THE EFFECT OF WATER AND GLUTEN ON THE WATER MOBILITY, GELATINIZATION AND RETROGRADATION OF BREAD AND STARCH GELS

by

XIN WANG

(Under the Direction of William L. Kerr)

ABSTRACT

The effects of water and gluten on the water mobility in bread and retrogradation of starch gels were studied using pulsed ^{1}H NMR.

Breads were made with three levels of water content and three levels of gluten content. Model system was used on starch-gluten gels. Distributed exponential analysis of the transverse relaxation curves showed no significant difference with different gluten content, indicating decrease of the mobility of water associated with starch due to gelatinization, so that gluten addition did not affect the total water mobility of the bread.

Starch-gluten gels with different gluten content were measured to study the effect of gluten on starch retrogradation. The T_2 values of all the starch-gluten samples decreased much less than the starch gels, but there was no significant difference among gels with different gluten contents. This suggested that gluten retarded starch retrogradation, but further addition of gluten did not further decrease retrogradation rate.

INDEX WORDS: Bread, Starch, Gluten, Gel, Water mobility, NMR, T_2, Gelatinization, Retrogradation

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DEDICATION

This thesis is dedicated to

My parents:

Mr. Xuezhen Wang and Mrs. Xiuqin Lu

And my sister:

Ms. Ye Wang

For constant support, encouragement, and unconditional love.

To

My beloved husband

Jianwei Wang

For his patience, help and love.

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CHAPTER 1

INTRODUCTION

Gluten is a major component in wheat flour, comprising 10-14% of the total weight of flour, and is a protein prepared by gentle washing of a flour-water dough in an excess of water or dilute salt solution to remove most of the starch and soluble material (Pomeranz, 1988). When making a bread, gluten forms an increasingly extensible and fragile network in the presence of water during the kneading process and traps the gases produced during fermentation (Callejo, 1999). The gluten proteins play an important role in subsequent stages of the breadmaking process and in the structure of the finished product.

The gluten component in bread has an important effect on the water behavior in bread, thus further influences starch gelatinization and retrogradation. The presence of gluten decreased the starch gelatinization enthalpy and increased the gelatinization temperature (Eliasson, 1983). The presence of gluten might also affect starch retrogradation, thus influencing bread staling. Hypotheses on the role of gluten in bread staling range from gluten having an anti-firming effect, or no effect on firming, to gluten-starch interactions being essential for bread firming (Callejo, 1999; Kim and D'Appolonia, 1977; Martin et al., 1991).

The interaction of water with the components in bread strongly influences the physical and structural properties of bread. It has been recognized that water relationship affects starch gelatinization and retrogradation. Various fractions of water in bread dough

have properties that differ from each other, or from those of bulk water (Kuntz and Kauzmann, 1974).

Various methods have been used to study starch gelatinization and retrogradation, including X-ray diffraction, differential scanning calorimetry (DSC), dynamic mechanical thermal analysis (DMTA), and a variety of nuclear magnetic resonance (NMR) spectroscopy techniques. Among them, pulsed ^{1}H NMR has been widely used to study the dynamic behavior of water in foods by observing proton relaxation phenomenon (Ablett, 1992).

Knowledge about changes in the molecular dynamics of water caused by its interaction with other macromolecules in flour helps increase understanding and control of the behavior of flour containing foods.

This thesis is divided into four chapters. Chapter 2 is a literature review which describes gluten, starch, starch gelatinization, starch retrogradation, bread staling, bound water and free water, water mobility, pulsed proton NMR, and previous studies related to this research.

In Chapter 3, the effect of water and gluten levels on the water mobility in white bread and starch gels is studied using pulsed proton NMR.

In Chapter 4, the effects of gluten on retrogradation and water mobility in wheat starch gels is investigated by pulsed ^{1}H NMR.

Chapter 5 summarizes and concludes of the studies reported in Chapter 3 and 4.

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CHAPTER 2

LITERATURE REVIEW

2.1. Gluten

Gluten is the major protein in the endosperm of wheat, making up 80-90% of the protein in the flour. The traditional method of gluten preparation involves gentle washing of a flour-water dough in an excess of water or a dilute salt solution to remove most of the starch and soluble material until the water-insoluble protein of flour is obtained as a rubbery mass. The dry solids contain 75-85% protein, depending on the thoroughness of washing (Pomeranz, 1988).

Gluten consists of two major fractions: gliadins, which are soluble in alcohol, and glutenins, which are not soluble in either alcohol or salt solutions. Gliadins consist of 40 distinct polypeptides (Wrigley, 1988). Existing in the form of single-chain polypeptides, gliadins have an average molecular weight of 40,000 and exhibit little or no disulfide bonding between chains (Hoseney, 1986) The glutenin fraction consists of protein chains cross-linked by intermolecular disulfide bonds to form linear, concatenated multi-polymers with molecular weights ranging from 100,000 to the millions. The physical properties of the two fractions differ when hydrated: gliadins behave like a viscous liquid, while glutenins behave like cohesive elastic solid (Wall, 1979).

The rheological properties of the gluten (the combination of its viscous, elastic and cohesive properties) are responsible for the bread making quality of wheat flour, and the two fractions, gliadins and glutenins, contribute to gluten's viscoelastic characteristics. The plastic properties of dough are acquired during the kneading process,

when the gluten forms an increasingly expansible yet fragile network. The dough properties depend on the flour quality and the overall process. The gluten proteins play an important role in subsequent stages of the breadmaking process and in the structure of the finished product: they are sufficiently expansible, yet resilient enough to be able to stretch with the dough and trap the gases produced during fermentation. These properties need to be conserved during baking (Callejo, 1999).

2.2. Starch

Starches are the major storage polysaccharides in foods of plant origin. The major botanical and commercial sources of starches are cereals, tubers, roots, and pulses. Native and modified starches serve as important ingredients of many fabricated foods. Starches are α-glucans composed of two different homopolymers of D-glucose — amylose and amylopectin. Amylose has traditionally been considered to be a linear polymer composed of glucopyranose units linked through α-D- (1→4) glycosidic linkages. Amylopectin is a branched polymer with one of the highest molecular weights known among naturally occurring polymers. It contains short chains of (1-4)-α-linked D-glucan chains with (1-6)-α-linked branches (Karim et al., 2000).

Starches exist naturally in the form of discrete granules within plant cells. These granules may be viewed as partially crystalline and partially amorphous polymeric systems (Blanshard, 1987; Slade & Levine, 1989). The crystalline character of the granules of common starches arises from the organization of the amylopectin molecules within the granules, while amylose largely makes up the amorphous regions, which are randomly distributed between the amylopectin clusters (Blanshard, 1987; Zobel, 1988).

Granules from different starch sources vary in size and shape. Cereal starch granules are smaller and contain more residual of protein and lipid; in some species, such

as wheat, there is a bimodal distribution of granule size, with larger lecticular granules as well as smaller spherical granules (Swinkels, 1985). Variations in the ratio of the polysaccharides, their properties and interaction with other components can explain any difference in the swelling behavior of granules and functionality of starches.

2.3. Starch gelatinization

When the starch-water suspension is heated, irreversible changes occur starting at a specific temperature, the gelatinization temperature, which occurs between 50 and 70^{o}C, and is characteristic of each type of starch. The starch absorbs 20-40 g of water/g of starch, and the viscosity of the suspension rises steeply. At the same time, a part of the amylose diffuses out of the granule and goes into solution; the swollen granules are enriched in amylopectin. As a direct result of granule swelling, there is an increase in starch solubility, paste consistency and paste clarity (Belitz and Grosch, 1999). Gelatinization can be defined as a phase transition of the starch granules from an ordered state to a disordered state, which involves loss of crystallinity, loss of anisotropic order and birefringence, and hydration of starch (Donovan, 1979; Zobel and Stephen, 1995). The gelatinization temperature is actually a temperature range over which the gelatinization process occurs, and is dependent on several factors including the type of starch or the presence of salts, sugars, and lipids, as well as any chemical modifications (Pomeranz, 1991).

Gelatinization of starch is a basis for many types of food production. Processes such as the baking of bread, the gelling of pie fillings, the production of pasta products, the fabrication of starch-based snack foods and the thickening of sauces are all dependent on proper gelatinization of starch to produce a desirable texture or consistency of the end product (Juhani and Chokyun, 1978). A starch paste can be described as a two-phase

system composed of a dispersed phase of swollen granules and a continuous phase of leached amylose (Hermansson and Svegmark, 1996). On cooling of concentrated starch paste, starch gels quickly develop as leached starch links swollen starch granules together. The amounts of leached amylose and starch concentration are important factors for gelation. It was observed that amylose gelatinizes at concentrations above ~1.5% (w/w), and noted that solubilization of sufficient quantities of amylose was necessary for the gelation of starch. Typically, this requires a minimum of 6% (w/w) total starch for gelation (Ott and Hester, 1965). On the other hand, at very high starch concentrations, there is insufficient water to gelatinize all granules, leading to limited leakage of amylose out of the granules. If most of the amylose remains inside the swollen granules, amylose cannot form a continuous gel phase outside the granules (Hermansson and Svegmark, 1996).

2.4. Starch retrogradation

Cooking or processing normally causes starch gelatinization. The behavior of gelatinized starches on cooling and storage, generally termed retrogradation, is of great interest to food scientists and technologists since it profoundly affects quality, acceptability and shelf life of starch-containing foods (Biliaderis, 1991). Starch molecules in pastes or gels are known to associate upon aging, leading to crystallite formation and accompanied by gradual increases in rigidity and phase separation between polymer and solvent (syneresis). Recrystallization or retrogradation of starch gels is characterized by changes in many physical and nutritional phenomena, such as hardness, water absorptive capacity, opacity, enzyme hydrolysis, and soluble starch content of starch gels, and contributes to defects in quality of starch-containing foods (Hermansson and Svegmark, 1996).

Several researchers proposed a mechanism for retrogradation of starch gel. Using X-ray diffraction, Miles et al. (1985) proposed that amylose was responsible for short-term irreversible development of gel structure and crystallinity in a starch gel, while amylopectin was involved in development of long-term reversible crystallization. Orford et al. (1987) showed that gels (10-40% w/w) from pea, potato, wheat, and maize starches showed increases in shear modulus over 7 days. The initial rate of development of stiffness of the gels followed the order: pea>maize>wheat>potato, and was related to the amylose solubilized during gelatinization. The initial gelation was not reversed upon heating to 100^{o}C. There was also a long-term increase in gel stiffness that was thermally reversible. With increase in concentration, the latter process became more important.

Many methods have been used to study starch retrogradation. Rheological or textural measurements monitor alterations in physical properties; enzyme assays measure the resistance to hydrolysis; DSC analysis measures the enthalpy of fusion of recrystallized starch molecules; X-ray diffraction determines the degree and type of crystallinity developed in retrograded starch (Kalichevsky et al., 1990; Russel, 1987; Ward et al., 1994; Colwell et al., 1969a; Tsuge et al., 1990; Teo and Seow, 1992; Roulet et al., 1988).

Several factors have been known to affect starch retrogradation, including starch source, ratio of amylose to amylopectin, heating and storage temperature, salts and sugars, lipids, and chemical modifications (Hoover, 1995). Water is also an important factor to starch retrogradation and water relationships have been an issue in the study of starch retrogradation. In general, retrogradation of starch gels proceeds faster with water contents in the range of 30-60% (Liu et al., 1998). Hellman et al. (1954) showed that the most intense “B” x-ray pattern is obtained for wheat starch gels with water content of

47% to 50% (w/w). Zeleznak and Hoseney (1986) have shown using differential scanning calorimetry (DSC) measurements that the maximum extent of crystallization for wheat starch occurs, respectively, at 50% and 60%. Orford et al. (1987) and Biliaderis and Tonogai (1991) observed a linear relationship between starch concentration and storage modulus (G') during storage of cereal, tuber, and legume starch gels. The increase in G' was negligible at a concentration of 20% w/w. However, 30% and 40% (w/w) gels of these starches showed a marked increase in G'.

2.5. Bread staling

Bread consists predominantly of proteins, starch and water in the approximate ratio of 1:6:5.

Freshly baked bread can be easily recognized by its sensory attributes: crispy crust, soft crumb, and appealing aroma. During aging, crumb firmness significantly increases, crispness of the bread crust decreases, and the bread loaf loses its fragrance, assuming a stale flavor.

Bread staling has been the subject of a number of studies focused on understanding its intrinsic mechanism and with the promise of modifying dough recipes to increase shelf life. Numerous studies on bread staling have shown that starch retrogradation upon aging plays a major role in causing bread firmness (Hertz, 1965; Zobel, 1973). Cornford et al. (1964) studied the relationship between elastic modulus, time and temperature in bread crumb. The results showed that the limiting modulus increased faster as temperatures were lowered towards the freezing point. This evidence emphasized the importance of the negative temperature coefficient of the staling process, and the fact that crystallization, a physical process involving a more ordered arrangement of molecules, was the principle factor involved in crumb firmness. Several studies

(Axford, 1968; McIver, 1968; Colwell, 1969b) detected starch retrogradation in bread crumb or in starch gels upon aging using DSC analysis. The results also indicated that bread staling is basically characterized by the retrogradation of the starch component of the crumb. A direct comparison of bread firmness with crystallinity of starch by X-ray diffraction showed that, for a given firmness measured at room temperature, there was a higher level of crystallinity for bread stored at 4^{o}C than 21^{o}C (Wright, 1971). These results again demonstrated that the firming of bread at constant moisture levels is controlled by the degree of crystallinity of the starch.

Although starch retrogradation plays a major role in bread staling, changes in other components and their interactions with starch may also play an important role in the staling process. Numerous studies have been done on the effect of amylose, amylopectin, pentosans, moisture content, lipids, storage temperature, anti-staling agents, and loaf volume on bread staling (Kim, 1977a; Gil et al., 1997; Rogers et al., 1988; Maleki et al., 1980; Armero and Collart, 1998). However, there is considerable uncertainty about the role of protein in bread staling.

For many years, the theory of Schoch and French (1947), associating bread staling (firming) solely with starch retrogradation, was generally accepted as fact. However, Martin et al. (1991) presented evidence suggesting that starch retrogradation and bread firming are not related. They postulated that firming is caused by entanglements and relatively weak cross-links (hydrogen bonds) between protein fibrils and starch remnants. This implies that protein is essential to the staling process and bread with low or no protein content would therefore have a low or zero firming rate. This assumption appears to contradict previous studies showing that decreasing protein content in flour, or reconstituted flour, increases the rate of firming (Steller, 1938; Bechtel, 1954; Prentice et

al., 1954). Kim and D'Appolonia (1977b) suggested that protein had no direct role in firming, but only diluted the starch in bread and thereby reduced the firming rate. Alternatively, the low specific loaf volume of low protein bread could explain the high firming rate, as Axford et al. (1968) have shown that decreasing the specific loaf volume increases the rate of firming. Erlander and Erlander (1969) postulated that the retrogradation of starch may be inhibited by protein, and that the ratio of starch to protein in the dough is critical in determining the rate of firming.

