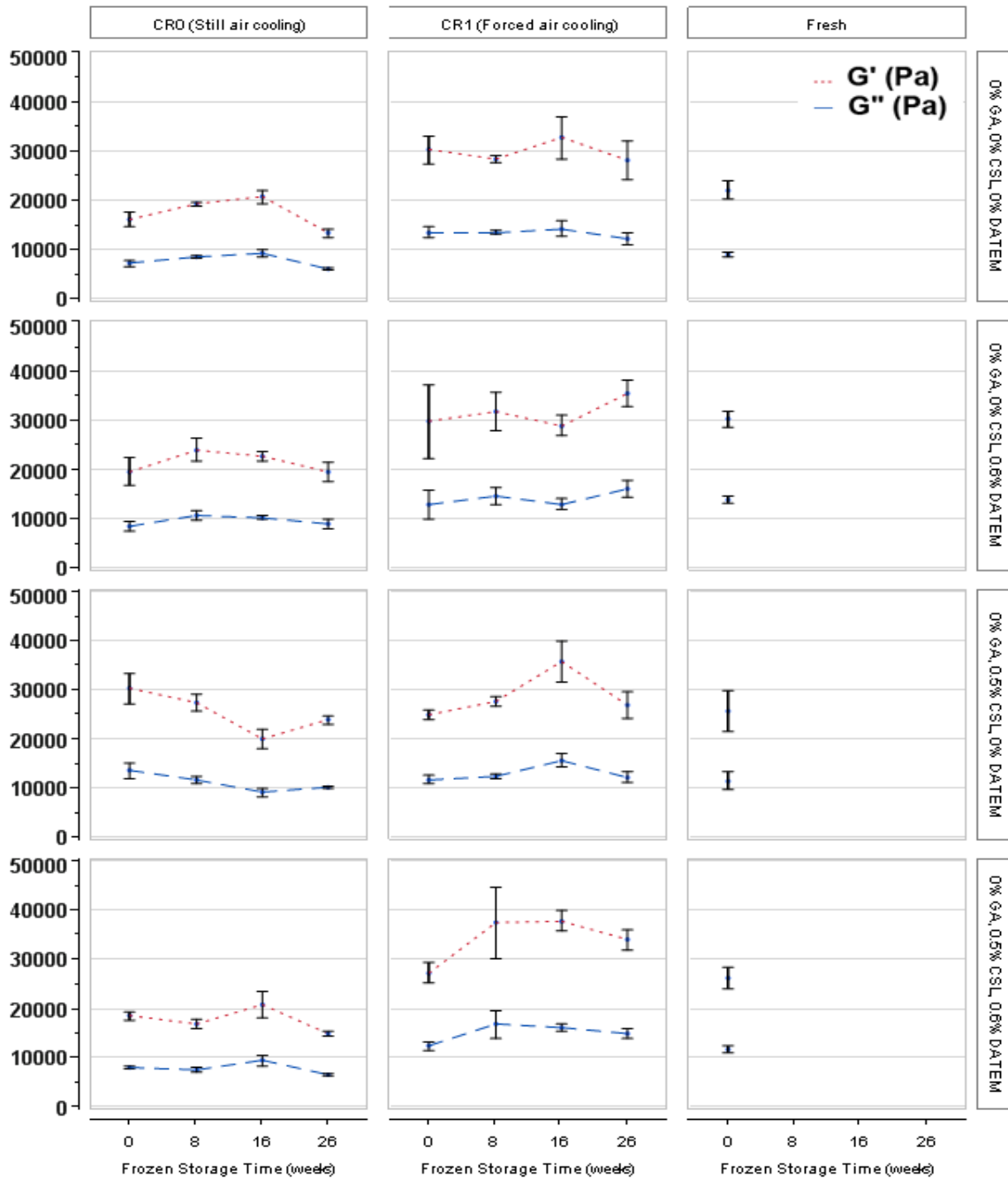
**Figure 4-20** Storage modulus ( $G'$ ), loss modulus ( $G''$ ), and phase angle ( $\delta$ ) versus frequency for fresh dough made with 3% GA, 0.5% CSL, 0.6% DATEM at 5 °C and 25 °C. GA: Gum Arabic; CSL: Calcium stearoyl-2-lactylate, DATEM: Diacetyl-tartaric acid ester of monoglycerides



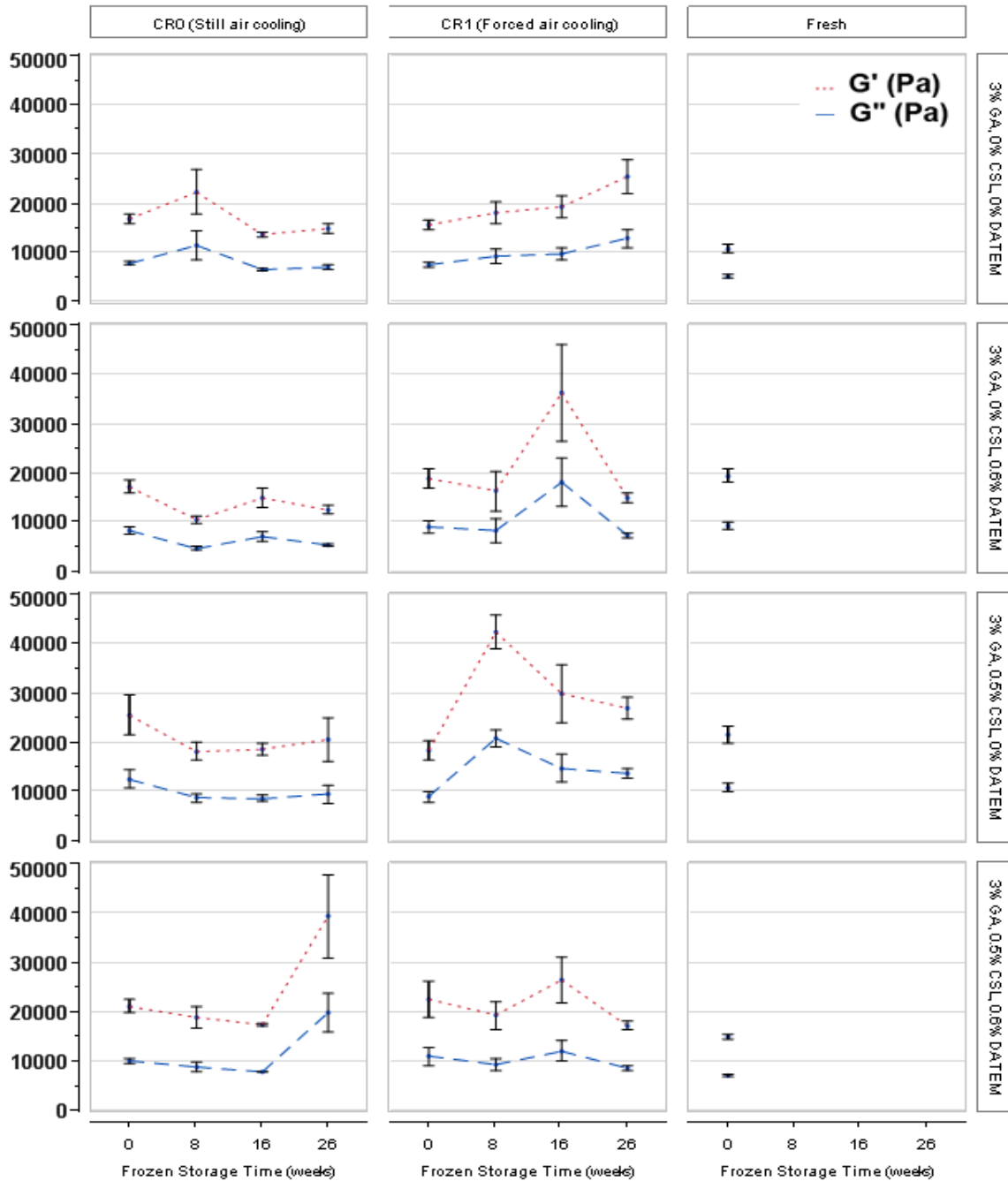
**Figure 4-21** Effect of frozen storage time and cooling rate on storage modulus ( $G'$ ) and loss modulus ( $G''$ ) measured at 0.002 Hz and 5 °C for dough made with eight formulations (continued to Figure 4-22) obtained using different % (w/w) of GA, CSL, and DATEM. GA: Gum Arabic; CSL: Calcium stearoyl-2-lactylate, DATEM: Diacetyl-tartaric acid ester of monoglycerides. The error bars indicate one standard error from mean obtained with triplicate measurements



**Figure 4-22** Effect of frozen storage time and cooling rate on storage modulus ( $G'$ ) and loss modulus ( $G''$ ) measured at 0.002 Hz and 5 °C for dough made with eight formulations (continued from Figure 4-21) obtained using different % (w/w) of GA, CSL, and DATEM. GA: Gum Arabic; CSL: Calcium stearoyl-2-lactylate, DATEM: Diacetyl-tartaric acid ester of monoglycerides. The error bars indicate one standard error from mean obtained with triplicate measurements



**Figure 4-23** Effect of frozen storage time and cooling rate on storage modulus ( $G'$ ) and loss modulus ( $G''$ ) measured at 1.62 Hz and 25 °C for dough made with eight formulations (continued to Figure 4-24) obtained using different % (w/w) of GA, CSL, and DATEM. GA: Gum Arabic; CSL: Calcium stearoyl-2-lactylate, DATEM: Diacetyl-tartaric acid ester of monoglycerides. The error bars indicate one standard error from mean obtained with triplicate measurements



**Figure 4-24** Effect of frozen storage time and cooling rate on storage modulus ( $G'$ ) and loss modulus ( $G''$ ) measured at 1.62 Hz and 25 °C for dough made with eight formulations (continued from Figure 4-23) obtained using different % (w/w) of GA, CSL, and DATEM. GA: Gum Arabic; CSL: Calcium stearoyl-2-lactylate, DATEM: Diacetyl-tartaric acid ester of monoglycerides. The error bars indicate one standard error from mean obtained with triplicate measurements

## Chapter 5

### **MANUSCRIPT II: Effects of functional additives, cooling rates, and frozen storage time on wheat bread quality attributes**

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Bread quality improvements using functional...

## 5.1 Abstract

The effects of functional ingredients such as gum arabic (GA), calcium stearoyl-2-lactylate (CSL), and diacetyl tartaric acid ester of monoglycerides (DATEM) in combination with frozen storage time (0–26 weeks) and two different cooling rates on wheat bread quality attributes such as baked specific volume (BSV), textural profile analysis (TPA) parameters (cohesiveness, gumminess, and chewiness), and crust textural parameters (compressive force at break and modulus of elasticity) were studied. GA, CSL, and DATEM were added to improve the quality attributes and counteract the detrimental effects of frozen storage so that the quality attributes of bread made with frozen dough were comparable to the quality attributes of bread made with fresh dough. Two cooling rates (CR0 or still air cooling and CR1 or forced air cooling), four frozen storage times (0, 8, 16, and 26 weeks) along with two levels of GA, CSL, and DATEM were studied for analyzing main and interaction effects on quality attributes of bread. Increasing frozen storage time decreased the BSV along with most of the quality attributes when compared to breads made with fresh dough. Cohesiveness, gumminess, and chewiness for bread crumb made with fresh or frozen dough was significantly affected by interactions among GA, CSL, and DATEM. Bread crust texture showed a significant dependence on levels of functional ingredients along with processing parameters.

**Keywords:** wheat bread quality, frozen storage, crumb, crust, emulsifier



**Practical Application:** The study suggests that different textural quality attributes of wheat bread made using frozen dough can be improved by adding certain combinations of emulsifiers and/or gums.

## 5.2 Introduction

Bread is one of the oldest staple foods in the world. For baked food manufacturers to compete in the marketplace and provide customers a variety of baked products at a low cost, there has been centralization of dough manufacturing facilities (Best 1995; Frank 2008). Due to centralization, more than 50 % of in-store supermarket bakeries as well as retail stores use frozen dough. However, it has a short shelf-life of six to eight weeks (Berglund and others 1991). A significant decrease in the quality of the baked product occurs when dough is frozen and stored for long period of times (Inoue and Bushuk 1996; Bot 2003; Giannou and Tzia 2007). If frozen dough is used, it is important to ensure that the quality of the resulting bread is similar to that produced using fresh dough. This has led to great interest in studies relating to frozen dough in the last decade (Rosell and Gómez 2007).

The redistribution of water within dough during freezing (MacRitchie 1976; Räsänen and others 1997; Lu and Grant 1999; Ribotta and others 2001), release of glutathione from yeast cells during frozen storage (Wolt and D'Appolonia 1984a, b; Chen and Schofield 1996), and limited re-absorption of water during thawing leading to 'drip loss' (Nesvadba 2009) are the main reasons for deterioration in dough quality. Aforementioned reasons lead to changes in the ice crystal size, water binding capacity of gluten and starch, reduction of disulphide bonds in gluten — decreasing the gas holding capacity of gluten during proofing/baking (resulting in an increase in proof

time). Cooling rate and frozen storage time affects the formation of ice crystals and water redistribution and are very important in determination of the quality of the baked product made from the frozen dough (Ribotta and others 2001; Zounis and others 2002; Giannou and Tzia 2007; Yi and Kerr 2009b).

Effects of wheat cultivar, cold pre-treatment, pre-fermentation time, temperature fluctuations during frozen storage, freezing rate, frozen storage temperature/time, and functional additives such as hydrocolloids, emulsifiers, enzymes, fiber, ice structuring proteins, and amino acids on frozen dough quality, bread volume, and crumb and crust structure have been studied in past. Functional additives used in these studies were sodium stearoyl lactylate (SSL), calcium stearoyl-2-lactylate (CSL), diacetyl-tartaric acid ester of monoglycerides (DATEM), gluten, guar gum, xanthan, carrageenan, carboxymethylcellulose, hydroxypropylmethylcellulose (HPMC), polysorbate 80 (PS80 or Tween 80), transglutaminase, waxy wheat flour, and vegetable shortening (Wolt and D'Appolonia 1984a, b; Ribotta and others 2001; Ribotta and others 2004; Koh and others 2005; Matuda and others 2005; Matuda and others 2006; Caballero and others 2007; Dodić and others 2007; Huang and others 2008; Phimolsiripol and others 2008a; Phimolsiripol and others 2008b; Lorenzo and others 2009; Shon and others 2009; Xu and others 2009; Yi and others 2009a; Yi and Kerr 2009a, b; Yi and others 2009b; Le-Bail and others 2010; Polaki and others 2010). SSL and diacetyl tartaric acid decreased the effects of frozen storage on rheological

properties. However, they were ineffective in reducing the proofing time of dough. It was observed in these studies that the use of some of these ingredients decreased the effects of frozen storage on its rheological properties and resulted in greater bread volume and more open crumb structure as compared to formulation without these ingredients. For example, Ribotta and others (2001) observed that frozen dough with DATEM, gluten, and guar gum resulted in bread with greater volume and more open crumb structure.

Although a considerable amount of research work on the effects of various ingredients on the quality of the frozen wheat dough has been performed, very few studies have been conducted to understand the combined effect of various functional additives such as gum arabic (GA), CSL, and DATEM along with processing parameters such as cooling rate and frozen storage time on the quality of the baked. The current study was performed to investigate the combined effects of functional additives and processing parameters on baking performance in fresh and frozen dough. The quality parameters studied were based on textural profile analysis (TPA) for crumb texture, puncture test for crust texture, and baked specific volume (BSV).

