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The Effect of Chemical Modification on the Molecular Mobility, Texture, and
Retrogradation of Starch-Water Suspensions

(Under the direction of WILLIAM LLOYD KERR)

The effects of chemical modification by hydroxypropylation and cross-linking on the molecular dynamics of starch-water suspensions at low moisture levels or in the gel state were studied. The relationships with textural properties and retrogradation were also investigated.

Wheat starch was hydroxypropylated with propylene oxide (MS of 0.05, 0.12, and 0.18) or cross-linked with phosphorus oxychloride at the concentrations of 0.03%, 0.1%, and 0.2%, respectively. Pulsed ^1H NMR was used to determine moisture content and to study molecular mobility of wheat starch suspensions at low moisture content. Moisture determination by NMR agreed well with gravimetric determinations ($R^2 = 0.99$). Proton relaxation associated with starch molecules increased with increasing water content and molar substitution of hydroxypropyl group, indicating increased mobility of starch chains. However, mobility was not significantly affected by cross-linking. Transverse proton relaxation associated with water in control (alkali treated), hydroxypropylated and cross-linked starch showed two distinct populations in the a_w range of 0.53 to 0.93, suggesting that treatment with alkaline reagents may cause structural changes in starch granules.

Gels were formed from native and hydroxypropylated starches at concentrations between 25 and 40%. For all gels, two distinct water fractions were identified using distributed exponential analysis of CPMG results. For the less mobile fraction, T_{2a} ranged from 2~6ms while for the more mobile state, T_{2b} ranged from 20~200ms. Values of both T_{2a} and of T_{2b} increased with increasing moisture content and hydroxypropyl M.S.

Control experiments indicated that more mobile states could be associated with both the extragranular amylose gel, as well as intragranular amylopectin-rich gel. The less mobile state was associated only with granule remnants. Gel hardness decreased significantly with increasing moisture content, and with increasing hydroxypropylation. Cohesiveness increased with degree of hydroxypropylation. Results from multi-regression analysis between hardness and NMR parameters showed that the mobility and amount of water in the less mobile state was most related to gel hardness.

During storage of starch gels, decrease in water mobility accompanied retrogradation. Greatest decrease was observed with native starch, less with alkali treated starch, and even less with hydroxypropylated starch.

INDEX WORDS: Wheat starch, Moisture sorption isotherm, NMR, Molecular mobility, Water mobility, Texture, Hydroxypropylation, Cross-linking, Hardness, Retrogradation, Gel, Amylose, Amylopectin

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MOBILITY, TEXTURE, AND RETROGRADATION OF
STARCH-WATER SUSPENSIONS

by

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B. S., Korea University, Korea, 1992

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DEDICATION

This dissertation is dedicated to

My parents:

Mr. Ui-Haeng Choi and Mrs. Kwang-Chang Kim

and Family

For constant support, encouragement, and

unconditional love.

To

My beloved wife

Ji-Yeon Chun

For her patience and encouragement.

AND

To

My beloved son

Seung-Beom Choi, who is a constant source of joy and inspiration

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CHAPTER I

INTRODUCTION

Chemical modification of starches has been used to provide parent starches with improved or specific properties to extend their usefulness in food or industrial applications. The types of chemical modification are generally classified as acid hydrolysis, oxidation, acetylation, hydroxypropylation, and cross-linking. Chemically modified starches have markedly altered physicochemical properties, depending on the type of functional groups employed and the degree of substitution (Rutenberg and Solarek, 1984; Kim et al., 1992).

In the food industry, cross-linked and hydroxypropylated starches are extensively used to thicken, stabilize, and texturize food systems. Cross-linked starches minimize or prevent granule rupture during cooking, thus providing starches with better ultimate viscosity and short, noncohesive, paste texture, in contrast to the cohesive, gummy textures associated with unmodified starch dispersions (Wurzburg, 1986). Cross-linked starches are used in the preparation of pie fillings, bread, pudding, baby foods, soups and sauces, gravies, salad dressing, etc., and for retorted foods and aseptic processing (Wurzburg, 1986). Hydroxypropylation effectively retards the gelling and retrogradation tendencies of starch gels, increases water holding capacity, and improves the freeze-thaw stability or cold storage stability (Hoover et al., 1988; Liu et al., 1999; Yeh and Yeh, 1993). Hydroxypropylation reduces the gelatinization temperature of starch.

Hydroxypropylated starches are used in white creamer, cream soups, frozen foods (Tuschhoff, 1986).

The interaction of water with starch strongly influences the physical and structural properties of starch and involve molecular motion of both water and starch chains. It has been recognized that molecular mobility in food systems is related to processibility, product properties, quality, stability, and safety of the system (Kou et al., 1999). In current practice, several techniques including X-ray diffraction, differential scanning calorimetry (DSC), dynamic mechanical thermal analysis (DMTA), and a variety of nuclear magnetic resonance (NMR) spectroscopy techniques have been used to characterize the structure and dynamics of food polymers (Farhat et al., 1996; Wu et al., 1992; Kalichevsky et al., 1992). Among them, NMR has been widely used to specify the rates and amplitudes of motions of food polymers by observing nuclei (^1H , ^2H , ^{13}C , or ^{17}O) relaxation phenomenon (Ablett et al., 1993).

Knowledge about changes in the molecular dynamics of starch and water molecules caused by chemical modification are important, not only for understanding the altered functional properties but also for providing better control of modification processes and for developing chemically modified starch with desired properties.

This dissertation is divided into six chapters. Chapter II is a literature review which describes starch molecules, starch gelatinization, texture of starch gels, retrogradation, molecular mobility, pulsed proton NMR, and previous studies related to this research.

In Chapter III, pulsed proton NMR techniques were developed to identify and quantify the molecular motion of starch chains and water molecules of starch suspensions

at low moisture levels. In addition, the techniques were also employed to determine water content through analysis of free induction decay (FID) curves.

In Chapter IV, we investigated the effects of hydroxypropylation and cross-linking on water absorptive capacity and molecular mobility of starch and water molecules of starch suspension at low moisture levels.

In Chapter V, the textural properties and water binding properties of hydroxypropylated starch gels at high starch concentration were investigated. The relationship between water binding properties and textural properties were analyzed.

Retrogradation and changes in water mobility of hydroxypropylated starch gel during storage at 5°C were investigated in chapter VI. A summary and conclusion of the four studies reported in Chapter III through Chapter VI are presented in chapter VII.

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CHAPTER II

LITERATURE REVIEW

Starch

Starch is a major hydrocolloid used extensively in the food industry as a carbohydrate source as well as a thickening, texturizing, and emulsifying agent (Luallen, 1985). In plant tissues starch is present in the form of water insoluble granules. The granules contain ordered regions, which are semi-crystalline, and show birefringence. Most starches consist of a mixture of two polysaccharides, amylose and amylopectin. Amylose is an essentially linear molecule consisting of (1-4)- α -linked D-glucan chains. Amylopectin is a highly branched molecule. It contains short chains of (1-4)- α -linked D-glucan chains with (1-6)- α -linked branches (Manners, 1989). Chemical and physical properties of starches, including the molecular weights and the fine structures of amylose and amylopectin, vary with the botanical source (Takeda et al., 1984; Hizukuri, 1985).

Commercial starches can be classified into three groups (Swinkels, 1985). The first group is composed of tuber (potato), root (tapioca, arrowroot, and sweet potato), and pith (sago) starches. The second group includes cereal starches (corn, wheat, sorghum, and rice). The third group comprises the waxy starches (waxy maize, waxy sorghum, waxy rice) obtained from cereals, but having physical properties similar to those of root starches. Starches from different plant sources consist of different ratios of amylose to amylopectin (Pomeranz, 1991). In cereal, the amylose content is about $23 \pm 3\%$ (Hoseney, 1994). Starches with 100% amylopectin are called waxy starches. Therefore,

corn, sorghum, and rice containing about 100% amylopectin are called waxy corn, waxy sorghum, and waxy rice, respectively (Hoseney, 1994).

Granules from different starch sources also 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 bimodal distribution of granule size, with larger lenticular granules as well as smaller spherical granules (Swinkels, 1985). Variations in the ratio of these polysaccharides, their properties and interaction with other components can explain differences in the swelling behavior of granules and functionality of starches.

Starch gelatinization

Gelatinization has been defined as a phase transition of the starch granules from an ordered state to a disordered state, which takes place during heating in excess water. This transition involves loss of crystallinity, loss of anisotropic order (birefringence) and hydration of starch (Donovan, 1979 and Zobel and Stephen, 1995). These changes in the structure of the starch granules are accompanied by separation of amylose and amylopectin; amylose leach out of the granules. The swollen granules are enriched in amylopectin. 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, and chemical modification (Pomeranz, 1991).

Starch gels

The gelling ability of starch as well as the viscous nature of starch paste is important to the manufacture of food products. The control of gel properties is indispensable for quality control and the development of starchy food. 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 Svegmarm, 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. Ott and Hester (1965) observed amylose gelation 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. On the other hand, at very high starch concentration, 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 Svegmarm, 1996).

Textural and mechanical properties of gels

The textural or mechanical properties of starch gels depend mainly on the rheological properties of the continuous phase, the volume fraction of the swollen granules, the deformability of swollen granules, and interaction between the dispersed and continuous phase (Eliasson, 1986; Ring et al., 1987). For concentrated starch gels it was found that addition of swollen granules increased the elastic modulus of the amylose

gel (Ring, 1985). Heating at high temperature ($>125^{\circ}\text{C}$) and /or in the presence of shear causes swollen granules to be disrupted, changes the rheological and structural character of starch gels, thus leading to decrease in gel stiffness (Svegmark and Hermansson, 1992). Han and Hamaker (2000) showed, by reconstituting insoluble swollen granules with soluble starch at various levels, that resultant gels were firmer and had a fibrous structure by increasing amounts of added soluble starch.

Water plays an important role in textural and mechanical properties of starch gels and starch-based foods. Much research has been conducted on the role of moisture content, water binding properties and water mobility on mechanical properties of gels. Inaba et al. (1994) investigated the textural and mechanical properties of starch gels prepared at various water contents, through analysis of compression and decompression tests. They reported that the mechanical parameters of compressive force and work increased as the starch concentration increased, while resiliency and compressibility were not dependent on starch concentration. Chung and Lee (1991) investigated the relationship between texture of starch-based foods and water binding as measured by DSC (unfreezable water at -30°C). They demonstrated that bound water in surimi gels prepared with potato starch, and with and without pregelatinization, was highly correlated with compressive force and inversely correlated with expressible moisture. Ruan et al. (1997) investigated textural attributes and water mobility of various types of cooked rice using compression test and ^1H NMR spectroscopy. The authors found that hardness of cooked rice was highly correlated with spin-spin relaxation constants of protons ($P<0.01$), which is regarded as a measure of rotational molecular mobility of water in the samples.

Starch Retrogradation

Gelatinized starch returns to an insoluble or aggregated form over a period. This phenomenon, called retrogradation, has been defined as the changes which occur in a starch paste or gel on ageing (Hoover, 1995; Hermansson and Svegmarm, 1996). 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, soluble starch content of starch gels, contributing to defects in quality of starch-containing foods (Hermansson and Svegmarm, 1996).

A mechanism for retrogradation of starch gel was proposed by several researchers. 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) reported that the initial rate of development of gel stiffness (retrogradation) was correlated with the amount of amylose solubilized during gelatinization. Initial gel stiffness decreased in pea, maize, wheat, and potato starch (in descending order), which correlate with soluble amylose content. Ring et al. (1987) have shown that elastic modulus (G') of starch gels continued to increase for several days. The long-term increase in rigidity was reversible upon heating to 100°C, but the short-term development was irreversible.

There have been several methods to study starch retrogradation based on the associated changes in specific physicochemical properties. These include enzymatic assays that measures the resistance to hydrolysis, rheological measurements that determine hardness, shear or elastic moduli, DSC analysis that measures the enthalpy of fusion of recrystallized starch molecules, and X-ray diffraction that determines the degree and type of crystallinity

developed in retrograded starch (Takahashi et al., 1989; Kalichevsky et al., 1990; Russel, 1987; Ward et al., 1994; Colwell et al., 1969; Tsuge et al., 1990; Teo and Seow, 1993; 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 modification (Orford et al., 1987; Biliaderis and Tonogari, 1991; Seow et al., 1992; Wu and Seib, 1990; Hoover and Sosulski, 1986; Morikawa and Nishinari, 2000). Water is also an important factor on 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 and Thompson, 1998). Ali and Bhattacharya (1976), using an equilibrium moisture content (EMC) method, studied the starch retrogradation in parboiled rice. The authors found the EMC of soaked and parboiled rice fell appreciably upon starch retrogradation during storage, indicating that water holding capacity of the rice decreased as rice became firmer during storage. They also noticed that the extent of starch retrogradation was dependent on the water content and storage. Hellen et al. (1954) showed that the most intense “B” X-ray pattern is obtained for wheat starch gel with water content of 47% to 50% (w/w). Zeleznak and Hosney have shown by DSC measurements that the maximum extent of recrystallization for wheat starch occurs at 60% moisture. Furthermore, in wheat starch gels the enthalpy of retrogradation decreased to zero in 10% and 80% starch concentration. Orford et al. (1987) 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' .

Several controversial hypotheses have been developed on the dynamics and migration of water molecules in starch gel and starch-based food during ageing. 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. Based on DSC measurements, Whistler and Daniel (1985) suggested that water be expelled from the starch matrix as starch molecules aggregated. Stone (1952) noticed that the crisp crust of bread became softer and more leathery during storage, probably owing to migration of water from the crumb, or possibly owing to absorption of moisture from the atmosphere. Ruan et al (1996) and Chen et al. (1997) investigated changes in water states in bread and starch-based food during storage using magnetic resonance imaging (MRI) and NMR techniques. They observed a redistribution between distinguishable fractions of water with different mobility as a result of retrogradation. They also noticed that moisture migrated from the crumb to the crust, which was associated with the firming of the crumb.

Chemical Modification

The use of unmodified starch in foods is limited by its physical and chemical properties. Functional groups have been introduced into starches by chemical modification in order to provide starch with improved or specific properties to extend their usefulness in foods or industrial applications. The chemically modified starches have markedly altered physicochemical properties, compared to their parent starches, primarily depending on the molar substitution (M.S.) or the degree of substitution and the type of functional groups employed (Rutenberg and Solarek, 1984).

Types of chemically modified starches

Cross-linked starch. Cross-linking reinforces the hydrogen bonds in the granule with chemical bonds, which act as bridges between molecules. As a result, when the cross-linked starch is heated in water, the granules stay intact to varying degrees by chemical bridges. Cross-linked starch is resistant to high temperature, low pH and high shear (Rutenberg and Solarek, 1984; Inagaki and Seib, 1992; Reddy and Seib, 1999).

Cross-linked starches have been developed to minimize or prevent granule rupture during cooking, thus providing starches that give better ultimate viscosity and short, noncohesive, pasty texture, in contrast to the cohesive, gummy textures associated with unmodified starch dispersions (Wurzburg, 1986). Rutledge et al. (1972) cross-linked rice starch with epichlorohydrin and reported that cross-linked rice starch showed resistance to overcooking and suitable for canning. They found the cross-linked rice had solid loss of 5.2% after retorting at 115.6°C for 60 min, 78% less than obtained from conventional parboiled samples.

The extent of cross-linking that is allowed in modified food starches is controlled by regulation (CFR, 1995); treatment is limited to a maximum of 0.1% phosphorus oxychloride, based on dry weight of starch. Therefore, most cross-linking reactions produce a relatively low degree of cross-linking, but are sufficient to strengthen the outer envelope of the starch granule and to affect the rheological properties. However, cross-linking with this amount of reagents may actually contribute little to changes in the appearance of the intact starch granule (Wurzburg, 1986) and water sorption isotherms (Chilton and Collison, 1974).