Water relationships have long been an issue in the study of bread staling. The hypothesis that simple drying out might be solely responsible for staling was refuted nearly 150 years ago when Boussingault (1852) demonstrated that bread might stale without appreciable loss of moisture. However, loss of moisture from the loaf may accelerate reactions leading to bread staling (MacMasters, 1961). The effect of moisture migration or redistribution within bread during staling and starch retrogradation has been studied by numerous researchers. Leung et al. (1983) proposed that, as starch changes from the amorphous state to the more stable crystalline state, water molecules become immobilized as they are incorporated into the crystalline structure, resulting in a decrease in mobility of water during the firming process. Whistler and Daniel (1985) suggested that water was expelled from the starch matrix due to retrogradation. This has been supported by polymer theory and DSC results indicating that freezable water in the amorphous matrix migrates to crystalline hydrates of B-type wheat starch, where the water becomes unfreezable (Slade and Levine, 1989). Willhoft (1971) and Breaden and Willhoft (1971) reported that gluten underwent a transformation resulting in the release of water, which became absorbed by retrograding starch.

2.6. Bound water and free water

Water is a very important component in foods impacting quality attributes, shelf stability, textural properties, and processing (Levine and Slade, 1991). The ability to form hydrogen bonds with other water molecules and macromolecules is the most important nature of water, and is responsible for the unusual physical properties and functionality of water. Like other molecules, water molecules are constantly in motion. However, because of its bonding with other molecules, the mobility of water in heterogeneous environments such as foods is far more complex. The theory proposed by Ling (1962) has been accepted by many researchers for many years: hydrophilic groups on macromolecules (e.g. $–NH_2$ and –OH) may form hydrogen bonds with nearby water molecules, which, due to polarization caused by hydrogen bonding, in turn will be more inclined to form hydrogen bonds with water molecules in the next layer, and so on. These associated water molecules have restricted motion compared with pure water, and are called bound water. In general, bound water is the water in a food or biological system whose properties differ detectably from those of pure water (Kuntz and Kauzmann, 1974).

The physical state of water in food system is believed to play an important role in the structural, physical, chemical and sensory properties of foods. The concepts of bound and free water have been used to describe the states of water, but the physical meaning of these descriptions is controversial as the levels measured depend on the technique used. Bound water is usually defined as the moisture level at which a significant discontinuity in behavior, such as in freezing, dehydration, solvent properties, spectroscopic properties, or binding energy is observed. Leung and Steinberg (1979) determined the water binding properties of some common food constituents using water sorption, dehydration, freezing

and Nuclear Magnetic Resonance (NMR) methods. Since NMR is very sensitive to water mobility, bound water in food polymers was often characterized by NMR spin-spin relaxation time (Steinberg and Leung, 1975; Nagashima and Suzuki, 1984; Richadson et al., 1986). A two-phase, fast exchange model of free water and bound water usually fits well for systems in the high-moisture range (Schmidt and Lai, 1991; Richardson et al., 1987a,b; Callaghan et al., 1983). By analysis of the Carr-Purcell-Meiboom-Gill (CPMG) relaxation curves from wheat starch-water suspensions, Botlan et al. (1998) determined bound water content in wheat starch suspensions with moisture contents in the range of 0.14 to 0.95 g of water per g starch using time domain NMR spectroscopy. It was determined that there was a constant bound water content of 0.17g. Water content in the range of 0.17 to 0.42g was weakly bound, and acted as potentially free water. By applying sorption isotherm and pulsed NMR techniques, Tanteeratarm et al. (1990) successfully distinguished the polymer and capillary water associated with the proteins, which were defined as the first layer of water above the monolayer region and the next, respectively.

2.7. Water mobility

Water in foods can be described by three properties or parameters: water content, water activity, and water dynamic mobility (Li et al., 1998). Because of the straightforward meaning and ease of determination, water content has been a universal parameter, and has been related to biological, chemical, and physicochemical reactions. However, water exists in different states in biopolymers such as foods. It is thought that water molecules in different states function differently.

In the late 1950s, the microbiologist Walter Scott (Scott, 1957) suggested that water activity could provide a better and more reliable measure of availability of water.

Since then, a_w has been widely accepted by many food and biological scientists and has been used as a primary guideline for the safety and quality control of food, biological and pharmaceutical products (Schwimmer, 1980; Troller, 1980; Troller 1989).

In recent years, the theoretical and practical limitations of a_w have been voiced by many food scientists (Frank, 1991a,b; Slade and Levine, 1991). The measurement of a_w of a food system is based on the assumption that the food system is in its equilibrium state, which is true only for infinitely dilute systems. Unfortunately, most food systems usually are non-equilibrium systems and, during processing and storage, are maintained or brought into a state of thermodynamic instability. Therefore a concept rooted in equilibrium thermodynamics, such as a_w, cannot fully represent most food systems. Another defect of a_w is that, when different solutes are used to lower the a_w of a system, the response of the system in terms of the reaction rates or stability is different. This suggests that measurement of a_w is insensitive to the solute-solute and solute-water interactions, which, in fact, have a profound impact on the reaction kinetics of the system.

Many researchers have found that the mobility of water, as measured by NMR and other techniques, is related to the availability of water in complex systems (Richardson et al., 1986; Richardson et al., 1987a; Mora-Gutierrez and Gaianu, 1989; Kakalis et al., 1990; Lai and Schmidt, 1990; Hills et al., 1990; Schmidt and Lai, 1991; Lai and Schmidt, 1993). The higher the mobility of water, the higher the availability of the water.

2.8. NMR

Nuclear magnetic resonance (NMR) spectroscopy has seen increased use in the field of food science. NMR spectroscopy is based on the magnetic properties of atomic nuclei. Most of the nucleus has positive charge and can spin like a small magnet. When

placed in a static magnetic field, the nucleus spins align themselves with the field and precess around it. If we apply a second magnetic field in the form radio frequency (RF) pulse at the same frequency as the precession of the nuclei, the spins can resonate and absorb energy from this second field. By absorbing energy, they increase their angle of precession. The pulse, which is switched on long enough to cause a precession angle to increase from the equilibrium starting point (angle 0) to 90^{o}, is called a 90^{o} pulse. Similarly, a pulse causing 180^{o} rotation is called a 180^{o} pulse. After the pulse is turned off, the spins will lose their energy and return to their equilibrium position, a process defined as relaxation. During this process, the NMR instrument detects the signals as dying away.

Many nuclei, such as ^{1}H, ^{2}H, ^{13}C, ^{15}N, and ^{17}O, have resonance behavior that can be detected through NMR spectroscopy. Among these nuclei, ^{1}H is the most commonly used, primarily because of its high natural abundance and presence in the majority of food and biological samples.

Two kinds of relaxation processes can be used to characterize the signal decay: ***spin-lattice*** or ***longitudinal*** (T_1) relaxation and ***spin-spin*** or ***transverse*** (T_2) relaxation. The time constants describing these exponential relaxation processes are known as relaxation times. Since the relaxation time is dependent on the chemical and physical environments surrounding the nucleus, the relaxation time constant is a fundamental property of the sample (Ruan and Chen, 1998; Ablett, 1992). Therefore, by analyzing T_1 and T_2 of a sample, we can study the chemical and physical properties of the sample. A long T_1 or T_2 indicates a slow relaxation rate; a short T_1 or T_2 indicates a fast relaxation rate.

Longitudinal relaxation (T_1) is the process by which the spins return to equilibrium after a pulse due to loss of energy to the surrounding nucleus. It describes the magnetization along the z axis (M_z). It is difficult to detect by a single 90^o RF pulse, therefore, T_1 can be determined by a multiple 180^o -τ- 90^o sequence with varying delay time (τ). This process is called ***inversion recovery***. Transverse relaxation (T_2) describes the decay of the transverse component of magnetization (M_{xy}) after a 90^o RF pulse. The decay curve is normally termed free induction decay (FID). Because the magnetic field is not perfectly homogeneous, the Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence, which is 90x-(t-180y-τ-echo)$_n$, is used to determine spin-spin (T_2) relaxation (Ruan and Chen, 1998). The relaxation decays determined by a single 90^o pulse sequence usually give information about the fast decay components, typically solid component, while those determined by the CPMG sequence is used to obtain information about relatively slow decay components (liquid-like components) (Fullerton and Cameron, 1988).

The relaxation behavior of the proton is normally exponential. The relaxation time constant can be obtained from the relaxation decay curve using mono-exponential, multi-exponential, or distributed exponential fitting models (Ruan and Chen, 1998). The mono- or multi-exponential fitting model can provide useful information, however, since solid and water molecules may exist in an infinite number of states in heterogeneous food systems, they maybe oversimplified. Provencher (1982) suggested that the relaxation behavior in complex systems may be described better by using the distributed exponential fitting model based on the Contin algorithm.

The proton in water molecules is the most often used nucleus in NMR experiments on water. Many studies directly link the observed T_2 with water mobility.

For example, one approach shows that the rate of transverse relaxation (T_2^{-1}) due to homonuclear dipolar interactions varies as:

$$T_2^{-1} = 0.1A[6J(0,\tau_c) + 10J(\omega_o,\tau_c) + 4J(4\omega_o,\tau_c) \quad (1a)$$

where the spectral density function is given by

$$J(n\omega_o,\tau_c)=\frac{\tau_c}{1+n\omega^2\tau_c^2} \quad (1b)$$

and τ_c is the autocorrelation time associated with molecular motions. In general, faster moving molecules have shorter times over which they interact to exchange spin energy. Thus, higher mobility systems generally have longer T_2 values. Low mobility systems, and particularly solids, have relatively shorter T_2 values. Rotational and vibrational motions are usually faster than translational motions, and thus have a greater effect on the observed T_2. The pulsed NMR technique is fast and sensitive, and, because the data are electronic signals collected in real time, it is possible to automate measurements. NMR requires little sample preparation, and is non-destructive and non-invasive to the sample. It can be used to determine the amount of different fractions of water having different mobility, moisture and fat content (Ruan and Chen, 1988).

A number of researchers have utilized NMR to study the state of water in bread. Leung et al. (1983) used deuterium (^2H) NMR to study bread staling and found that T_2 decreased over time. Chen et al. (1997) monitored the changes in crumb firmness, T_2, and proton intensity of three categories of water in the crumb during storage. It was found that several NMR parameters correlated with the firming process in the bread

crumb, and could be related to macroscopic and microscopic migration of water during staling.

Seow et al. (1996) monitored the changes in FID curves of bread, corn starch gel, and rice cupcake during storage. A high correlation between the firmness and normalized NMR solid phase signal was found. He concluded that the NMR solid phase signal may be used as an index of staling of aging starch gels and starch-based products. Ruan (1998) found that the T_2 value was highly correlated with the firming process in the starch-based systems. Different fractions of water with distinguishable molecular mobility were also identified.

Through analysis of FID, T_1, and T_2 curve, NMR has also been used to characterize total water content, water binding capacity, starch chain mobility, water mobility, gelatinization, retrogradation and structural properties of starch in the granule and gel state (Ruan and Chen, 1998; Botlan et al., 1998; Wu and Eads, 1993; Farhat et al. 2000).

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CHAPTER 3

THE EFFECT OF WATER AND GLUTEN LEVELS ON WATER MOBILITY IN WHITE BREAD AND STARCH GELS AS STUDIED BY PULSED ^{1}H NMR[1]

[1]Wang, X., Choi, S.G. and Kerr, W.L. Submitted to Lebensmittel-Wissenschaft und-Technoligie, 04/22/2003.

3.1. Abstract

Three levels of water and three levels of gluten were used to study the effects of water and gluten on the water mobility in white bread using ^{1}H NMR relaxometry. The solid contents are same when gluten is added. Changes in the transverse relaxation time constant (T_2) were related to water mobility. The three levels of water addition in bread dough resulted in three different levels of water content in bread. Gluten levels made no difference in bread moisture content. Distributed exponential analysis showed two distinct regions of T_2, associated with multiple domains of water in the bread crumb. There was no significant difference in peak T_2 values with different gluten content, but significant differences were observed with different water content. The results suggested that water associated with starch increased significantly after heating, the mobility of water associated with starch decreased dramatically because of gelatinization. To further investigate the effect of gluten on starch gelatinization, NMR measurements were made directly on model systems containing starch and various gluten amounts. The starch-gluten gels had higher T_2 values than the starch only gels, which indicated less swelling of starch granules and absorption of water. This was attributed to less water available to starch in the presence of gluten.

3.2. Introduction

Three essential ingredients of white bread are flour, air and water. The wheat flour contains about 70% starch and 10-14% protein (Pomeranz, 1988). The gluten proteins make up 80-90% of the protein in the flour. The traditional method of gluten preparation involves gentle washing of a flour-water dough in an excess of water or dilute salt solution to remove most of the starch and soluble material, until the water-insoluble protein of flour is obtained as a rubbery mass. Based on solubility, gluten can be

classified into two groups: gliadins, which are soluble in alcohol, and glutenins, which are not soluble in either alcohol or salt solutions.

During the kneading process, gluten forms an increasingly extensible and fragile network in the presence of water and traps the gases produced during fermentation. The ability of gluten to form crosslinked networks allows the formation of the viscoelastic dough in bread (Callejo et al., 1999).

The dough water content ranges between 40 and 46% (Gil, 1997). Doughs do not form at water contents below 35%, and are excessively fluid and difficult to handle at water contents above 50%. Water comprises about 35% of the baked bread. The water added to the flour fulfills four functions; it dissolves soluble molecules, activates enzymes, brings about the formation of new bonds between the macromolecules in the flour, and alters the rheological properties of the dough.

The physical state of water in food systems is believed to play an important role in the structural, physical, chemical and sensory properties of foods. However, the location and behavior of water during dough making and bread baking is not fully understood.

Various fractions of water in bread dough have properties that differ from each other, or from those of bulk water (Kuntz and Kauzmann, 1974). Some water is unfreezable (Leung and Steinberg, 1979), cannot be easily removed by drying, or has unusually short NMR relaxation times (Leung et al., 1979). This has led several researchers to suggest that some portion of water is bound within the dough matrix. Leung and Steinberg (1979) studied the water binding properties of some common food constituents by water sorption, dehydration, freezing, and NMR methods, and defined the bound water as the moisture level at which a significant discontinuity in freezing, dehydration, and NMR signal occurred. However, others have questioned the use of the

term “bound water” (Belton, 1990). Another viewpoint is that various regions of water in bread have different rates of molecular motion, and that these rates are significantly slower than those for bulk water (Engelsen et al., 2001).

Bushuk and Winkler (1957) reported that water is bound more to starch than to gluten, due to the higher water sorption capacity of starch. Through separation of the components of dough and measuring the moisture content of each component during baking and storage, Willhoft (1971) suggested that there was more water associated with the gluten than with the starch in the dough, and that the water moves from the gluten to the starch during conventional baking. Using differential thermal analysis (DTA) and differential scanning calorimetry (DSC), several researchers found that the bound water content in dough was independent of flour protein content, added chemicals, amount of damaged starch, and mixing time (Davis and Webb, 1969; Daniels, 1975; Bushuk and Mehrotra, 1977a, b, c).

Pulsed nuclear magnetic resonance (NMR) has been used extensively to study the properties of water in biological systems (Kuntz et al., 1977). By measuring the longitudinal (T_1) and transverse (T_2) relaxation times of protons, NMR can be related to the molecular mobility of water. Leung et al. (1976) found two species of water molecules with different T_2 values in hydrated cornstarch samples, and attributed these to a mobile and a bound water fraction. Leung et al. (1979) also found two water populations with different T_2 values in wheat flour dough, indicating different water mobilities. Using deuteron relaxation pulsed NMR, Leung et al. (1983) studied water binding of wheat flour doughs and breads. Hard wheat and soft wheat flour doughs showed similar increases in T_1 and T_2 with increasing moisture content.