## **5.3 Materials and Methods**

### **5.3.1 Materials**

Commercial organic whole wheat flour (The King Arthur Flour Company, Inc., Norwich, VT) with 14.0 % protein content and 1.5 % ash was used to formulate dough. Active dry yeast (Fleischmann's Yeast, Chesterfield, MO), vegetable oil (ConAgra Foods, Inc., Omaha, NE), and non-iodized salt (Morton International, Inc., Chicago, IL) were used for all formulations. Active dry yeast was obtained from a single vendor and kept in air-tight containers until use. The hydrocolloid used for the current research work was Gum arabic (GA) and was obtained from Colloides Naturels, Inc., Bridgewater, NJ. The emulsifiers used were calcium stearoyl-2-lactylate (CSL) and diacetyl-tartaric acid ester of monoglycerides (DATEM) and were obtained from Caravan Ingredients, Lenexa, KS and Danisco Inc., New Century, KS respectively. Rapeseeds used for BSV measurements of bread were obtained from Specialty Commodities, Inc., Fargo, ND.

### **5.3.2 Design of experiments**

The seven formulation and processing parameters and their levels used in the full factorial design for the design of experiments in this study are listed in Table 5-1.

Breads obtained by treatments using this full factorial design were tested using TPA and puncture test on a Universal Testing Machine. These final baked products were also

tested for two more quality attributes — BSV and cook yield. Figure 5-1 shows the flow diagram representing bread preparation and experimental techniques used to collect data on the breads. All bread samples in this study were prepared using either fresh dough or frozen-thawed dough. Bread obtained using fresh dough was prepared by baking dough after mixing, molding, and 10 min fermentation at  $25 \pm 1$  °C. Therefore, fresh dough refers to the dough samples that were never cooled or kept in frozen storage. As shown in Figure 5-1, bread prepared using fresh dough samples for all dough formulations were studied using similar techniques for quality attributes and compared to bread prepared using frozen-thawed dough. The bread prepared using dough samples which were frozen-thawed were obtained by fermenting dough for 10 min at  $25 \pm 1$  °C (immediately after mixing), then freezing at the desired cooling rate (CR0: Still air cooling or CR1: Forced air cooling) followed by frozen storage for the desired time (0, 8, 16, or 26 weeks), thawing at 5 °C for 24 hrs, and then baking. The bread with treatment combinations for frozen storage time point “FT0: 0 week” with CR0 or CR1 indicates that it was prepared by baking dough samples cooled at the desired cooling rate of CR0 or CR1, frozen to a stable temperature below  $-18$  °C in the freezer (24 hrs of frozen storage time resulted in a stable internal temperature of  $-18$  °C), and thawed at 5 °C for 24 hrs. In this study these treatments are referred to as 0 week treatments with cooling rate of either CR0 or CR1. Bread prepared with dough without GA, CSL, or DATEM (Bread 1 in Table 5-2) was used as the reference dough for

comparison with the rest of the bread treatments. Bread quality attributes were analyzed using crumb and crust texture along with loaf volume. Triplicate measurements were performed for all tests to report the means and standard errors.

### **5.3.3 Dough formulation**

For all dough formulations, the ratio of wheat flour : yeast : oil : salt was 50:2:1:1. This ratio was calculated from the base recipe: whole wheat flour (4200 g, 61.35 % w/w), yeast (168 g, 2.45 % w/w), salt (84 g, 1.23 % w/w), vegetable shortening (84 g, 1.23 % w/w), and water (2310 g, 33.74 % w/w) as used by Zounis and others (2002). Breads were prepared using eight dough formulations obtained using two levels of CSL, DATEM, and GA (full factorial design) as presented in Table 5-2. To study the effect of emulsifiers, bread prepared using dough with no GA, CSL, or DATEM (Bread 1 in Table 5-2) was used as the reference bread for comparison with rest of the bread treatments.

### **5.3.4 Dough preparation and its freezing**

As indicated in Table 5-1, two processing variables were studied: cooling rate and frozen storage time. 500 g of starter dough was prepared for each of the eight treatments of dough formulations. A classic stand mixer (KitchenAid, K45SSWH 4.3L, St. Joseph, MI) with a bowl (of volume 4½ quarts) and a dough hook with six speed levels (settings available were stir, 2, 4, 6, 8, and 10) was used to mix all the dough

ingredients. A moderate mixing rate of “M1: #4” (planetary speed: 135 rpm and beater speed: 442 rpm) was used. Yeast is more susceptible to freeze damage with an increase in fermentation/resting time and therefore, minimizing the fermentation time before freezing is important (Rosell and Gómez 2007). Hence, starter samples were set aside for only 10 min at  $25 \pm 1$  °C to minimize yeast activity before freezing (Dodić and others 2007).

A heavy duty commercial chest freezer (model # FFC13C3AW2, Electrolux Home Products, Inc., Cleveland, OH) operating at an internal temperature of approximately  $-22$  °C was used for cooling and frozen storage of dough. CR0 for dough was achieved by keeping the dough inside the freezer under still air conditions. CR1 was achieved using a small table fan (8" High velocity personal fan, Wal-Mart stores, Inc., Bentonville, AR 72716) kept inside a separate chamber in the freezer. To achieve CR1, samples were kept at the centerline of air flow generated by the fan set at its highest speed. Air speed at the centerline of the fan at its highest speed setting was 0.1 m/s. Frozen samples were sealed in a plastic zip lock freezer bag (after the core reached  $-18$  °C) to prevent any moisture exchange between the frozen dough and the ambient air in the freezer during frozen storage. Temperature at the center of sample was recorded for the first 20 hrs (enough time to freeze sample cores to  $-18$  °C) to differentiate between the cooling rates of dough samples in the freezer. Temperature acquisition was performed



using a Digi-Sense Scanning Thermocouple Thermometer (Model # 92800-10, Cole-Parmer Instrument Co., Niles, IL) attached with 12" temperature probes.

### 5.3.5 Cooling rates used for dough sample preparations

All doughs were cooled at two different rates (CR0: using still air and CR1: using forced air). The temperature drop at the center of the starter dough from room temperature to  $-5\text{ }^{\circ}\text{C}$  relates to the sensible heat extracted from dough before extraction of latent heat of fusion occurs. A significant difference ( $\sim 1$  hr) was observed between CR0 and CR1 in the time taken for the center of dough to reach  $-5\text{ }^{\circ}\text{C}$ . In addition, a significant difference ( $\sim 2$  hrs) was observed in the time required for extraction of latent heat of fusion between the two cooling rates. Cooling rates (also described as Freezing rates or Fr) were calculated as the ratio of the difference between the initial and final temperature to the time duration in hours (International Institute of Refrigeration 1986; Havet and others 2000):

$$\text{Fr} = \frac{T_2 - T_1}{t_2 - t_1} \quad (5-1)$$

where  $T_1$  is the temperature at  $t_1 = 0$  and  $t_2$  is the time when  $T_2$  first reaches  $-5\text{ }^{\circ}\text{C}$ . For CR0 or still air cooling, Fr was  $6.8\text{ }^{\circ}\text{C/hr}$  and for CR1 or forced air cooling Fr was  $12.1\text{ }^{\circ}\text{C/hr}$ .

### **5.3.6 Thawing, molding, proofing, and baking of dough**

After the 500 g starter dough had been cooled (at CR0 or CR1) and frozen (for 0, 8, 16, or 26 weeks), it was thawed at 5 °C for 24 hrs. For fresh samples (no cooling or frozen storage), molding, proofing, and baking were done after 10 min of fermentation time. For molding, the 500 g starter dough (fresh or frozen-thawed) was divided into three (for triplicate measurements) 120 g samples and each placed into a rectangular cubical mold of (~6 cm × 3 cm × 3 cm). The dough samples in the molds were proofed at 25 ± 1 °C for 60 min. Proofed samples were taken out of the molds and placed on an aluminum foil in a tray and baked in a convection baking oven (FBC212/1600W, Krups North America Inc., Closter, NJ) at 230 °C for 15 min.

### **5.3.7 Determination of baked specific volume (BSV)**

Baked samples were placed under ambient conditions to let them cool for 1 hr before BSV was measured. Cooling the baked samples in an open environment minimizes any condensation that might accumulate on the crust (and thus change crust structure). BSV was measured using rapeseed displacement method — AACC 10-05 (AACC 1995) which is a common technique for measuring volumes of irregular solids (Datta and others 2007). In this method, first the tapped bulk density of rapeseeds is determined by filling a glass container of known mass ( $m_{\text{container}}$ ) and volume ( $V_{\text{container}}$ ) uniformly with

rapeseeds through tapping and smoothing the surface with a ruler, and measuring the mass of the rapeseed ( $M_{\text{seeds}}$ ) in the container. Then, the baked sample ( $m_{\text{sample}}$ ) and rapeseeds are placed together into the same container. The container is again tapped and the surface is smoothed with a ruler. Tapping and smoothing is continued until a constant total weight ( $m_{\text{total}}$ ) is reached between consecutive measurements, while rapeseeds are filled into the container. The bulk volume ( $V_b$ ) and BSV of the baked product is calculated as follows:

$$\rho_{\text{seeds}} = \frac{M_{\text{seeds}}}{V_{\text{container}}} \quad (5-2)$$

$$m_{\text{seeds}} = m_{\text{total}} - m_{\text{sample}} - m_{\text{container}} \quad (5-3)$$

$$V_{\text{seeds}} = \frac{m_{\text{seeds}}}{\rho_{\text{seeds}}} \quad (5-4)$$

$$V_b = V_{\text{container}} - V_{\text{seeds}} \quad (5-5)$$

$$\text{BSV} = \frac{V_b}{m_{\text{sample}}} \quad (5-6)$$

where  $M$  or  $m$  denotes the mass of the sample or the seeds (as indicated by the subscript) and  $\rho_{\text{seeds}}$  is the bulk density for the rapeseeds.

To avoid any further significant moisture exchange between baked sample and its ambience after BSV measurements, baked samples were immediately wrapped into zipped plastic bags and kept at room temperature for 24 hrs until TPA and puncture test measurements were performed.

### 5.3.8 Textural profile analysis (TPA) for crumb texture of baked product

TPA was performed after 24 hrs of baking the samples to study the textural properties of the crumb. The baked and then cooled samples, as described in earlier section, were cut into cylindrical discs (~16 mm diameter and 2.2 cm height) from the bottom section of the bread. These samples were used to determine the hardness (N), cohesiveness (no units), gumminess (N), and chewiness (N·cm) using a Universal Testing Machine (model 5542, Instron, Corp., Canton, MA) by performing the two bite “texture profile analysis” method (Peleg 1976; Bourne 1978; Bourne 2002; Manohar and Rao 2002) with a sample compression of 80 % and crosshead speed of 100 mm/min. The following parametric definitions are used for TPA:

$$\text{Cohesiveness} = (A2/A1) \quad (5-7)$$

$$\text{Gumminess} = (\text{Hardness}) \times (\text{Cohesiveness}) = (\text{Hardness}) \times (A2/A1) \quad (5-8)$$

$$\text{Chewiness} = (\text{Gumminess}) \times (\text{Springiness}) = (\text{Hardness}) \times (A2/A1) \times (\text{Springiness}) \quad (5-9)$$

where A1 and A2 are the areas under the force-deformation curves (Figure 5-2) of the first and second bites, respectively. Hardness is the force measurement at the second major peak of the first bite (at the end of the first down stroke), and “springiness” is the width (deformation units) of the second bite curve. Triplicate measurements were performed for each bread treatment and mean and standard errors were reported.

### **5.3.9 Puncture test for analysis of crust of baked product**

Puncture test was performed after 24 hrs of baking the samples to study the textural properties of the crust. The baked and then cooled samples, as described in earlier section, were punctured using a plunger (0.6 cm diameter) in a Universal Testing Machine (model 5542, Instron, Corp., Canton, MA). The crosshead speed for the test was 100 mm/min. Maximum force or the compressive load (in N) at the breakpoint of crust and the modulus of elasticity (in MPa) of the crust (for 1-4 mm segment of compression for bread crust before break) were reported as mean and standard error obtained using triplicate measurement.

#### **5.3.10 Statistical analysis**

The effects of functional ingredients (GA, CSL, and DATEM) and processing parameters (cooling rates and frozen storage times) on properties such as BSV, cohesiveness, gumminess, chewiness, compressive load at break for crust, and modulus of elasticity for crust were analyzed with one-way analysis of variance (ANOVA) and generalized linear model (to study main and interaction effects) using JMP® 8.0 software (SAS Institute Inc., Cary, NC) at a significance level of 0.05. One-way ANOVA was applied across eight dough formulations and across nine different combinations of cooling rates and frozen storage times. Tukey-Kramer honestly significant difference (HSD) tests

were performed for all pair-wise comparisons of means when significant differences existed ( $p$ -value < 0.05) in one-way ANOVA tests. All means reported in this study were obtained using triplicate measurements and the error bars presented in the tables are one standard error from the mean values.

## **5.4 Results and Discussion**

### **5.4.1 Effects of functional additives and processing parameters on BSV**

Within fresh breads, compared across eight different formulation treatments, BSV was significantly influenced by GA as shown in Table 5-9. No other factors showed a main or interaction effect on the BSV. This can also be observed in Table 5-3 where bread made with 3 % w/w GA with or without DATEM or CSL had lower BSV than the rest of the breads. The BSV values were in the range of 1.53–2.42 cm<sup>3</sup>/g and are comparable to BSV values found in literature (Havet and others 2000; Ozge Keskin and others 2004; Datta and others 2007; Sumnu and others 2007). Bread 1 (no functional ingredients added) made using fresh dough had the highest BSV among all fresh breads. This was only comparable to BSV of breads made with CSL or DATEM (bread 4 and bread 5) or that made with CSL and DATEM combined (bread 7). This shows that GA had a negative effect on the BSV when it was present in bread with or without CSL or DATEM.