Cross-linking also effects thermal properties, solubility, and swelling power of starch. Inagaki and Seib (1992), who studied firming behavior of bread crumb with cross-linked waxy barley starch substituted for wheat starch, reported that the swelling power of cross-linked waxy barley starch declined as the level of cross-linking increased. Liu et al. (1999) found that cross-linking reduced the soluble starch that leached out of the swollen granules on cooking, and reduced solubility from the granules in cold dimethyl sulfoxide. Studies on thermal properties of cross-linked starches using differential scanning calorimetry (DSC) have shown that gelatinization and glass transition (T_g) temperature was not effected by cross-linking, but the enthalpy (ΔH) associated with gelatinization of starch increased with increasing level of cross-linking (Yeh and Yeh, 1993; Liu et al. 1999).

Using ^{31}P NMR spectroscopy Lim and Seib (1993) showed that phosphate groups on potato starch by chemical modification mainly take place on 6-OH terminal of the glucose moiety, while the endogeneous phosphate groups occur on the 6-OH and 3-OH of glucose moiety. Jane et al. (1992) found that more amylopectin than amylose molecules were cross-linked.

Hydroxypropylated starch. Hydroxypropyl groups are hydrophilic in nature and when introduced into starch granules, weaken the internal bond structure holding the granule together, leading to an increase in accessibility of starch granules to water. This reduction in bond strength is reflected in starch pasting temperature (Wurzburg, 1986).

Hydroxypropylation effectively retards the gelling and retrogradation tendencies of starch, increases water-holding capacity, improves the freeze-thaw stability of starch-based products, and reduces tensile strength but increases elongation of starch films

(Tuschhoff, 1986). Liu et al. (1999) compared the textural attributes of unmodified fresh starch gel with those of starch gel hydroxypropylated by propylene oxide. They reported the hardness of starch decreased with increasing levels of hydroxypropylation, indicating formation of weak gels. Perera and Hoover (1999) investigated retrogradation properties of hydroxypropylated potato starch during storage, based on turbidity measurement, X-ray diffraction analysis, thermal analysis by DSC, and scanning electron microscopy. They found that hydroxypropylation decreased the rate and extent of increase in turbidity, which is affected by the interplay between: (1) steric effects imposed by hydroxypropyl groups on chain aggregation and (2) aggregation of granule remnant. They reported that the X-ray pattern of hydroxypropylated potato starch was similar to that of unmodified potato starch ('B' type X-ray pattern), but that the X-ray intensity decreased. They also reported that the retrogradation endotherm (monitored by differential scanning calorimetry) appeared only after 7 days in hydroxypropylated starches while it occurred after 2 days in unmodified starch. In addition, the enthalpy of retrogradation decreased with increasing degree of hydroxypropylation.

The freeze-thaw stability of hydroxypropylated potato starch was investigated by Kim et al. (1992a, 1992b, 1993) and Wu and Seib (1990) through analysis of rheological changes and syneresis determination. They reported that with increasing degree of substitution of hydroxypropyl groups, changes in both complex modulus (G^*) and phase angle (δ) indicative of destabilization were delayed. In addition, starch paste syneresis was delayed after several freeze-thaw cycles.

Recently, Perera et al. (1997) used X-ray diffraction to show that hydroxypropylation caused profound changes in physical arrangement of starch chains

within the amorphous and crystalline domains of starch granules. These changes were shown to alter granule crystallinity, swelling power, thermal properties and amount of amylose leached from granules. Liu et al. (1999) and Perera et al. (1997) reported that hydroxypropylation increased the swelling power of starch and the soluble starch which leach out of the swollen granules on cooking. DSC studies by several researchers (Seow and Thevamalar, 1993; Hoover et al., 1988) have shown that incorporation of hydroxypropyl groups into the starch molecules lowers thermal transition temperatures (T_o , T_p , and T_c) and overall enthalpy (ΔH_G) associated with gelatinization. Kim and Eliasson (1993) studied the effect of hydroxypropylation on thermal properties of starch at low and intermediate moisture levels (21.7% and 44.0%(w/w)). They observed biphasic transitions, indicative of a gelatinization endotherm (G) and a melting endotherm (M_1), and found that with increasing degree of hydroxypropylation, the enthalpy of the G-endotherm decreased while the enthalpy of the M_1 -endotherm increased. They stated that hydroxypropylation acts as an internal plasticizer and destabilizes the amorphous regions of the starch granules.

The degree of molar substitution (M.S.) indicates the average number of sites per anhydroglucose unit on which there are substituent groups (Wurzburg, 1986). Thus, if one hydroxyl on each of anhydroglucose units in a starch has been etherfied with hydroxypropyl group, the M.S is 1. If all three hydroxyls are etherfied, M.S is 3. Most of the commercially available food modified starches have low M.S values ranging up to about M.S of 0.1, which would represent on average 1 substituent group per every 10 anhydroglucose units (Wurzburg, 1986). The formula for calculating the M.S of a starch

derivative will vary depending upon the molecular weight and functionality of substituent group; for hydroxypropylated starch,

$$MS = [162 \times (\% \text{ hydroxypropyl} / 58)] / [100 - (57/58 \times \% \text{ hydroxypropyl})]$$

Hoover et al. (1988) investigated the digestibility of pea starch hydroxypropylated with molar substitutions (MS) ranging from 0.04 to 0.12. They found that the digestibility with α -amylase decreased with increasing MS up to the level of 0.08. Further increases in MS caused an increase in digestibility, which was greater than that observed for unmodified starch. The results were confirmed by scanning electron microscopy on hydrolyzed starch.

At the molecular and granular levels, Steeneken (1984) reported that the amylose in the etherified (hydroxypropylated) granular potato starch (M.S. 0.01) was 1.5 time more reactive than the amylopectin, which means that 33% of the amylopectin is inaccessible to reaction. Hood and Mercier (1978) and Biliaderis (1982) suggested that substituents on the amylose are distributed over the whole molecules, while in amylopectin, the substituents are distributed mainly in amorphous region of amylopectin rich in α -1,6 linkages. Kim et al. (1992) used light microscopy to show that the central region of the potato starch granule is where hydroxypropyl groups are mainly distributed. Based on measurements by high resolution ^1H NMR spectra, Xu and Seib (1997) determined the distribution of hydroxypropyl groups in eight hydroxypropylated wheat and corn starches with M.S values ranging from 0.05 to 0.23, and found that the frequency of the hydroxypropyl substitution was; OH (2), 67%~78%; OH (3), 15%~29%; and OH (6), 2%-17%.

Molecular Mobility of Complex System

Molecular mobility in food systems (i.e., water mobility, solids mobility, and glass transition temperature of food system) has a pronounced influence on many important aspects of food properties, quality, stability, and safety (Kou et al., 1999; Chen et al., 1997; Li et al., 1996; Slade and Levine, 1991). Water and solids mobility in food systems is often related to such phenomena as diffusion, viscosity, and glass transition. When a food is cooled and/or reduced in water content so that all or part of it is converted to glassy state, molecular mobility is greatly reduced and the food becomes more stable (Fennema, 1996).

Several techniques, including differential scanning calorimetry (DSC), dynamic mechanical thermal analyzer (DMTA), and nuclear magnetic resonance (NMR) spectroscopy, have been used to characterize the molecular dynamics of starch – water systems. Zeleznak and others (1987) used differential scanning calorimetry (DSC) to determine glass transition temperatures (T_g) of wheat starch at low moisture contents (13-22%, dry basis). They found that the glass transition temperature, at which there is a sharp change in heat capacity (ΔC_p) due to increase in molecular motion of the system, decreased from 90 to 25°C as water content increased from 13 to 22%. Kalichevsky et al. (1992) investigated the glass transition of amorphous amylopectin containing between 10 and 22% water using DSC, DMTA, and NMR. From DMTA measurements, storage modulus (E') and $\tan \delta$ were monitored as a function of temperature to determine the glass transition. From NMR experiments, proton NMR relaxation time after a 90° pulse was recorded as a function of temperature. They found that T_g determined by NMR was 20-30 °C lower than that determined by DSC and DMTA. They suggested that T_g values

obtained from DSC and DMTA could be based on polymer motions at the macroscopic level to characterize the overall mobility of the system, while the Tg determination using NMR could be based on polymer motions at the microscopic level.

Mobility of Starch Chains

Food mechanical and rheological properties often depend on the flexibility of macromolecular networks. In such networks, the rates of making and breaking of network bonds, and the flexibility of intervening molecules or their segments are important (Morris, 1990). Chain segments near covalent cross-links, in crystallites, or in associated chain forms are expected to be relatively immobile or even rigid.

The mobility of macromolecules including starch molecules has been determined by several types of NMR techniques and its relationship to physical and functional properties have been studied. Callaghan et al. (1983), using ^{13}C NMR spectroscopy, determined the mobility of starch molecules was high when present in fresh pastes, dilute gels, or dissolved starch. The experiment consisted of a single short pulse at the carbon frequency and low-power irradiation at the proton frequency to eliminate ^1H - ^{13}C scalar coupling. To obtain ^{13}C signal from solid domains, line-narrowing techniques such as magic angle spinning or high-power ^1H decoupling were employed. However, such experiments are not routine.

Wu and Eads (1992 and 1993) quantified the amount and relative rigidity of immobilized starch chains in starch gels and granule states using high resolution ^1H cross relaxation NMR and wide-line NMR. They noticed the changes in amount and molecular dynamics of solid and semisolid domains of gelatinized starch during ageing.

In studies on caking phenomenon of powdered foods, Chung et al. (2000) reported that molecular motion of food solids, which was characterized by monitoring transeverse relaxation by one pulse sequence as a function of temperature, showed a strong relationship with caking phenomena.

Water Mobility

Water profoundly affects the textural, chemical, structural properties and stability of food systems. Water in food can be described by three properties or parameters: water content, water activity, and water dynamic mobility (Li et al., 1998).

Water content has been a universal parameter in relation to biological, chemical, and physicochemical reactions, because of its straightforward meaning and ease of determination. However, water exists in different states in biopolymers such as foods. It is thought that water molecules in different states function differently.

Water activity (a_w) has been used extensively as an empirical measure of the degree of water “availability” (Bone et al., 1975; Franks, 1982). It has been widely used as a primary guideline for the quality and safety control of food. In recent years, the theoretical and practical limitation of a_w have been suggested by several researchers (Franks, 1991; Slade and Levine, 1991; van der Berg and Bruin, 1981). The measurement of a_w of a food system is based on the assumption that it is in an equilibrium state, which is true only for infinitely dilute systems. Most food systems are usually non-equilibrium systems, and during processing and storage, brought into a state of thermodynamic instability. Therefore, a_w concept rooted in equilibrium thermodynamics cannot fully represent most food systems. Another defect of the approach 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. It has been suggested that water activity is not the parameter that directly affects food stability (van den Berg and Bruin, 1981; Slade and Levine, 1991; Franks, 1982). Rather, it has been recognized by many that food properties are more related to the dynamic molecular mobility “state” of water in foods (van den Berg and Bruin, 1981).

The dynamic behavior of water has been studied using nuclear magnetic resonance (NMR) spectroscopy (Hills et al., 1989, 1990; Belton et al., 1991, Yakubu et al., 1990, 1991, 1993; Chinachoti, 1993; Chinachoti et al., 1991). The slowing down of water motion at low water content has been reported to be associated with “bound” water arising from hydrogen bonding (Leung et al., 1983; d’Avignon et al., 1988). In terms of water molecular sorption, monolayer and multilayer water have been attributed to bound water, but they are poorly defined without a direct measurement of the molecular dynamics. In a higher moisture system, water is expected to move freely and undergo rapid motion, with a rotational correlation time τ_c at a picosecond scale (Bryant and Shirley, 1980; Bryant, 1987). Bound water in food polymers was often characterized by its NMR spin-spin relaxation time (Steinberg and Leung, 1975; Nagashima and Suzuki, 1984; Richardson 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, 1983)

Pulsed ^1H Nuclear Magnetic Resonance (NMR)

The use of nuclear magnetic resonance (NMR) spectroscopy has seen an increase in the field of food science. NMR spectroscopy is based on the magnetic properties of atomic nuclei. Any nucleus, such as ^1H , ^2H , ^{13}C , ^{15}N , ^{17}O , ^{23}Na , ^{31}P , and ^{39}K , with a non-zero quantum number ($I \neq 0$), when placed in a magnetic field, can absorb and emit energy through electromagnetic radiation, which can be detected by NMR spectroscopy. The ^1H nucleus, is the most commonly used in NMR, primarily because of the ease of observation and its high natural abundance in biological samples. In pulsed NMR, a sequence of radio frequency (rf) pulses, which are turned on for only a short time, is used to generate the magnetization signal. When the rf field is directed perpendicular to the main magnetic field (M_0), it will rotate the magnetization vector away from the rest state. The rf field is turned on long enough so that the magnetization vector is rotated 90° , or any degree of angle as needed. After the rf pulse is turned off, the rotated magnetization vector will return back to its rest state, a process commonly termed “relaxation”.

The relaxation behavior of the magnetization is dependent on the chemical and physical environments surrounding the nucleus. Thus, the relaxation decay is a fundamental property of the sample (Ruan and Chen, 1998a and Ablett, 1992). Therefore, pulsed NMR can be used to clarify the physical structure of food by analyzing NMR decay signals. The NMR decay rate is related to dynamics of the nucleus at molecular levels (molecular mobility), and the signal intensity is proportional to the number of nuclei present (Ablett, 1992).

Relaxation of the magnetization is characterized by two processes, namely, spin-lattice or longitudinal (T_1) relaxation and spin-spin or transverse (T_2) relaxation. Spin-

lattice relaxation will result in the restoration of the longitudinal component of the magnetization (M_z). Since magnetization along the z axis is difficult to detect by using a single 90° RF pulse, T_1 can be determined by running multiple 180° - τ - 90° sequence with varying delay time (τ). Spin-spin relaxation describes the decay of the transverse component of magnetization (M_{xy}) caused by a 90° RF pulse. The decay curve is normally termed “free induction decay” (FID). Due to inhomogeneity of magnetic field in low resolution NMR, the Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence, which is 90_x -(t - 180_y - τ -echo) $_n$, is also used to determine spin-spin (T_2) relaxation (Luan and Chen, 1998). The relaxation decays determined by single pulse (90°) sequence is useful to obtain information about the fast decay component, typically solid component, while those determined by the CPMG sequence give information about relatively slow decay components (liquid-like components) (Fullerton and Cameron, 1988).

Proton relaxation is normally exponential, and the relaxation time constants can be determined from the relaxation decay curve using mono-exponential, multi-exponential (discrete spectrum), or distributed exponential (continuous spectrum of relaxation) fitting model (Luan and Chen, 1998c). The mono- or multi-exponential fitting model has been able to provide some useful information, but are over-simplified. Distributed exponential fitting model based on the “Contin” algorithm (Provencher, 1982), may describe best the relaxation behavior in complex systems because solid and water molecules may exist in an infinite number of states in heterogeneous food systems. However, there may be dominant states as governed by the physical and chemical structure of the system. The relaxation time or relaxation rate of both spin-lattice and

spin-spin relaxation are related to mobility of the molecules in the system. In general, a longer relaxation time indicates a high mobility of the observed molecules.