By measuring spin-spin relaxation times and the amount of unfreezable water using pulsed NMR, Wynne-Jones and Blanshard (1986) investigated the changes in the state of water with time in gels and bread. They found the heated gluten gel showed a greater binding of water than the starch gel. Chen et al. (1997) found three components (at 10 μs, 280 μs, and 2.5 ms) in bread, associated with fractions of water with different degrees of mobility. Cherian and Chinachoti (1997) used ^{2}H and ^{17}O NMR to study gluten in the glassy and rubbery states. Kou et al. (2000) studied mobility in waxy cornstarch using wide-line ^{1}H NMR. Tang et al. (2001) used proton NMR to investigate changes in states of water in potato starch during gelatinization or acid hydrolysis. Several domains of protons were identified and how they change with processing was shown.

Using solid-state NMR, Li et al. (1996) examined the proton relaxation in the rotating frame for waxy cornstarch, wheat gluten, and mixtures of both. They studied the effects of moisture contents and heating on the starch and gluten molecular mobility. The data suggested that wheat gluten has a stronger affinity for water molecules, as the water in gluten remained immobile while water in starch was more mobile.

Umbach et al. (1992) investigated the water self-diffusion coefficients of starch-gluten-water mixtures measured by pulsed gradient spin-echo nuclear magnetic resonance. They found a higher diffusion coefficient of water molecules in starch-water mixtures than in gluten-water mixtures before heating. It was concluded that small amounts of water in dry starch were very tightly associated, but additional water did not interact with starch, thus remaining quite mobile. Once further hydrated, water in gluten led to more water-protein interaction, thus becoming more immobile.

Limited reports were found on the effects of gluten level on the properties of water in bread. The objective of this study was to investigate the effects of water and gluten on the mobility and domains of water in fresh white bread using NMR techniques. NMR measurements were also made directly on starch-gluten gels to further investigate the effects of gluten on starch gelatinization.

3.3. Materials and methods

3.3.1. Experimental design

White pan bread was made with a Breadman Plus TR845 bread machine (Salton, Inc., Mt. Prospect) using the standard white bread cycle for a 1lb loaf: mix and knead for 37 mins, proof for 88 mins, and bake for 45 mins. All-purpose flour with a 10.8% protein content was supplied by Bay State Milling Company (Quincy, Mass). Gluten was purchased from King Arthur Flour Company (Norwich, Vermont). A 3*3 factorial experiment was used to study the effects of water and gluten content, each with three levels and three replicates. In total, 27 breads were made. The three levels of water added to the dough were 180g, 220g, and 260g per 345g of flour. With the three levels of protein, enough flour was replaced with gluten to bring the final protein concentration to 11.2%, 13%, and 14.2%, respectively. This was similar to the protein content of flour used for making bread (Charles, 1990).

Additional ingredients, added prior to mixing, included 5.3g NaCl, 15g sucrose, 9.1g yeast (Fleischmann's #2139 instant yeast, Canada), 10.2g vegetable oil, and 5g nonfat dry milk solids (Nestle USA, Inc., Solon). Granular lecithin (12.6g)(King Arthur Flour Inc., Norwich, VT) was added to the dough to limit bread staling. After baking, the bread was left to stand at 25^{o}C for 2 hours. The bread was sliced into 1 cm slices with a Model 1612E slicing machine (Hobart Corp., Troy, OH). The moisture content of the

bread crumb was calculated from the difference in weight after vacuum-drying at 75°C for 48 hours (AOAC, 1995). Water activity was measured using an Aqua Lab Series 3 water activity meter (Decagon Devices, Inc., Pullman, WA).

3.3.2. Gel preparation

Model systems composed of starch gels and starch-gluten gels were also analyzed as part of this study to achieve further information on the effect of gluten.

Samples of native wheat starch 0.5g (Sigma Chemical Co., St. Louis, MO) were weighed into a glass vial (3cm × 1cm diameter) and fitted with a screw cap. Distilled water (0.8g or 1.3g) was added to the vial to make suspensions with 38.5% and 27.8% starch concentration. The starch-water suspension was vortexed for 20s. The starch-gluten-water mixtures were prepared by the same method except that 0.050g, 0.075g and 0.100g wheat gluten were added. The same amount of starch was removed to keep the total solid content constant, which resulted 10%, 15% and 20% of gluten content in total solids. These were chosen to mimic the ratio of gluten and starch in flour used in the bread samples of previous study. Samples containing only gluten were prepared by substituting 0.5g starch with gluten. The vials were sealed, placed on a rotating turntable and heated in microwave oven (Daewoo, Seoul, South Korea) for 35s at 800W to obtain homogeneous gels. After heating, the sample temperature was $92^{o}C \pm 3^{o}C$. Gels were formed as the samples were allowed to cool to $25^{o}C$ for 1 hour. Fresh gels were immediately measured by NMR.

3.3.3. NMR measurements

Proton relaxation measurements were made using a 20 MHz ^{1}H NMR spectrometer (Resonance Instruments, Whitney, UK). Approximately 0.5 g bread crumb samples were taken from the center of the bread slices. Three replicates were taken from

each loaf of bread. Each sample was placed in a 18mm diameter NMR tube, then covered with parafilm. Transverse (T_2) relaxation curves were developed using the CPMG pulse sequence: $90x\text{-}(\tau\text{-}180y\text{-}\tau\text{-}echo)_n$ (Meiboom and Gill, 1958). Acquisition parameters were set to a 90^o pulse of 4.2 μs and a recycle delay of 2s. A pulse spacing (τ) of 100 μs was chosen to exclude the fast decaying solid-like signal. All measurements were made at $25^oC\pm0.1$. The temperature was controlled by the internal temperature control system in NMR spectrometer. Relaxation curves obtained from CPMG sequences were analyzed using a distributed exponential routine. Based on the contin algorithm of Provencher (1982), this routine calculates a distribution of T_2 terms that best describe the data:

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

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

3.3.4. Statistical analysis

The effects of water content and gluten content on the water content, water activity and NMR data of the bread crumb were analyzed by two-way analysis of variance at a level of significance of 0.05. Mean values were compared using the LSD method with a level of significance of 0.05. All samples were analyzed in triplicate. SAS software was used for data analysis (SAS Institute, INC., 1990).

3.4. Results and discussion

3.4.1. The effect of water and gluten content on moisture content and water activity

Table 1 shows the effect of initial added water and gluten content of the loaf on the moisture content and water activity of baked bread crumb. Moisture content ranged from 0.544 to 0.786g H_2O/g of solids. There were significant differences in moisture content of the crumb with different levels of added water to the bread dough. During baking, some water was lost through evaporation, but the moisture content of the final product, bread crumb, was still different for each of the three water levels added to the dough. Water activity (a_w) values ranged from 0.951 to 0.970. A_w values were significantly different for samples prepared from 180g and 260g of water. The gluten content made no difference in moisture content or water activity of the bread crumb. These results are in agreement with previously reported data (Every et al., 1998). With five levels of gluten addition, all resulting bread crumbs had approximately the same moisture content.

3.4.2. The effect of water and gluten content on T_2

Distributed exponential analyses of the data are shown in Figure 1a-c. In general, two distinct regions of T_2 distributions were observed for all samples, one at relatively short times (30-600 μs) and one at relatively long times (1-60 ms). The multiple components model in baked products has been observed by several researchers. Ruan et al. (1996) reported a two-component model in sweet rolls, consisting of a peak T_2 in the μs range and a peak T_2 in the ms range. Chen et al. (1997) found three fractions of water in fresh bread crumb, one with a peak T_2 at 8-12 μs, one at 280-320 μs, and one at 2.0-2.6 ms, which they attributed to regions of differing water mobility. The component at 8-12 μs was attributed to water strongly associated with starch and gluten through hydrogen bonding. Engelsen et al. (2001) also found three components in bread; however, these

were measured during the baking process. They attributed the shortest T_2 component to water associated with protein, the next shortest component to water associated with starch and pentosans, and the longest component to diffusive exchange of water between starch and protein fractions.

The occurrence of two decay regions indicates the presence of multiple domains of water in the bread crumb. These can be defined as regions in which spin-spin relaxation rates are distinct. Many studies directly link the observed T_2 with water mobility. For example, one approach (Hills, 1998) shows that the rate of transverse relaxation (T_2^{-1}) due to homonuclear dipolar interactions varies as:

$$T_2^{-1} = 0.1A[6J(0,\tau_c) + 10J(\omega_o,\tau_c) + 4J(4\omega_o,\tau_c) \quad (2a)$$

where the spectral density function is given by

$$J(n\omega_o,\tau_c)=\frac{\tau_c}{1+n\omega^2\tau_c^2} \quad (2b)$$

τ_c is the autocorrelation time associated with molecular motions. In general, faster moving molecules have shorter times over which they interact to exchange spin energy. Thus, higher mobility systems generally have longer T_2 values. Low mobility systems, and particularly solids, have relatively much shorter T_2 values. Transverse relaxation is also enhanced by chemical exchange of protons from water to –OH, NH_2, or –SH groups on starch, sugars or wheat proteins. The short time constant domain in Figures 1a-c may be associated with water in lower mobility regions; the long time constant domain may be associated with water in high mobility regions.

To understand the nature of the water domains, it is informative to understand the microstructure of bread. Microscopic studies by Bechtel et al. (1978) showed that the protein in bread forms a continuous network of smooth, thin strands devoid of the vacuoles seen in the unbaked dough. Much of the starch was seen to be gelatinized and distributed as long fibrous strands interwoven with the protein network. Thin fibrils connected the dispersed starch with the protein network.

The peak T_2 values in each region are shown in Table 2. The peak T_2 values ranged from 170~220 μs in less mobile region, and from 3~6 ms in more mobile region. Statistical analysis of the effect of moisture content and gluten content showed that gluten content made no difference in peak T_2 values of each region, but that there was a significant difference due to moisture content. The peak T_2 values of the longer component increased with moisture content; the peak T_2 values of the shorter component increased when going from 180 to 220 g of added water.

Before heating, the bread dough with low gluten content did not absorb as much water as the dough with high gluten content, and appeared more sticky and wet. Umbach et al. (1992) found that when equivalent amounts of starch or gluten were mixed with water at relatively high moisture content, the starch-water mixture was liquid and the gluten-water mixture was not. In addition, the water self-diffusion coefficient decreased as the amount of gluten added to starch-water mixture increased. This indicated that gluten binds or entraps water more effectively than starch, so that water molecules have lower mobility. However, the water mobility did not decrease with gluten content in baked bread samples, as we observed in our experiment. Our results suggest that after heating, starch granules absorbed water during gelatinization so that the mobility of water associated with starch decreased dramatically. The mobility of water associated with

starch and gluten were very similar, therefore, the gluten addition did not affect the total water mobility of the bread within the gluten content and moisture content ranges used in this experiment. Since most of the water was associated with gluten before heating, it is also suggested that a redistribution of water occurred from gluten to starch. Willhoft (1971) observed that moisture bound by hydrated gluten was released during baking, and starch absorbed the free water from the gluten-starch interface. He suggested that a mild transformation occurs in the gluten structure, both during heat treatment and subsequent storage. This transformation may be associated with an increase in the extent of denaturation of the protein, and may involve a change of the protein configuration.

The integrated areas under each peak are shown in Table 3. The areas under the peak with shorter T_2 values have no difference either due to moisture content or gluten content, but the areas under the peak with longer T_2 values increased with moisture content. Since the integrated area in each region represent the total number of protons, this result indicated that either moisture content or gluten content did not make any difference in the number of protons detected in the less mobile region, but the number of protons increased with moisture content in the more mobile region. This is what we expected because

To further investigate the effect of gluten on the starch gelatinization, a model system was used directly on starch and gluten gels. It is hard to compare the starch-water system before heating and after heating because they are different systems, one is dispersion and the other is gel. However, since the bulk water in starch suspension is absorbed by the starch after heating, the mobility of water should be decreased. Using pulsed NMR, Lelievre and Mitchell (1975) studied starch gelatinization and found that,

when the temperature of a starch suspension was increased, water mobility was decreased, indicating the adsorption of water to starch molecules.

Figure 2 shows the distributed T_2 relaxation plots for heated fresh starch and gluten gels at solid concentrations of 27.8% and 38.5%. Two distinct regions of T_2 distributions were observed for all samples, one at relatively short times (0.5-10ms) and one at relatively long times (9-220ms). Both of the T_2 values were higher than the bread samples because it was gel and had higher moisture content.

Multiple domains of proton relaxation have also been observed in starch granules and starch gels. Tang et al. (2000) identified three water populations inside potato starch granules. These were assigned to water in the amorphous growth rings as well as water in the semi-crystalline lamellae and in the hexagonal channels of B-type amylopectin crystals. Comparing starch chain mobility obtained by replacing water by D_2O with water mobility in gelatinized potato starch, Tang et al. (2001) identified that one of the three peaks, which was at ca. 5 ms, corresponded to water inside the granules, since the peak area increased when temperature was raised to 60^oC, presumably reflecting the swelling of the granules. Choi and Kerr (2001) studied native and hydroxypropylated wheat starch gels using ^1H NMR. They observed two distinct water fractions in the gels at ca. 2-6 ms and at 10-300 ms, the latter being dependent on gel concentration. Control experiments showed that the longer T_2 region could be associated with both the continuous amylose-rich gel and the intragranular amylopectin-rich gel, while the shorter T_2 region was associated only with granule remnants. Our results are very similar to those of Choi and Kerr (2002).

Figure 3a,b show the T_2 values from less mobile (low T_2) and more mobile (high T_2) regions of the fresh starch-gluten gels with different gluten contents at solid

concentrations of 27.8% and 38.5%. For both of the less mobile and more mobile regions, the T_2 values of the starch only samples were lower than the starch-gluten mixtures at both solid contents. Since T_2 was lowered during starch gelatinization due to starch absorption of more water, the higher T_2 of the starch-gluten mixture indicated that starch swelled less and absorbed less water in the presence of gluten, probably due to less water available to the starch. This concurs with results by Eliasson (1983). He studied the influence of gluten on wheat starch gelatinization by differential scanning calorimetry (DSC) and found that the presence of gluten decreased the starch gelatinization enthalpy and increased the gelatinization temperature. He attributed this to a decreasing amount of water available for the starch. For both the less mobile and more mobile regions, the T_2 of the starch gels was higher than the gluten gels, but there was no significant difference among the T_2 values of starch-gluten mixtures with different gluten contents. This was consistent with the results in our bread study. These results showed that when gluten and starch were mixed together, a redistribution of water occurred between them during heating. Gluten released some water and starch absorbed more water. Willhoft (1971) also observed the moisture bound by hydrated gluten was released during baking, and that starch absorbed free water from the gluten-starch interface.

The T_2 areas from less mobile (low T_2) and more mobile (high T_2) regions of the fresh starch-gluten gels with different gluten contents at solid concentration of 27.8% and 38.5% are shown in Figure 4a,b. At less mobile regions, the T_2 areas of the starch gels were very similar to the gluten gels. There was no significant difference among the T_2 areas of starch-gluten mixtures with different gluten contents, and the T_2 areas of the starch only and gluten only gels are lower than all the starch-gluten gels. At more mobile regions, the T_2 areas of the starch only and gluten only gels are higher than all the starch-

gluten gels. The sum of the two areas represents the total number of protons in the sample. Therefore, the total water protons detected were the same for all samples at the same moisture content. This indicated that more water molecules were associated in the less mobile region of the starch-gluten gels than the gels containing only starch, while less water molecules were associated in the more mobile region of the starch-gluten gels. Since starch granules swell less in the presence of gluten and less water was incorporated into the granules, the more mobile region of the starch-gluten gel might correspond to the water molecules associated with the starch granules.