Therefore, CSL or DATEM could significantly improve the BSV when the dough is not

frozen. Since BSV is a key quality attribute for pan breads, CSL or DATEM can play an important role in improving the overall quality for pan bread producers. In another study (Asghar and others 2007), increased absorption of water with addition of GA (added at 3 % w/w) was observed using farinograph and mixograph measurements, which improved certain quality attributes of pizza dough. However, the quality of pizza dough was based on empirical parameters (such as water absorption capacity, arrival time, dough development time, departure time, and tolerance index measured using farinograph) and was not based on the BSV or loaf volume. In another study (Kim and others 2008), locust bean gum was shown to negatively affect CO<sub>2</sub> release and increase the hardness of bread. Thus, different hydrocolloids have different effects on the BSV of baked products (made from fresh dough) and must be investigated experimentally before they could be used as a functional ingredient. As observed from Table 5-9, when dough is cooled under still air conditions (CR0) and stored frozen, GA and frozen storage time were shown to have a significant effect on BSV. With forced air cooling (CR1) of dough, most of the interactions, as shown in Table 5-9, affected the BSV. For bread 1, with no functional ingredients added, BSV decreased with increasing frozen storage time and was significantly lower at the end of 16 and 26 weeks. This is in accordance with earlier studies showing a decrease in BSV with increasing frozen storage of dough (Bail and others 1999; Carr and Tadini 2003; Sharadanant and Khan 2003; Dodić and others 2007). Since the BSV values for bread made with fresh dough

(bread 4 — GA only, bread 5 — DATEM only, and bread 7 — CSL and DATEM combined) were not significantly different from the BSV value for bread 1 (with no added functional ingredients), analyzing how the addition of GA, DATEM or both improves BSV with frozen storage time is of importance. As observed from Table 5-3, the BSV of bread 5 (with only DATEM added) at CR1 was not significantly different up to 16<sup>th</sup> week, after which it was significantly lower. Bread 4 (with only CSL) at CR1 did not show any improvements in BSV with frozen storage time since the bread made after 8 weeks or longer frozen storage time resulted in a significant decrease in BSV when compared to that made with fresh dough. Bread 7 (with CSL and DATEM added) had an increase in BSV up to the 8<sup>th</sup> week, which was an intermediate time between that for breads 4 and 5. Thus, DATEM alone proved to have an effect of increasing BSV for longer frozen storage times as compared to CSL alone or when compared to CSL and DATEM combined. Therefore, it might be concluded that when DATEM is added to dough, the BSV of the bread made from frozen dough can be similar to the BSV of the bread made from fresh dough (without DATEM). Similar effects of DATEM have been observed in some studies (Sahlstrøm and others 1999; Xiujin and others 2007). DATEM acts as a dough strengthener and improves the strength of the dough matrix and hence holds gases such as CO<sub>2</sub> generated during proofing and baking. It has also been associated with lipid interaction in addition to their interaction with gluten and starch (Jacobsberg and others 1976). Dough strengtheners such as DATEM and CSL exert their



strengthening effects on the dough matrix during yeast fermentation, handling, proofing, and baking and are believed to form a liquid layer of lamellar structure in the interphase between gluten and starch (Stampfli and Nersten 1995).

#### **5.4.2 Effects of functional additives and processing parameters on crumb textural properties**

The textural properties of bread crumb were analyzed using three different quality attributes obtained from TPA — cohesiveness, gumminess, and chewiness (Peleg 1976; Bourne 1978; Bourne 2002; Manohar and Rao 2002).

*Cohesiveness.* Cohesiveness may be physically related to the strength of internal bonds in a food material and is the amount of sample deformation before rupture when biting with ones molar teeth (Carr and Tadini 2003). Cohesiveness obtained using instrumental techniques has been positively correlated with sensory attributes such as smoothness and soft center area in earlier studies (Gámbaro and others 2006). As observed from Table 5-10, the cohesiveness for bread made with fresh dough was significantly affected by CSL and DATEM and all interactions between GA, CSL, and DATEM. GA did not have any main effect on the cohesiveness of bread when added alone. However, it had a significant interaction effect with other added functional additives. A significant decrease in number of main and interaction effects is seen when the dough is cooled at CR0 or CR1 and stored under frozen conditions. In fact, only the

main effects of GA and DATEM were observed for slower cooling (CR0). For CR1, the interaction between CSL and frozen storage time along with main effects of functional additives was significant. This implies that when the dough is cooled and stored under frozen conditions, only one functional additive (CSL) interacts with frozen storage time in significantly decreasing the detrimental effect of frozen storage on the cohesiveness of the bread crumb. As seen in Table 5-4, the cohesiveness of bread 1 (with no added functional additives) was highest and was not significantly different from the result for bread 2 (added with all functional additives) or bread 4 (only CSL used). Hence it is concluded that CSL, when used alone, might improve the cohesiveness of bread made from fresh dough. However, its interaction with DATEM and GA in fresh dough was significant and decreased the cohesiveness of the final baked product. As expected from the interaction effects for cohesiveness, there are very few significantly different values for cohesiveness with increasing frozen storage times or between the cooling rates for a particular dough formulation as presented in Table 5-4. This is consistent with another study (Asghar and others 2009) performed using modified whey protein concentrates in which the cohesiveness improved with inherent wheat protein content and with added whey protein concentrates.

*Gumminess.* The interaction analysis between processing and formulation parameters shows that gumminess was significantly affected by all processing and formulation parameters, as presented in Table 5-10. For CR0, main effects of GA and

DATEM and interaction effect between CSL and DATEM were significant. However, at a higher cooling rate (CR1), in addition to main and interaction effects seen for CR0, it was observed that interaction of DATEM with all factors was significant in influencing gumminess. Therefore, at higher cooling rates, DATEM plays an important role in determination of gumminess. As presented in Table 5-5, bread 1 (made using fresh dough with no added functional ingredients), had significantly higher gumminess than rest of the bread formulations within the fresh category. Thus, addition of any functional additive (GA, CSL, or DATEM), results in improvement of gumminess. However, among these, it was observed that with addition of GA in any combination except when all three functional additives were present, the gumminess values were the lowest and statistically similar. In most cases, it is observed from Table 5-5 that the addition of GA in any combination with CSL or DATEM significantly lowered the values of gumminess over the frozen storage time and across cooling rates. In an earlier study (Asghar and others 2009), detrimental effects of frozen storage time on gumminess has been reported. However, it was observed that adding functional ingredients such as modified whey protein concentrates had beneficial effects. As gumminess takes into account hardness of a bread crumb, higher values of gumminess are not desirable in a bread product at a given cohesiveness value. Gumminess has been negatively correlated with sensory quality attributes such as soft center and smoothness of bread crumb (Gámbaro and others 2006). In another study (Gómez and others 2008), hardness was

correlated to gumminess and a high correlation was observed between the two. This may be explained due to presence of hardness in the equation (5-8) used to obtain gumminess.

*Chewiness.* Chewiness of a food material is physically related to energy required to disintegrate a solid food to a state ready for swallowing. In sensory terms, it is the number of chews required to masticate a sample at one chew per second at a constant rate of application of force, to reduce it to a consistency suitable for swallowing (Carr and Tadini 2003). It has been negatively correlated to sensorial attributes of soft center and smoothness (Gámbaro and others 2006). Table 5-10 shows that, for the bread made using fresh dough, all but the main effect of CSL can be attributed to significant changes in the chewiness of the bread. However, when cooled at CR0, all main effects due to GA, CSL, and DATEM, and only the interaction due to CSL and frozen storage time were significant. In addition, it was observed that this same interaction between CSL and frozen storage time was not observed at a higher cooling rate, where all other main effects and interaction effects were seen. This may be attributed to the negative effects to the gluten matrix strength due to larger ice crystal formation in a slow cooling process, and might be large enough to overcome any beneficial effects produced due to functional additives. Therefore, a higher cooling rate and functional additives might result in desirable chewiness characteristics in the final baked product. As observed from Table 5-6, the chewiness values with addition of functional additives within the

fresh category (first row) decreased significantly when compared to rest of the treatments in that category. In addition, bread 2 (that contains all three functional additives) showed a significant decrease in chewiness for both cooling rates (CR0 and CR1) from 0 to 26 weeks. In a study (Carr and Tadini 2003) it was observed that vegetable shortening reduced the crumb firmness and chewiness due to their softening effect in the bread. However, firmness and chewiness increased with frozen storage time, showing a detrimental effect of longer frozen storage times.

#### **5.4.3 Effects of functional additives and processing parameters on textural attributes of crust**

*Compressive load at break for bread crust.* One of the key attributes which affects the sensory quality of many food materials is the amount of force applied to the food material to deform the food and is physically correlated to the hardness or firmness of the food. Firmness is defined as the force necessary to attain a given deformation or the force required to compress a substance between the incisor teeth (Carr and Tadini 2003). Bread crust has been less extensively studied when compared to bread crumb. In an attempt to understand the sensory attributes affecting the overall quality of bread, studying firmness or hardness of the bread crust is of great importance. In this research work, we have studied the crust firmness by measuring the amount of force required to break the crust of bread and by calculating the modulus of elasticity (related to

compressive extension of crust from 1 to 4 mm). Table 5-11 presents the significant main and interaction effects of functional ingredients and processing parameters on compressive load at break. For bread made of fresh dough, the compressive load was affected by main effects of GA, CSL, and DATEM and by the interaction effects of GA and CSL. For CR0, all main and interaction effects except the main effect of frozen storage time, interaction effect of CSL and DATEM, and interaction effect of DATEM and frozen storage time, were significant in determining the effects on compressive load at break. For CR1, all main effects and interactions between GA and CSL, GA and frozen storage time, and CSL and frozen storage time were significant. Table 5-7 indicates that bread 1 (with no functional ingredients added) experienced the highest amount of compressive load at break among all treatments in breads made with fresh dough. For bread 2 (with GA, CSL, and DATEM) and bread 5 (with only DATEM), there is a decrease in the compressive load at break with increasing frozen storage time. However, for all other bread formulations, values for compressive load at break remained significantly close to the initial values. It can thus be concluded that DATEM has a significant effect in increasing softness of the crust, which is a desirable characteristic for certain baked products such as pan bread. Thus, it was seen that dough requiring frozen storage can be formulated using a specific combination of DATEM (either alone or with GA and CSL together) to negate the detrimental effects of frozen storage. It has been seen in previous studies (Primo-Martin and others 2007; Yi and Kerr 2009a) that bread crust

structure and its color are affected by factors such as cooling rates of dough and structural composition (starch crystallinity) of dough, which affects crispness, staling, and overall quality of certain breads such as crispy rusk. Other studies (Sharadanant and Khan 2003; Giannou and Tzia 2007; Yi and Kerr 2009a), found that the crust color was darker for breads produced after longer frozen storage times and stored at low temperatures ( $-30\text{ }^{\circ}\text{C}$  and  $-35\text{ }^{\circ}\text{C}$ ).

*Modulus of elasticity for bread crust.* To understand staling behavior of breads, elasticity and firmness, along with other quality attributes of bread crumbs, has been studied to a much greater extent, than elasticity or firmness of crust (Baik and Chinachoti 2000, 2003; Gray and Bemiller 2003; Gómez and others 2008). It has been shown that the deterioration of textural characteristics during staling included loss of elasticity and increased firmness of crumb. Elasticity is defined as the ability of bread to return to its original dimensions after being compressed and is correlated with bread crumb structure such as cell wall rigidity (Gil and others 1999). In this study, the modulus of elasticity (MPa) of the crust is calculated based on the stress and strains obtained at 1 and 4 mm compressive extension in the puncture test.

The interaction and main effects of functional ingredients and processing parameters in influencing the modulus of elasticity were analyzed (Table 5-11). In case of bread made using fresh dough, the modulus of elasticity was affected mainly by addition of GA and the interaction between GA and DATEM. It was also observed that a

considerable number of main and interaction effects were observed for CR1 as compared to that for CR0. This may be attributed to much higher detrimental effects caused due to larger ice crystals produced during a slower cooling rate, which leads to a loss in ability of functional ingredients to cause significant changes in elasticity. It can be seen from Table 5-8 that in breads made using fresh dough, there was no significant improvement in elasticity of the crust with addition of any combination of functional ingredients. Similarly, with increasing frozen time or varying cooling rates, there was no significant change in modulus of elasticity for the crust. Other functional ingredients such as enzymes (pentosanase and lipase), cysteine, and L-ascorbic acid may be considered for reducing firmness and reducing the loss of recoverability of the crust, which considerably affects staling of bread (Sebesic and others 1981; Gil and others 1999; Gámbaro and others 2006).

## **5.5 Conclusions**

Functional additives (GA, CSL, and DATEM) and processing parameters (cooling rates and frozen storage time) used in preparation of dough affects various important quality attributes of the baked product. These quality attributes, which determine the sensory and the consumer acceptability of breads (BSV, cohesiveness, gumminess, chewiness, firmness of crust, and modulus of elasticity of crust), were studied. These attributes significantly varied and were dependent on levels of functional additives and their



interactions with cooling rates and frozen storage times. Longer storage times resulted in lower BSV. With increasing frozen storage time, DATEM, when added as the only functional ingredient, improved the BSV. DATEM also proved to be a significant factor in improving the softness of the crust. These effects of DATEM might be attributed to its dough strengthening effects, which leads to a stronger dough matrix and results in higher retention of CO<sub>2</sub> and therefore an improved crumb and crust structure. CSL, when used alone in fresh dough, improved cohesiveness of the baked product. Specific combinations of DATEM with CSL and GA showed significantly better results for improving other textural attributes of bread crumb such as gumminess and chewiness. Therefore, improving quality attributes of a baked product made using frozen dough is feasible by prioritizing the desired quality attributes in bread and by using a specific combination of functional additives targeted to improve these attributes.

Understanding the interactions between the functional ingredients (GA, CSL, and DATEM) and dough components such as gluten and starch at macromolecular level remains to be investigated in further studies.

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**Table 5-1** Formulation and processing variables and their levels for design of experiments in this study

| Variables  | Levels | Values of different levels <sup>a</sup>  |
|--|--------|--|
| Water content  | 1      | W1: 33.74 %  |
| Mixing rate  | 1      | M1 (setting: # 4 on mixer i.e. planetary speed: 135 rpm & beater speed: 442 rpm)           |
| Cooling rate   | 3      | Fresh (no cooling or frozen storage), CR0: still air cooling, CR1: forced air cooling      |
| Frozen storage time                                    | 4      | FT0: 0 week (for cooling and immediate thawing), FT1: 8 week, FT2: 16 weeks, FT3: 26 weeks |
| Gum Arabic (GA)  | 2      | GA0: 0 %, GA1: 3 %   |
| Calcium stearoyl-2-lactylate (CSL)                     | 2      | CSL0: 0 %, CSL1: 0.5 %   |
| Diacetyl-tartaric acid ester of monoglycerides (DATEM) | 2      | DATEM0: 0 %, DATEM1: 0.6 %   |

<sup>a</sup>All the % values are in w/w basis

**Table 5-2** Eight bread formulations obtained using full factorial design of experiments with variables described in Table 5-1

| <b>Formulation</b>          | <b>Water</b> | <b>Flour</b> | <b>Yeast</b> | <b>GA</b> | <b>CSL</b> | <b>DATEM</b> | <b>Oil</b> | <b>Salt</b> |
|-----------------------------|--------------|--------------|--------------|-----------|------------|--------------|------------|-------------|
| <i>Bread 1</i> <sup>a</sup> | 34.25        | 62.26        | 1.00         | 0.00      | 0.00       | 0.00         | 1.25       | 1.25        |
| Bread 2                     | 34.25        | 58.32        | 1.00         | 3.00      | 0.50       | 0.60         | 1.17       | 1.17        |
| Bread 3                     | 34.25        | 59.38        | 1.00         | 3.00      | 0.00       | 0.00         | 1.19       | 1.19        |
| Bread 4                     | 34.25        | 61.78        | 1.00         | 0.00      | 0.50       | 0.00         | 1.24       | 1.24        |
| Bread 5                     | 34.25        | 61.68        | 1.00         | 0.00      | 0.00       | 0.60         | 1.23       | 1.23        |
| Bread 6                     | 34.25        | 58.89        | 1.00         | 3.00      | 0.50       | 0.00         | 1.18       | 1.18        |
| Bread 7                     | 34.25        | 61.20        | 1.00         | 0.00      | 0.50       | 0.60         | 1.22       | 1.22        |
| Bread 8                     | 34.25        | 58.80        | 1.00         | 3.00      | 0.00       | 0.60         | 1.18       | 1.18        |