NMR has been used to characterize hydration properties of starch in terms of total water content, water binding capacity, starch chain mobility water mobility, gelatinization and retrogradation, and structural properties of starch in the granule and gel state, through the analysis of FID, T_1 , and T_2 curves (Brosio and Nola, 1982; Ruan and Chen, 1998; Le Botlan et al., 1998; Mousseri et al., 1974; Wu et al., 1992; Wu et al., 1993; Kalichevsky et al., 1992; Ruan et al., 1996, 1999; Farhat et al., 1996; Farhat et al., 2000; Blanshard et al., 1986; Li et al., 1998; Yakubu et al., 1993; Hills et al., 1990; Bogarcheva et al., 2001; Tang et al., 2000; Ohtsuka et al., 1994).

The determination of moisture content by pulsed ^1H NMR is based on the fact that the NMR proton signals from the solid and liquid components in a system, following a 90° pulse of exciting radiofrequency radiation, decay at significantly different rates. The spin-spin relaxation time (T_2) for protons in the solid phase is in the range of tens of microseconds due to the rigid molecular lattice which facilitates energy transfer while that in the liquid phase is very much longer in the range of milliseconds. This well-known principle has been also applied in the oils and fats industry for determination of solid fat contents.

Tang et al (2000) investigated the distribution and dynamic state of water in native potato, maize, and pea starch granules, through analysis of water proton relaxation determined by T_2 CPMG experiments and deuterium lineshape. They found that in A-type (cereal starch) maize starch there was only one intragranular population of water, whereas in B-type (tuber starch) potato starch there were two intra-granular water

populations. This is because that the smaller granule size in A-type starch permits faster exchange averaging of water between the amorphous growth rings and semi-crystalline lamella. It leads to one population of water within the starch granule. On the other hand, one population in B-type starch (potato) was assigned to water in amorphous regions and another to water in amorphous regions within the semi-crystalline. They suggested that NMR water proton relaxation and deuterium lineshape analysis can provide a simple method for measuring water distribution inside native starch granules and for distinguishing A, B, and C type starches.

Kalichevsky et al. (1992) in studies on the glass transition of amorphous amylopectin containing water content of 10 to 22% (w/w) as a function of temperature observed a small discontinuity in T_1 or T_2 as the material passes from the less mobile glassy state to the more mobile rubbery state, due to increase in segmental and molecular mobility of the system.

Using time domain NMR spectroscopy, Le Botlan et al. (1998) determined bound water in wheat starch suspensions with moisture contents in the ranges of 0.14 to 0.95g water per g starch. They suggested that there was constant bound water content of 0.17g. Water content in the range of 0.17 to 0.42 was weakly bound, and acted as “potentially free” water.

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CHAPTER III
¹H NMR STUDIES OF MOLECULAR MOBILITY IN LOW MOISTURE
STARCH¹

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ABSTRACT

Pulsed ^1H NMR was used to determine moisture content and to study molecular mobility of wheat starch suspensions at low moisture content. Dry samples were equilibrated to water activity values between 0 and 0.93. Moisture determination by NMR agreed well with gravimetric determinations ($R^2 = 0.99$). Transverse proton relaxation (T_2) of the systems were identified using distributed exponential routine from one pulse and Carr-Purcell-Meiboom-Gill (CPMG) experiments. The relaxations were resolved into different time domains in the range of μs and ms . Proton relaxation associated with starch molecules increased linearly from 7.1 to 10.1 μs as a_w increased from 0 to 0.93, suggesting increased mobility due to plastisizing effects of water. The proton relaxation associated with water molecules showed a linear increase up to a_w of 0.69 and deviation from linearity above a_w of 0.81. Such deviation may be attributed to enhancement of relaxation by exchange between water molecules in different states. Results indicated that NMR technique could be employed to determine moisture content of starch suspension and be used to identify and quantify the molecular motion of starch chains and water molecules of starch suspensions at low moisture levels.

INTRODUCTION

Starch is an important plant polysaccharide used to thicken fluid foods, provide gelling in semi-solid foods, and to help maintain stable emulsions while also serving as a source of complex carbohydrate (Luallen, 1985). The physical and structural properties of starch are strongly dependent on molecular interactions with water. Such interactions with water are complex, as water serves as both a solvent and plasticizer, and is involved in regions of hydration. Several macroscopic phenomena associated with the uptake of water in dry starches have been studied. The gelation of starch is the most obvious, and involves the uptake of water, and subsequent disruption of starch granules. However, the pasting properties and absorption of water by starch granules are also of interest. For example, absorption isotherms have been developed for several starch varieties (Boki and Ohno, 1991). The amount of water “bound”, immobilized, or unavailable to freezing has also been studied by a variety of techniques (Leung and Steinberg, 1979; Li et al., 1998)

The structure of starch granules has been studied by electron microscopy, x-ray diffraction, polarized light microscopy, differential scanning calorimetry, and neutron scattering (Bogracheva et al., 1998; Gallant et al., 1997). The arrangement and mobility of water within starch granules has been more difficult to study. Time-domain nuclear magnetic resonance has been useful for understanding water relations in starch. Based on the observation of multiple relaxation times, early studies indicated that more than one population of water existed in starch (Lechert, 1976; Mousseri et al., 1974; Leung et al., 1979). These have routinely been interpreted in terms of water mobility or “bound” water. Using ^2H and ^{17}O NMR spectroscopy, Richardson et al observed different regions

of water mobility in cornstarch for starch concentrations between 10 and 70% (1987a) and between 71 and 96% (1987b). Le Botlan et al. (1998) determined bound water in wheat starch suspensions with moisture content between 0.14 and 0.95g H₂O/g starch. They suggested that there was a constant bound water content of 0.17g H₂O/g starch and “potentially free” water in the range of 0.17 to 0.42 H₂O/g starch.

Recent studies have provided more refined models of moisture distribution in starch, and have addressed the issues of polymer mobility and the role of water in plasticizing food polymers. This has coincided with current interest in how molecular mobility of food polymers influence diffusion-limited properties of foods (Fennema, 1996). CPDAS solid state NMR has distinguished crystalline and amorphous “rubbery” regions in starch (Morgan et al., 1995). Kulik et al (1994) used 2-D solid state NMR to study short-range interactions of water with starch. Liquid state ¹H NMR techniques have also proven useful. Tang et al. (2000) used ¹H NMR relaxometry and diffusometry to show that at least 3 water populations exist in water-saturated beds of potato starch granules, and assigned these to amorphous growth regions, semi-crystalline lamella, and “channel” water. These authors also measured the fast-decaying components in free induction decay experiments, and showed these to be associated with starch molecule mobility. Kou et al. (2000) used wide-line ¹H NMR to study molecular mobility in cornstarch at 10-25.6% moisture, and found that both T₂^{*} and T₂ components indicated decreasing mobility with increased starch concentration. They also found that increased mobility was associated with the onset of transitions from the glassy to rubbery state. Ruan et al. (1999) have made similar observations based on ¹H NMR analysis of starch hydrolysates.

There are still relatively few reports in which both moisture and polymer mobility have been studied in intact starch granules, particularly in wheat starch. In this paper, we report on ^1H NMR studies of molecular mobility in wheat starch after absorption of moisture to water activity levels between 0 and 0.93. Free induction decay (FID) measurements were used to assess total moisture. Mathematical analysis of rapidly relaxing components following single-pulse experiments was used to assess polymer mobility. To limit the effects of field inhomogeneity on relatively slowly relaxing protons on water, a CPMG spin echo sequence was used to study water mobility.

MATERIALS AND METHODS

Sample preparations

Native wheat starch was obtained from Sigma Chemical Co. (St. Louis, MO), and dried at 35°C for 3 days under vacuum (660 mm Hg). Samples (approximately 1g each) were put into polystyrene weighing dishes (2 in.* 2 in., Fisher Scientific Co.) and further dehydrated in a vacuum dessicator over P₂O₅ for 7 to 10 days until a constant weight was attained.

The dehydrated samples were equilibrated at 25°C over various saturated salt solutions with known relative vapor pressures (RVP): P₂O₅ (0), LiCl (0.11), KCH₃CO₂ (0.23), MgCl₂ (0.33), K₂CO₃ (0.43), Mg(NO₃)₂ (0.53), KI (0.69), (NH₄)SO₄(0.81), and KNO₃ (0.93). Equilibrium moisture content (% db) was calculated from the weight gain after no further change in weight occurred.

NMR Measurements

Proton relaxation measurements were made using a 20 MHz NMR spectrometer (Resonance Instruments, Whitney, UK). Equilibrated samples (approximately 0.5g each) were placed into glass vials (50mm height* 10mm diameter) and returned to the relative vapor pressures (RVP) an additional 24 h prior to analysis. The vials with samples were placed in 18mm diameter NMR tubes which were then covered with parafilm to limit moisture loss. All measurements were made immediately at 25°C ± 0.1

FID measurements. Free induction decay curves were measured using a 90° pulse of 3.8 µsec. The transverse NMR signals were collected every 1µs for a total of 512 data points. A total of 8 scans were taken with a recycle delay (RD) of 2 s. Moisture content of each sample was calculated as described by Ruan and Chen (1998):

$$\% \text{Moisture} = f \frac{S_{70}}{S_{10} - S_{70}} \times 100 \quad (1)$$

where S_{10} is the signal at $t=10 \mu\text{s}$ (due to all protons) and S_{70} is the signal at time $70 \mu\text{s}$ (after the contribution from solid protons had decayed).

As data from the single-pulse experiment could be collected over the µs domain, the rate of decay of rapid components was used to measure mobility of starch side chains. These were separated from other components by using a distributed exponential routine (WinDXP, Resonance Instruments, Whitney, UK), and assuming the FID is best fit by a Gaussian model (Ruan and Chen, 1998):

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

where g_i are the values of the exponential distribution at time t_i , f_j are the pre-exponential weighting factors, and $T_{2,j}^*$ are the time constants. Based on the “contin” routine of Provencher (1982), the DXP routine calculates a distribution of T_{2}^* terms that best describe the data. We use the term T_{2}^* as the single-pulse experiment does not limit relaxation due to magnetic field inhomogeneity, while subsequent CPMG sequences used to assess water mobility do. For rapidly decaying components, however, T_{2}^* approaches T_2 , the true transverse relaxation time constant (Fullerton and Cameron, 1988; Ruan et al,

1999). In general, distributed analysis showed one or more peaks describing regions with similar time constants. Curves derived from various samples were compared as to time at maximum peak height and peak width. In addition, the area under a given peak was integrated to estimate the proportion of protons in a given mobility region.

CPMG relaxation measurements.

Transverse relaxation (T_2) time in the ms time domain was identified using the Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence: $90x-(\tau-180y-\tau\text{-echo})n$ (Meiboom & Gill, 1958). The 90 to 180 pulse spacing (τ) was set to 50 μ s. Relaxation curves obtained from the CPMG sequence were analyzed as described above using a distributed exponential routine (WinDXP, Resonance Instruments, Whitney, UK).

Statistical analysis

The data were analyzed using the SAS program (SAS Institute, INC., 1990). Analysis of variance (ANOVA) was used to determine the significance of variable effects on mobility of both starch chain and water molecules. Significance of difference was defined as $p < 0.05$.

RESULTS AND DISCUSSION

Moisture content by FID

Figure 1 shows the normalized free induction decay (FID) curves for wheat starch suspensions at various a_w values from 0 to 0.93. The dry basis moisture content of samples equilibrated at various a_w values was calculated using Equation 1. A comparison of moisture content determined by NMR and gravimetric methods is shown in Figure 2, and shows that the methods were well correlated ($R=0.99$) with a coefficient of $f = 0.676$. In general, moisture content based on FID measurement had lower standard deviation, indicating that this technique may be very useful for measurement of moisture in low moisture starch systems.

Figure 3 shows adsorption isotherms at 25°C for the wheat starch suspensions. The data showed a typical Type II isotherm and was well-fit by the GAB model (Bell and Labuza, 2000), with $k = 0.753$, $c = 17.513$, and a “monolayer” moisture content of $m_o = 8.617$ gH₂O/100 g dry starch.

Transverse relaxation from single-pulse experiments

The transverse relaxation data determined after a single 90° pulse (Figure 1) was analyzed using a distributed exponential routine (Figure 4). In general, the transverse relaxation data was well fit with a distributed exponential model; standard errors for such fits were 0.01 or less. For all samples, except that at $a_w = 0$, the distributed exponential fit showed two distinct components. A rapidly decaying component with maximum time constant (T_{2s}^*) in the range 7 to 10 μ s, and a more slowly decaying component with

maximum time constant (T_{2m}^*) in the range 160 to 300 μs . Components decaying within 40-60 μs are characteristic of nuclei associated with biopolymers (Rugraff et al., 1996; Le Botlan and Helie-Fourel, 1995). For the sample equilibrated at $a_w = 0$, only one rapidly decaying component was observed. This suggests that T_{2s}^* is primarily associated with the relaxation behavior of protons from starch molecules, while T_{2m}^* is associated with water protons and exchangeable $-\text{OH}$ groups. This hypothesis is further supported by the observations on the areas under the T_{2s}^* curves, and the areas under the T_2 curves obtained from the CPMG pulse sequence (see following section). That is, the area (I_{2s}^*) under the T_{2s}^* component was taken as a measure of total starch, while the area (I_2) under the T_2 curve obtained by the CPMG sequence was taken as a measure of total moisture. Figure 5 shows that the ratio of the integrated signal intensity I_2 / I_{2s}^* was highly correlated with the moisture to solids ratio ($R^2=0.95$).

Tang et al (2000) found components at approximately 20 μs and 80 μs for saturated beds of corn starch granules. As the peak at 80 μs disappeared upon freezing, they attributed this peak to residual HOD, while the peak at 20 μs was attributed to $-\text{CH}$ groups on starch. Ruan et al. (1999) studied maltodextrins, and found that the T_{2s}^* component was relatively constant (ca 12 μs) at temperatures below the glass transition temperature (T_g), but steadily increased to higher values at $T > T_g$. Kalichevsky et al (1992) observed similar results with amorphous amylopectin. Chung et al (2000) found that the changes in T_{2s}^* versus temperature for several food powders were highly associated with physical changes, including agglomeration, water vaporization, and caking behavior.

In our studies, we found that T_{2s}^* increased from 7.1 to 10.3 μs as the water content increased from 0 to 28.86 gH₂O/100 g dry starch ($a_w = 0$ to 0.93). This is seen in Figure 3 in which the relaxation rate, given by $R_{2s}^* = 1/T_{2s}^*$, is plotted versus a_w .

R_{2s}^* decreases steadily as a_w increases, suggesting greater mobility of starch protons with increased moisture. Even with no moisture, there is evidence of starch mobility. The increase in T_{2s}^* (decrease in R_{2s}^*) with moisture can be attributed to the plasticizing effects of water on starch. Water is known to increase the molecular mobility of many biopolymer, in particular by increasing the effective free volume for polymer motions (Levine and Slade, 1986; Roos and Karel, 1990; Zeleznak et al., 1987). For example, Zeleznak and others (1987) used DSC to show that the glass transition temperature (T_g) decreased from 90 to 25°C as water content increased from 13 to 22%. While both T_g as measured by DSC, and transverse relaxation as measured by T_{2s}^* , give measures of mobility, it is not entirely clear what the specific motions are that each method emphasizes. Tang et al (2000) concluded that T_{2s}^* was primarily dependent upon motions in amylose-rich amorphous regions.

It is of interest that there were no obvious transition in R_{2s}^* as a function of a_w or moisture content. DSC measurements of glass transition temperatures indicated that all of samples, except the sample at $a_w=0.93$, were in the glassy state. The sample at $a_w=0.93$ (28.86 gH₂O/100 g dry starch) had a T_g of 19.2°C. As samples were analyzed at $T=25^\circ\text{C}$, the difference $T-T_g$ was evidently not large enough to cause a major increase in starch mobility.