3.5. Conclusion

The effects of water and gluten content on the water mobility in white bread were investigated using ^{1}H NMR relaxometry. Two distinctive regions of T_2 were observed, associated with multiple domains of water in the bread crumb. There was no significant difference in peak T_2 values with different gluten content, but significant differences were observed with different water content. It is suggested that after heating, starch granules absorbed water during gelatinization so that the mobility of water associated with starch decreased dramatically. The mobility of water associated with starch and gluten were very similar, therefore, the gluten addition did not affect the total water mobility of the bread within the gluten content and moisture content ranges used in this experiment. NMR measurements were made directly on starch gels with different gluten content to further investigate the effect of gluten on starch gelatinization. The starch-gluten gels had higher T_2 values than the gels containing only starch, which indicated less swelling of starch granules and absorption of water. This was attributed to less water available to starch in the presence of gluten.

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Table 3.1. The effect of initial added water and gluten content on the moisture content and water activity of bread crumb

Initial water added (g)	**Moisture content (g water/g solids)***		
	1.5% gluten	***4.5% gluten***	***7.8% gluten***
180	0.550[a]	0.555[a]	0.544[a]
220	0.652[b]	0.667[b]	0.665[b]
260	0.775[c]	0.775[c]	0.786[c]
	Water activity*		
	1.5% gluten	***4.5% gluten***	***7.8% gluten***
180	0.951[a]	0.954[a]	0.956[a]
220	0.959[a]	0.953[a]	0.964[a]
260	0.970[b]	0.963[b]	0.962[b]

*Values followed by different letters are significantly different at $P<0.05$.

Table 3.2. The effect of initial added water and gluten content on the peak T_2 values (measured from CPMG, less mobile and more mobile region) of bread crumb

Initial water added (g)	**T_2 (μs) (less mobile region)***		
	1.5% gluten	***4.5% gluten***	***7.8% gluten***
180	177.45[a]	165.89[a]	196.11[a]
220	188.67[b]	219.67[b]	220.22[b]
260	205.33[b]	205.11[b]	199.67[b]
	T_2 (ms) (more mobile region)*		
	1.5% gluten	***4.5% gluten***	***7.8% gluten***
180	3.03[a]	3.14[a]	3.03[a]
220	3.53[b]	3.71[b]	3.71[b]
260	4.45[c]	4.95[c]	5.22[c]

*Values followed by different letters are significantly different at $P<0.05$.

Table 3.3. The effect of initial added water and gluten content on the T_2 area (measured from CPMG, less mobile and more mobile region) of bread crumb

Initial water added (g)	**T_2 area (less mobile region)***		
	1.5% gluten	***4.5% gluten***	***7.8% gluten***
180	1093.33[a]	1016.11[a]	978.68[a]
220	1142.11[a]	912.89[a]	998.33[a]
260	1069.33[a]	1113.56[a]	802[a]
	T_2 area (more mobile region)*		
	1.5% gluten	***4.5% gluten***	***7.8% gluten***
180	7721.11[a]	7592.78[a]	7412.56[a]
220	8305.22[b]	8150.89[b]	8446.22[b]
260	9127.89[c]	9078.33[c]	8972.33[c]

*Values followed by different letters are significantly different at $P<0.05$.

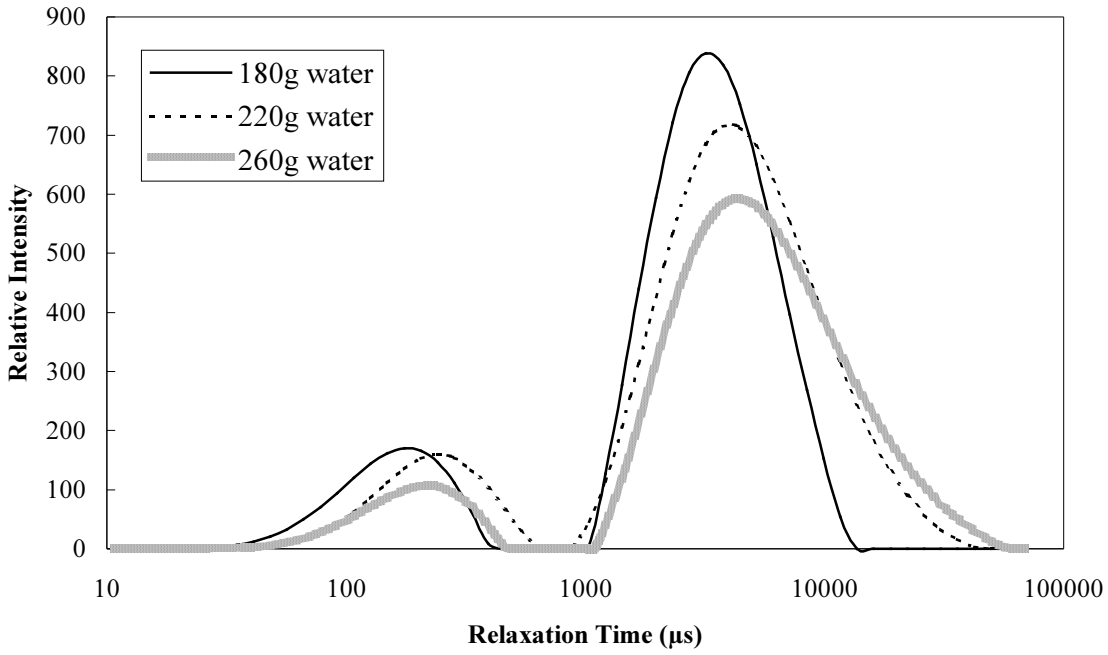

Figure 3.1a. Distributed T_2 relaxation plots for bread crumbs with initial addition of 180g, 220g and 260g water and 1.5% gluten. Final moisture contents were 0.550, 0.652, 0.775 g H_2O/g solids in the bread crumb, and final protein content was 11.2%.

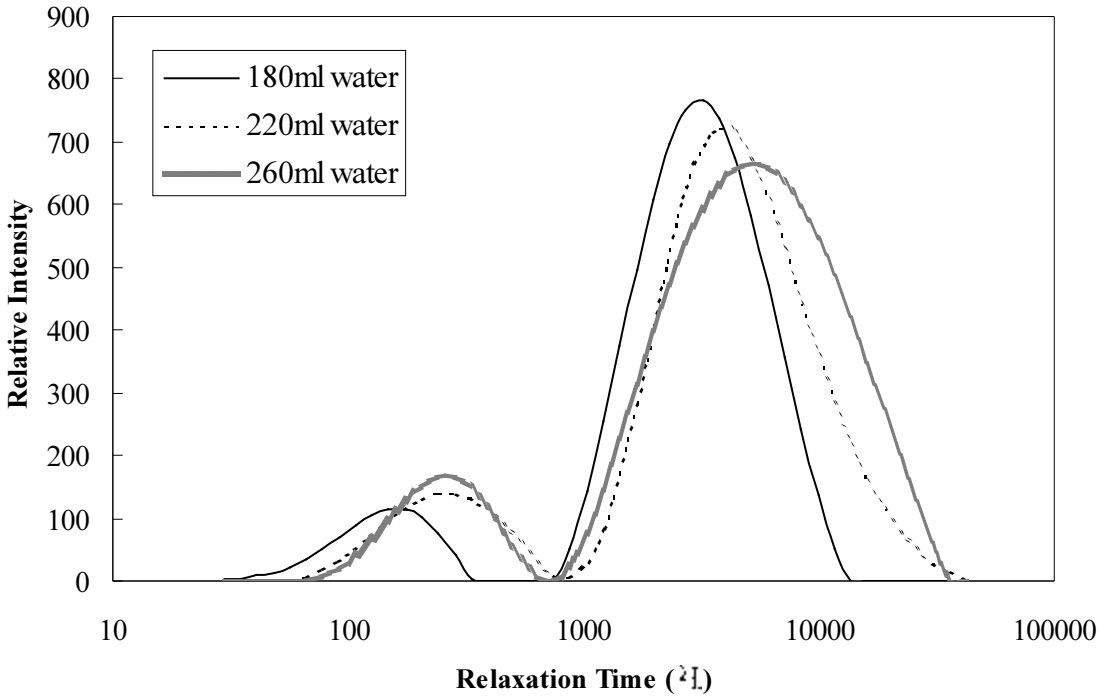

Figure 3.1b. Distributed T_2 relaxation plots for bread crumbs with initial addition of 180g, 220g and 260g water and 4.5% gluten. Final moisture contents were 0.550, 0.652, 0.775 g H_2O/g solids in the bread crumb, and final protein content was 13%.

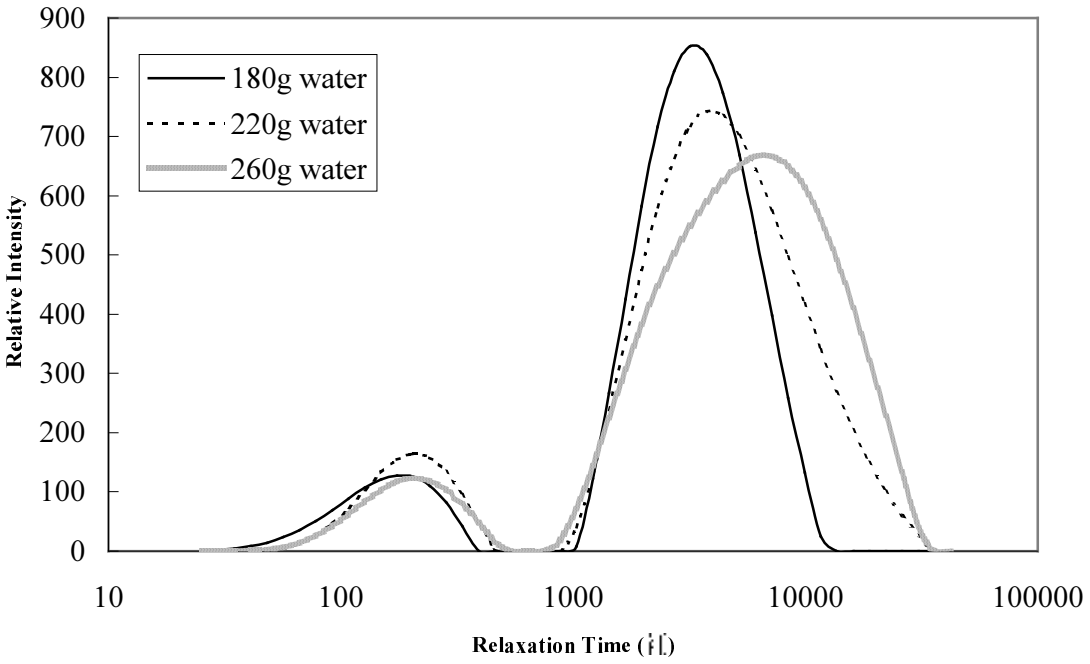

Figure 3.1c. Distributed T_2 relaxation plots for bread crumbs with initial addition of 180g, 220g and 260g water and 7.8% gluten. Final moisture contents were 0.550, 0.652, 0.775 g H_2O/g solids in the bread crumb, and final protein content was 14.2%.

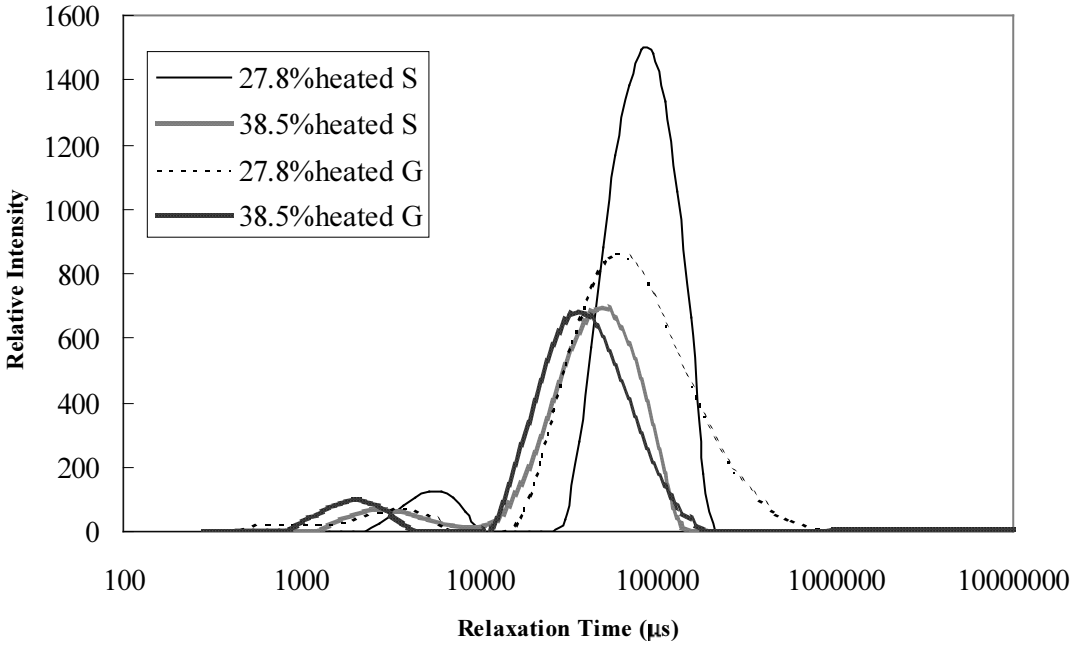

Figure 3.2. Distributed T_2 relaxation plots for heated fresh starch and gluten gels at solid concentrations of 27.8% and 38.5%.

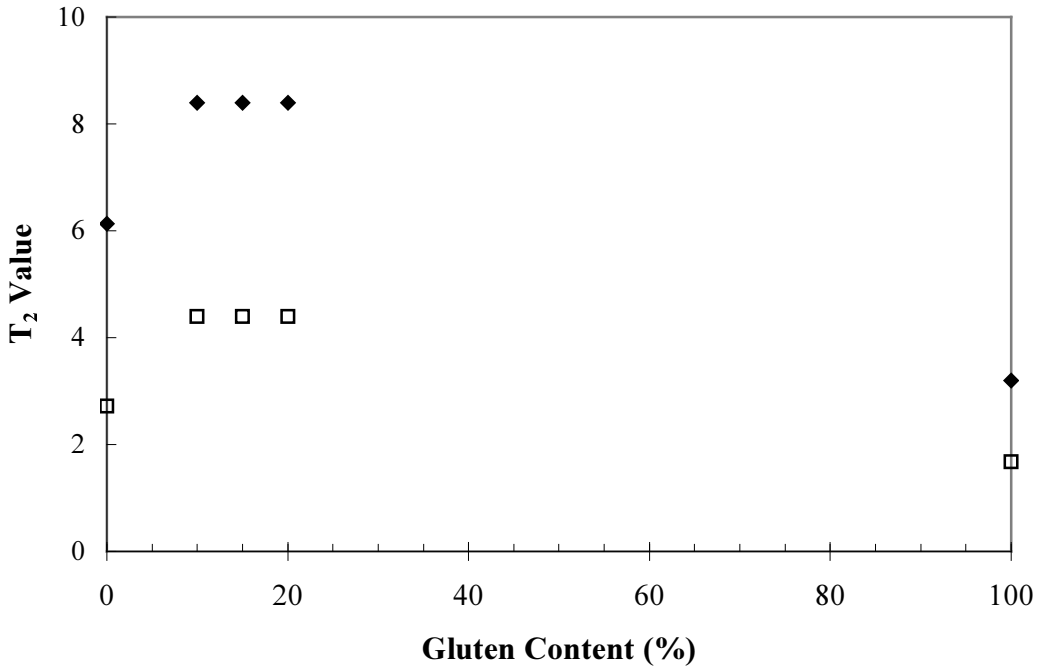

Figure 3.3a. T_2 values from less mobile (low T_2) regions of the fresh starch-gluten gels with different gluten contents at solid concentration of 27.8%(♦) and 38.5% (⊏).