<sup>a</sup> Bread 1 has no emulsifiers in it

**Table 5-3** Baked specific volume (BSV in cm<sup>3</sup>/g) for breads made with different levels of Gum Arabic (GA), Calcium stearoyl-2-lactylate (CSL), Diacetyl-tartaric acid ester of monoglycerides (DATEM), frozen storage time, and cooling rates

|                             |                       | Bread treatment <sup>a</sup> |                               |                              |                             |                            |                              |                             |                              |
|-----------------------------|-----------------------|------------------------------|-------------------------------|------------------------------|-----------------------------|----------------------------|------------------------------|-----------------------------|------------------------------|
| Frozen storage time (weeks) | Cooling rate notation | Bread 1                      | Bread 2                       | Bread 3                      | Bread 4                     | Bread 5                    | Bread 6                      | Bread 7                     | Bread 8                      |
|                             |                       | 0% GA, 0% CSL, 0% DATEM      | 3% GA, 0.5% CSL, 0.6% DATEM   | 3% GA, 0% CSL, 0% DATEM      | 0% GA, 0.5% CSL, 0% DATEM   | 0% GA, 0% CSL, 0.6% DATEM  | 3% GA, 0.5% CSL, 0% DATEM    | 0% GA, 0.5% CSL, 0.6% DATEM | 3% GA, 0% CSL, 0.6% DATEM    |
|                             | Fresh                 | 2.37 ± 0.09 <sup>ak</sup>    | 2.06 ± 0.03 <sup>bck</sup>    | 2.01 ± 0.03 <sup>ck</sup>    | 2.42 ± 0.05 <sup>ak</sup>   | 2.36 ± 0.06 <sup>ak</sup>  | 2.02 ± 0.01 <sup>ck</sup>    | 2.29 ± 0.07 <sup>abk</sup>  | 2.01 ± 0.02 <sup>ck</sup>    |
| 0                           | CR0                   | 2.21 ± 0.01 <sup>akl</sup>   | 1.87 ± 0.01 <sup>bcdklm</sup> | 1.62 ± 0.01 <sup>dm</sup>    | 1.95 ± 0.06 <sup>bcl</sup>  | 2.04 ± 0.1 <sup>abcl</sup> | 1.87 ± 0.07 <sup>bcdkl</sup> | 2.1 ± 0.03 <sup>abkl</sup>  | 1.8 ± 0.04 <sup>cdlm</sup>   |
|                             | CR1                   | 2.1 ± 0.07 <sup>aklm</sup>   | 1.99 ± 0.03 <sup>abkl</sup>   | 1.66 ± 0.03 <sup>cm</sup>    | 2.24 ± 0.06 <sup>ak</sup>   | 2.24 ± 0.02 <sup>akl</sup> | 1.79 ± 0.09 <sup>bckl</sup>  | 2.12 ± 0.06 <sup>akl</sup>  | 1.73 ± 0.04 <sup>bcmn</sup>  |
| 8                           | CR0                   | 2.1 ± 0.04 <sup>aklm</sup>   | 1.53 ± 0.11 <sup>do</sup>     | 1.89 ± 0.07 <sup>abckl</sup> | 2.01 ± 0.05 <sup>abl</sup>  | 2.06 ± 0.04 <sup>al</sup>  | 1.69 ± 0.07 <sup>cdl</sup>   | 2.03 ± 0.04 <sup>abkl</sup> | 1.73 ± 0.03 <sup>bcdmn</sup> |
|                             | CR1                   | 2.1 ± 0.04 <sup>aklm</sup>   | 1.73 ± 0.03 <sup>cmno</sup>   | 1.8 ± 0.02 <sup>bclm</sup>   | 1.96 ± 0.02 <sup>abcl</sup> | 2.12 ± 0.04 <sup>akl</sup> | 1.76 ± 0.09 <sup>ckl</sup>   | 2.02 ± 0.1 <sup>abkl</sup>  | 1.76 ± 0.01 <sup>cmn</sup>   |
| 16                          | CR0                   | 1.84 ± 0.1 <sup>abm</sup>    | 1.84 ± 0.05 <sup>abklm</sup>  | 1.71 ± 0.04 <sup>blm</sup>   | 2 ± 0.05 <sup>al</sup>      | 1.98 ± 0.02 <sup>al</sup>  | 1.71 ± 0.02 <sup>bl</sup>    | 1.9 ± 0.03 <sup>abl</sup>   | 1.7 ± 0.04 <sup>bmn</sup>    |
|                             | CR1                   | 1.93 ± 0.05 <sup>ablm</sup>  | 1.74 ± 0.03 <sup>bcmno</sup>  | 1.65 ± 0.04 <sup>cm</sup>    | 1.91 ± 0.02 <sup>abl</sup>  | 2.09 ± 0.06 <sup>akl</sup> | 1.65 ± 0.05 <sup>cl</sup>    | 1.93 ± 0.05 <sup>abl</sup>  | 1.96 ± 0.01 <sup>akl</sup>   |
| 26                          | CR0                   | 1.99 ± 0.03 <sup>alm</sup>   | 1.59 ± 0.04 <sup>dno</sup>    | 1.72 ± 0.05 <sup>bcdlm</sup> | 1.85 ± 0.01 <sup>abcl</sup> | 1.98 ± 0.06 <sup>al</sup>  | 1.72 ± 0.04 <sup>bcdl</sup>  | 1.87 ± 0.08 <sup>abl</sup>  | 1.62 ± 0.03 <sup>cdn</sup>   |
|                             | CR1                   | 2 ± 0.1 <sup>alm</sup>       | 1.78 ± 0.04 <sup>ablmn</sup>  | 1.68 ± 0.06 <sup>blm</sup>   | 1.91 ± 0.05 <sup>abl</sup>  | 2.02 ± 0.03 <sup>al</sup>  | 1.68 ± 0.02 <sup>bl</sup>    | 1.85 ± 0.02 <sup>abl</sup>  | 1.73 ± 0.04 <sup>bmn</sup>   |

<sup>a</sup> Values across columns (with superscripts a, b, c, d) and rows (with superscripts k, l, m, n, o) not connected by same letter are significantly different (*p*-value < 0.05). Values represent mean and standard error obtained by triplicate measurements

**Table 5-4** Cohesiveness of crumb for breads made with different levels of Gum Arabic (GA), Calcium stearyl-2-lactylate (CSL), Diacetyl-tartaric acid ester of monoglycerides (DATEM), frozen storage time, and cooling rates

|                             |                       | Bread treatment <sup>a</sup> |                              |                             |                              |                            |                             |                             |                            |
|-----------------------------|-----------------------|------------------------------|------------------------------|-----------------------------|------------------------------|----------------------------|-----------------------------|-----------------------------|----------------------------|
| Frozen storage time (weeks) | Cooling rate notation | Bread 1                      | Bread 2                      | Bread 3                     | Bread 4                      | Bread 5                    | Bread 6                     | Bread 7                     | Bread 8                    |
|                             |                       | 0% GA, 0% CSL, 0% DATEM      | 3% GA, 0.5% CSL, 0.6% DATEM  | 3% GA, 0% CSL, 0% DATEM     | 0% GA, 0.5% CSL, 0% DATEM    | 0% GA, 0% CSL, 0.6% DATEM  | 3% GA, 0.5% CSL, 0% DATEM   | 0% GA, 0.5% CSL, 0.6% DATEM | 3% GA, 0% CSL, 0.6% DATEM  |
| 0                           | Fresh                 | 0.29 ± 0.01 <sup>al</sup>    | 0.26 ± 0.01 <sup>abkl</sup>  | 0.24 <sup>bco</sup>         | 0.27 <sup>abn</sup>          | 0.25 ± 0.01 <sup>bl</sup>  | 0.25 ± 0.01 <sup>bk</sup>   | 0.2 <sup>cp</sup>           | 0.24 <sup>bn</sup>         |
|                             | CR0                   | 0.33 ± 0.02 <sup>akl</sup>   | 0.25 ± 0.01 <sup>cdklm</sup> | 0.29 <sup>abcdkl</sup>      | 0.3 ± 0.02 <sup>abclmn</sup> | 0.31 ± 0.01 <sup>abk</sup> | 0.27 ± 0.01 <sup>bcdk</sup> | 0.32 <sup>abklm</sup>       | 0.24 <sup>dn</sup>         |
|                             | CR1                   | 0.33 ± 0.01 <sup>abkl</sup>  | 0.26 ± 0.02 <sup>ckl</sup>   | 0.29 ± 0.01 <sup>bckl</sup> | 0.36 <sup>ak</sup>           | 0.32 <sup>abk</sup>        | 0.26 ± 0.01 <sup>ck</sup>   | 0.3 ± 0.01 <sup>bclmn</sup> | 0.26 <sup>clm</sup>        |
| 8                           | CR0                   | 0.03 ± 0.02 <sup>ak</sup>    | 0 <sup>dm</sup>              | 0.01 ± 0.01 <sup>bck</sup>  | 0.01 ± 0.01 <sup>akl</sup>   | 0.02 ± 0.01 <sup>abk</sup> | 0.01 <sup>ck</sup>          | 0 <sup>co</sup>             | 0 <sup>ckl</sup>           |
|                             | CR1                   | 0.33 ± 0.01 <sup>akl</sup>   | 0.25 <sup>cklm</sup>         | 0.27 <sup>bclmn</sup>       | 0.32 <sup>aklm</sup>         | 0.31 ± 0.01 <sup>ak</sup>  | 0.27 ± 0.01 <sup>bk</sup>   | 0.32 <sup>ak</sup>          | 0.26 <sup>bclm</sup>       |
| 16                          | CR0                   | 0.32 ± 0.01 <sup>abkl</sup>  | 0.26 <sup>dekl</sup>         | 0.26 <sup>demn</sup>        | 0.35 ± 0.02 <sup>ak</sup>    | 0.27 <sup>cdel</sup>       | 0.28 <sup>bcdk</sup>        | 0.3 <sup>bcmno</sup>        | 0.25 <sup>emn</sup>        |
|                             | CR1                   | 0.36 ± 0.01 <sup>ak</sup>    | 0.27 <sup>cdk</sup>          | 0.28 <sup>cdklm</sup>       | 0.35 <sup>akl</sup>          | 0.31 <sup>bk</sup>         | 0.28 <sup>ck</sup>          | 0.33 <sup>ak</sup>          | 0.25 <sup>dmn</sup>        |
| 26                          | CR0                   | 0.35 ± 0.01 <sup>ak</sup>    | 0.25 <sup>eklm</sup>         | 0.26 <sup>dno</sup>         | 0.28 <sup>cdmn</sup>         | 0.31 <sup>bk</sup>         | 0.29 <sup>ck</sup>          | 0.32 ± 0.01 <sup>bkl</sup>  | 0.28 ± 0.01 <sup>ckl</sup> |
|                             | CR1                   | 0.35 ± 0.01 <sup>ak</sup>    | 0.24 ± 0.01 <sup>elm</sup>   | 0.27 <sup>dln</sup>         | 0.31 <sup>bcklmn</sup>       | 0.34 <sup>abk</sup>        | 0.27 ± 0.01 <sup>dk</sup>   | 0.29 <sup>cdno</sup>        | 0.28 <sup>dk</sup>         |

<sup>a</sup> Values across columns (with superscripts a, b, c, d) and rows (with superscripts k, l, m, n, o) not connected by same letter are significantly different (*p*-value < 0.05). Values represent mean and standard error obtained by triplicate measurements

**Table 5-5** Gumminess (in N) of crumb for breads made with different levels of Gum Arabic (GA), Calcium stearoyl-2-lactylate (CSL), Diacetyl-tartaric acid ester of monoglycerides (DATEM), frozen storage time, and cooling rates

|                             |                       | Bread treatment <sup>a</sup> |                             |                               |                              |                              |                              |                              |                               |
|-----------------------------|-----------------------|------------------------------|-----------------------------|-------------------------------|------------------------------|------------------------------|------------------------------|------------------------------|-------------------------------|
| Frozen storage time (weeks) | Cooling rate notation | Bread 1                      | Bread 2                     | Bread 3                       | Bread 4                      | Bread 5                      | Bread 6                      | Bread 7                      | Bread 8                       |
|                             |                       | 0% GA, 0% CSL, 0% DATEM      | 3% GA, 0.5% CSL, 0.6% DATEM | 3% GA, 0% CSL, 0% DATEM       | 0% GA, 0.5% CSL, 0% DATEM    | 0% GA, 0% CSL, 0.6% DATEM    | 3% GA, 0.5% CSL, 0% DATEM    | 0% GA, 0.5% CSL, 0.6% DATEM  | 3% GA, 0% CSL, 0.6% DATEM     |
|                             | Fresh                 | 29.06 ± 0.3 <sup>ak</sup>    | 22.82 ± 1.41 <sup>bkl</sup> | 15.73 ± 0.97 <sup>dekl</sup>  | 23.71 ± 1.77 <sup>bm</sup>   | 20.12 ± 0.58 <sup>bcdl</sup> | 17.08 ± 0.2 <sup>cde</sup>   | 21.13 ± 0.27 <sup>bckl</sup> | 13.94 ± 0.24 <sup>elm</sup>   |
| 0                           | CR0                   | 24.84 ± 1.34 <sup>al</sup>   | 10.45 ± 1.6 <sup>em</sup>   | 18.05 ± 0.49 <sup>bck</sup>   | 23.35 ± 0.18 <sup>am</sup>   | 20.63 ± 0.19 <sup>abl</sup>  | 10.72 ± 0.82 <sup>dem</sup>  | 22.64 ± 1.23 <sup>ak</sup>   | 15.17 ± 0.41 <sup>cdklm</sup> |
|                             | CR1                   | 24.51 ± 0.26 <sup>al</sup>   | 12.89 ± 1.28 <sup>dm</sup>  | 15.12 ± 0.1 <sup>bcdl</sup>   | 18.25 ± 0.38 <sup>bno</sup>  | 24.04 ± 1.09 <sup>ak</sup>   | 13.69 ± 0.93 <sup>cdlm</sup> | 18.24 ± 0.51 <sup>blm</sup>  | 17.85 ± 1.63 <sup>bck</sup>   |
| 8                           | CR0                   | 16.96 ± 0.16 <sup>amn</sup>  | 11.89 ± 0.39 <sup>bm</sup>  | 15.05 ± 0.74 <sup>al</sup>    | 16.56 ± 0.3 <sup>ano</sup>   | 16.08 ± 0.52 <sup>am</sup>   | 15.25 ± 0.78 <sup>al</sup>   | 15.32 ± 0.95 <sup>amn</sup>  | 16.67 ± 0.24 <sup>akl</sup>   |
|                             | CR1                   | 14.41 ± 0.31 <sup>bn</sup>   | 13.37 ± 0.29 <sup>bm</sup>  | 13.29 ± 0.49 <sup>blm</sup>   | 18.23 ± 1.11 <sup>ano</sup>  | 17.69 ± 0.45 <sup>alm</sup>  | 13.3 ± 0.72 <sup>blm</sup>   | 13.37 ± 0.54 <sup>bn</sup>   | 14.53 ± 0.62 <sup>bklm</sup>  |
| 16                          | CR0                   | 15.95 ± 0.43 <sup>bcdn</sup> | 18.9 ± 0.84 <sup>abl</sup>  | 15.72 ± 0.15 <sup>bcdkl</sup> | 14.29 ± 0.93 <sup>cdo</sup>  | 17.58 ± 0.5 <sup>bclm</sup>  | 21.46 ± 1.29 <sup>ak</sup>   | 17.9 ± 0.43 <sup>abm</sup>   | 13.63 ± 0.64 <sup>dln</sup>   |
|                             | CR1                   | 19.31 ± 0.38 <sup>cm</sup>   | 23.54 ± 0.58 <sup>ak</sup>  | 13.58 ± 0.39 <sup>dln</sup>   | 19.63 ± 0.22 <sup>bcnm</sup> | 19.89 ± 0.59 <sup>bcl</sup>  | 22.4 ± 1.16 <sup>abk</sup>   | 15.5 ± 0.2 <sup>dnm</sup>    | 12.73 ± 0.56 <sup>dm</sup>    |
| 26                          | CR0                   | 24.75 ± 0.23 <sup>bl</sup>   | 13.55 ± 0.23 <sup>cdm</sup> | 11.61 ± 0.48 <sup>dm</sup>    | 29.02 ± 1.08 <sup>al</sup>   | 15.17 ± 1.04 <sup>cm</sup>   | 14.68 ± 0.54 <sup>clm</sup>  | 14.13 ± 0.16 <sup>cdn</sup>  | 13.37 ± 0.33 <sup>cdln</sup>  |
|                             | CR1                   | 23.78 ± 0.78 <sup>bl</sup>   | 12.42 ± 0.28 <sup>em</sup>  | 14 ± 0.41 <sup>delm</sup>     | 38.43 ± 1.63 <sup>ak</sup>   | 17.88 ± 0.43 <sup>clm</sup>  | 13.4 ± 0.2 <sup>delm</sup>   | 13.05 ± 0.45 <sup>den</sup>  | 16 ± 0.46 <sup>cdklm</sup>    |