The relaxation behavior of the more mobile component (T_{2m}^*) was associated with water, and in general the integrated signal intensity (I_{2m}^*) of this component increased with moisture content. However, this more mobile component is susceptible to decay through field inhomogeneity, and several researchers have noted that it is not a reliable measure of water mobility (Botlan and Helie-Fourel, 1995; Tang et al., 2000; Kalichevsky et al., 1992). Thus, we performed CPMG experiments on all samples to study transverse relaxation (T_2) in the millisecond domain.

Transverse relaxation from CPMG experiments

To further investigate transverse relaxation behavior of the more mobile components associated with water, the transverse relaxation data were also obtained using the CPMG sequence. The resulting curves were analyzed using a distributed exponential routine (Figure 6). From the CPMG measurements, only one major population of spins was observed. We found that it was important to set an appropriate 90-180 pulse spacing (τ) in order to obtain useful results. At $\tau > 100 \mu\text{s}$, proton relaxation was quite noisy or indeterminate for relatively low moisture samples (<10%), while at $\tau < 50 \mu\text{s}$, relaxation was affected by protons from the starch molecules.

For samples equilibrated at $a_w = 0$, no signal was detected, indicating that protons associated with starch molecules did not affect relaxation behavior determined by the CPMG sequence. The maximum relaxation constant T_2 increased (R_2 decreased) with increasing moisture content (Figure 3). The one exception to this was for samples equilibrated at $a_w = 0.81$, which had T_2 (0.93 ms) lower than those at either $a_w = 0.69$ ($T_2 = 1.67$ ms). In general, the change in T_2 , and presumably water mobility were greater

than those for T_{2s}^* . Richardson et al (1987) showed that for corn starch concentrations between approximately 25 and 240 g solids/mole D_2O , R_2 increased steadily from about 1 to 14 s^{-1} . They attributed this to a “Region III” moisture regime in which multilayer water is being formed. They modified the isotropic two-state model with fast exchange and proposed that the relaxation rates in this region were given by:

$$R_{2,obs} = P_B R_B + P_F R_F + P_M R_M \quad (3)$$

where R is the relaxation rate and P the fraction of water in each state, and the subscripts B, F, and R refer to “bound”, “free”, and “multilayer” water, respectively. Our results are similar to those of Richardson et al (1987) although in our case R_2 ranged from 0.4 to 6 s^{-1} (Figure 7). As we studied 1H rather than 2H nuclei, differences may be due to the chemical exchange of protons between water molecules in different states as typically observed with 1H NMR (Yakubu et al., 1993)

Whether the increase in R_2 with moisture is evidence for “bound” or “free” water is disputable. However, the fact that the processes have a single time constant regime (Figure 6), and that the T_2 values increase with moisture, indicate that an equation such as Equation 3 is operative. In other words, multiple regions of water are indicated, however the time required for water to diffuse between regions is short to T_2 .

CONCLUSION

The moisture content of starch suspension was discerned by FID measurements and agreed well with gravimetric determinations. The proton relaxation associated with starch molecules increased linearly from 7.1 to 10.1 μs as a_w increased from 0 to 0.93, suggesting increased mobility due to plasticizing effects of water. The proton relaxation associated with water molecules showed a linear increase up to a_w of 0.69 and deviation from linearity above a_w of 0.81. Such deviations may be attributed to enhancement of relaxation by exchange between water molecules in different states

The NMR data reinforce the concept of several regions of hydration of water molecules shown by similar measurements made on sorption isotherm curve and the concept of plasticization of water. In addition, results indicate that NMR technique could be powerful to determine moisture content of starch suspension and give useful information on molecular motion of both starch and water at low moisture levels.

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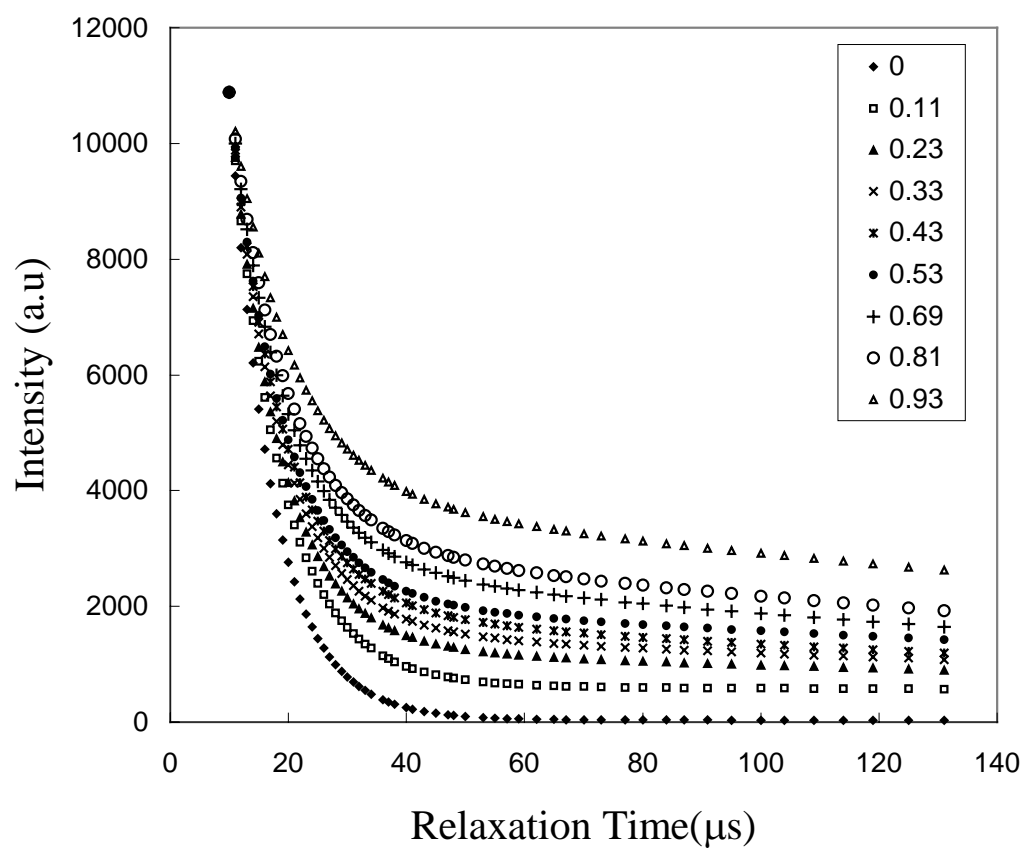


Figure 1- Normalized FID curves for wheat starch suspensions at different a_w .

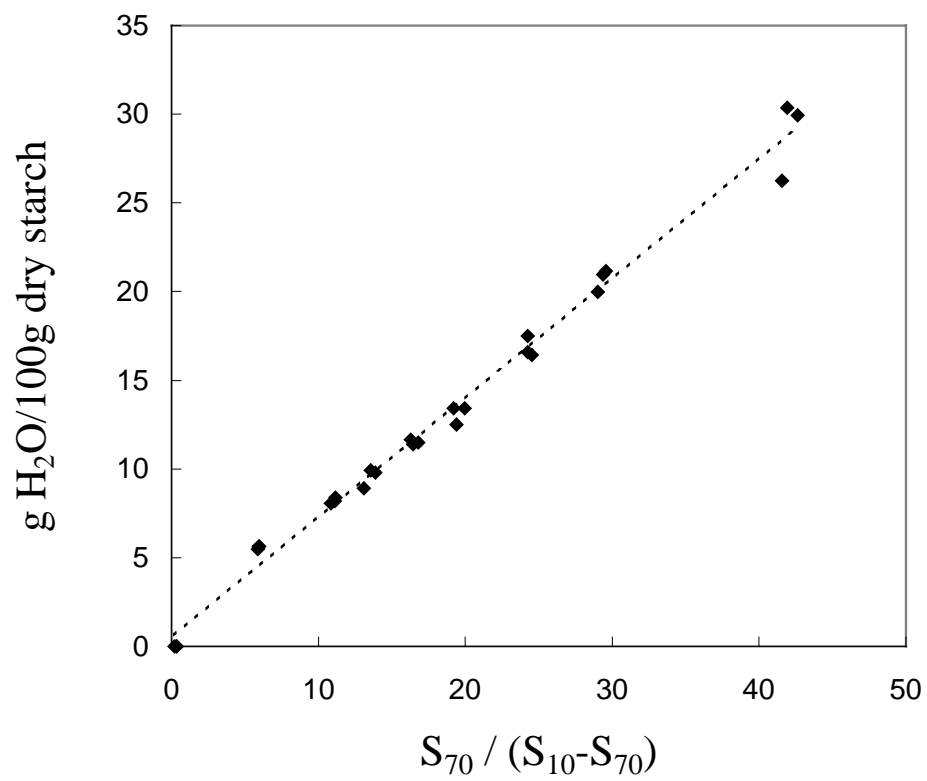


Figure 2- Correlation between gravimetric and NMR measurements for determining moisture content.

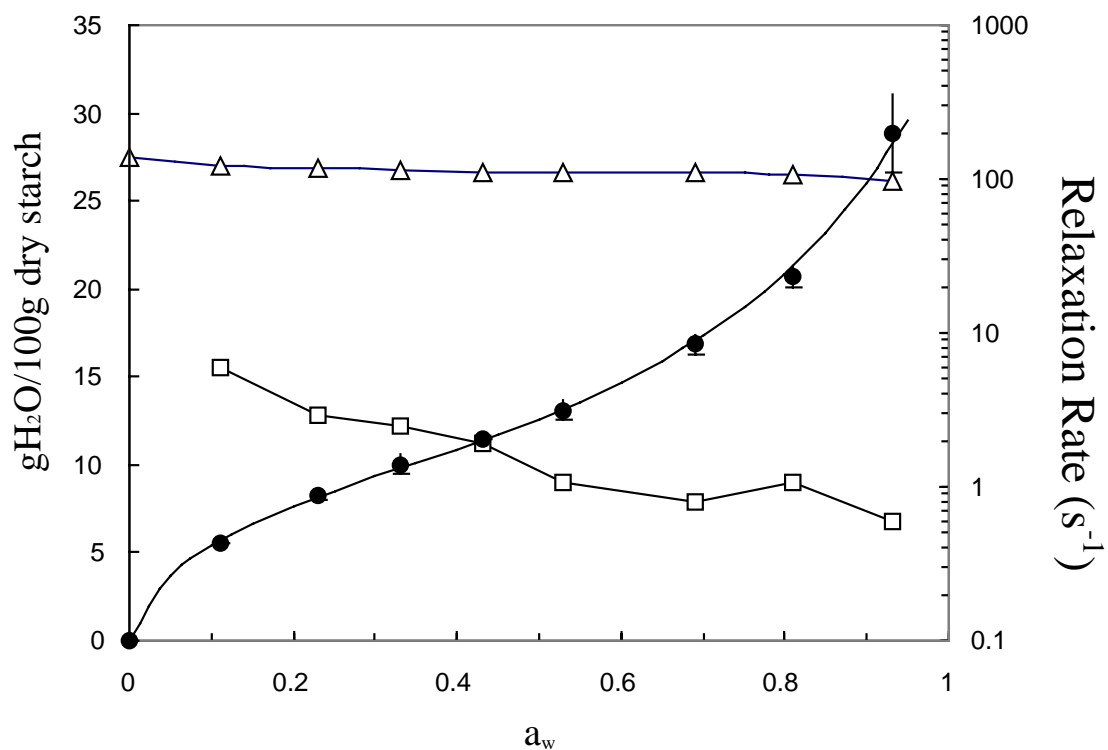


Figure 3- Moisture content and relaxation rate of starch suspensions as a function of a_w : ●-Moisture content, △-relaxation rate of solid-like component from single pulse sequence, and □-relaxation rate of proton from CPMG sequence.

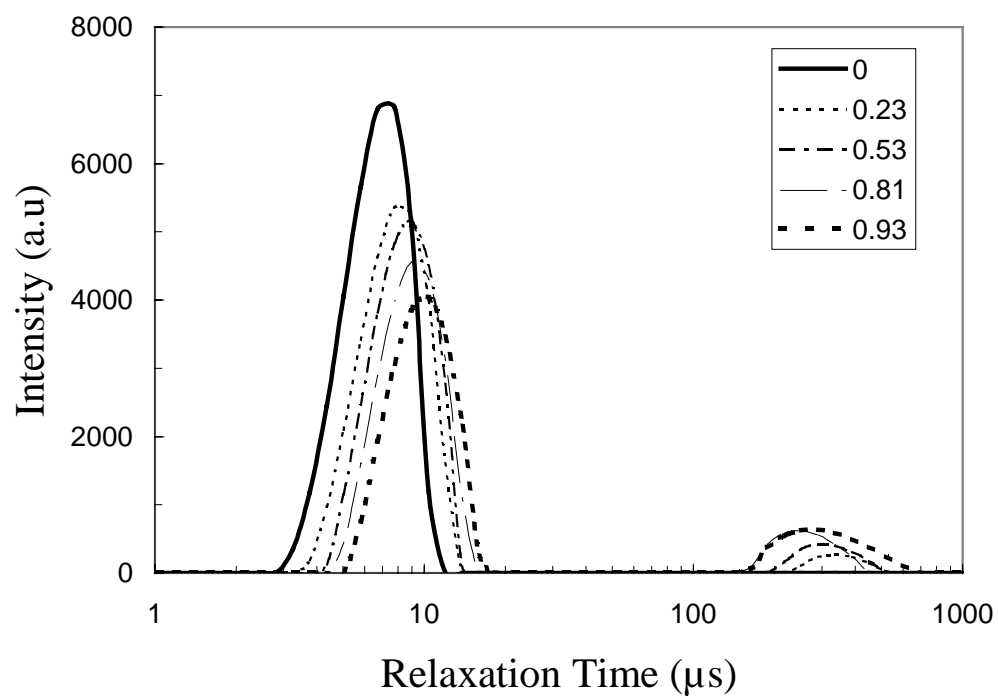


Figure 4- Distributed T_2 values curves for wheat starch suspensions measured by single-pulse sequence experiment.

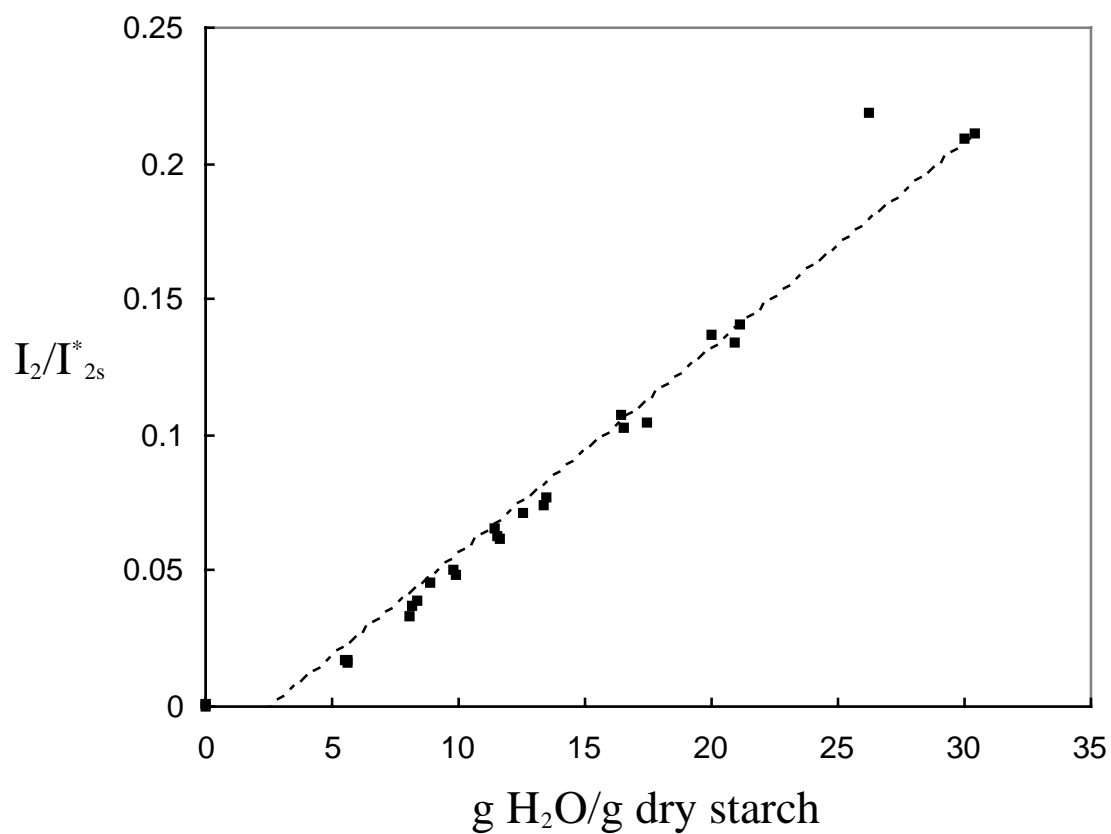


Figure 5- Correlation between the ratio of integrated signal intensity (I_2/I_{2s}^*) and the moisture to solid ratio.