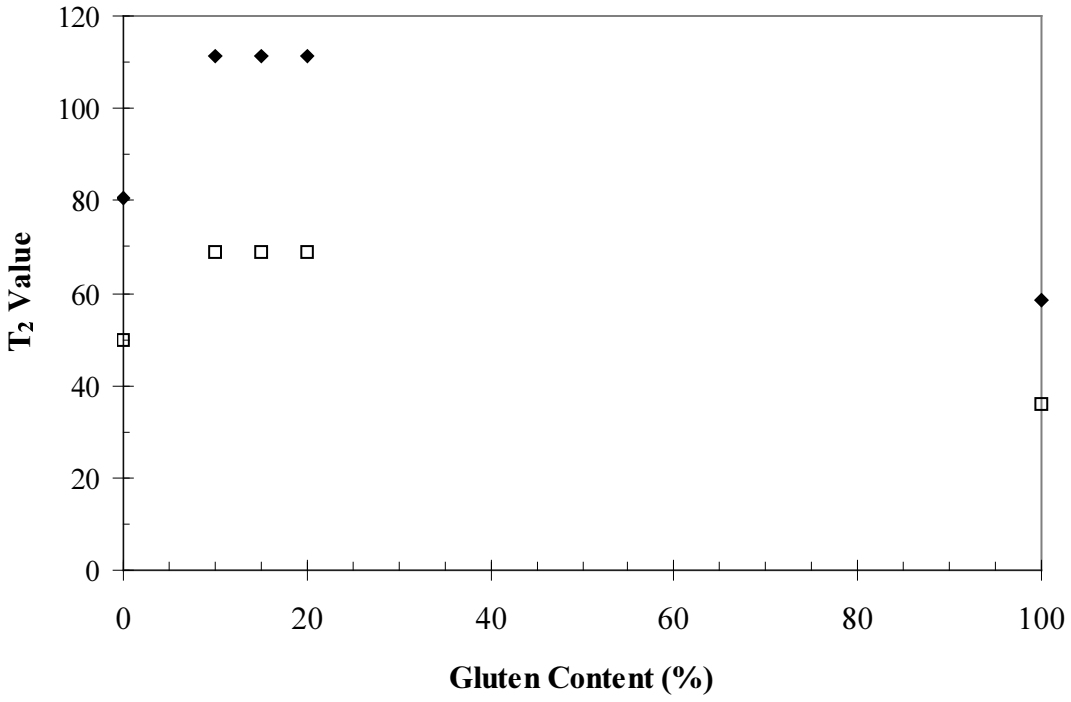

Figure 3.3b. T_2 values from more mobile (high T_2) regions of the fresh starch-gluten gels with different gluten contents at solid concentration of 27.8%(♦) and 38.5% (⌐).

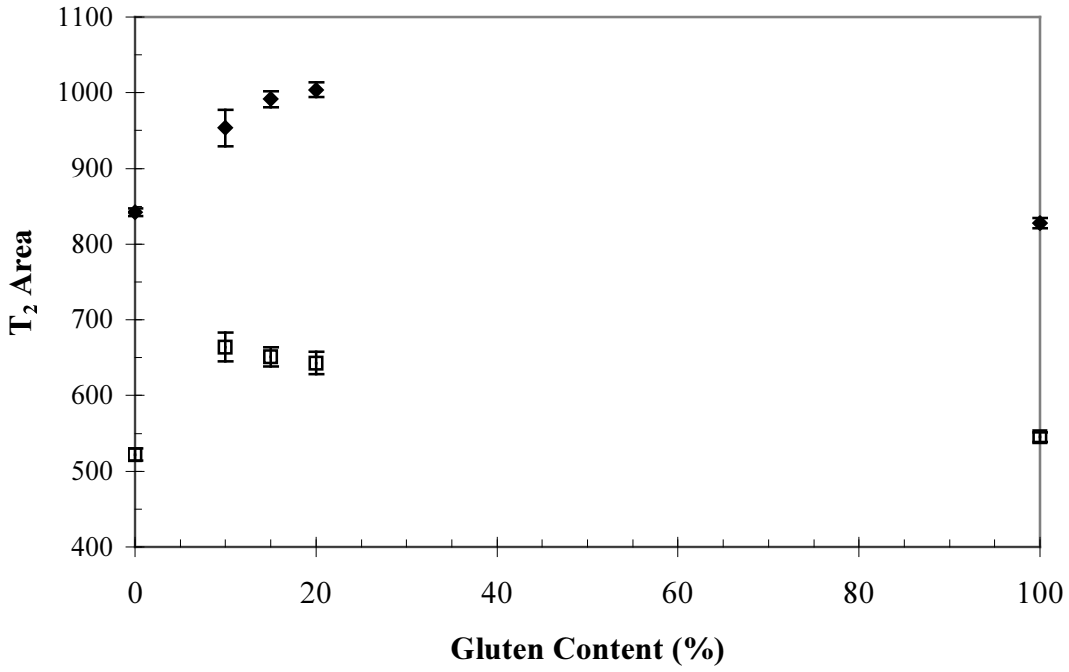

Figure 3.4a. T_2 areas from less mobile (low T_2) regions of the fresh starch-gluten gels with different gluten contents at solid concentration of 27.8%(♦) and 38.5% (⊏).

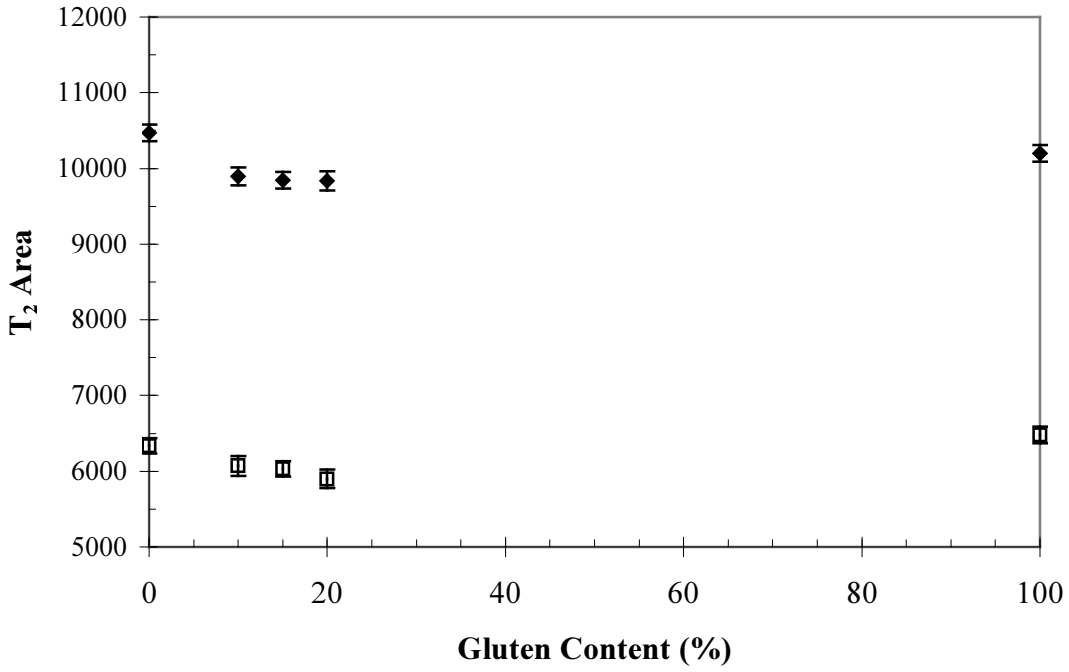

Figure 3.4b. T_2 areas from more mobile (high T_2) regions of the fresh starch-gluten gels with different gluten contents at solid concentration of 27.8%(♦) and 38.5% (⊏).

CHAPTER 4

THE EFFECT OF GLUTEN CONTENT ON RETROGRADATION AND WATER MOBILITY IN WHEAT STARCH GELS USING PULSED ^{1}H NMR[1]

[1]Wang, X., Choi, S.G. and Kerr, W.L. Submitted to *Journal of Food Science*, 04/22/2003.

4.1. Abstract

The effect of gluten on starch retrogradation at 5°C was studied using ^{1}H NMR relaxometry. Gels were made from gluten and starch at 27.8 and 38.5% solids and with gluten comprising either 10, 15, or 20% of the solids. Changes in the transverse relaxation time constant (T_2) were related to water mobility. Mono-exponential studies showed that, in general, gluten retarded starch retrogradation. T_2 values in gluten gels also decreased during storage, indicating that gluten undergoes some structure change. Distributed exponential studies showed that two distinct regions of T_2 were observed in all samples. During aging, the peak values of both regions shifted to lower values for all the gels. Starch samples showed the most significant shift, and gluten showed the least. The three levels of gluten addition in starch-gluten gels produced similar shifts. For all the samples, the signal intensity of the less mobile region decreased more dramatically than that of the more mobile region during storage. It was suggested that gluten retards water loss in the granule remnants.

4.2. Introduction

Starch-containing foods are normally cooked by heating in the presence of water. When heated over a temperature range, starch granules undergo an order-disorder phase transition called gelatinization (Hoover, 1995). This phase transition is a non-equilibrium process associated with the diffusion of water into the granule, hydration and swelling of the starch granules, uptake of heat, loss of crystallinity, and leaching of amylose from the granules. On cooling, the starch chains in the gelatinized paste associate, leading to the formation of a more ordered structure. Retrogradation of starch gels is characterized by changes in many physical and nutritional properties, such as hardness, water absorptive

capacity, opacity, enzyme hydrolysis and soluble starch content gels, and contributes to defects in the quality of starch-containing foods (Hermansson and Svegmark, 1996).

Several factors have been known to affect starch retrogradation, including the starch source, ratio of amylose to amylopectin, heating and storage temperature, salts and sugars, lipids, and chemical modification (Hoover, 1995). Water relationship also affects starch retrogradation. Leung et al. (1983) proposed that as starch changes from an amorphous to a more crystalline state, water molecules become immobilized by incorporation into the crystalline structure, resulting in a decrease in water mobility during storage. However, Whistler and Daniel (1985a) made differential scanning calorimetry (DSC) measurements of unfreezable water during storage and suggested that water was expelled from the starch matrix due to re-association of starch molecules. Willhoft (1971) and Breaden and Willhoft (1971) reported that gluten underwent a transformation resulting in the release of water, which became absorbed by retrograding starch.

Based on T_1 or T_2 relaxation studies, pulsed ^1H NMR has been useful for identifying water molecules with different degrees of rotational freedom, as well as quantifying the relative amounts of water in regions of differing water mobility. It has been observed that water mobility and fractions of water in different environments are highly correlated with the degree of starch retrogradation (Ruan et al., 1998; Farhat et al., 2000; Wynne-Jones and Blanshard, 1986). Teo and Seow (1992) showed that pulsed NMR could be used to monitor starch retrogradation based on measurements of the signal intensity from protons in the solid-like component in starch gels. As recrystallization proceeded during aging of a starch gel, the proportion of the solid-like component in the system increased. Teo and Seow (1992) also studied staling of bread and rice cupcake

using NMR, and found the increase in "solid-like" signal was significantly correlated with firmness. Wynne-Jones and Blanshard (1986) reported a reduction in the T_2 value of wheat starch gels during storage, indicating an overall decrease in mobility of the water molecules. Choi and Kerr (2001) found that the greatest decreases with time in T_2 occurred for native wheat starch. Alkali-treated starch showed less change over time, while hydroxypropylated starch showed even less.

It is well known that starch retrogradation plays a major role in bread staling, but other components in bread also affect staling rate. Hypotheses on the role of gluten in bread staling range from gluten having an anti-firming effect, or no effect on firming, to gluten-starch interactions being essential for bread firming (Callejo et al., 1999; Kim and D'Appolonia, 1977; Martin et al., 1991). Eliasson (1983) investigated the effect of gluten on the aging of starch gels using DSC. He found that the addition of gluten led to a decrease in the extent of starch crystallization during aging at 21^{o}C. Every et al. (1998) found that breads with different gluten content had similar firming rates, indicating that protein might have some role in firming. Little research has been published regarding the influence of gluten on the changes in water relations during starch aging.

The objective of this research was to investigate the direct effects of gluten on the dynamic states of water in starch gels during storage, and to further increase understanding of the influence of gluten on bread staling.

4.3. Materials and methods

4.3.1. Gel preparation

Wheat starch was purchased from Sigma Chemical (St. Louis, MO). Samples (0.5g) were weighed into glass vials (3cm × 1cm diameter) that were then fitted with screw caps. Distilled water (0.8g or 1.3g) was added to each vial to make starch

suspensions with 38.5% and 27.8% solids concentration. The starch-water suspension was vortexed for 20s. The starch-gluten-water mixtures were prepared by replacing 0.05g, 0.075g, or 0.1g of the starch with wheat gluten (King Arthur Flour Co., Norwich, VT), thus keeping the same solids concentration. The gluten content in starch-gluten gels was either 10, 15, or 20% of the total solid weight, similar to those typically found in flour used to make bread. In addition, samples without starch were prepared by using 0.5g gluten only. The sealed containers were placed on a rotating turntable and heated in a microwave oven (Daewoo, Seoul, South Korea) for 35s at 800W in order to form homogeneous gels. After heating, the sample temperature was $92^{o}C \pm 3^{o}C$. Gels were formed as the samples were allowed to cool to $25^{o}C$ over 1 hour. Fresh gels were immediately measured by NMR. For storage studies, gels were kept at $5^{o}C \pm 2^{o}C$ in a laboratory refrigerator and removed periodically for NMR analysis. All samples were equilibrated to $25^{o}C$ for 1 hour prior to measurement.