<sup>a</sup> Values across columns (with superscripts a, b, c, d) and rows (with superscripts k, l, m, n, o) not connected by same letter are significantly different (*p*-value < 0.05). Values represent mean and standard error obtained by triplicate measurements

**Table 5-6** Chewiness (in N·cm) of crumb for breads made with different levels of Gum Arabic (GA), Calcium stearoyl-2-lactylate (CSL), Diacetyl-tartaric acid ester of monoglycerides (DATEM), frozen storage time, and cooling rates

|                             |                       | Bread treatment <sup>a</sup> |                             |                             |                            |                              |                               |                             |                              |
|-----------------------------|-----------------------|------------------------------|-----------------------------|-----------------------------|----------------------------|------------------------------|-------------------------------|-----------------------------|------------------------------|
| Frozen storage time (weeks) | Cooling rate notation | Bread 1                      | Bread 2                     | Bread 3                     | Bread 4                    | Bread 5                      | Bread 6                       | Bread 7                     | Bread 8                      |
|                             |                       | 0% GA, 0% CSL, 0% DATEM      | 3% GA, 0.5% CSL, 0.6% DATEM | 3% GA, 0% CSL, 0% DATEM     | 0% GA, 0.5% CSL, 0% DATEM  | 0% GA, 0% CSL, 0.6% DATEM    | 3% GA, 0.5% CSL, 0% DATEM     | 0% GA, 0.5% CSL, 0.6% DATEM | 3% GA, 0% CSL, 0.6% DATEM    |
| 0                           | Fresh                 | 0.97 ± 0.05 <sup>ak</sup>    | 0.61 ± 0.05 <sup>bck</sup>  | 0.42 ± 0.03 <sup>cdkl</sup> | 0.67 ± 0.09 <sup>bl</sup>  | 0.55 ± 0.05 <sup>bcdlm</sup> | 0.47 ± 0.05 <sup>bcdklm</sup> | 0.48 ± 0.03 <sup>bcdl</sup> | 0.35 ± 0.03 <sup>dlmn</sup>  |
|                             | CR0                   | 1.06 ± 0.18 <sup>ak</sup>    | 0.27 ± 0.07 <sup>cl</sup>   | 0.52 ± 0.04 <sup>bckl</sup> | 0.69 ± 0.03 <sup>abl</sup> | 0.72 ± 0.05 <sup>abklm</sup> | 0.25 ± 0.03 <sup>cm</sup>     | 0.71 ± 0.07 <sup>abk</sup>  | 0.37 ± 0.01 <sup>bclmn</sup> |
|                             | CR1                   | 0.77 ± 0.1 <sup>abkl</sup>   | 0.36 ± 0.04 <sup>cdl</sup>  | 0.46 ± 0.01 <sup>cdkl</sup> | 0.6 ± 0.02 <sup>bcl</sup>  | 0.95 ± 0.04 <sup>ak</sup>    | 0.35 ± 0.03 <sup>dlm</sup>    | 0.47 ± 0.03 <sup>cdl</sup>  | 0.48 ± 0.06 <sup>cdkl</sup>  |
| 8                           | CR0                   | 0.79 ± 0.03 <sup>akl</sup>   | 0.27 ± 0.02 <sup>cl</sup>   | 0.6 ± 0.12 <sup>abk</sup>   | 0.6 ± 0.05 <sup>abl</sup>  | 0.56 ± 0.05 <sup>ablm</sup>  | 0.53 ± 0.03 <sup>abkl</sup>   | 0.4 ± 0.02 <sup>bcl</sup>   | 0.56 ± 0.03 <sup>abk</sup>   |
|                             | CR1                   | 0.53 ± 0.02 <sup>abl</sup>   | 0.31 ± 0.02 <sup>dl</sup>   | 0.46 ± 0.02 <sup>bckl</sup> | 0.63 ± 0.02 <sup>al</sup>  | 0.56 ± 0.02 <sup>ablm</sup>  | 0.34 ± 0.06 <sup>cdlm</sup>   | 0.35 ± 0.02 <sup>cdl</sup>  | 0.36 ± 0.02 <sup>cdlmn</sup> |
| 16                          | CR0                   | 0.45 ± 0.01 <sup>abl</sup>   | 0.38 ± 0.01 <sup>bl</sup>   | 0.43 ± 0.04 <sup>abkl</sup> | 0.5 ± 0.09 <sup>abl</sup>  | 0.45 ± 0.01 <sup>abm</sup>   | 0.66 ± 0.11 <sup>ak</sup>     | 0.54 ± 0.02 <sup>abkl</sup> | 0.31 <sup>bm</sup>           |
|                             | CR1                   | 0.76 ± 0.09 <sup>akl</sup>   | 0.57 ± 0.03 <sup>abk</sup>  | 0.35 ± 0.03 <sup>bl</sup>   | 0.79 ± 0.04 <sup>al</sup>  | 0.71 ± 0.13 <sup>aklm</sup>  | 0.7 ± 0.05 <sup>ak</sup>      | 0.52 ± 0.04 <sup>abkl</sup> | 0.3 ± 0.01 <sup>bn</sup>     |
| 26                          | CR0                   | 1.1 ± 0.07 <sup>ak</sup>     | 0.34 ± 0.02 <sup>cl</sup>   | 0.35 ± 0.02 <sup>cl</sup>   | 0.78 ± 0.05 <sup>bl</sup>  | 0.46 ± 0.04 <sup>cm</sup>    | 0.5 ± 0.01 <sup>cklm</sup>    | 0.52 ± 0.08 <sup>ckl</sup>  | 0.4 ± 0.02 <sup>clmn</sup>   |
|                             | CR1                   | 1.17 ± 0.08 <sup>abk</sup>   | 0.27 ± 0.01 <sup>dl</sup>   | 0.45 ± 0.02 <sup>cdkl</sup> | 1.24 ± 0.21 <sup>ak</sup>  | 0.8 ± 0.07 <sup>bckl</sup>   | 0.46 <sup>cdklm</sup>         | 0.34 ± 0.03 <sup>dl</sup>   | 0.44 ± 0.02 <sup>cdklm</sup> |

<sup>a</sup> Values across columns (with superscripts a, b, c, d) and rows (with superscripts k, l, m, n, o) not connected by same letter are significantly different (*p*-value < 0.05). Values represent mean and standard error obtained by triplicate measurements

**Table 5-7** Compressive load at break (in N) of crust for bread made with different levels of Gum Arabic (GA), Calcium stearoyl-2-lactylate (CSL), Diacetyl-tartaric acid ester of monoglycerides (DATEM), frozen storage time, and cooling rates

|                             |                       | Bread treatment <sup>a</sup> |                             |                            |                             |                             |                             |                             |                             |
|-----------------------------|-----------------------|------------------------------|-----------------------------|----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| Frozen storage time (weeks) | Cooling rate notation | Bread 1                      | Bread 2                     | Bread 3                    | Bread 4                     | Bread 5                     | Bread 6                     | Bread 7                     | Bread 8                     |
|                             |                       | 0% GA, 0% CSL, 0% DATEM      | 3% GA, 0.5% CSL, 0.6% DATEM | 3% GA, 0% CSL, 0% DATEM    | 0% GA, 0.5% CSL, 0% DATEM   | 0% GA, 0% CSL, 0.6% DATEM   | 3% GA, 0.5% CSL, 0% DATEM   | 0% GA, 0.5% CSL, 0.6% DATEM | 3% GA, 0% CSL, 0.6% DATEM   |
|                             | Fresh                 | 17.56 ± 0.64 <sup>al</sup>   | 6.37 ± 0.16 <sup>ekl</sup>  | 7.04 ± 0.04 <sup>emn</sup> | 9.97 ± 0.07 <sup>clm</sup>  | 13.56 ± 0.19 <sup>bk</sup>  | 8.53 ± 0.07 <sup>dno</sup>  | 9.27 ± 0.2 <sup>cdn</sup>   | 6.77 ± 0.17 <sup>ekl</sup>  |
| 0                           | CR0                   | 17.33 ± 0.62 <sup>al</sup>   | 4.84 ± 0.18 <sup>emn</sup>  | 8.22 ± 0.12 <sup>dl</sup>  | 10.91 ± 0.39 <sup>ckl</sup> | 13.19 ± 0.11 <sup>bk</sup>  | 4.66 ± 0.2 <sup>ep</sup>    | 9.85 ± 0.06 <sup>cmn</sup>  | 7.29 ± 0.04 <sup>dkl</sup>  |
|                             | CR1                   | 14.53 ± 0.17 <sup>am</sup>   | 7 ± 0.64 <sup>efk</sup>     | 9.47 ± 0.2 <sup>ck</sup>   | 8.05 ± 0.15 <sup>dno</sup>  | 6.27 ± 0.26 <sup>fo</sup>   | 12.89 ± 0.07 <sup>bk</sup>  | 9.35 ± 0.17 <sup>cdn</sup>  | 6.44 ± 0.11 <sup>fl</sup>   |
| 8                           | CR0                   | 13.21 ± 0.81 <sup>amn</sup>  | 6.78 ± 0.12 <sup>ckl</sup>  | 7.23 ± 0.17 <sup>cm</sup>  | 11.23 ± 0.17 <sup>bk</sup>  | 11.65 ± 0.26 <sup>abl</sup> | 10.86 ± 0.43 <sup>bl</sup>  | 12.54 ± 0.09 <sup>abk</sup> | 4.55 ± 0.21 <sup>dn</sup>   |
|                             | CR1                   | 9.93 ± 0.23 <sup>bop</sup>   | 6.38 ± 0.16 <sup>dekl</sup> | 5.55 ± 0.18 <sup>eo</sup>  | 11.84 ± 0.49 <sup>ak</sup>  | 10.43 ± 0.15 <sup>bm</sup>  | 8.04 ± 0.08 <sup>co</sup>   | 6.97 ± 0.09 <sup>cdop</sup> | 5.48 ± 0.17 <sup>em</sup>   |
| 16                          | CR0                   | 8.24 ± 0.13 <sup>ap</sup>    | 5.67 ± 0.15 <sup>clm</sup>  | 7.29 ± 0.16 <sup>bm</sup>  | 8.21 ± 0.14 <sup>ano</sup>  | 5.37 ± 0.17 <sup>cdo</sup>  | 7.69 ± 0.14 <sup>abo</sup>  | 7.95 ± 0.1 <sup>ao</sup>    | 4.73 ± 0.05 <sup>dmm</sup>  |
|                             | CR1                   | 11.32 ± 0.57 <sup>ano</sup>  | 5.61 ± 0.1 <sup>clm</sup>   | 6.44 ± 0.04 <sup>cn</sup>  | 8.32 ± 0.2 <sup>bn</sup>    | 11.49 ± 0.26 <sup>al</sup>  | 5.54 ± 0.14 <sup>cp</sup>   | 6.29 ± 0.06 <sup>cp</sup>   | 4.02 ± 0.08 <sup>dn</sup>   |
| 26                          | CR0                   | 12.88 ± 0.11 <sup>amn</sup>  | 6.14 ± 0.11 <sup>ekl</sup>  | 7.26 ± 0.1 <sup>dm</sup>   | 7.04 ± 0.18 <sup>deo</sup>  | 9.61 ± 0.12 <sup>cmn</sup>  | 9.95 ± 0.17 <sup>clm</sup>  | 11.56 ± 0.33 <sup>bkl</sup> | 6.92 ± 0.37 <sup>dekl</sup> |
|                             | CR1                   | 21.07 ± 0.95 <sup>ak</sup>   | 4.3 ± 0.16 <sup>dn</sup>    | 9.21 ± 0.05 <sup>bck</sup> | 9.65 ± 0.18 <sup>bm</sup>   | 9.37 ± 0.16 <sup>bcn</sup>  | 9.27 ± 0.14 <sup>bcmn</sup> | 10.81 ± 0.42 <sup>blm</sup> | 7.64 ± 0.13 <sup>ck</sup>   |

<sup>a</sup> Values across columns (with superscripts a, b, c, d) and rows (with superscripts k, l, m, n, o) not connected by same letter are significantly different (*p*-value < 0.05). Values represent mean and standard error obtained by triplicate measurements