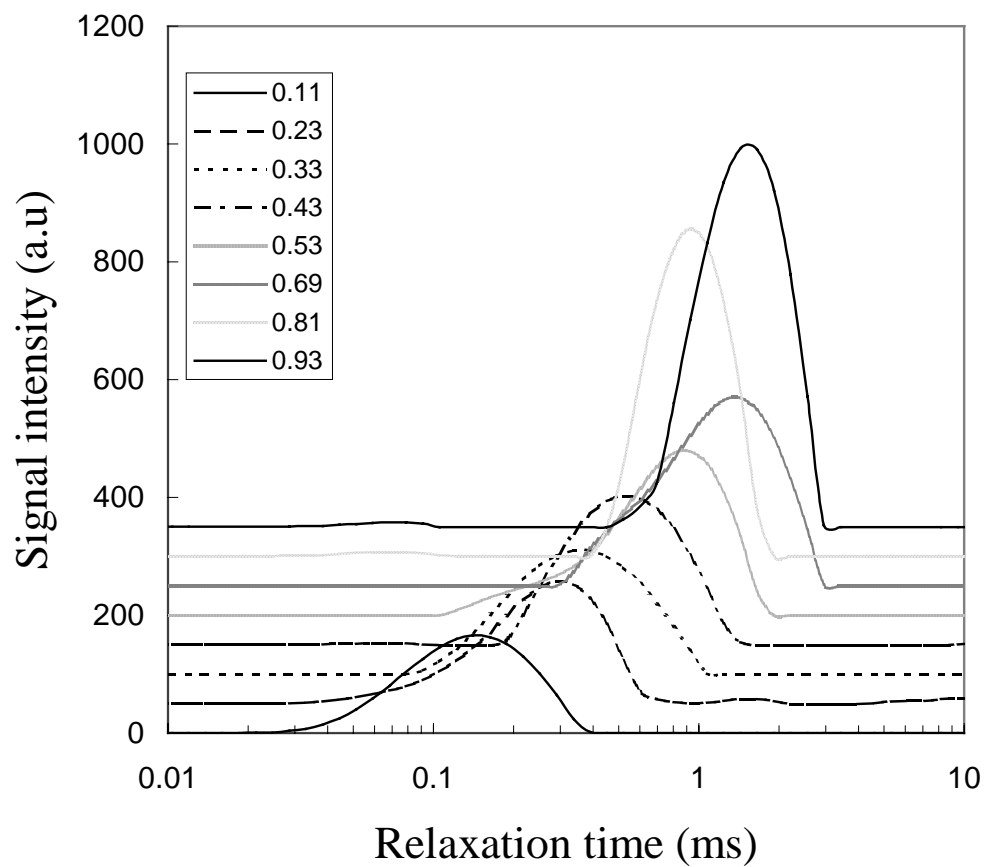


Figure 6- Distributed T_2 values for wheat starch suspensions measured by CPMG sequence experiment.

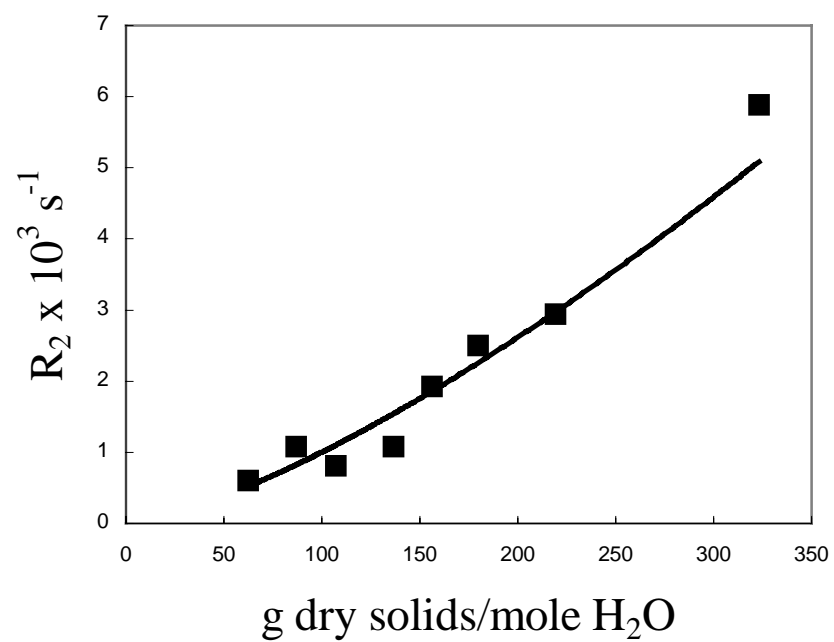


Figure 7- NMR relaxation rate from CPMG sequence as a function of moisture content.

CHAPTER IV
EFFECTS OF CHEMICAL MODIFICATION OF WHEAT STARCH ON
MOLECULAR MOBILITY AS STUDIED BY PULSED ^1H NMR¹

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ABSTRACT

The effects of hydroxypropylation and cross-linking on the molecular mobility of wheat starch suspensions at low moisture were investigated by a pulsed ^1H NMR. Wheat starch was hydroxypropylated with propylene oxide (MS of 0.05, 0.12, and 0.18) or cross-linked with phosphorus oxychloride in the concentration of 0.03%, 0.1%, and 0.2%, respectively. Starch samples were equilibrated over 0 ~ 0.93 a_w . Transverse proton relaxation (T_2) of the systems were obtained from single-pulse and CPMG sequence experiments and were analyzed by distributed exponential fitting. Proton relaxation associated with starch molecules increased with increasing molar substitution of hydroxypropyl group, indicating increased mobility of starch chains. Proton relaxation was not significantly affected by cross-linking. Transverse proton relaxation associated with water in control (alkali treated), hydroxypropylated and cross-linked starch showed two distinct populations in the a_w range of 0.53 to 0.93. In general, water mobility in both regions increased as a_w or hydroxypropyl MS increased, while cross-linking at 0.03% and 0.1% produced little change in water mobility.

INTRODUCTION

Starch is a major food biopolymer found in fruits, vegetables, and cereals. It is used in a variety of foods for gelling and viscosity enhancement, dusting and moisture control, as a molding agent, and as a basis for extruded products. The physical and structural properties of starch are strongly dependent on the interaction of starch molecules with water. For example, during gelation water helps swell the starch granule, extract soluble amylose, and hydrate interior crystalline amylopectin regions. In recent years, there has been increased interest in how water plasticizes food polymers and enhances molecular mobility (Levine and Slade, 1986; Roos and Karel, 1990; Rolee and LeMeste, 1999). The molecular mobility of water and food solids has been related to important diffusion-limited properties of foods (Fennema, 1996; Kou et al., 2000). It has also been recognized that molecular dynamics can influence the processability, product properties, quality, and safety of foods (Kou et al., 1999; Slade and Levine, 1991; Chung et al., 2000).

Chemical modification by hydroxypropylation or cross-linking has been used to improve the properties of native starches used in food. Chemically modified starches have markedly altered physicochemical properties, primarily depending on the degree of substitution and the type of functional group applied (Rutenberg and Solarek, 1984). Hydroxypropylation effectively retards retrogradation, decreases pasting temperature, increases water holding capacity, and improves the freeze-thaw stability of starch gels (Tuschhoff, 1986). Cross-linking of starch with phosphorous oxychloride has been used to improve resistance to high temperature, low pH and high shear, while providing a short

texture to cooked products. Cross-linking reinforces the hydrogen bonds in the granule with chemical bonds which act as bridges between molecules (Wurzberg, 1986).

Several techniques have been used to characterize the structure and dynamics of food polymers including X-ray diffraction, vibrational spectroscopy, differential scanning calorimetry (DSC), dynamic mechanical thermal analysis (DMTA), and nuclear magnetic resonance (NMR) spectroscopy (Farhat et al., 1996; Wu et al., 1992; Kalichevsky et al., 1992; Ablett et al., 1993; Ruan et al., 1999). Among them, NMR has been widely used to specify the rates and amplitudes of motions of food polymers and water molecules through study of the relaxation of specific nuclei (^1H , ^2H , ^{13}C , or ^{17}O) in both solid and liquid states.

NMR has been used to study several phenomena related to starch hydration including total water content (Brosio et al., 1978; Ruan and Chen, 1998); bound water capacity (Le Botlan et al., 1998; Mousseri et al., 1974); starch chain mobility (Wu et al., 1992; Wu et al., 1993; Kalichevsky et al., 1992); water mobility (Li et al., 1998; Yakubu et al., 1993; Hills et al., 1990); starch gelatinization and retrogradation (Cheetham and Tao, 1998; Ruan et al., 1996; Farhat et al., 1999; Wynne-Jones and Blanshard, 1986); and structure of starch in granule and gel states (Bogarcheva et al., 2001; Tang et al., 2000; Ohtsuka et al., 1994)

Several researchers have studied the dynamics of water and starch chains in unmodified starch granules. Richardson et al (1987a, 1987b) studied different regions of water mobility in cornstarch at concentrations between 10 and 96% solids. Le Botlan et al. (1998) measured “bound” water in wheat starch suspensions with moisture content between 0.14 and 0.95 gH₂O/g starch. Solid state NMR was used to distinguish crystalline and rubbery amorphous regions of starch (Wu et al, 1993), while Kulik et al (1994) used 2-D solid

state NMR to observe short-range interactions of water with starch. Liquid state NMR has also been used to study both water and starch chain mobility, through combinations of both single-pulse and multiple-pulse techniques. Tang et al. (2000) used pulsed ^1H NMR to show that three populations of water exist in water-saturated beds of potato starch granules. They were also able to use rapidly decaying components of single pulse experiments to monitor the mobility of starch chains. Kou et al. (2000) used wide-line ^1H NMR to study molecular mobility in cornstarch at 10-25.6% moisture. They also found that fast-decaying components were associated with starch, while more slowly decaying components were related to domains of water mobility.

Limited research work has been published on the influence of hydroxypropylation and cross-linking on the molecular dynamics of starch suspensions. The objective of this study was to investigate the effects of chemical modification by hydroxypropylation and cross-linking on molecular motion of both starch and water in low moisture wheat starch using NMR techniques.

MATERIALS AND METHOD

Chemical modification

Cross-linking of wheat starch with phosphorus oxychloride (POCl_3) was accomplished essentially as described by Wu and Seib (1990). Native wheat starch (Sigma Chemical Co., St. Louis, MO) was lyophilized for 48 hrs, and then kept over phosphorous pentoxide (Sigma Chemical Co., St. Louis, MO) to ensure maximum dryness. Dry sample (200 g) was suspended in 440 ml of distilled water containing 20 g Na_2SO_4 , then the pH adjusted to 11.3 with 1N NaOH. The mixture was placed in 500 ml screw cap jars and maintained at 35°C in a controlled temperature water bath. Aliquots (35 μl , 120 μl , or 240 μl) of phosphorus oxychloride (Acros Chemical Co., NJ) were added to the jars. The reaction proceeded for 2 hrs with agitation, and was terminated by adjusting the pH to 5.5 using 1 N HCl. The suspension was washed with distilled water and centrifuged several times for 15 min at 3200 g.

Hydroxypropylation of wheat starch was accomplished as described by Kim et al. (1992). Dry sample (200 g) was suspended in 440 ml of distilled water containing 20 g Na_2SO_4 , then the pH adjusted to 11.3 with 1N NaOH. The mixture was placed in 500 ml screw cap jars and maintained at 35°C in a controlled temperature water bath. Aliquots (8 ml, 16 ml, or 25 ml) of propylene oxide (Acros Chemical Co., NJ) were added to the jars. The reaction proceeded for 24 hrs with agitation, and was terminated by adjusting the pH to 5.5 using 1 N HCl. The suspension was washed with distilled water and centrifuged several times for 15 min at 3200 g.

Control wheat starch was prepared with the same reagents and procedure, but without addition of propylene oxide or phosphorus oxychloride. This was to ensure that any measured

changes were due to hydroxypropylation or cross-linking and not from effects of pH change or heating. The hydroxypropyl content was determined by the spectrophotometric method of Johnson (1969). The molar substitution (MS) was defined as moles of substituent per mole of anhydroglucose unit. The cross-linked wheat starch was designated as Xa, Xb, and Xc (Table 1) and the hydroxypropylated wheat starch was designated as Ha, Hb, and Hc.

Sorption isotherms

Modified starch samples were dried at 35°C for 3 days under vacuum (660 mm Hg). Triplicate pre-dried samples (approximately 1g each) were put into 2 inch hexagonal polystyrene weighing dishes (Fisher Scientific Co.) and further dehydrated in a vacuum desiccator over P₂O₅ for 7 to 10 days until no further loss in weight was measured.

The dehydrated samples were equilibrated in vacuum desiccators containing various saturated salt solutions of known relative vapor pressures (RVP) ranging from 0 to 0.93 at 25°C; P₂O₅ (0), LiCl (0.11), KCH₃CO₂ (0.23), MgCl₂ (0.33), K₂CO₃ (0.43), Mg(NO₃)₂ (0.53), KI (0.69), (NH₄)SO₄ (0.81), and KNO₃ (0.93). Equilibrium was assumed when there was no further change in weight. Equilibrium moisture content (% db) was calculated from the gain in weight.

NMR Measurements

Proton relaxation measurements were made using a 20 MHz ¹H NMR spectrometer (Resonance Instruments, Whitney, UK). Approximately 0.5g samples were removed from the isopiestic chambers and placed into glass vials (50mm height* 10mm diameter), then immediately returned to the chambers. After an additional 24 hour equilibration period, the

vials with samples were placed in 18mm diameter NMR tubes and then covered with parafilm. Transverse (T_2) relaxation curves were developed using both single-pulse and CPMG pulse sequences: $90_x-(\tau-180_y-\tau\text{-echo})_n$ (Meiboom and Gill, 1958). Acquisition parameters were set to a 90° pulse of $4.1 \mu\text{s}$ and a recycle delay of 2 s. The 90° to 180° pulse spacing (τ) was set to $50 \mu\text{s}$. All measurements were made at $25^\circ\text{C} \pm 0.1$. Relaxation curves obtained from both the single-pulse and CPMG sequences were analyzed using a distributed exponential routine. Based on the “contin” algorithm of Provenchar (1982), this routine calculates a distribution of T_2 terms that best describe the data:

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

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.