4.3.2. NMR measurement

Proton relaxation measurements were made using a MARAN 20 MHz NMR spectrometer (Resonance Instruments, Whitney, UK). Each glass vial containing a gel specimen was placed in a 18mm diameter NMR tube, which was then covered with parafilm. All measurements were made at $25^{o}C \pm 0.1^{o}C$. Transverse (T_2) relaxation curves were developed using the Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence: $90x\text{-}(t\text{-}180y\text{-}\tau\text{-}echo)_n$ (Meiboom and Gill, 1958). Acquisition parameters were set to a 90^{o} pulse of 4.2 μs, relaxation delay of 6 s, and τ of 300 μs. Relaxation curves obtained from the CPMG sequence were analyzed using a single exponential fitting model:

$$S = Ae^{-t/T_2} \qquad (1)$$

Where T_2 describes the rate of change of the signal S, and A is a pre-exponential factor dependent upon the number of protons. Relaxation curves obtained from CPMG sequences were also analyzed using a distributed exponential routine. Based on the "contin" algorithm of Provencher (1982), this routine calculates a distribution of T_2 terms that best describe the data:

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

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

To compare retrogradation behavior over time for gluten-starch mixtures, normalized changes in T_2 values and signal intensity were made using the following formulae:

$$\Delta T_2(t) = \frac{T_{2,0} - T_{2,t}}{T_{2,0}} \quad (3)$$

$$\Delta S(t) = \frac{S_0 - S_t}{S_0} \quad (4)$$

Where $T_{2,0}$ and $T_{2,t}$ are the time constants at time 0 and time t, respectively, and S_0 and S_t are the signal intensities at time 0 and time t, respectively.

4.3.3. Statistical analysis

Five different kinds of gels were developed consisting of gels containing only starch, gels containing only gluten, and starch-gluten gels with gluten contents of either 10, 15, or 20% of total solids. For each kind of gel, two concentrations of solids (27.8% and 38.5%) were studied. For each treatment, three replicates were analyzed. Analysis of variance and the LSD (SAS institute, Cary, NC) were used to test for differences among

samples at a given time and for differences over time. Non-linear regression techniques were used to fit the Avrami equation (Avrami, 1940) to plots of normalized T_2 and signal intensity versus time.

4.4. Results and discussion

4.4.1. Transverse relaxation

The transverse relaxation decay from the CPMG used on starch gels originates from water inside the gels (Farhat et al., 2000). The proton relaxation in a single region is normally exponential, and the relaxation time constants of heterogeneous systems can be determined from the decay curve using mono-exponential, multi-exponential, or distributed exponential fitting (Ruan and Chen, 1998). In this study, mono-exponential fitting (Equation 1) was used to evaluate the overall effects of starch retrogradation on water during storage. A distributed exponential model (Equation 2) was used to investigate specific changes during storage of water residing in different regions of the starch gel structure.

4.4.2. Mono-Exponential model

Figure 1a-b shows changes in T_2 during storage of starch gels, gluten gels, and starch-gluten gels with 10, 15, or 20% gluten at solid concentrations of 27.8% and 38.5%. In general, T_2 decreased most during the first five days, then changed more gradually up until ten days. No significant differences in T_2 were measured between days 10 through 15. Roulet et al. (1988) studied starch retrogradation using compression modulus, x-ray diffraction patterns, and dynamic mechanical moduli and observed similar signal changes over time. The decrease in T_2 over time indicated that water molecules *in toto* became less mobile during storage. Using an FID sequence, Teo and Seow (1992) found a decrease in the liquid-like signal and an increase of the solid-like signal. Using cross-

relaxation NMR, Wu and Eads (1993) showed that about 25-40% starch gels became immobilized during storage. Reasons for changes in water mobility have been postulated by several researchers. Whistler and Daniel (1985) showed that the amount of unfreezable water in starch gels decreased during storage and attributed this to water expelled from the starch matrix as starch molecules reassociated. Leung et al. (1983) proposed that as starch changes from an amorphous to a crystalline state during retrogradation, the water molecules become immobilized as they are incorporated in crystalline regions. Choi and Kerr (2001) proposed that the water near the polymer had reduced motion because of the decreased mobility of the starch chain segments during storage. The apparent T_2 was reduced since the overall T_2 and the bound water, bulk water have the relationship:

$$\frac{1}{T_2} = \frac{P_B}{T_{2B}} + \frac{P_A}{T_{2A}} \tag{5}$$

where P_A and P_B are the fractions of, and T_{2A} and T_{2B} the time constants associated with water near, and far from, the polymer surface, respectively.

The extent and rate of change in T_2 over time for 38.5% starch gels was higher than for 27.8% gels. In general, the greater the starch concentration, the greater the extent and rate of change. This can be attributed to increased cross-link density with increasing starch concentration. The starch only samples had the greatest change among the five treatments. All of the starch-gluten gels changed much less than the starch only samples. This suggested that addition of gluten decreased starch retrogradation. Several researchers found similar results. Callejo et al. (1999) measured bread firmness during storage and found that addition of gluten decreased the firmness in the bread at higher moisture content. Eliasson (1983) studied the effects of gluten on starch retrogradation using DSC. He found that the presence of gluten led to a decrease in the extent of starch

crystallization during aging at 21^{o}C. He also found that gluten addition decreased the starch gelatinization enthalpy and increased the gelatinization temperature. He attributed this to the smaller amount of water available to the starch when gluten is present. Martin et al. (1991) provided evidence to show that the more swollen the starch granule, as measured by the hydration capacity of crumb, the more rapid the firming rates of bread crumb. From our previous work in last chapter, we found that gluten binds much more water than starch before heating. Less water was available for the starch granule to swell during heating, thus decreasing the rate of starch retrogradation.

Figure 1a,b show that for the gluten only gels, the relaxation time constant decreased somewhat over time. This indicated that gluten also undergoes a mild water structure change during storage. Willhoft (1971) observed a re-distribution of moisture between the starch and gluten fractions during storage. He postulated that the hydrated protein of natural crumb undergoes a mild, first-order transformation during baking and subsequent storage. It was concluded that bread staling involved, in addition to starch retrogradation, an irreversible modification in the structure of the gluten. In molecular terms, the transformation may be associated with an increase in the extent of denaturation of the protein, and possibly involve a change of the protein configuration.

No significant difference was found in the retrogradation rate amongst the three levels of gluten addition, indicating that adding more gluten did not further decrease starch recrystallization. Every et al. (1998) measured the firmness of bread with different gluten levels during storage, and did not find any significant difference in the firming rate due to increased gluten concentration. As the higher gluten content samples have less starch, they may be expected to have a lower retrogradation rate than the low gluten content samples. However, this was not supported by our results. We suggest that, in

addition to the decreased retrogradation rate, the gluten transformation during storage resulted in a rigidification or firming process. This effect was minor, however, compared with the decreasing retrogradation rate effect. The total effect of gluten in bread was to retard bread staling.

4.4.3. Distributed exponential model

Figure 2 a, b show plots of continuous distributions of T_2 for pure wheat starch gels at concentrations of 27.8% and 38.5% held at 5^{o}C over 15 days. Two distinct regions of T_2 distributions were observed for all samples using distributed exponential fitting, one at relatively short times (approximately 0.5-10ms) and one at relatively long times (9-220ms). Similar results were found by Choi and Kerr (2001). Both regions represented relatively short T_2 values compared with freely mobile water, which typically has a T_2 of about 2 seconds. This indicated that the water molecules interacted with the starch macromolecules so as to restrict water mobility. Current theories on composite starch gel microstructure suggest that amylose is solubilized from the granule during gelatinization and forms the continuous gel matrix (Ring and Stainsby, 1982; Eliasson and Bohlin, 1982). Embedded within this matrix are swollen granule remnants, which are composed primarily of branched amylopectin (Han et al., 2000; Hibi, 1998).

At 27.8% starch concentration, the peak T_2 values of the more mobile region were 80, 68 and 42 ms on 0, 1, and 10 days of storage. The peak T_2 values of the less mobile region were 6.1, 3.76, 1.97 ms on 0, 1, and 10 days of storage. Both of the T_2 regions of the 38.5% starch concentration shifted to lower values, and the distribution became narrower during storage, indicating that water mobility became more restricted as the starch chains aggregation proceeded during aging.

Figure 3a-c show the plots of continuous distributions of T_2 for starch-gluten gels at a solid concentration of 27.8%. For all of the three levels of gluten addition, the peak T_2 values of the two regions shifted to the lower values too, but the extent of the changes were much smaller than the starch only samples. This indicated that the presence of gluten retarded the starch retrogradation. There was no significant difference in the extent of the peak shifts among the three levels of gluten addition.

The plots of continuous distributions of T_2 for gluten gels at concentrations of 27.8% and 38.5% are shown in Figure 4 a, b. Two distinct regions of T_2 distributions were also observed for gluten gels. At both gluten concentrations, the peak T_2 values decreased a little bit over storage. This indicated that gluten undergoes a mild water structure change during storage, resulting restricted water mobility.

The integrated signal intensity of each region shows the water population. The change in integrated signal intensity of the two regions during storage for starch, gluten and starch-gluten gels are shown in Figure 5 a, b and Figure 6 a, b. At both solid concentrations, the integrated signal intensity of all the samples decreased over time. This is in agreement with results reported by Choi and Kerr (2001) and Ishirguro et al. (2000). They found water loss in different starch gel types during storage. Willhoft (1971) also observed a release of water from gluten during heating and storage. The driving force for the release of water by the gluten appears to be provided not by the starch but by the gluten. The changes in intensity for the more mobile region were significant but not large, and there were no significant differences amongst the starch, gluten and the starch-gluten samples. The changes in intensity for the less mobile region, however, were much more dramatic. The starch only samples showed the greatest changes, and gluten samples showed the least. In general, the three levels of gluten addition samples showed smaller

changes than the starch gels; however, there was no significant difference among the three levels. Choi and Kerr (2001) studied isolated amylose gels and granule remnants using distributed exponential fitting. They found that the less mobile region was associated with water in granule remnants; the decrease in signal intensity in the less mobile region indicates that water was expelled from swollen granule remnants. Using particle size analysis, Morikawa and Nishinari (2000) also reported that the average particle size of swollen granules decreased as starch retrogradation proceeded. The more dramatic decreases in integrated signal intensity of the less mobile regions indicated gluten addition decreased water loss from the swollen granule remnants during starch retrogradation. This can be attributed to less swelling of the starch granules in the presence of gluten.

4.4.4. Retrogradation kinetics

For the starch retrogradation, it has been shown that the rate at which the amorphous phase changes to an ordered structure can be expressed by the Avrami equation (Avrami, 1940):

$$\theta = \exp(-kt^n) \qquad (5)$$

Where θ is the fraction of uncrystallized material remaining after time t, k is the crystal growth rate constant, and n is an integer varying from 1 to 4 that is characteristic of the mode of nucleation. We modified Equation 5 to 6 to test whether the changes in T_2 of the less mobile water fraction is described by Avrami kinetics

$$\ln \theta_{T_2} = \ln\left[\frac{T_{2,\infty} - T_{2,t}}{T_{2,\infty} - T_{2,0}}\right] = -kt^n \qquad (6)$$

where $T_{2,0}$ is the initial average value, $T_{2,}$ the average value at long storage time, and $T_{2,t}$ the value at time t. The data for all gels was fitted to equation 6 using non-linear regression. The data was fitted very well in all cases, with R^2 over 0.98. Values for

Avrami exponent and time constant were calculated form the fitted curves and are shown in Table 1. Values of n for samples containing only starch were about 0.6-0.8. This is in good agreement with the results from Teo and Seow (1992) and Choi and Kerr (2001). At each starch concentration, the Avrami exponents (n) for starch-gluten gels were smaller than those for starch gels, implying that addition of gluten might affect the nature of nucleation of starch during recrystallization. However, there was not much difference in n values among three levels of gluten, indicating that the basic mechanism of starch retrogradation was not affected by gluten content. Kim S.K. and D'Appolonia B.L. (1977b) showed similar results. At each starch concentration, the rate of retrogradation for starch-gluten gels was smaller than starch gels, but there was not much difference among the three levels of gluten addition. In addition, as the starch concentration increased, retrogradation proceeded faster.

4.5. Conclusion

To further increase the understanding of the influence of gluten on bread staling, ^{1}H NMR measurements were made on starch and gluten gels to study the effect of gluten on starch retrogradation. Changes in the transverse relaxation time constant (T_2) were related to water mobility. T_2 values of all the samples decreased during 15 days of storage. All of the starch-gluten gels changed much less than the starch only samples, indicating gluten retarded starch retrogradation. The three levels of gluten addition in starch-gluten gels produced similar changes. T_2 values in gluten gels also decreased during storage, indicating gluten also underwent some structure change during storage, which resulted in firming, therefore, further addition of gluten did not decrease the retrogradation rate. Two distinct regions of T_2 were observed in all samples. For all the samples, the signal intensity of the less mobile region decreased more dramatically than that of the more

mobile region during storage. It was suggested that gluten retards water loss in the granule remnants.

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Table 4.1. Avrami constants (Equation 6) for starch and starch-gluten gels held up to 15 days.

% Starch	Sample	Avrami Exponent (n)	Time constant (k)	R^2
27.8	Starch	0.69	0.47	0.99
	10% Gluten	0.60	0.37	0.99
	15% Gluten	0.56	0.41	0.99
	20% Gluten	0.61	0.42	0.99
38.5	Starch	0.89	0.71	0.99
	10% Gluten	0.80	0.53	0.98
	15% Gluten	0.75	0.49	0.99
	20% Gluten	0.75	0.54	0.99

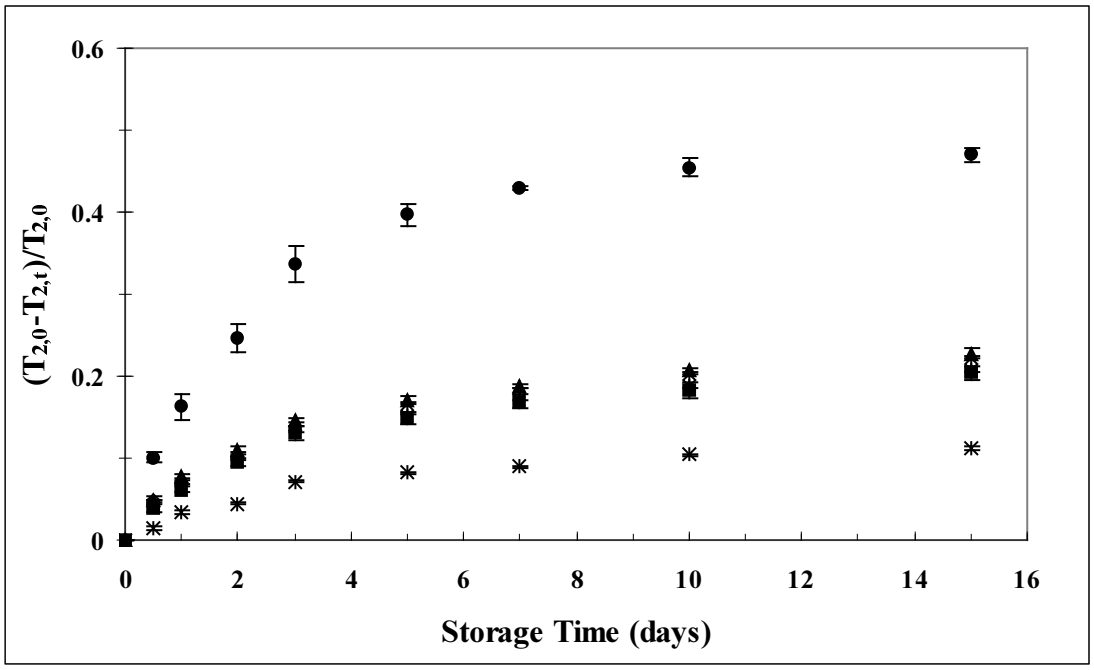

Figure 4.1a. Normalized changes in transverse relaxation time (T_2) (fitted by mono-exponential fitting) for wheat starch (●), gluten (⁎), starch-gluten gels (■-10% gluten, ◊-15% gluten, ▲-20% gluten) at starch concentration of 27.8%.