**Table 5-8** Modulus of Elasticity (in MPa) of bread crust made with different levels of Gum Arabic (GA), Calcium stearoyl-2-lactylate (CSL), Diacetyl-tartaric acid ester of monoglycerides (DATEM), frozen storage time, and cooling rates

|                             |                       | Bread treatment <sup>a</sup> |                             |                            |                                |                               |                              |                              |                             |
|-----------------------------|-----------------------|------------------------------|-----------------------------|----------------------------|--------------------------------|-------------------------------|------------------------------|------------------------------|-----------------------------|
| Frozen storage time (weeks) | Cooling rate notation | Bread 1                      | Bread 2                     | Bread 3                    | Bread 4                        | Bread 5                       | Bread 6                      | Bread 7                      | Bread 8                     |
|                             |                       | 0% GA, 0% CSL, 0% DATEM      | 3% GA, 0.5% CSL, 0.6% DATEM | 3% GA, 0% CSL, 0% DATEM    | 0% GA, 0.5% CSL, 0% DATEM      | 0% GA, 0% CSL, 0.6% DATEM     | 3% GA, 0.5% CSL, 0% DATEM    | 0% GA, 0.5% CSL, 0.6% DATEM  | 3% GA, 0% CSL, 0.6% DATEM   |
|                             | Fresh                 | 0.17 ± 0.01 <sup>ablm</sup>  | 0.13 ± 0.01 <sup>abkl</sup> | 0.14 <sup>abl</sup>        | 0.16 ± 0.01 <sup>abklm</sup>   | 0.17 ± 0.02 <sup>akl</sup>    | 0.16 ± 0.01 <sup>ablmn</sup> | 0.18 <sup>aklm</sup>         | 0.12 ± 0.01 <sup>bkl</sup>  |
| 0                           | CR0                   | 0.16 ± 0.01 <sup>almn</sup>  | 0.15 <sup>ak</sup>          | 0.13 ± 0.01 <sup>abl</sup> | 0.16 ± 0.01 <sup>aklm</sup>    | 0.14 <sup>alm</sup>           | 0.1 <sup>bo</sup>            | 0.16 ± 0.01 <sup>almn</sup>  | 0.14 <sup>abk</sup>         |
|                             | CR1                   | 0.17 ± 0.01 <sup>bclm</sup>  | 0.13 ± 0.01 <sup>dki</sup>  | 0.2 ± 0.01 <sup>bk</sup>   | 0.13 <sup>cdmn</sup>           | 0.11 ± 0.01 <sup>dm</sup>     | 0.27 <sup>ak</sup>           | 0.12 ± 0.01 <sup>dn</sup>    | 0.12 <sup>dklm</sup>        |
| 8                           | CR0                   | 0.18 ± 0.01 <sup>bcl</sup>   | 0.14 <sup>cdk</sup>         | 0.13 <sup>dl</sup>         | 0.17 ± 0.01 <sup>bcdkl</sup>   | 0.15 ± 0.01 <sup>bcdklm</sup> | 0.19 ± 0.01 <sup>abl</sup>   | 0.22 ± 0.01 <sup>ak</sup>    | 0.09 ± 0.01 <sup>emn</sup>  |
|                             | CR1                   | 0.13 ± 0.01 <sup>abcnm</sup> | 0.12 <sup>bcdklm</sup>      | 0.12 ± 0.01 <sup>cdl</sup> | 0.15 ± 0.01 <sup>abcklmn</sup> | 0.15 <sup>abklm</sup>         | 0.15 ± 0.01 <sup>amn</sup>   | 0.14 ± 0.01 <sup>abcnm</sup> | 0.1 ± 0.01 <sup>dln</sup>   |
| 16                          | CR0                   | 0.12 ± 0.01 <sup>abn</sup>   | 0.1 ± 0.01 <sup>ablmn</sup> | 0.11 ± 0.01 <sup>abl</sup> | 0.13 ± 0.01 <sup>almn</sup>    | 0.11 ± 0.01 <sup>abm</sup>    | 0.14 <sup>an</sup>           | 0.14 ± 0.02 <sup>amn</sup>   | 0.09 <sup>bm</sup>          |
|                             | CR1                   | 0.15 <sup>blmn</sup>         | 0.1 <sup>dnm</sup>          | 0.12 <sup>bcl</sup>        | 0.18 <sup>ak</sup>             | 0.19 <sup>ak</sup>            | 0.1 ± 0.01 <sup>cdo</sup>    | 0.13 ± 0.01 <sup>bm</sup>    | 0.08 <sup>dn</sup>          |
| 26                          | CR0                   | 0.15 <sup>blmn</sup>         | 0.11 <sup>clmn</sup>        | 0.13 ± 0.01 <sup>bcl</sup> | 0.11 <sup>cn</sup>             | 0.16 <sup>bklm</sup>          | 0.16 ± 0.01 <sup>bm</sup>    | 0.21 ± 0.02 <sup>akl</sup>   | 0.12 ± 0.01 <sup>bckl</sup> |
|                             | CR1                   | 0.35 <sup>ak</sup>           | 0.09 <sup>en</sup>          | 0.18 ± 0.01 <sup>ck</sup>  | 0.16 <sup>cdklm</sup>          | 0.16 ± 0.01 <sup>cdkl</sup>   | 0.17 ± 0.01 <sup>clm</sup>   | 0.2 <sup>bkl</sup>           | 0.14 <sup>dk</sup>          |

<sup>a</sup> Values across columns (with superscripts a, b, c, d) and rows (with superscripts k, l, m, n, o) not connected by same letter are significantly different (*p*-value < 0.05). Values represent mean and standard error obtained by triplicate measurements



**Table 5-9** Significant interactions among Gum Arabic (GA), Calcium stearoyl-2-lactylate (CSL), and Diacetyl-tartaric acid ester of monoglycerides (DATEM), and frozen storage time for baked specific volume (BSV)

|                                    | BSV     |         |         |
|------------------------------------|---------|---------|---------|
|                                    | Fresh   | CR0     | CR1     |
| <b>GA</b>                          | <.0001* | <.0001* | <.0001* |
| <b>CSL</b>                         | 0.7246  | 0.1949  | 0.1033  |
| <b>DATEM</b>                       | 0.3769  | 0.4666  | 0.0021* |
| <b>Frozen Storage Time</b>         | .       | <.0001* | <.0001* |
| <b>GA × CSL</b>                    | 0.4875  | 0.1504  | 0.0099* |
| <b>GA × DATEM</b>                  | 0.1681  | 0.4019  | 0.1049  |
| <b>GA × Frozen Storage Time</b>    | .       | 0.2649  | 0.003*  |
| <b>CSL × DATEM</b>                 | 0.5724  | 0.7705  | 0.1173  |
| <b>CSL × Frozen Storage Time</b>   | .       | 0.589   | 0.0098* |
| <b>DATEM × Frozen Storage Time</b> | .       | 0.3889  | 0.8373  |

\* *p*-value < 0.05 indicates that the main effect or interaction was significant

**Table 5-10** Significant interactions among Gum Arabic (GA), Calcium stearoyl-2-lactylate (CSL), and Diacetyl-tartaric acid ester of monoglycerides (DATEM), and frozen storage time for textural profile analysis (TPA) parameters: cohesiveness, gumminess, and chewiness

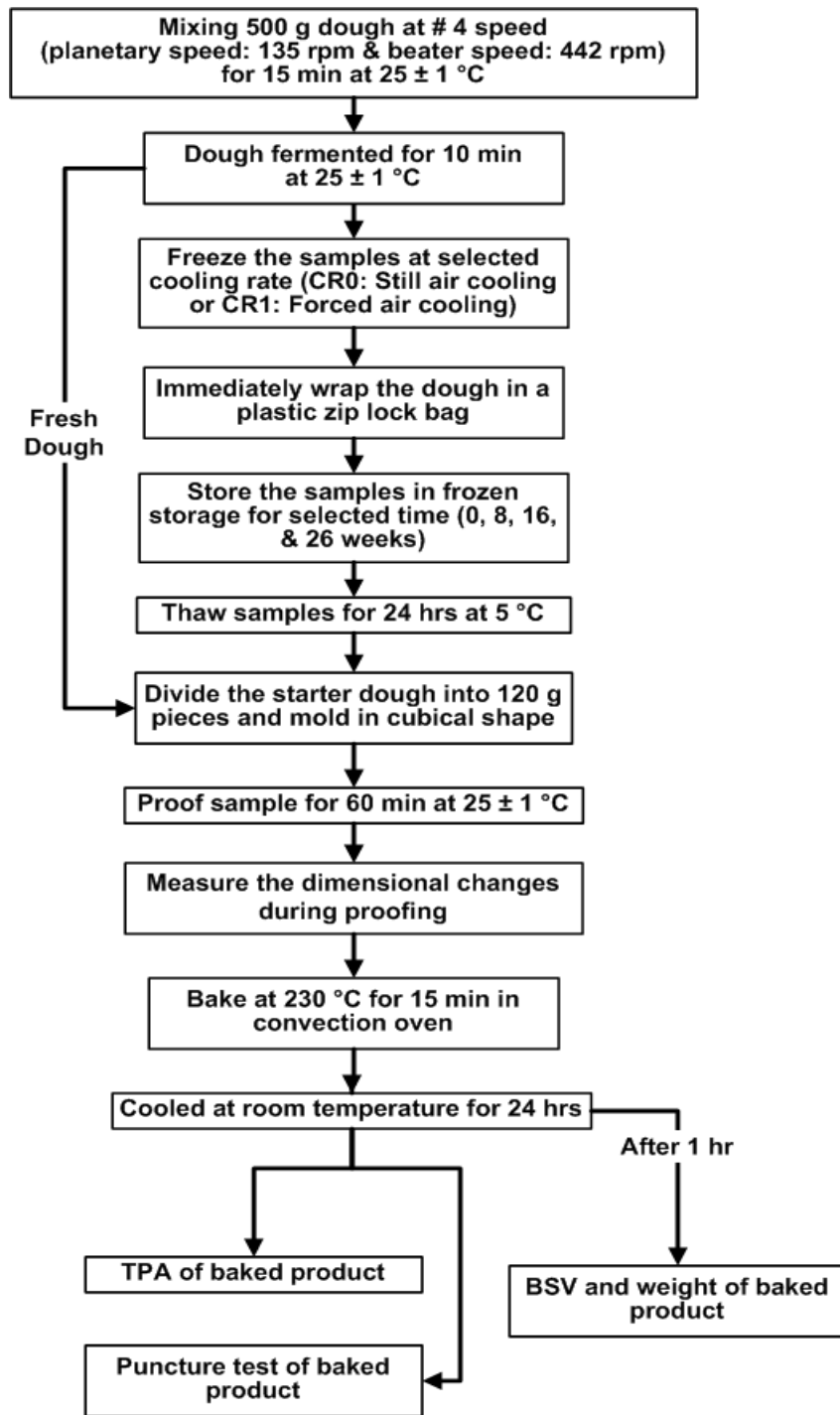
|                                    | Cohesiveness |          |          | Gumminess |          |          | Chewiness |          |          |
|------------------------------------|--------------|----------|----------|-----------|----------|----------|-----------|----------|----------|
|                                    | Fresh        | CR0      | CR1      | Fresh     | CR0      | CR1      | Fresh     | CR0      | CR1      |
| <b>GA</b>                          | 0.1168       | <0.0001* | <0.0001* | <0.0001*  | <0.0001* | <0.0001* | <0.0001*  | <0.0001* | <0.0001* |
| <b>CSL</b>                         | 0.0172*      | 0.0593   | 0.0432*  | 0.0097*   | 0.9215   | 0.9326   | 0.553     | 0.0201*  | 0.0158*  |
| <b>DATEM</b>                       | <0.0001*     | <0.0001* | <0.0001* | 0.0014*   | 0.0023*  | 0.0127*  | <0.0001*  | <0.0001* | <0.0001* |
| <b>Frozen Storage Time</b>         | .            | 0.6266   | 0.9502   | .         | 0.5417   | 0.1357   | .         | 0.4545   | 0.0028*  |
| <b>GA × CSL</b>                    | <0.0001*     | 0.3695   | 0.9042   | <0.0001*  | 0.7469   | 0.2504   | <0.0001*  | 0.3102   | 0.0073*  |
| <b>GA × DATEM</b>                  | <0.0001*     | 0.9715   | 0.2092   | <0.0001*  | 0.1249   | 0.0016*  | <0.0001*  | 0.2025   | 0.0098*  |
| <b>GA × Frozen Storage Time</b>    | .            | 0.558    | 0.7988   | .         | 0.3494   | 0.2216   | .         | 0.2067   | 0.0119*  |
| <b>CSL × DATEM</b>                 | 0.1766*      | 0.8451   | 0.9345   | <0.0001*  | 0.5994   | 0.004*   | 0.0009*   | 0.4447   | 0.0019*  |
| <b>CSL × Frozen Storage Time</b>   | .            | 0.6296   | 0.0033*  | .         | 0.0083*  | 0.0034*  | .         | 0.0165*  | 0.1686   |
| <b>DATEM × Frozen Storage Time</b> | .            | 0.1492   | 0.5264   | .         | 0.0653   | 0.0006*  | .         | 0.2294   | <0.0001* |

\**p*-value < 0.05 indicates that the main effect or interaction was significant

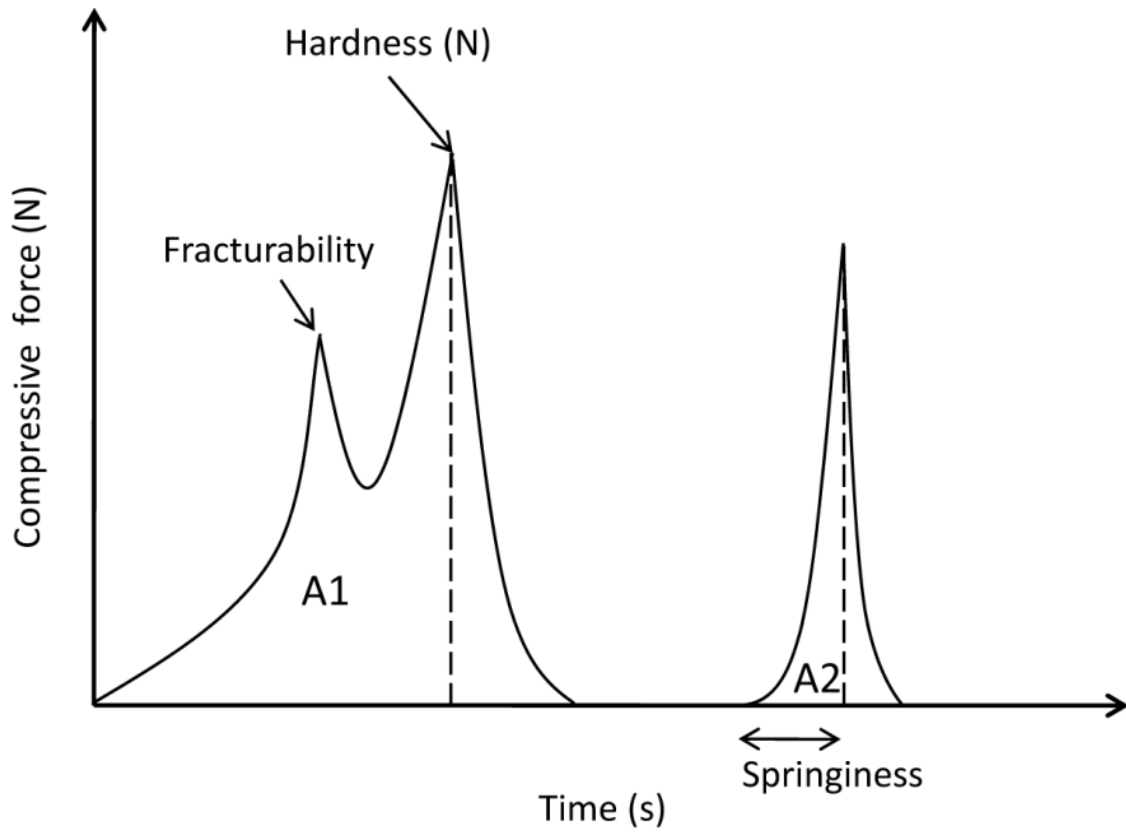
**Table 5-11** Significant interactions among Gum Arabic (GA), Calcium stearoyl-2-lactylate (CSL), and Diacetyl-tartaric acid ester of monoglycerides (DATEM), and frozen storage time for compressive load at break of crust and modulus of elasticity

|                                    | Compressive load at break |          |          | Modulus of Elasticity |          |          |
|------------------------------------|---------------------------|----------|----------|-----------------------|----------|----------|
|                                    | Fresh                     | CR0      | CR1      | Fresh                 | CR0      | CR1      |
| <b>GA</b>                          | <0.0001*                  | <0.0001* | <0.0001* | <0.0001*              | <0.0001* | 0.0005*  |
| <b>CSL</b>                         | <0.0001*                  | 0.1365   | 0.0087*  | 0.2502                | 0.0042*  | 0.3186   |
| <b>DATEM</b>                       | <0.0001*                  | 0.0002*  | <0.0001* | 0.1876                | 0.5931   | <0.0001* |
| <b>Frozen Storage Time</b>         | .                         | 0.0123*  | 0.1817   | .                     | 0.2441   | 0.0121*  |
| <b>GA × CSL</b>                    | <0.0001*                  | 0.0129*  | <0.0001* | 0.2176                | 0.8278   | 0.0163*  |
| <b>GA × DATEM</b>                  | 0.0712                    | 0.1442   | 0.5438   | 0.0125*               | 0.004*   | 0.039*   |
| <b>GA × Frozen Storage Time</b>    | .                         | <0.0001* | 0.0004*  | .                     | 0.7024   | <0.0001* |
| <b>CSL × DATEM</b>                 | 0.262                     | 0.0323*  | 0.1685   | 0.9607                | 0.0331*  | 0.6272   |
| <b>CSL × Frozen Storage Time</b>   | .                         | 0.0063*  | 0.0012*  | .                     | 0.901    | 0.0008*  |
| <b>DATEM × Frozen Storage Time</b> | .                         | 0.4948   | 0.7724   | .                     | 0.8843   | 0.8519   |