Statistical analysis

Seven treatment groups were considered in this experiment including control wheat starch (subject to reaction conditions but without propylene oxide or phosphorus oxychloride), 3 levels of hydroxypropylation, and 3 levels of cross-linking. Starch suspensions were prepared at nine different RVP. All samples were analyzed in triplicate. SAS software was used for data analysis (SAS Institute, INC., 1990). The

general linear model ANOVA was utilized to test for effects of treatments. Significance of difference was defined as at $p < 0.05$.

RESULTS AND DISCUSSION

Sorption isotherms for hydroxypropylated and cross-linked starch

Moisture sorption isotherms of control, hydroxypropylated, and cross-linked starch at 25°C are shown in Figure 1a and 1b. No significant differences existed between any of the starch samples at $a_w < 0.53$. For hydroxypropylated starch at $a_w > 0.53$, the equilibrium moisture contents were significantly higher than for control starch or any other samples at equivalent a_w . Such increase in water sorptive capacity of hydroxypropylated starch at higher a_w was more pronounced with increasing molar substitution. The higher water sorptive capacity may be attributed to structural changes in starch granules by hydroxypropylation. Kim et al. (1992) observed changes in the granular structure of hydroxypropylated potato starch with varying molar substitution (M.S) using light microscopy, and found that hydroxypropyl groups were mainly distributed in the central region of the starch granules, and specifically in amorphous regions. The bulky hydroxypropyl groups decrease hydrogen bonding between starch chains and increase starch chain flexibility (Seow and Thevamalar, 1993). Presumably, this more open structure allows more water within the interior of the granule.

In contrast, 0.03% and 0.1% cross-linked starch showed no significant difference in water absorptive capacity at all a_w ranges as compared to control starch. However, 0.2% cross-linked starch had slightly decreased moisture at $a_w > 0.43$ as compared to control starch ($p < 0.05$). Most cross-linking reactions produce a relatively low degree of cross-linking sufficient to strengthen the outer envelope of starch granules (Wurzberg, 1995). Cross-linking at levels less than 0.1%, although having a substantial effect on properties such as granule swelling, paste viscosity, and retrogradation (Rutenberg and

Solarek, 1984; Inagaki and Seib, 1992; Reddy and Seib, 1999), may actually contribute little to water sorption properties (Chilton and Collison, 1974)

Transverse relaxation from single-pulse experiments

Transverse relaxation decay curves for control and modified starch as a function of a_w were obtained using a single-pulse free-induction-decay (FID) experiment. Typical distributed relaxation curve for control starch is shown in Figure 2. For all samples except for those equilibrated at $a_w = 0$, the distributed fits showed two distinct regions: one region had relaxation times in the range 7 to 20 μs and a peak maximum denoted by T_{2s}^* ; the other had relaxation times in the range 130 to 180 μs and peak maximum denoted by T_{2m}^* . While the FID decays through both spin-spin and other processes, it has been shown that for rapidly decaying components T_2^* approaches T_2 , the true transverse relaxation time constant (Fullerton and Cameron, 1988; Ruan et al, 1999). Samples equilibrated at $a_w = 0$, showed only a single component with a peak T_{2s}^* around 7-8 μs . Typically, rapid transverse decay (short T_2) is associated with relatively slow motion of biopolymers, and others have shown that the T_{2s}^* component is associated with starch segmental mobility (Tang et al., 2000; Fullerton and Cameron, 1988; Ruan et al, 1999; Rugraff et al., 1996). The fact that T_{2s}^* was still present in the absence of water supports this. The component described by T_{2m}^* is more likely associated with water (Tang et al., 2000; Fullerton and Cameron, 1988). However, as the decay in this region is more affected by field inhomogeneity, a CPMG pulse sequence was subsequently used to study this component.

In Figure 3, T_{2s}^* is plotted as a function of water activity. For all samples, T_{2s}^* increased with moisture content, indicating increased mobility of starch. This fits with our current understanding of water as a plasticizer of food biopolymers (Levine and Slade, 1986; Roos and Karel, 1990; Rolee and LeMeste, 1999). That is, water increases the free volume in which starch segments are free to move. The curves showed three general regions: rapid increasing T_{2s}^* over a_w 0-0.11; a more moderate increase over a_w 0.11-0.80; and a rapid increase at $a_w > 0.80$. Using ^2H and ^{17}O NMR relaxometry, Richardson et al. (1987) showed two regions of water in corn starch powders. Region B, at a_w between 0.11-0.23, showed rapid change in relaxation rates (that is, $1/T_2$), while region A, at a_w between 0.23 and 0.99, showed more gradual change in relaxation rates with a_w . They concluded that Region B is associated with “monolayer” water. Kou et al (2000) showed three regions when the percentage of mobile protons in corn starch, as measured by wide-line ^1H NMR spectra, were plotted versus water activity.

It is important to point out that our results are measurements of polymer mobility, while those of Richardson et al (1987) and Kou et al (2000) are of water mobility. However, the two types of motions are not independent. Water serves as the milieu in which starch chains are free to move. It has been well-documented that the amount of small molecular weight solvent determines the motional frequencies of polymers (Levine and Slade, 1986; Roos and Karel, 1990; Zeleznak et al., 1987). Our results also indicate that the motional freedom of the solvent also influences the motional freedom of the polymer. That is, the change in mobility of starch varied with moisture content and showed regions of change that were similar to changes in moisture mobility.

Figure 3a also shows the T_{2s}^* values for hydroxypropylated starch as a function of water activity. In general, hydroxypropylated starches had greater T_{2s}^* values than control starch at the same a_w , and the extent of the increase was greater with increasing MS of hydroxypropyl groups. This indicates that the mobility of starch chains increases with hydroxypropylation. These results are consistent with studies of hydroxypropylated starch using differential scanning calorimetry. Seow et al (1993) found that hydroxypropylated rice starch had lower transition temperatures and broadened endotherms associated with glass transitions and crystallite melting. They suggested that hydroxypropyl groups provide internal plasticization of starch in amorphous regions. This was attributed to the increased motion of side chains, and in particular, those containing flexible hydroxypropyl pendant groups. Using microphotography, Kim et al (1992) showed that hydroxypropylation occurs primarily in the central amorphous regions of the starch granule. It is important to note that this increased mobility is coincident with increased moisture content at a specific a_w .

Figure 3b shows T_{2s}^* values for cross-linked starches as a function of a_w . For cross-linked starches, there were no significant differences in T_{2s}^* values as compared to control starch. This indicates that cross-linking has little effect on internal motions of starch chains. Cross-linking with POCl_3 occurs to a lesser extent than does hydroxypropylation. In addition, Hood et al (1974) showed that cross-linking occurs only at the very outermost regions of the starch granule.

Transverse relaxation from CPMG experiment

Starch samples held at different a_w were also studied using CPMG techniques. A pulse spacing (τ) of 50 μs was chosen, so as to exclude the fast decaying component found in the single-pulse experiments. Distributed exponential analyses of the data are shown in Figure 4. ^1H relaxation in the millisecond region has been shown to be associated with water. In general, all T_2 values were less than 10 ms. As the T_2 of pure water was measured as ~ 2.0 s, these relatively short T_2 values indicate that water is greatly affected by interactions with starch. No peak was observed for samples at 0 a_w . This also shows that for moisture contents up to 30-35 g $\text{H}_2\text{O}/\text{g}$ dry starch, the time required for any domain of water to diffuse to the starch surface is short compared to T_2 .

For untreated (native) starch, only a single peak was observed. The peak T_2 values are plotted in Figure 4a as a function of a_w . In general, T_2 increased with moisture, ranging from 0.141 ms at 0.11 a_w (5.37 g $\text{H}_2\text{O}/\text{g}$ dry starch) to 1.502 ms at 0.91 a_w (28.8 g $\text{H}_2\text{O}/\text{g}$ dry starch). However, at a_w of 0.81 T_2 was lower than at a_w of 0.91, and not significantly different than that at 0.69 a_w . Zobel (1988) showed that wheat starch has an A-type powder x-ray diffraction pattern. Tang et al (2000) found that water-packed beds of A-type starch have only a single water population discernible by ^1H NMR. In contrast, type-B starch shows up to three water populations associated with amorphous growth rings, semi-crystalline lamella, and channel water, respectively. They concluded, that due to the smaller granule size, intra-granular water populations in type-A starch were in fast exchange, thus obscuring differences between different structural areas containing water.

The curves for control starch (that is, treated with Na₂SO₄/NaOH) and modified starches were different than those for untreated (native) starch. At $a_w < 0.43$, only a single distributed region was observed, while at $a_w > 0.43$, two regions were observed, which we describe by the peak values T_{2a} and T_{2b} . For samples at $a_w > 0.43$, the rapidly decaying (T_{2a}) component had a T_{2a} value in the range of 0.091-0.252 ms (Figure 5), which is similar to the single T_2 value observed for samples at 0.11 or 0.23 a_w . No significant differences were observed between control or hydroxypropylated starch for the rapid decaying component. In contrast, the change in the slower decaying (T_{2b}) component was greater as moisture level increased. In this case, T_{2b} varied from 0.122-0.141 ms at 0.11 a_w to 2.97(control) - 3.97(hydroxypropylated) ms at a_w 0.91.

The presence of two decay regions suggests there are multiple domains of water in the starch granules. These are defined as regions in which spin-spin relaxation rates are distinct. To be distinguishable, the diffusion of water between domains must be slow compared to the spin-spin relaxation rate. At $a_w < 0.43$, all samples showed a single T_2 which increased with moisture level. Several researchers have explained this in terms of a two-site fast exchange model (Le Botlan et al., 1998; Leung et al., 1976; Leung et al., 1979; Wynne-Jones and Blanshard, 1986)

$$\frac{1}{T_2} = \frac{P_B}{T_{2B}} + \frac{P_A}{T_{2A}} \quad (1)$$

where P_A and P_B are the fractions of, and T_{2A} and T_{2B} the time constants associated with each domain. In fact, more than two domains may exist. For example, Richardson et al (1987) postulated an additional term that accounted for “multilayer” water.

At $a_w > 0.43$, control and hydroxypropylated starch showed two mobility regions, while untreated starch showed only one region. The differences in control starch may be

interpreted in light of what is known about structural changes due to alkali treatment. Microscopic studies have shown that alkali-treated starch retains a compact granular structure similar to native starch, as well as well-organized growth rings (Kim et al., 1992). Perera et al. (1997) showed that some swelling occurs, amylose leaching is unaffected, the transition temperatures T_o , T_p , T_c are unchanged, and that the heat of gelatinization is slightly decreased. They argued that the double helices within amorphous regions were disrupted by alkali treatment, thus exposing more $-OH$ groups, while crystalline areas were not. X-ray diffraction studies also showed that crystalline areas were not disrupted, but did indicate that the type of crystal pattern was altered. Native wheat starch is an A-type starch, and Tang et al (2000) showed that all water populations were in fast exchange in A-type starch. In contrast, B-type starch showed up to three distinct water populations. They suggested this was due to the smaller granule size. Our results suggest that other factors are at work. That is, while the granule size is not markedly changed by alkali treatment, new domains of water were distinguished. As there is evidence that alkali treatment modifies the basic crystal structure of starch, it seems reasonable that water associated with this structure is less able to move freely between crystalline and non-crystalline regions. It is known, for example, that the A-type crystals consist of starch chains in a monoclinic lattice with approximately four water molecules. In contrast, B-type crystals have a more open hexagonal sub-cell with up to 36 tightly bound water molecules per unit cell (Perera et al, 1997).

At $a_w > 0.81$, T_{2B} values were higher for hydroxypropylated starch as compared to control (Figure 5). In addition, T_{2B} increased with the level of hydroxypropylation (Figure 6). In general, this indicates a greater degree of mobility for water in the

hydroxypropylated starch. Wootton and Manatsathit (1983) showed that hydroxypropyl groups disrupt the inter- and intramolecular hydrogen bonds, thus increasing the motional freedom of starch chains in amorphous regions. Increased starch chain mobility was also observed from our single-pulse studies on hydroxypropylated starch (Figure 3a). The exact nature of the increased moisture mobility seen in hydroxypropylated samples is uncertain. One simple interpretation is that the more open and flexible starch network allows more water within the region, and elicits larger distances over which water molecules must diffuse to interact with starch. This is consistent with our observation that more water was absorbed by hydroxypropylated starch at a specific water activity (Figure 1a).

Distributed exponential fits of CPMG data from cross-linked starch were indistinguishable from those of control starch, and thus are not shown. This is consistent with the notion that cross-linking primarily reinforces external regions of the granule, and thus have little effect on dynamic water properties in the interior of the granule, apart from those due to alkali treatment.

CONCLUSION

The effects of chemical modifications by hydroxypropylation and cross-linking on water sorption isotherms and molecular mobility of both starch and water molecules were studied by pulsed ^1H NMR techniques. The water absorption capacity of hydroxypropylated starch increased with increasing degree of molar substitution of hydroxypropyl group at a_w above 0.53. Cross-linking with phosphorus oxychloride at concentrations of 0.03% and 0.1% did not alter water sorption capacity, while crosslinking at 0.2% caused a slight decrease in water absorption at $a_w > 0.53$. Results from single-pulse experiments indicated that hydroxypropylation increased the molecular mobility of starch chains, due to internal plasticization of hydroxypropyl groups. The proton relaxation associated with water molecules in chemically modified starch showed two distinct populations in the a_w ranges from 0.53 to 0.93, suggesting that treatment with alkaline reagents may cause structural changes in starch granules.

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Table 1. The amount of chemical used to prepare cross-linked starches

Starch (200 g)	Na ₂ SO ₄	POCl ₃ ^a (%)
Control	10 g	
XA	10 g	0.03
XB	10 g	0.1
XC	10 g	0.2

^a : based on dry weight of starch

Table 2. The amounts of chemical used to prepare hydroxypropylated starch, the percent ratio of hydroxypropyl groups, and the molar substitution (M.S.) of hydroxypropyl groups.

Starch (200 g)	Na ₂ SO ₄ (g)	P.O. (mL)	H.P. (C ₃ H ₇ O)(%)	M.S. ^a
Control	10	0	0	0
HA	10	8	2.02	0.05
HB	10	16	4.11	0.12
HC	10	25	6.22	0.18

^aM.S.: molar substitution (moles of hydroxypropyl per anhydroglucose)

P.O.: propylene oxide

H.P.: hydroxypropyl group

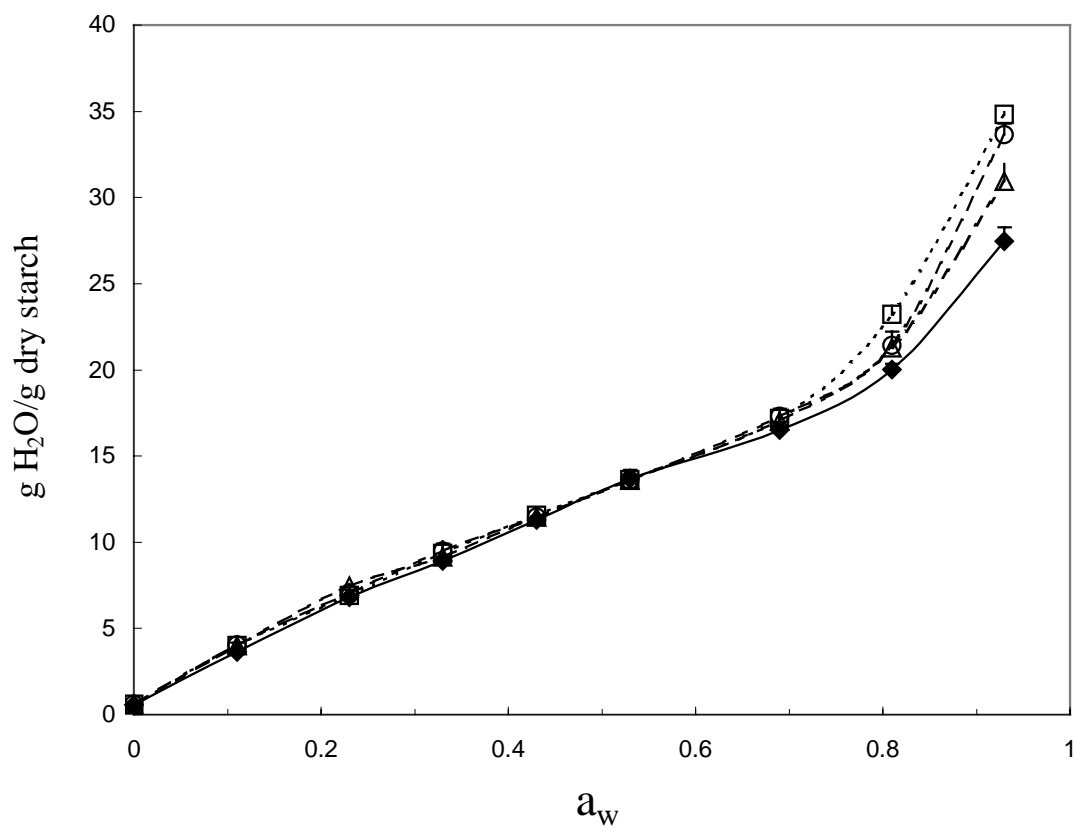


Figure 1a- Moisture sorption isotherms for control (◆) and hydroxypropylated (△-0.05 MS, ○-0.12 MS, and □-0.18 MS) wheat starches.