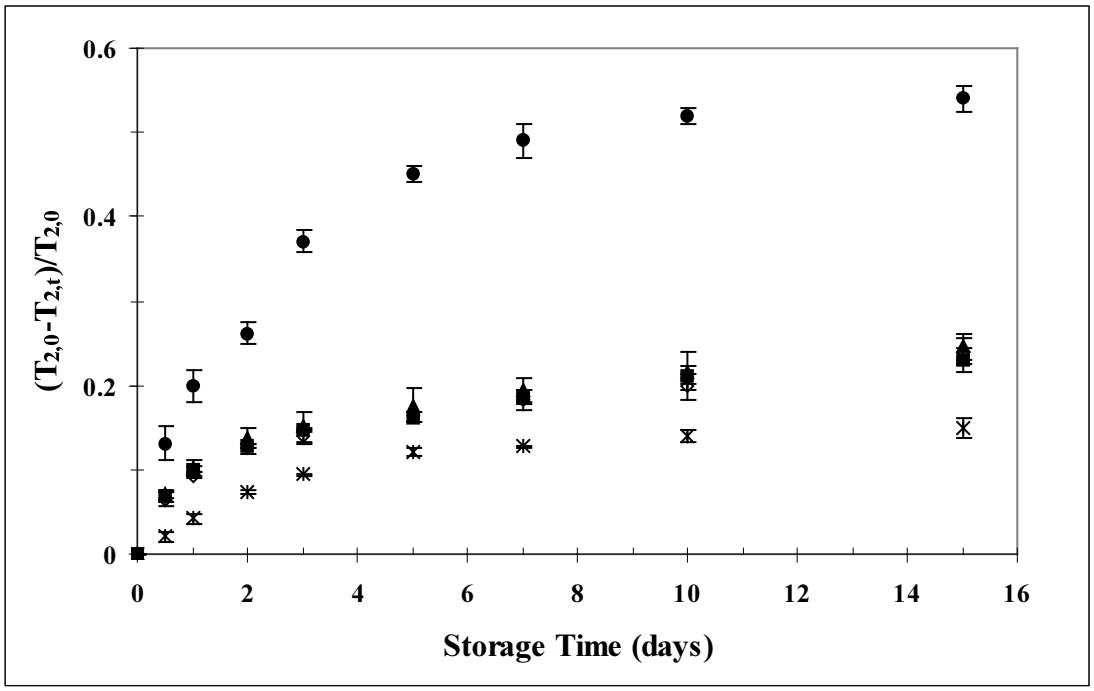

Figure 4.1b. Normalized changes in transverse relaxation time (T_2) (fitted by mono-exponential fitting) for wheat starch (●), gluten (∗), starch-gluten gels (■-10% gluten, ◊-15% gluten, ▲-20% gluten) at starch concentration of 38.5%.

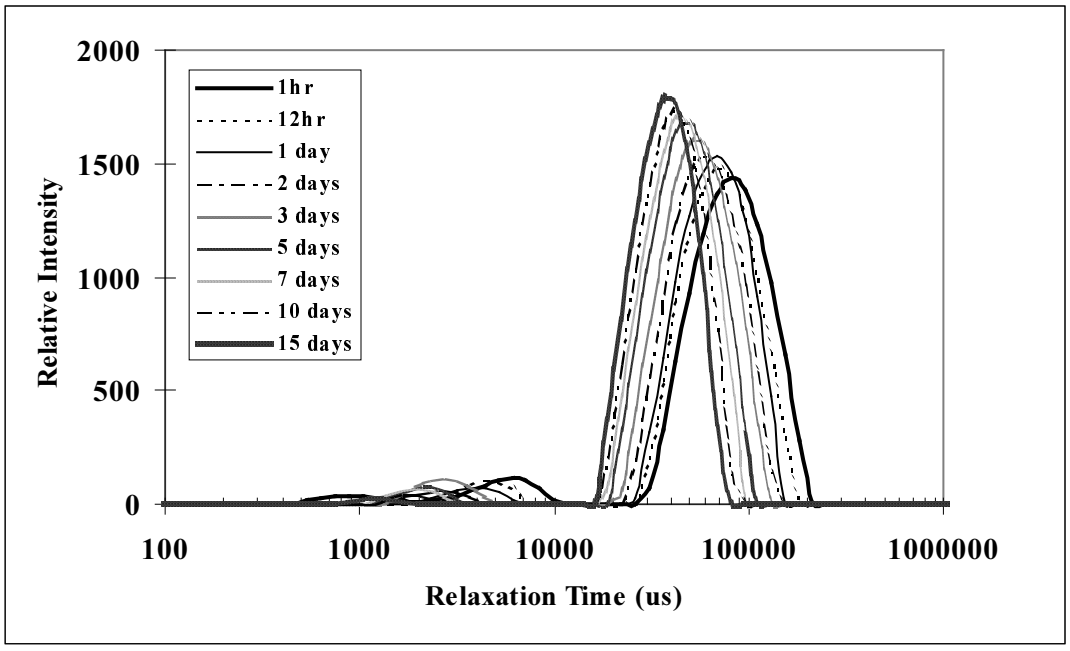

Figure 4.2a. Distributed exponential fits of T_2 over time for wheat starch gels at 27.8% concentration.

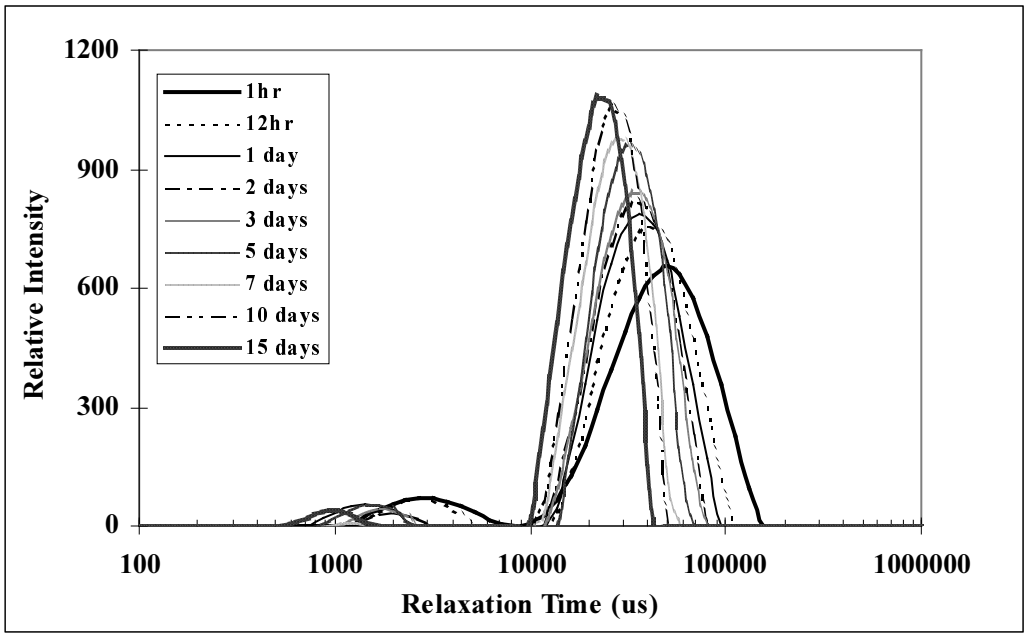

Figure 4.2b. Distributed exponential fits of T_2 over time for wheat starch gels at 38.5% concentration.

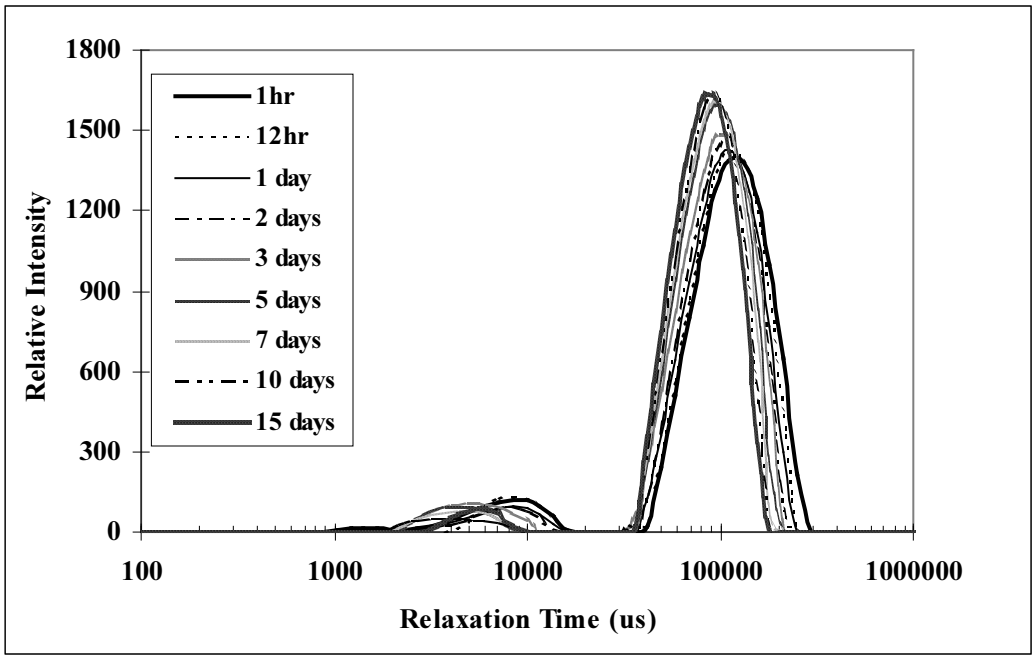

Figure 4.3a. Distributed exponential fits of T_2 over time for 10% gluten gels at 27.8% concentration.

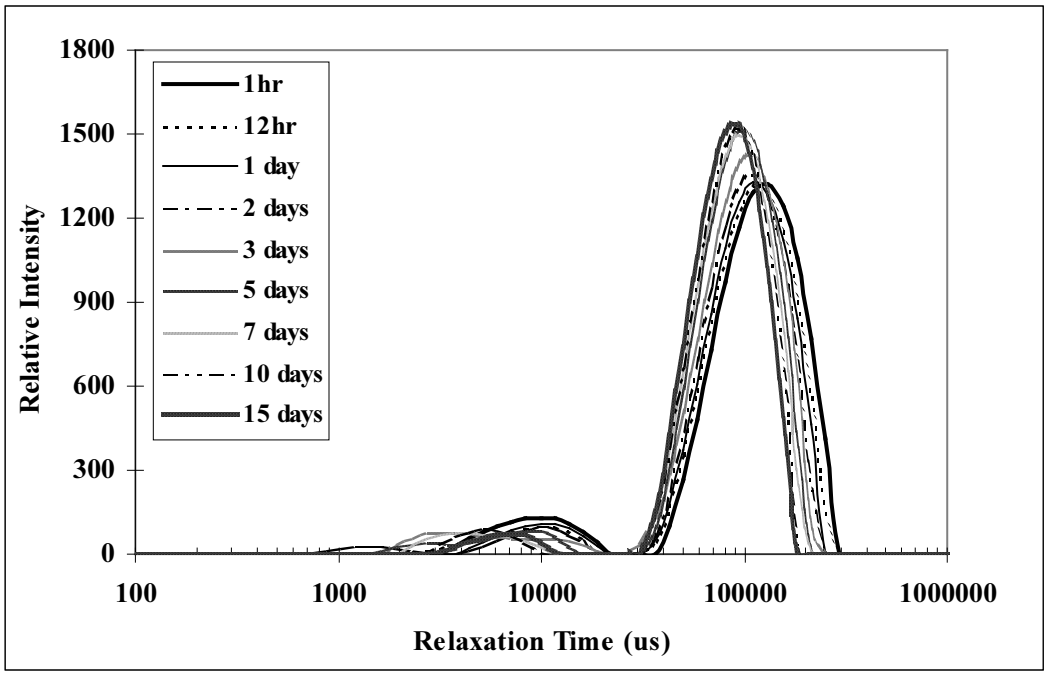

Figure 4.3b. Distributed exponential fits of T_2 over time for 15% gluten gels at 27.8% concentration.

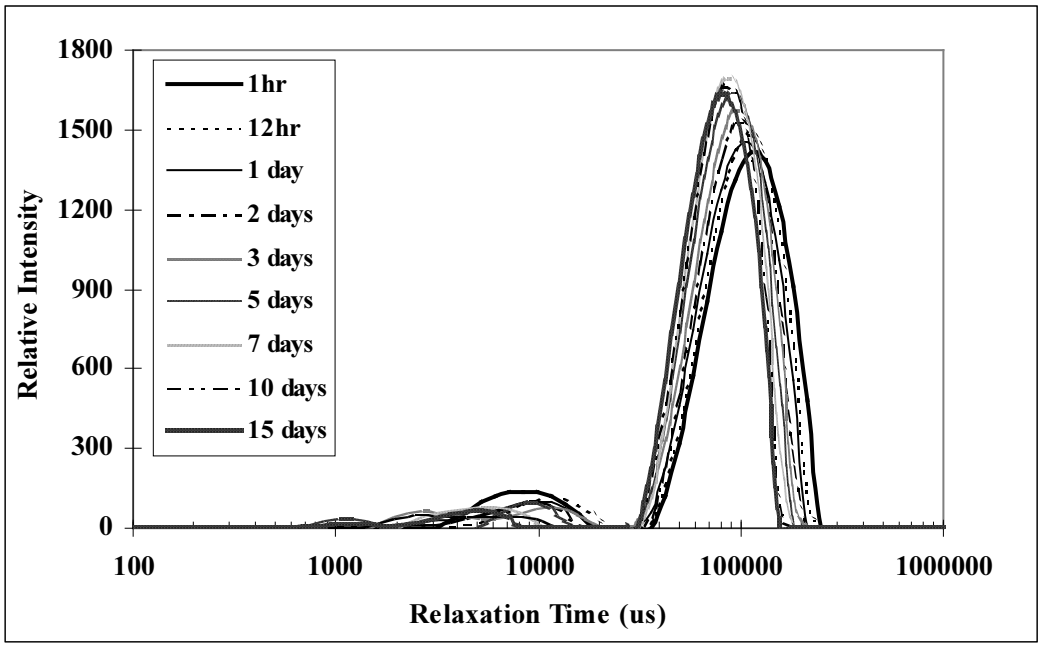

Figure 4.3c. Distributed exponential fits of T_2 over time for 20% gluten gels at 27.8% concentration.