\**p*-value < 0.05 indicates that the main effect or interaction was significant



**Figure 5-1** Flow diagram for preparation of bread and experimental tests involved to determine its quality attributes



**Figure 5-2** Typical shape of a TPA curve obtained using the Universal Testing Machine. Adapted from Bourne (2002)

## Chapter 6

# **Correlations Between Various Responses at Nano, Micro, and Macro Scales for Wheat Dough and Bread**

### **6.1 Introduction**

Foods are diverse and complex biological materials consisting of different solutes, minerals, vitamins, starches, proteins, and other complex macromolecules which occur at different scales within the food materials. For example, solutes, vitamins, and minerals exist at nano-scales. Starches and some bio-polymers may exist at micro-scale. Some quality attributes in foods may be dependent on macro-scale characteristics such as physical state and density of a material. Therefore, some quality attributes in foods (such as loaf volume, texture, and water holding capacity of bread) may be described as macro-scale phenomena due to presence of structures containing gases and bulk phases of oil and water (similar to structures present in foam). In most cases, macro-scale phenomena are dependent on the building blocks from lower scales such as nano- and micro- scale. Similar to the existence of structures or building blocks found in foods at different scales, various kinds of forces exist at nano-, micro-, and macro-scales between these building blocks, which largely determine the fundamental chemical and physiochemical properties of these building blocks. The correlations between the responses (measured output) and factors (inputs) for a food system obtained using

designed experiments, leads to understanding the responses and factors of that system. To understand the causes of observed behavior (mechanisms) in a food system, fundamental chemical and physicochemical properties of the food system are investigated and a more quantitative description and prediction using theoretical and mathematical models are developed. These understandings of the relationship between molecular and physicochemical properties of different components in a food system is important in *designing* and *fabricating* properties on a more systematic and rational basis (McClements 2007). In-addition, the detailed understanding of molecular and physicochemical properties of foods can assist in developing processes for production of these *designed* foods at industrial scales, while maintaining the integrity of desired quality attributes such as flavor, nutrition, and texture in the product.

In a study by Lin and others (2001), correlations between starch retrogradation and mobility of water were studied using differential scanning calorimetry (DSC) and nuclear magnetic resonance (NMR). In this study, a Pearson correlation coefficient ( $r$ ) analysis was performed between the average value of apparent amylase content and the exponent of the nonlinear regression. The study established the relationship between mobility of water and starch retrogradation and concluded that the decrease in mobility accompanies recrystallization of amylopectin. In another study (Haiduc and van Duynhoven 2005), correlations between porous and functional properties of food materials were determined using NMR relaxometry and multivariate analysis. The

study used oil-in-water emulsions stabilized by a protein which formed complex structures consisting of fat droplets dispersed in a porous protein phase. The study used the multiple linear regression (MLR) technique with NMR amplitudes (by transforming NMR decays into discrete domains or distributions) as predictors and hardness or water drainage of the model food system as responses. MLR coefficients correlated highly with  $T_2$  distribution components of about 20 ms and 200 ms, whereas, water drainage correlated with  $T_2$  distribution components of about 400 ms and 1800 ms.

Two-dimensional (2-D) hybrid pulse sequences can be used to estimate joint distribution of spin-lattice relaxation time ( $T_1$ ) and spin-spin relaxation time ( $T_2$ ) related to individual spin groups for resolving the heterogeneity of the whole spin system. Individual measurements of  $T_1$  or  $T_2$  do not allow correlating different relaxation modes since they cannot distinguish spin systems unless relaxation times of spin systems differ by more than a factor of approximately three (English and others 1991; Song and others 2002). In a 2-D correlation study (Qiao and others 2005), diffusion measurements were correlated to  $T_2$  to resolve different components of the diffusion of water in chives (*Allium schoenoprasum*) at different sites in the sample. Godefroy and Callaghan (2003) studied the 2-D correlations between NMR spin-relaxation time and diffusion coefficient to investigate water and oil dynamics in micro-emulsion systems.



Various 2-D correlations (between  $T_1$ ,  $T_2$ , and diffusion coefficient  $D$ ) provide important information (molecular interaction and diffusion behavior of molecules) regarding molecular dynamics in a system. For example,  $T_1$  vs.  $T_2$  correlation experiments allows the study of the dynamics of molecular species. In fluids, semi-solids, or fluids in porous media,  $T_2$  can be much shorter than  $T_1$  due to the presence of strong dipole-dipole interactions between spins.  $D$  vs.  $T_2$  correlations allow molecular motion to be distinguished from molecular interactions of spins in the system.  $D$  vs.  $D$  correlations can be obtained using pulsed gradient spin echo (PGSE) sequences with stimulated echoes separated by an exchange time where the gradient strengths of the two sequences are varied independently (Godefroy and Callaghan 2003).  $D$  vs.  $D$  correlation experiments provides evidence on the exchange between compartments with different diffusion behaviors within a system.

In a review (Song and Zheng 2007), detailed discussion of dynamic rheological properties of wheat flour dough and proteins was presented. It was noted in this review that farinograph, mixograph, and extensograph are the most common empirical techniques used to characterize dough rheology. These techniques are useful in providing practical information in a relatively short period of time and at a low cost when compared to dynamic rheological measurements. However, for determination of fundamental rheological behavior of dough properties — which is crucial for designing a system for processing dough at an industrial scale and for determining the quality of

the dough at different processing conditions — dynamic rheological measurements are important. Dynamic rheological properties of gluten have been used to study wheat quality. For example, poor quality wheat consists of gluten which has been rheologically characterized by less elastic and more viscous components as compared to gluten obtained from good quality wheat (Khatkar and others 1995; Song and Zheng 2007).

In a study (Khatkar and Schofield 2002b), correlation between a fundamental rheological property (storage modulus —  $G'$ ) obtained using a controlled-stress rheometer and a quality attribute (loaf volume) for baked product was studied. The loaf volume of baked product was correlated with the elastic modulus ( $G'$  at 25 Pa and 1 Hz) of dough made of wheat flour from different wheat cultivars and reconstituted using gluten from different cultivars. Dynamic rheological tests on flour dough failed to predict the baking potential of wheat cultivars. However,  $G'$  of gluten-water doughs correlated well ( $R^2 = 0.73$ ) with loaf volume (Khatkar and Schofield 2002b). In another study (Khatkar and Schofield 2002a), it was observed that starch fraction was largely responsible for the non-linear viscoelastic behavior of dough and adding starch to gluten caused a substantial narrowing of its linear viscoelastic range.

From the literature, it can be seen that considerable research work has been performed in correlating different properties of foods—attributable to nano- and micro-scale building blocks—to their quality attributes at macro-scale. Correlating properties of foods at different scales provides an insight into the qualitative

relationship between these properties. Correlations can possibly be used to predict, more rationally, the responses (outputs of a designed experiment) or the observed behavior of a property of the food material for a desired range of a factor (input). Therefore, to improve the understanding of the effects of various functional ingredients such as gum arabic (GA), calcium stearoyl-2-lactylate (CSL), and diacetyl-tartaric acid ester of monoglycerides (DATEM) and processing parameters such as frozen storage time and cooling rates on the quality of the wheat dough and the final baked product, this chapter discusses correlations between different responses and input factors. The main responses considered in this chapter are relaxation times ( $T_{12}$ ,  $T_{21}$ , and  $T_{22}$ ), self-diffusion coefficient of water ( $D$ ), viscoelastic properties ( $G'$  and  $G''$ ), baked specific volume (BSV), cohesiveness, gumminess, and chewiness. The input factors considered were concentrations of GA, CSL, and DATEM, frozen storage time (in weeks), and cooling rate. The details of measured response variables that are discussed in this chapter are presented in section 4.3 (“Materials and Methods”) and section 5.3 (“Materials and Methods”) of this dissertation.

## 6.2 Statistical analysis

### 6.2.1 Correlations between response variables

Pearson's correlation coefficients ( $r$ ) were calculated using JMP® 8.0 software (SAS Institute Inc., Cary, NC) by the pair-wise method between 26 response variables. These response variables were: BSV, D, Cohesiveness, Gumminess, Chewiness, Compressive load at break, Modulus of elasticity of crust,  $T_{11}$ ,  $T_{12}$ ,  $T_{21}$ ,  $T_{22}$ , Peak  $T_2$  for population 2, Peak area for population 2, Peak standard deviation for population 2, four phase angles ( $\delta$ ) for two frequencies (0.002 Hz and 1.62 Hz) and two temperatures (5 °C and 25 °C), four different  $G'$  values for two frequencies (0.002 Hz and 1.62 Hz) and two temperatures (5 °C and 25 °C), and four  $G''$  values for two frequencies (0.002 Hz and 1.62 Hz) and two temperatures (5 °C and 25 °C). Since this calculation yields a  $26 \times 26$  matrix, which is not easily readable, a color map was plotted for Pearson's correlation coefficients as shown in Figure 6-1. It can be seen from this figure that most of the high positive correlation values were at the bottom-right corner. These responses are the  $G'$  and  $G''$  values at 0.002 Hz and 1.62 Hz measured at 5 °C and 25 °C. Most of these values correlated well ( $r$  in the range of 0.72–1.00). This indicates that across all treatments, both frequency and temperature did not have a significant effect on the correlation coefficient between different  $G'$  and  $G''$  values, and that an increase in one viscoelastic modulus showed a corresponding increase in the other viscoelastic modulus. However,

as shown in Table 6-1, a similar trend was not observed for  $\delta$  values at 0.002 Hz and 1.62 Hz measured at 5 °C and 25 °C. The  $\delta$  values at 1.62 Hz correlated significantly at 5 °C and 25 °C ( $r = 0.82$ ) whereas,  $\delta$  values at 0.002 Hz did not significantly correlate between 5 °C and 25 °C ( $r = 0.41$ ). This may be attributed to a higher frequency enabling higher strains in the dough structure as compared to a lower frequency (low strain rates) where temperature significantly affects the overall structure of dough.

$T_{11}$  and  $T_{12}$  correlated moderately ( $r = 0.63$ ) whereas  $T_{21}$  and  $T_{22}$  correlated significantly ( $r = 0.91$ ). This shows that the two exponential decay components present in the spin-spin relaxation decay increase or decrease simultaneously, indicating that an increase in mobility of higher components corresponds to an increase in the mobility of the lesser mobile components. The peak  $T_2$  values for population 2 correlated positively ( $r = 0.39$ ) with the peak standard deviation for population 2. However, there was a negative correlation ( $r = -0.49$ ) between the peak  $T_2$  values for population 2 and peak area for population 2. Therefore, it may be suggested that as mobility increases, the heterogeneity in the dough sample increases and number of protons associated with that mobility decreases. This may be attributed to a greater diversity in the proton environment of the dough as mobility increases, resulting in movement of protons to other environments or populations within the dough system. There was no correlation ( $r = -0.04$ ) between the peak area of population 2 and peak standard deviation of population 2, suggesting that heterogeneity of the extra-granular region (population 2)

in the dough did not depend on the number of protons associated with that population. As can be seen from Figure 6-1, no significant correlations were observed for D with other response variables. However, high correlations were observed between gumminess and chewiness ( $r = 0.82$ ), and between compressive load at break and modulus of elasticity of crust ( $r = 0.75$ ), as shown in Table 6-1.

### **6.2.2 Relationship between quality parameters of baked product and response variables associated with nano- and micro-scales in wheat dough**

To understand the variability in response variables related to quality of final baked product (BSV, cohesiveness, chewiness, gumminess, compressive load at break, and modulus of elasticity of crust) with different response variables associated with dough ( $T_{11}$ ,  $T_{12}$ ,  $T_{21}$ ,  $T_{22}$ , D, G' and G" — which may be attributed to be nano- or micro-scale responses), preliminary data analysis was performed by plotting the final baked product quality parameters on y-axis and response variables associated with dough on x-axis. These scatter plots showed that when all observations and treatments were considered, CSL and DATEM did not significantly affect the relationship between the quality parameters of final baked product and dough response variables. However, GA significantly affected the relationship. This was clearly visible from the scatter plots where the quality parameters values were consistently higher at a given dough response variable, for dough samples with no GA as compared to those for dough

samples with GA (3 % w/w). Therefore, it was suggested that a full-factorial statistical model may be fitted for the quality response variables (to study effects of GA) using the variables which were controlled in the experiments. The controlled variables (refer to section 4.3 for details of controlled variables used in the experiments), as per the design of experiments, were CSL, DATEM, frozen storage time (weeks), and cooling rate. The % variability in a quality parameter, as affected by GA, was explained by the following equation:

$$\% \text{ variability} = \frac{SS(\text{Model})_{\text{with GA}} - SS(\text{Model})_{\text{without GA}}}{SS(\text{Total})} \quad (6-1)$$

where  $SS(\text{Model})_{\text{with GA}}$  is the *model sum of squares* (also known as *regression sum of squares* or *between sum of squares*) with GA and  $SS(\text{Model})_{\text{without GA}}$  is the *model sum of squares* without GA.  $SS(\text{Total})$  is the *total sum of squares* and from statistics (Ott and Longnecker 2001; Bower 2009), we know:

$$SS(\text{Total}) = SS(\text{Model})_{\text{with GA}} + SS(\text{Error})_{\text{with GA}} = SS(\text{Model})_{\text{without GA}} + SS(\text{Error})_{\text{without GA}} \quad (6-2)$$

where  $SS(\text{Error})_{\text{with GA}}$  is *error sum of squares* (also known as *within sum of squares*) with GA and  $SS(\text{Error})_{\text{without GA}}$  is *error sum of squares* without GA.