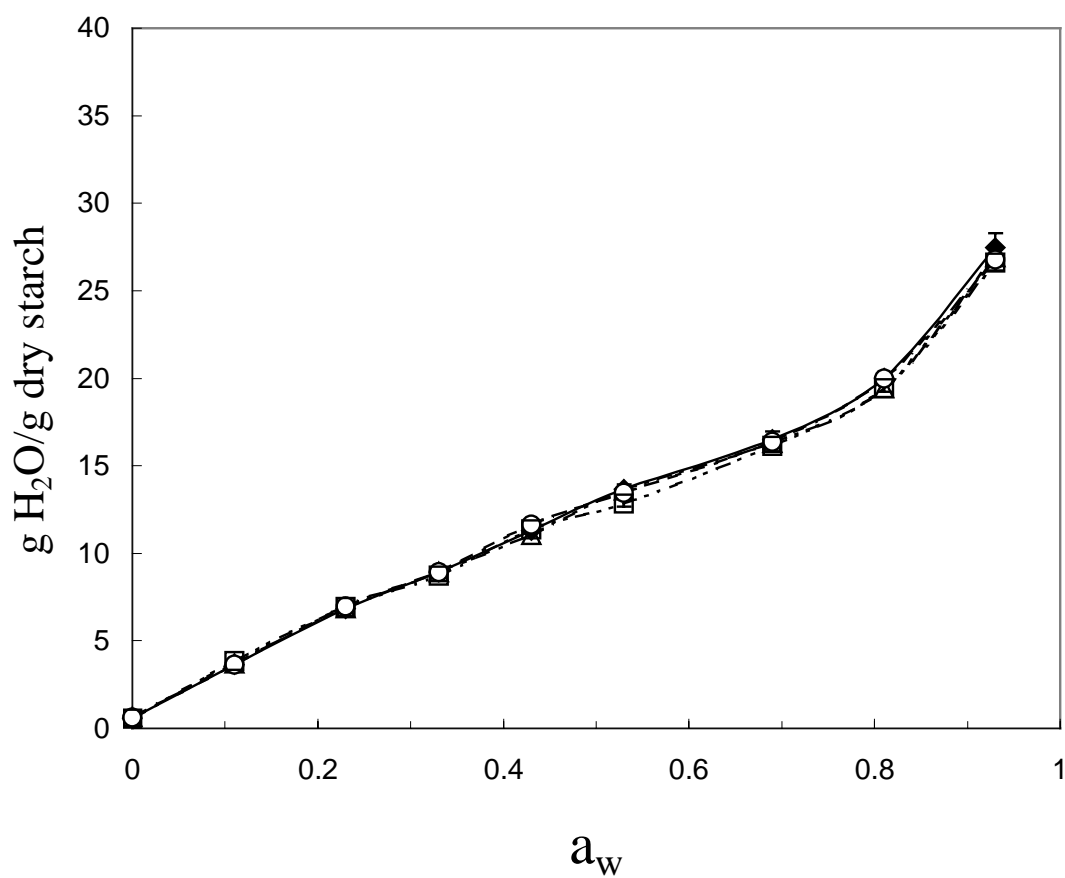


Figure 1b- Moisture sorption isotherms for control (◆) and cross-linked (△-0.03%, ○-0.1%, and □-0.2%) wheat starches.

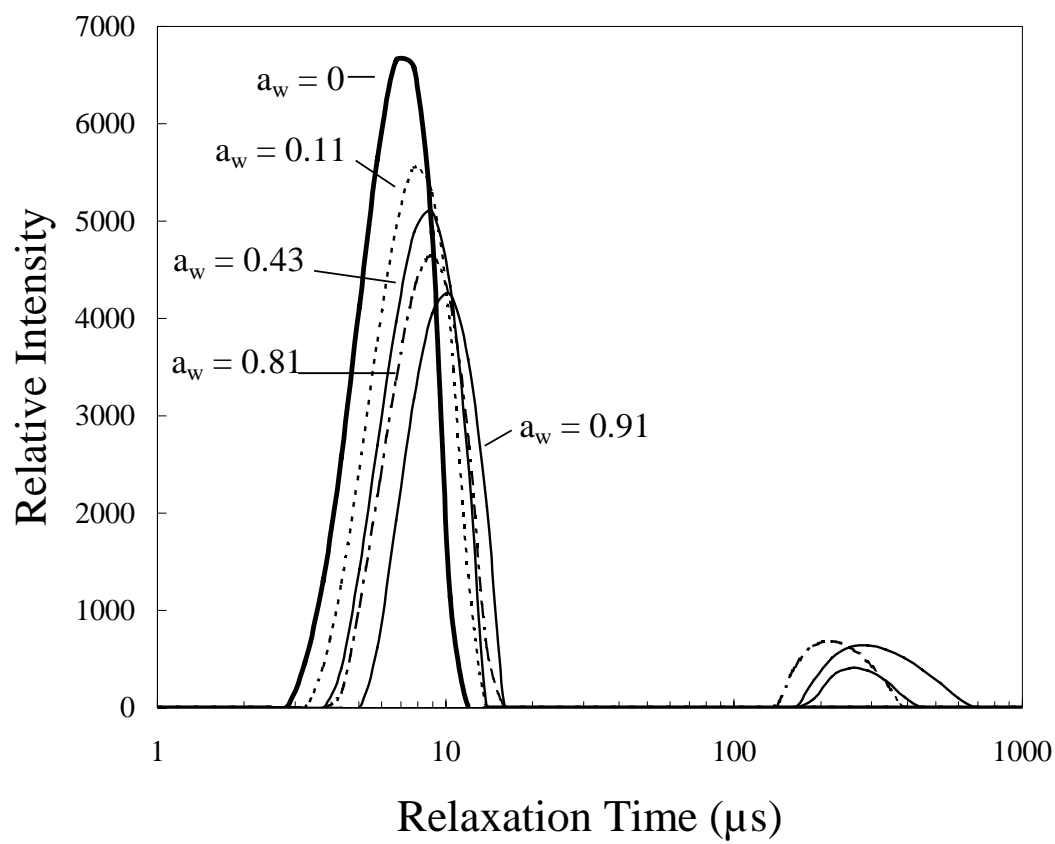


Figure 2- Typical T_2 distributed relaxation plots for control starch at different a_w .

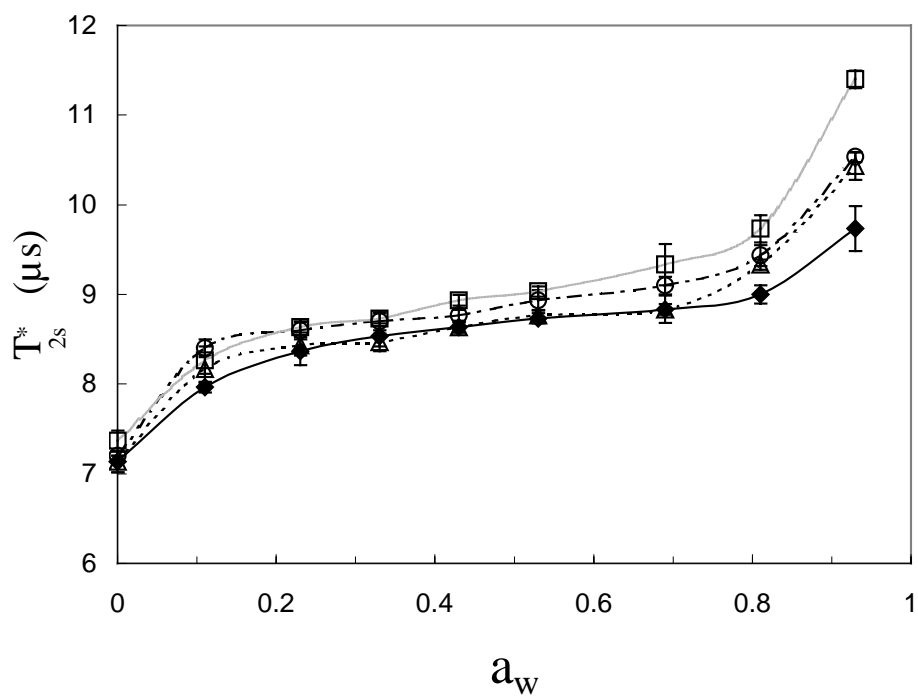


Figure 3a- The peak T_{2s}^* values for control (\blacklozenge) and hydroxypropylated (\triangle -0.05 MS, \circ -0.12 MS, and \square -0.18 MS) wheat starches as a function of a_w .

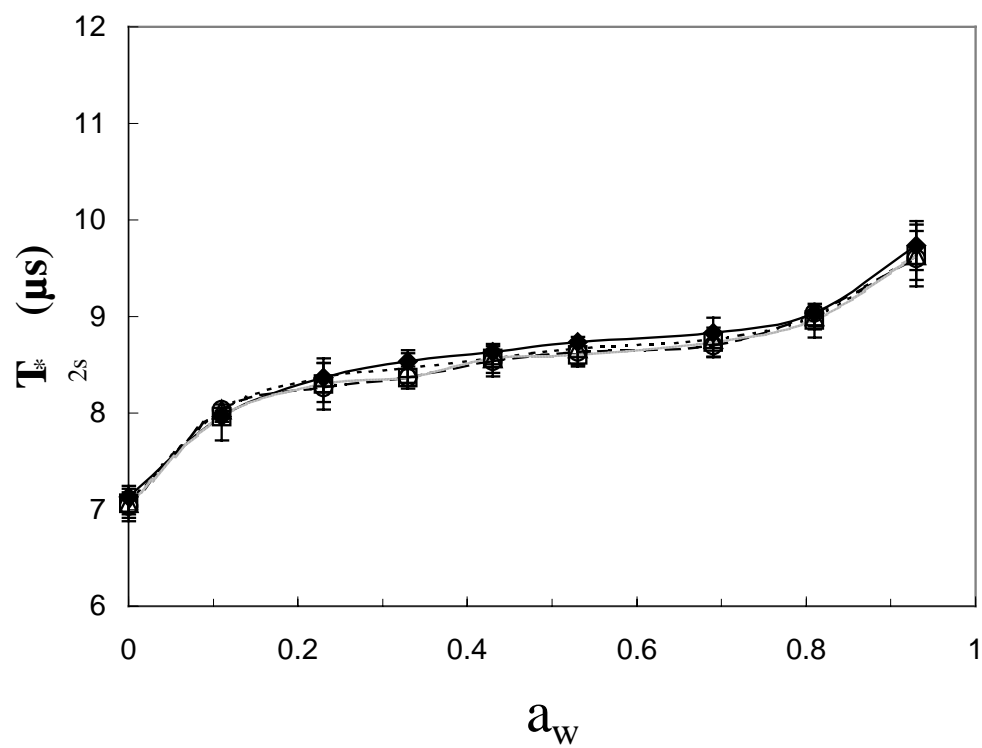


Figure 3b- The peak T_{2s}^* values for control (\blacklozenge) and cross-linked (\triangle -0.03%, \circ -0.1%, and \square -0.2%) wheat starches as a function of a_w .

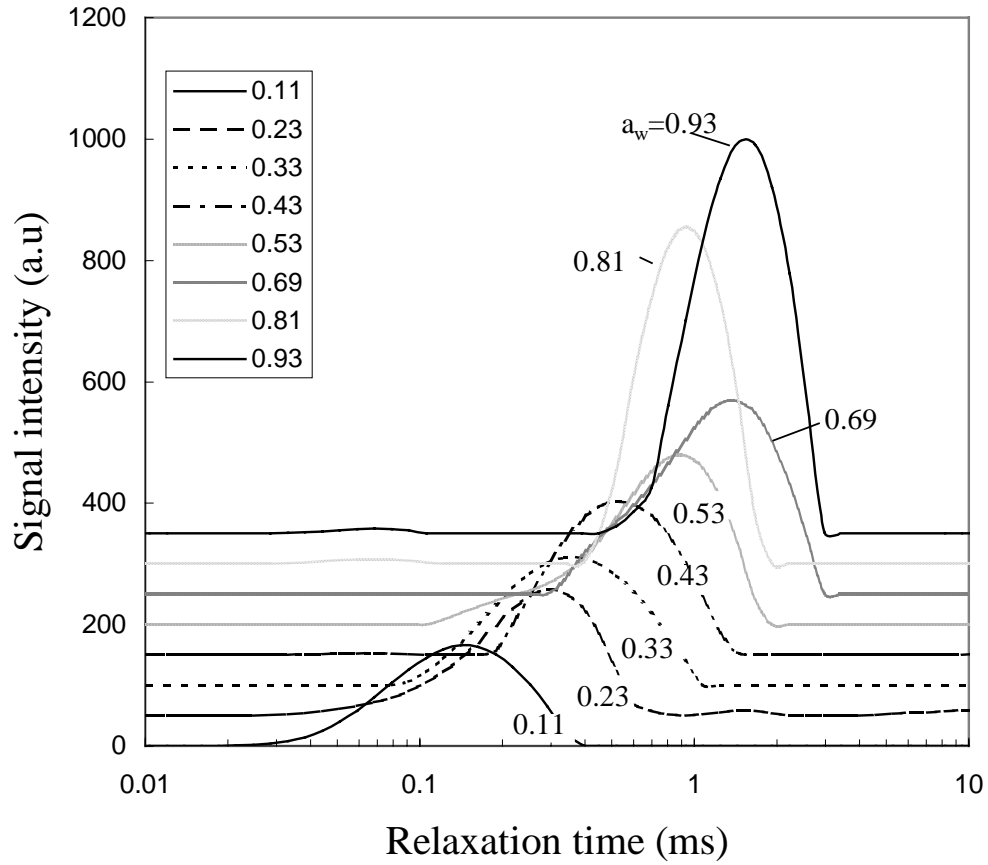


Figure 4a- Distributed T_2 relaxation plots for native wheat starch determined from CPMG sequence experiment.