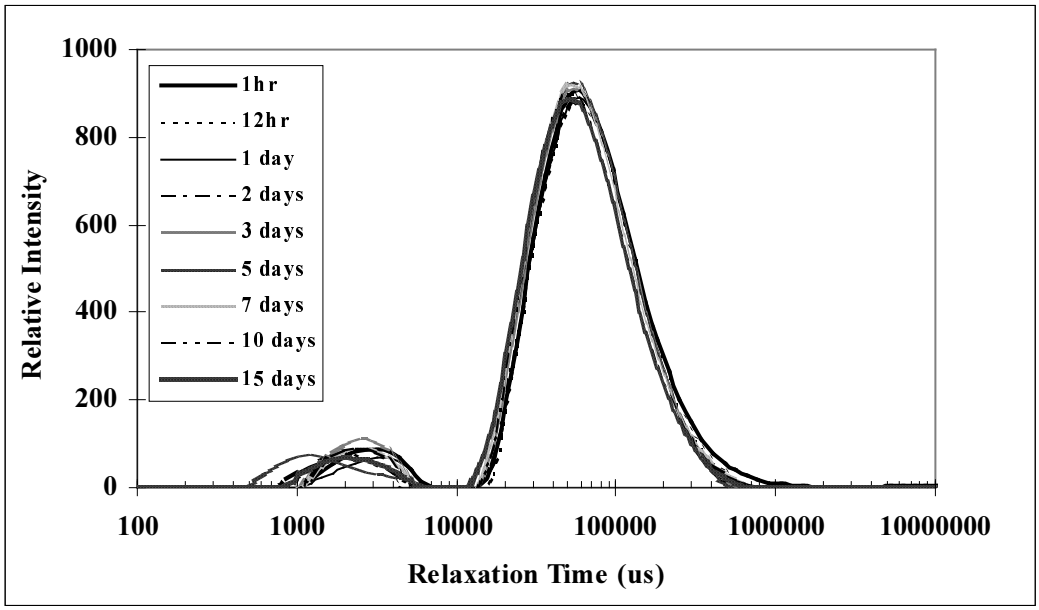

Figure 4.4a. Distributed exponential fits of T_2 over time for gluten gels at 27.8% concentration.

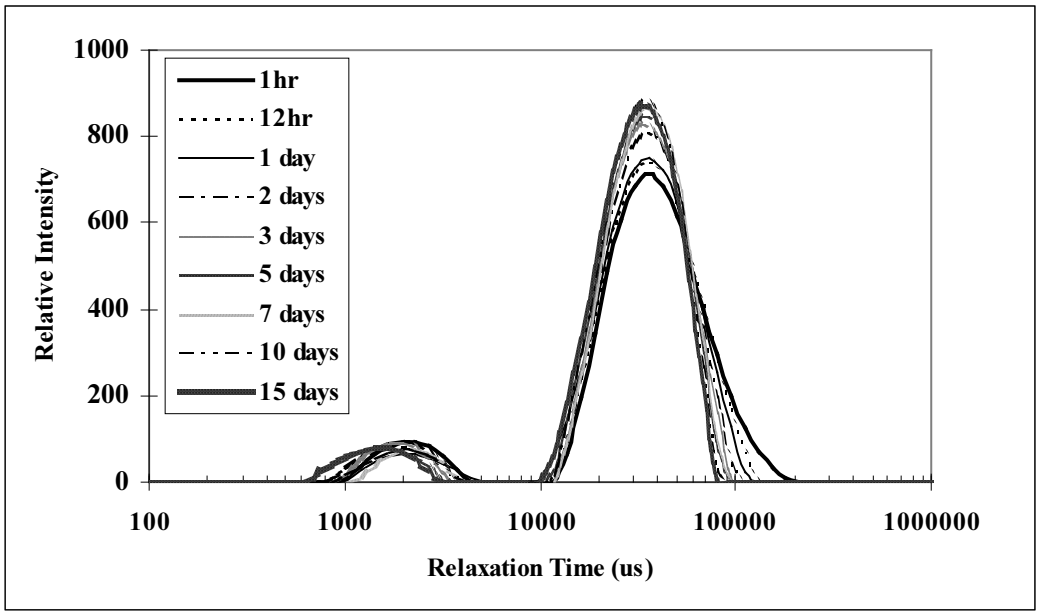

Figure 4.4b. Distributed exponential fits of T_2 over time for gluten gels at 38.5% concentration.

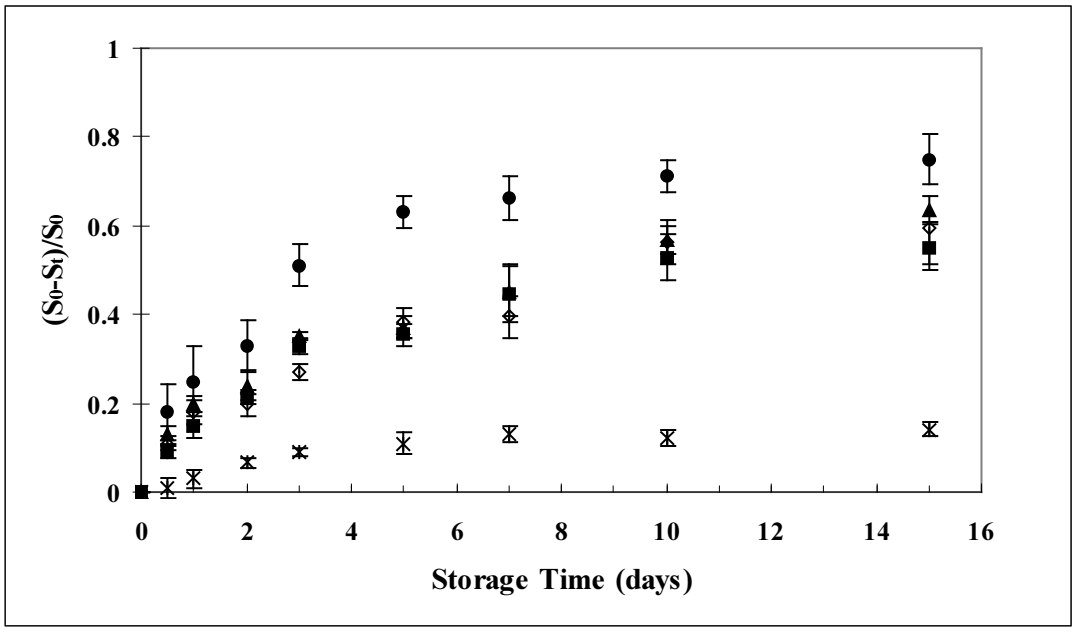

Figure 4.5a. Normalized signal intensities from less mobile (low T_2) regions of wheat starch (●), gluten (⁎), starch-gluten gels (■-10% gluten, ◊-15% gluten, ▲-20% gluten) at 27.8% concentration.

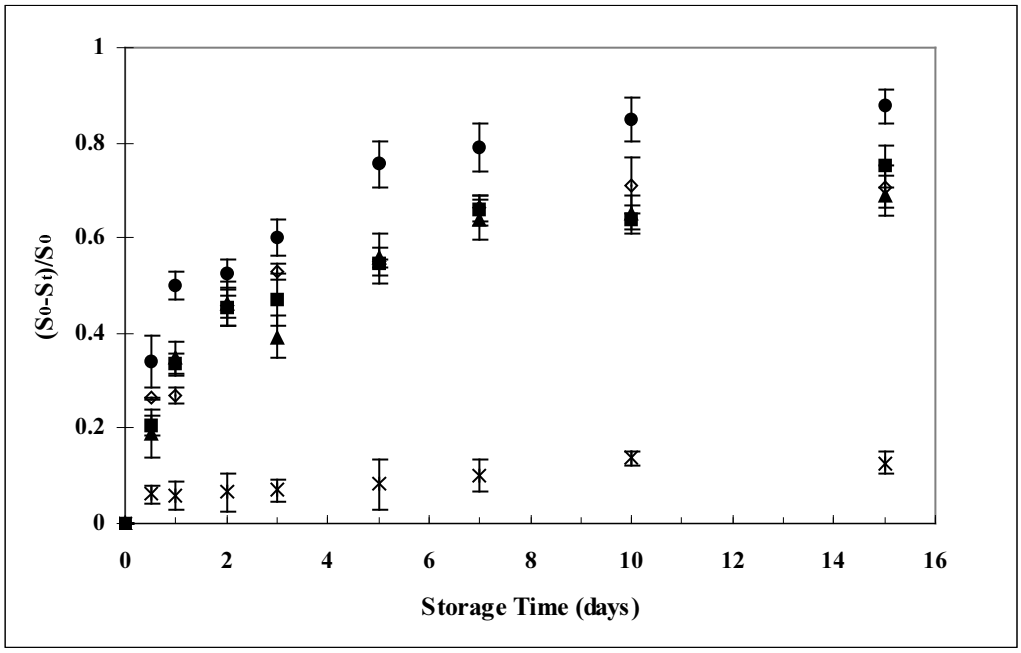

Figure 4.5b. Normalized signal intensities from less mobile (low T_2) regions of wheat starch (●), gluten (⁎), starch-gluten gels (■-10% gluten, ◊-15% gluten, ▲-20% gluten) at 38.5% concentration.

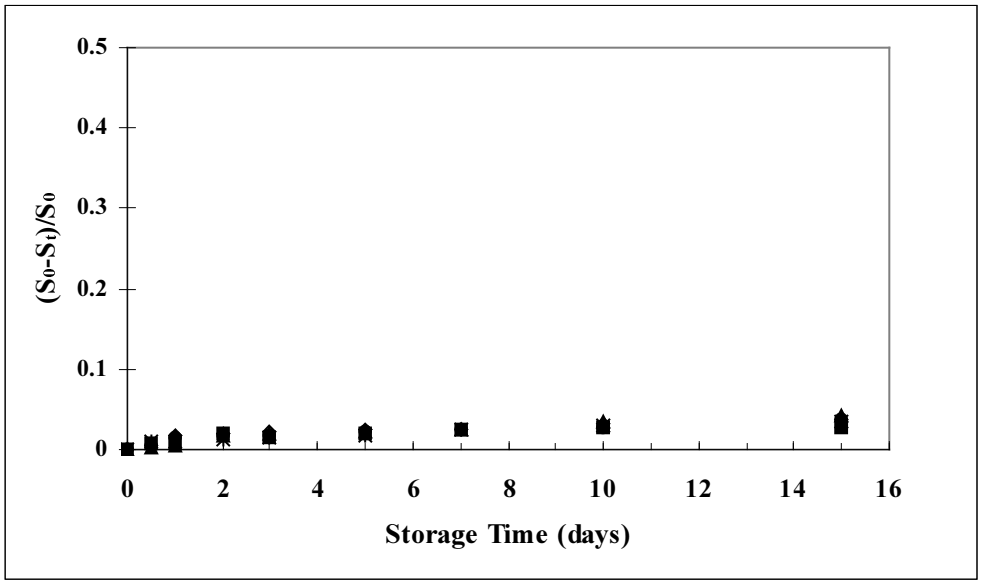

Figure 4.6a. Normalized signal intensities from more mobile (high T_2) regions of wheat starch (●), gluten (⁎), starch-gluten gels (■-10% gluten, ◊-15% gluten, ▲-20% gluten) at 27.8% concentration.

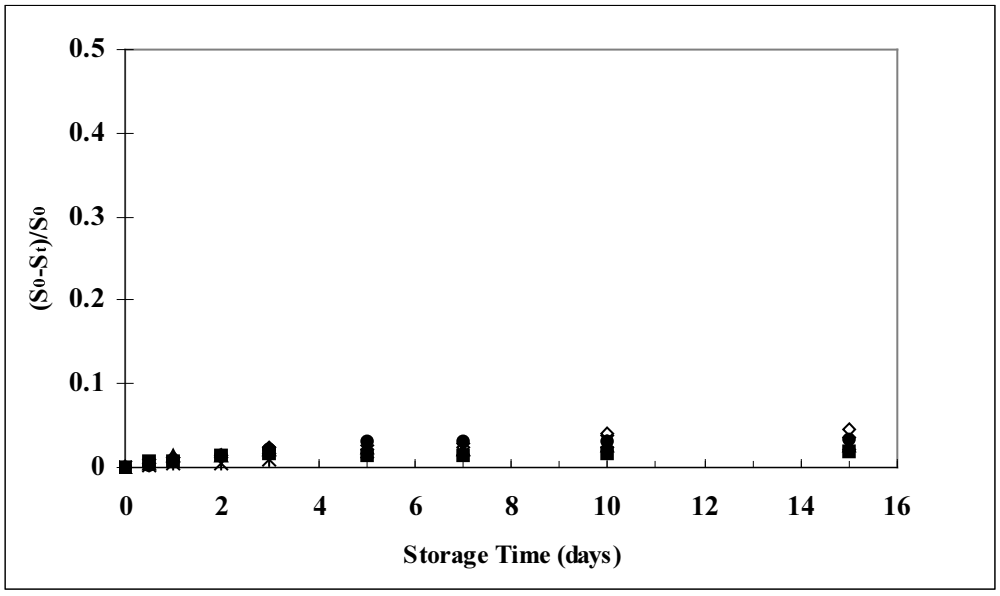

Figure 4.6b. Normalized signal intensities from more mobile (high T_2) regions of wheat starch (●), gluten (⁎), starch-gluten gels (■-10% gluten, ◊-15% gluten, ▲-20% gluten) at 38.5% concentration.

CHAPTER 5

SUMMARY AND CONCLUSIONS

The objective of this study was to evaluate the effect of water and gluten on the water mobility, gelatinization, and retrogradation of bread and starch gels.

In the first experiment, the effect of water and gluten levels on the water mobility in bread was studied using ^{1}H NMR. White breads were made with three levels of water and three levels of gluten. The three levels of water addition in bread dough resulted in three different levels of water content in bread. Gluten levels made no difference in bread moisture content. Distributed exponential analysis showed two distinctive regions of T_2, associated with multiple domains of water in the bread crumb. There were no significant differences in peak T_2 values with different gluten content, but significant differences were observed with different water content. It is suggested that water associated with starch increased significantly after heating; because of gelatinization, a redistribution of water occurred between starch and gluten. The mobility of water associated with both starch and gluten were very similar; therefore the gluten addition did not affect the total water mobility of the bread within the gluten content range used in this experiment. NMR measurements were made directly on starch gels with different gluten content to further investigate the effect of gluten on starch gelatinization. Two distinct regions of T_2 distributions were also observed for all samples. For both of the less mobile and more mobile regions, the T_2 values of the starch only samples were lower than the starch-gluten mixtures at both solid contents. This indicated less swelling of starch granules and absorption of water. There was no significant difference among the T_2 values of starch-

gluten mixtures with different gluten contents, which also indicated a redistribution of water occurring between starch and gluten during heating in that gluten released some water and starch absorbed more water.

The second study was performed using ^{1}H NMR relaxometry to investigate the effect of gluten on starch retrogradation at 5^{o}C. In general, T_2 values of all the samples decreased during fifteen days of storage, indicating a decrease in water mobility. The extent and rate of change in T_2 over time was higher at higher starch concentrations. The changes in gels containing only starch were the greatest among the five treatments. All of the starch-gluten gels changed much less than the starch only samples, indicating that gluten retarded starch retrogradation. The three levels of gluten addition in starch-gluten gels produced similar changes. Distributed exponential studies showed that two distinct regions of T_2 were observed in all samples. During aging, the peak values of both regions shifted to lower values for all the gels. Starch samples showed the most significant shift, and gluten showed the least. The three levels of gluten addition in starch-gluten gels produced similar shifts. T_2 values in gluten gels also decreased during storage, indicating gluten also underwent some structure change during storage, resulting in firming, therefore, further addition of gluten did not decrease the retrogradation rate. For all the samples, the signal intensity of the less mobile region decreased more dramatically than that of the more mobile region during storage. It was suggested that gluten retards water loss in the granule remnants.

In conclusion, the gluten component in flour made the starch granule swell less during heating, thus decreasing the extent of starch gelatinization, and further retarding starch retrogradation. This is probably due to decreased water availability to starch in the

presence of gluten. This provides better understanding and control of the behavior of flour containing foods during heating and storage.