% variability for different quality variables as explained by GA is presented in Table 6-2. It can be observed that the maximum % variability (55.5 %) due to GA is for cohesiveness and least % variability (35.8 %) due to GA is for modulus of elasticity of crust. However, it is important to note that for all the quality parameters studied, GA alone accounted for more than 35.8 % of variability in the model. This clearly supports

the observed behavior in the scatter plots described earlier in this section. Therefore, it may be concluded that presence of GA results in significant changes in quality parameters and may prove to be an essential functional ingredient for quality control of baked products.

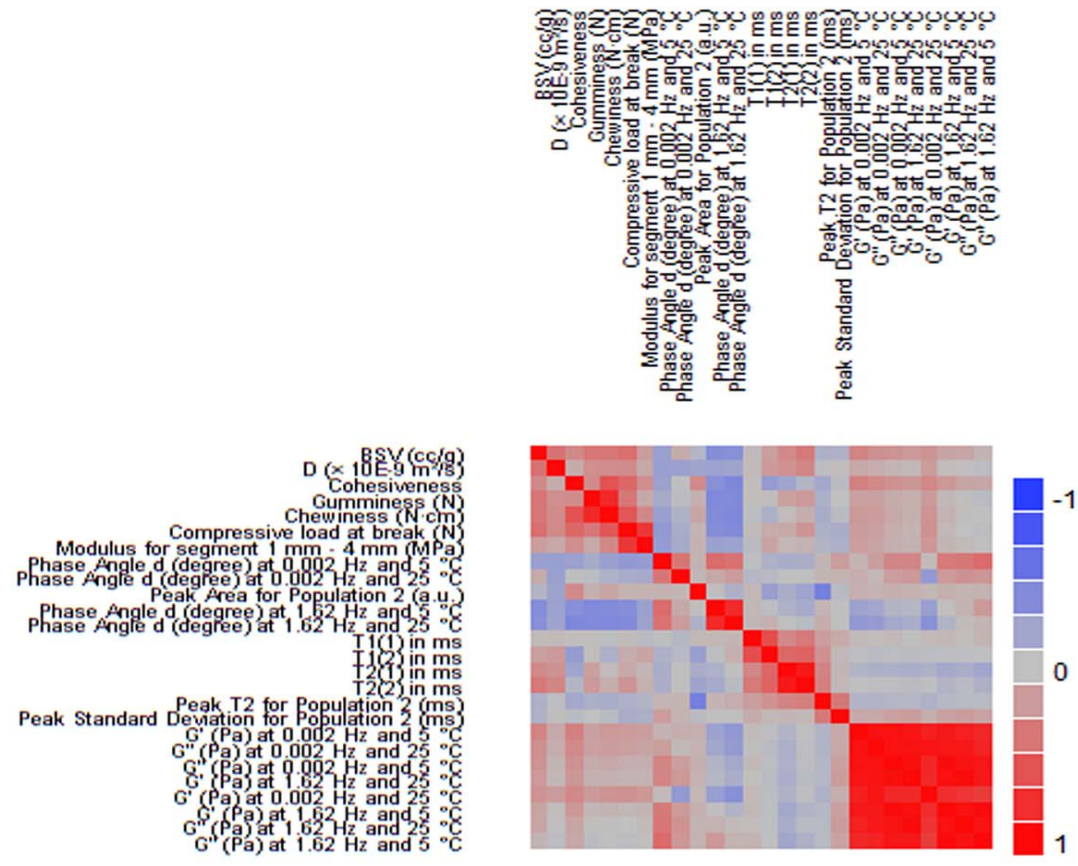


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**Figure 6-1** Color map of Pearson's correlation coefficients between 26 response variables. Red color corresponds to high positive correlation coefficients (close to +1) and blue corresponds to high negative correlation coefficients (close to -1)

**Table 6-1** Pearson's correlation coefficient matrix for certain response variables

|   | Chewi-<br>ness<br>(N·cm) | Modulus of<br>elasticity of<br>crust for<br>segment 1-4<br>mm (MPa) | Phase Angle $\delta$<br>(°) at 0.002 Hz<br>and 25 °C | Phase Angle $\delta$<br>(°) at 1.62 Hz<br>and 25 °C | T <sub>12</sub> (ms) | T <sub>22</sub> (ms) | Peak T <sub>2</sub> for<br>Population<br>2 (ms) | Peak Standard<br>Deviation for<br>Population 2 (ms) |
|---|--------------------------|---|--|---|----------------------|----------------------|---|---|
| Gumminess (N)                                       | <b><i>0.82</i></b>       | 0.13  | -0.03  | -0.41   | 0.02                 | 0.20                 | -0.13   | 0.00  |
| Compressive load<br>at break (N)                    | 0.58                     | <b><i>0.75</i></b>  | -0.15  | -0.41   | 0.11                 | 0.24                 | -0.12   | -0.14   |
| Phase Angle $\delta$ (°) at<br>0.002 Hz and 5 °C    | -0.25                    | -0.28   | <b><i>0.41</i></b>                                   | 0.42  | -0.12                | -0.13                | 0.06  | 0.19  |
| Phase Angle $\delta$ (°) at<br>1.62 Hz and 5 °C     | -0.40                    | -0.24   | 0.13   | <b><i>0.82</i></b>                                  | -0.02                | -0.16                | 0.07  | 0.00  |
| T <sub>11</sub> (ms)                                | -0.02                    | -0.04   | -0.07  | 0.06  | <b><i>0.63</i></b>   | 0.38                 | 0.18  | 0.11  |
| T <sub>21</sub> (ms)                                | 0.02                     | 0.05  | -0.01  | 0.00  | 0.50                 | <b><i>0.91</i></b>   | 0.33  | 0.06  |
| Peak Area for<br>Population 2 (a.u.)                | 0.14                     | 0.07  | 0.06   | -0.12   | -0.25                | -0.21                | <b><i>-0.49</i></b>                             | -0.04   |
| Peak Standard<br>Deviation for<br>Population 2 (ms) | -0.04                    | -0.12   | 0.07   | 0.00  | 0.05                 | 0.05                 | <b><i>0.39</i></b>                              | 1.00  |

Values in bold and italic are highest values in their respective row or column

**Table 6-2** Percentage variability in different quality response variables of baked product explained due to presence of gum arabic (GA) in the recipe

|  | <b>SS(Model)<sub>with GA</sub><sup>b</sup></b> | <b>SS(Model)<sub>without GA</sub><sup>b</sup></b> | <b>SS(Total)<sup>b</sup></b> | <b>% Variability due to GA<sup>c</sup></b> |
|--|--|---|------------------------------|--|
| <b>BSV (cc/g)<sup>a</sup></b>                                  | 7.64   | 3.03  | 9.67                         | 47.7                                       |
| <b>Cohesiveness</b>  | 0.23   | 0.07  | 0.29                         | 55.5                                       |
| <b>Chewiness (N·cm)</b>  | 7.70   | 2.78  | 11.69                        | 42.1                                       |
| <b>Gumminess (N)</b>   | 3338.72  | 1274.21   | 5441.00                      | 37.9                                       |
| <b>Compressive load at break (N)</b>                           | 1685.62  | 497.25  | 2331.21                      | 51.0                                       |
| <b>Modulus of elasticity of crust for segment 1–4 mm (MPa)</b> | 0.23   | 0.09  | 0.40                         | 35.8                                       |

<sup>a</sup> BSV, baked specific volume

<sup>b</sup> SS, sum of squares

<sup>c</sup>  $Df_{Total} = 215$ ,  $df_{Model\ with\ GA} = 39$ ,  $df_{Error\ with\ GA} = 176$ ,  $df_{Model\ without\ GA} = 19$ ,  $df_{Error\ without\ GA} = 196$ ; df: degree of freedom

## Chapter 7

# Conclusions and Recommendations for Future Work

### 7.1 Conclusions

The mobility of water in frozen-thawed dough was studied using nuclear magnetic resonance (NMR) by obtaining spin-lattice relaxation times ( $T_{11}$  and  $T_{12}$ ) and spin-spin relaxation times ( $T_{21}$  and  $T_{22}$ ) for the frozen-thawed samples. Diacetyl-tartaric acid ester of monoglycerides (DATEM) significantly affected the longer component of spin-spin relaxation time ( $T_{22}$ ) values at 0 week which were significantly different from the  $T_{22}$  values for fresh dough. Neither cooling rate nor frozen storage time had a significant effect on changing longer component of spin-lattice relaxation time ( $T_{12}$ ) values for all eight dough recipes. From the trends observed in the graphs for  $T_{12}$ , shorter component of spin-spin relaxation time ( $T_{21}$ ), and  $T_{22}$ , calcium stearoyl-2-lactylate (CSL) had no significant effect on reducing the relaxation time values, whereas, gum arabic (GA) and DATEM had significant effects in reducing the relaxation time values. Reduced relaxation time may be attributed to increased binding of water molecules to gluten matrix and starch granules. GA consists of hydrophilic molecules which bind water tightly and DATEM is a dough strengthener used to improve the gluten strength, leading to better retention of gases released during fermentation and proofing of dough. These

properties of DATEM and GA may be the cause for the observed decrease in overall mobility of water in frozen-thawed dough in this study. Spin-spin relaxation time ( $T_2$ ) distribution studies revealed that increasing cooling rate increased the number of protons and heterogeneity associated with population 2 ( $T_2$ : 5–30 ms) — the population of protons interacting with starch granules and emulsifiers/gum and known as extra-granular protons. Environmental scanning electron microscope (ESEM) micrographs show the presence of sheets in the gluten matrix of dough as compared to popularly believed strands or fibrous network, which has been confirmed by only one earlier study using ESEM for frozen dough. Dynamic rheological studies showed that the use of DATEM alone and freezing the dough at a higher cooling rate can result in improved viscoelastic properties for dough.

Quality attributes such as baked specific volume (BSV), cohesiveness, gumminess, chewiness, crust firmness, and modulus of elasticity of crust for final baked product, all of which affects its sensory and consumer acceptability, were measured for the baked product. The results in this study presented the main and interaction effects of functional ingredients and processing parameters. Longer frozen storage times resulted in low BSV and therefore, deteriorated the quality of the final baked product. DATEM, when added without GA or CSL, resulted in improved BSV with increasing frozen storage time. DATEM also proved to be a significant ingredient in improving the softness of the crust of the baked product. However, gumminess and chewiness

improved only with a specific combination of DATEM, CSL, and GA. It was observed from the results in this study that the interactions between functional ingredients and processing parameters is complex and all quality attributes of a baked product cannot be optimized at the same time in a single recipe. However, a specific combination of these functional ingredients may be used to improve the prioritized desired quality attributes in a baked product made from frozen-thawed dough.

Correlation studies between all the quality responses — measured to study the quality attributes for frozen-thawed dough and for the baked product — indicated the relationship between quality attributes at different scales (nano, micro, and macro). It was observed that  $T_{21}$  and  $T_{22}$  increased or decreased simultaneously, indicating that the increase in mobility of the higher mobile component corresponds to an increase in the mobility of the lesser mobile component. Results in this study suggested that as mobility increases, the heterogeneity in the dough sample increases and the number of protons associated with that population of protons decreases. This may be attributed to greater diversity in the proton environment of the dough as mobility increases, resulting in movement of protons to other environments or populations within the dough system. A correlation study between the quality parameters of baked product and quality response variables associated with wheat dough (at nano- and micro-scales), described using a statistical model, indicated that GA alone accounted for 35.8–55.5 % of variability in the quality attributes (BSV, cohesiveness, chewiness,



gumminess, compressive load at break, and modulus of elasticity for crust) of the baked product. This effect of GA may be explained based on either the segregative phase separation or water acting as a polymer plasticizer in the dough system.

This study aids in improving the understanding of interaction between emulsifiers/gums and processing parameters (cooling rates and frozen storage time) which may be used to improve the quality of baked product made from frozen wheat dough. The results shows the importance of designing and implementing a detailed study to determine the functional ingredients and processing parameters required for optimizing the desired quality attributes for a baked product made from frozen dough.

## 7.2 Recommendations for future work

Enzymes such as transglutaminase have shown to improve rheological properties and BSV of bread by re-establishing the damaged gluten network due to freezing of dough (Huang and others 2008). In another study (Caballero and others 2007), individual and synergistic effects of some gluten cross-linking enzymes (transglutaminase, glucose oxidase, and laccase) along with polysaccharide and gluten degrading enzymes ( $\alpha$ -amylase, xylanase, and protease) were studied. In this study, transglutaminase was observed to exert strengthening effects to the dough structure. Transglutaminase and glucose oxidase are known to improve the gluten network by promoting cross-linking between the gluten networks. In another study (Koocheki and others 2009), the effect of a combination of emulsifiers (lecithin, distilled monoglyceride, and diacetyl-tartaric acid esters of mono- and di-glycerides) with  $\alpha$ -amylase on rheological characteristics of wheat dough and quality of flat bread was studied. It was observed in this study that the highest improvement in firmness of the bread resulted due to lecithin. The effect of a combination of amylase and xylanase on texture of brown pan bread has been studied (Gámbaro and others 2006) and was shown to improve desired textural characteristics. Amylase breaks down the starches present in wheat dough to dextrin, thereby reducing the amount of amylose — responsible for firming (staling) of the crumb of the bread. Xylanase breaks down water-insoluble xylans that interrupt the gluten network. Phytase and asparaginase are used to improve the nutritional value of baked products.

Phytase is known to improve mineral bio-availability in bread by breaking down phytate — a compound that sequesters minerals. Asparaginase can be used to convert asparagine to aspartic acid, thereby reducing the formation of acrylamide — a carcinogen formed by reaction of asparagine with carbonyl groups present in sugars. Therefore, enzymes have shown to aid in improving texture, nutritional value, and overall quality of baked products and are considered “clean” for labeling purposes since they are considered as processing aids. Trehalose, a major storage carbohydrate in bakers’ yeast (*Saccharomyces cerevisiae*) is known to protect dough from various types of stresses such as dehydration, freezing, and autolysis (Kim and others 2008).

In future work, the effect of addition of enzymes and functional ingredients such as transglutaminase, lecithin, and trehalose on improving the quality of baked product made using frozen dough may be studied in combination with emulsifiers/gums. Since dough is a complex food, especially when considering all the ingredients used in this study (flour, water, emulsifiers, gums, yeast, salt, and oil), it may be useful to study a model system consisting of only flour and water to improve the understanding of the effects of other ingredients. This methodology may specifically be useful when considering NMR techniques, where signals arising in model systems (such as only flour and water mixtures), may be distinguished from signals arising due to presence of other ingredients (Doona and Baik 2007) to determine the effects of individual ingredients.

Two-dimensional (2-D) hybrid pulse sequences may be developed to determine joint distribution of spin-lattice relaxation time ( $T_1$ ) and spin-spin relaxation time ( $T_2$ ). This joint distribution is related to individual spin groups for resolving the heterogeneity of the whole spin system (English and others 1991; Song and others 2002). A 2-D pulse sequence may be developed to determine self-diffusion coefficients of water ( $D$ ) vs.  $T_2$  correlations (Godefroy and Callaghan 2003), which allow molecular motion to be distinguished from molecular interactions of spins in the system. Since dough is a complex food, the self-diffusion of water may be restricted — similar to that found in emulsion systems (such as butter and cheese). Therefore, developing a diffusion pulse program to identify these multiple  $D$  values and to correlate them with other NMR relaxometric parameters may be suggested for future work.

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