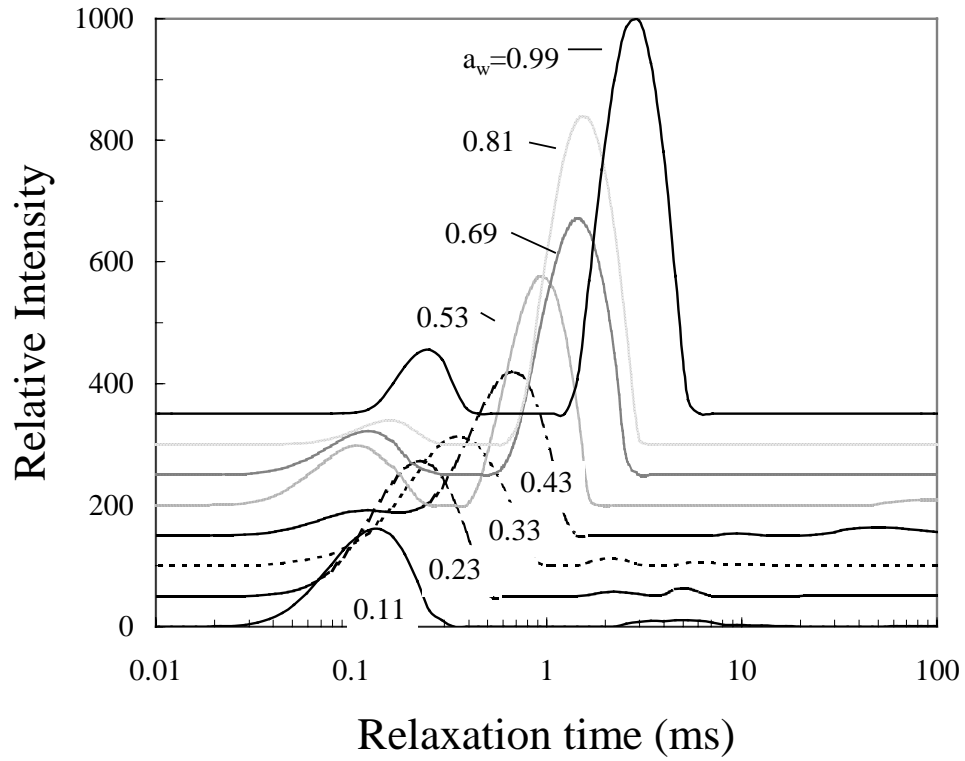


Figure 4b- Distributed T_2 relaxation plots for control starch determined from CPMG sequence experiment.

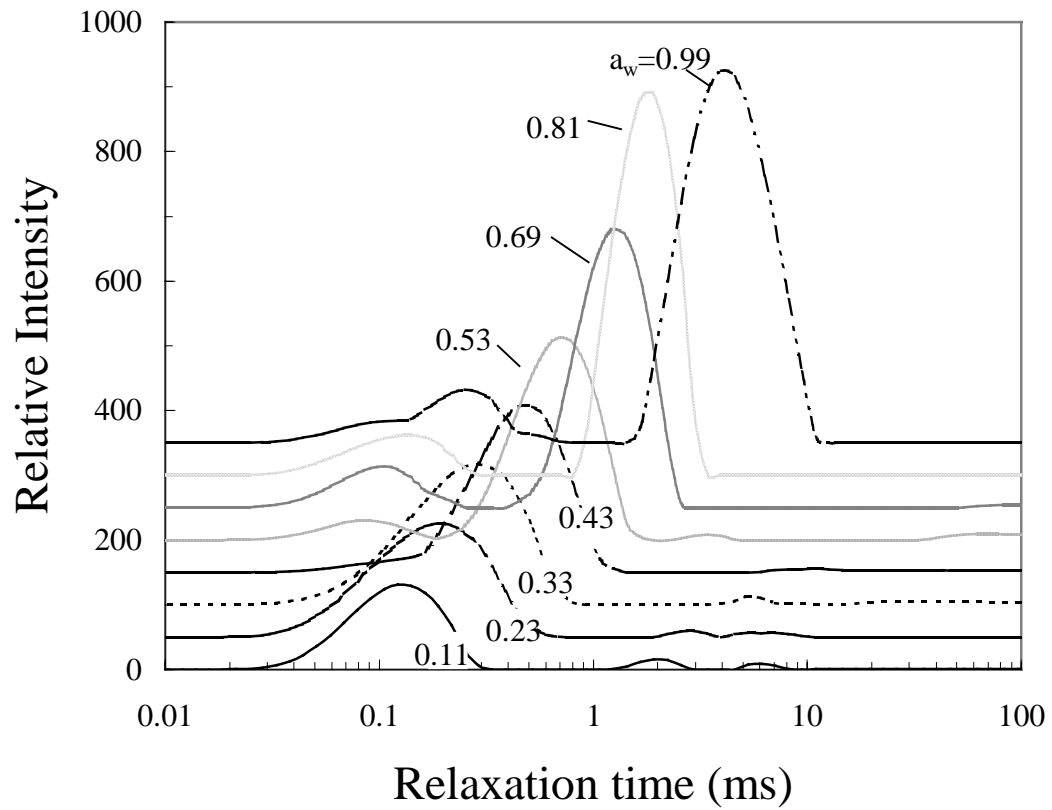


Figure 4c- Distributed T_2 relaxation curves for hydroxypropylated starch (HB), which was determined from CPMG sequence experiment.

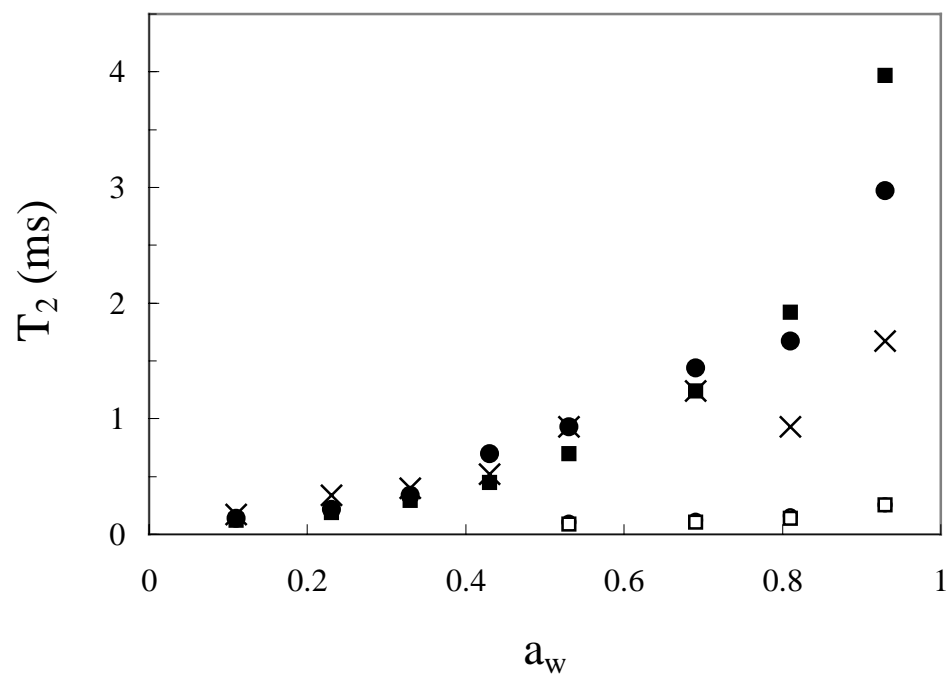


Figure 5- The peak T₂ values for native (×), control starch (more mobile component) (●), control starch (less mobile component) (○), 0.18 MS hydroxypropylated starch (more mobile component) (■), and 0.18 MS hydroxypropylated starch (less mobile component) (□).

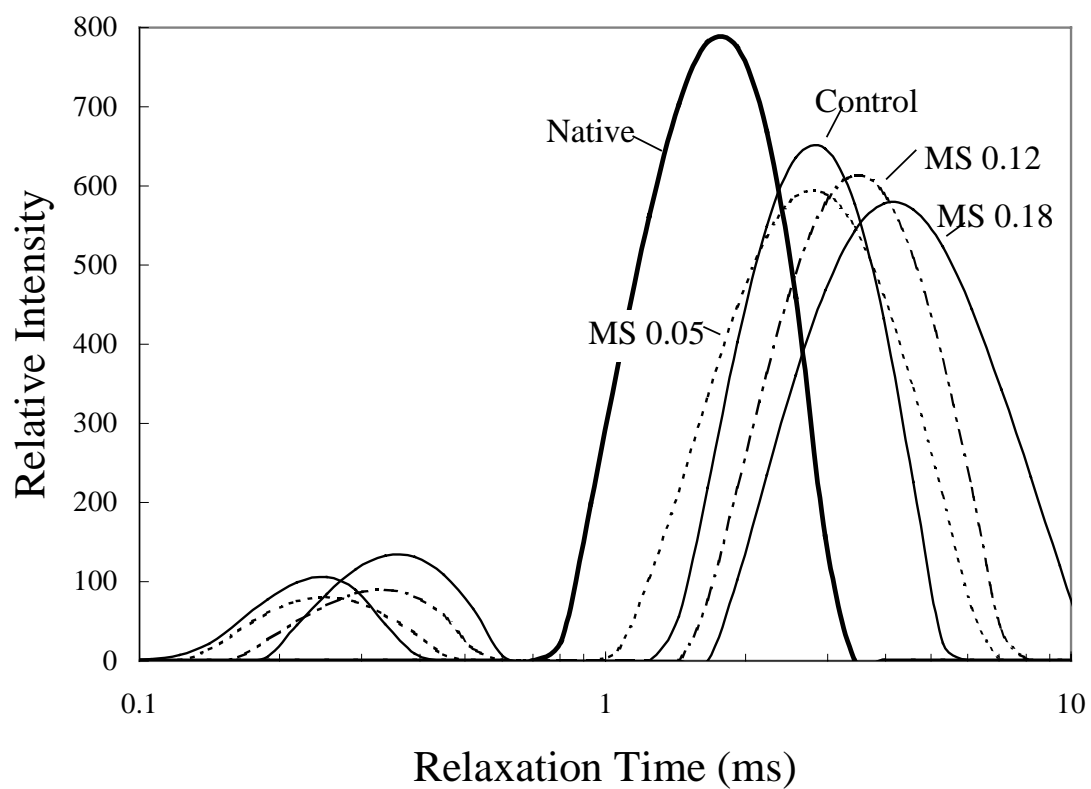


Figure 6- Distributed T₂ relaxation plots for native, control, and hydroxypropylated starch (0.05 MS, 0.12 MS, and 0.18 MS) at a_w of 0.93.

CHAPTER V

WATER MOBILITY AND TEXTURAL PROPERTIES OF NATIVE AND

HYDROXYPROPYLATED WHEAT STARCH GELS ¹

¹Choi, S. -G and W. L. Kerr. Submitted to Carbohydrate Polymers

ABSTRACT

The effect of wheat starch concentration and degree of hydroxypropylation on the water dynamics and textural attributes of starch gels was studied using ^1H NMR and texture profile analysis. Starches were prepared with molar substitution (M.S) of hydroxypropyl groups between 0.05 and 0.18. Gels were formed from modified starches at concentrations between 25 and 40%. For all gels, two distinct water fractions were identified using distributed exponential analysis of CPMG results. For the less mobile fraction, T_{2a} ranged from 2~6ms while for the more mobile state, T_{2b} ranged from 20~200ms. Values of both T_{2a} and of T_{2b} increased with increasing moisture content and hydroxypropyl M.S. Control experiments indicated that more mobile states could be associated with both the extragranular amylose gel, as well as intragranular amylopectin-rich gel. The less mobile state was associated only with granule remnants. Gel hardness decreased significantly with increasing moisture content, and with increasing hydroxypropylation. Cohesiveness increased with degree of hydroxypropylation. Results from multi-regression analysis between hardness and NMR parameters showed that the mobility and amount of water in the less mobile state was most related to gel hardness.

INTRODUCTION

Starch is one of the most important food biopolymers, and exhibits gelling properties that are used to control the texture and mechanical properties of many foods (Han and Hamaker, 2000). Starch is a mixture of two polysaccharides, the linear chain amylose and the heavily branched amylopectin. Heating starch in excess of water results in starch granule melting and water imbibition. It also causes irreversible swelling of granules to many times their original size, with the final size dependent upon the available space and the amount of amylose leaching out of the granules (Keetels et al., 1996).

Water is necessary to hydrate starch molecules and provide the fluid environment in which starch aggregates to form gels. Water also serves as a plasticizer, and thus influences the molecular mobility of starch molecules. Interactions of starch with water also determine how water is retained with the gel, for example whether water is “bound” or immobilized near the starch surface, and whether it is entrapped by the gel network. Several researchers have studied the molecular dynamics of water in starch systems.

Nuclear magnetic resonance spectroscopy (NMR) has been especially useful in this regard, as water may be studied through the use of ^1H , ^2H , and ^{17}O nuclear resonance. A wide variety of starches and physical states have been explored. In relatively low moisture waxy corn starch powders and suspensions, Li et al. (1998) measured a single relaxation component that increased with moisture content. Kou et al. (2000) found two components, one associated with starch, and one with water. Richardson et al. (1987a and b) analyzed a single NMR relaxation component to show three mobility regions existed with respect to water activity. Le Botlan et al (1998) found two relaxation

components in intact starch, one corresponding to “bound” water and one to unbound water. Tang et al (2000) studied the dynamic states of water in several types of starch granules. In B-type starch, they found three transverse relaxation regimes which were associated with water in amorphous growth rings, semi-crystalline lamella, and channels in crystalline amylopectin.

The textural and mechanical properties of starch gels depend mainly on the rheological properties of the continuous amylose phase, the volume fraction of the granules, the deformability of the granules, and the interaction between the dispersed and continuous phase (Eliasson, 1986; Ring et al., 1987). Water plays an important role in controlling these factors in starch gels and starch-based foods. Several researchers have studied the role of water, water holding capacity, and water mobility in determining the texture of starch-based foods. Keetels et al. (1996) suggested that increased moisture content caused greater deformation of swollen granules and a lower-strength amylose network, leading to decreased gel firmness. Inaba et al. (1994) investigated the textural and mechanical properties of starch gels prepared at various moisture contents. They reported that compressive force and work increased with starch concentration, while resiliency and compressibility did not depend on starch concentration. Chung and Lee (1991) demonstrated that the “bound” water (unfreezable water at -30°C) of surimi gels prepared with potato starch, with and without pregelatinization, was highly correlated with compressive force and inversely related to expressible moisture. Ruan et al. (1997) used ^1H NMR spectroscopy and texture analysis to study various types of cooked rice. They found that the hardness of cooked rice was highly correlated with spin-spin relaxation constants of protons ($P < 0.01$).

Chemical modification of starch by hydroxypropylation has been used to improve its functional properties. Hydroxypropylation reduces gelatinization temperature, decreases gel firmness, and increases water holding capacity (Hoover et al., 1988; Liu et al., 1999; Yeh and Yeh, 1993), as hydroxypropyl groups are hydrophilic in nature and weaken the internal bond structure holding the granule together. Hydroxypropylation also prevents water in the starch gel from separating through syneresis when subjected to freeze-thaw cycles (Eliasson and Kim, 1992). Seow and Thevamalar (1993) reported that the introduction of hydroxypropyl groups in starch molecules acted as an internal plasticizer and reduced the energy of gelatinization. Liu et al. (1999) reported that hydroxypropylation increased swelling power and solubility in starch pastes.

The objective of this study was to investigate the effects of hydroxypropylation and starch concentration on water mobility and textural attributes of wheat starch gels. Time domain ^1H NMR was used to study dynamic water properties in gels, while texture profile analysis was used to study various textural attributes. To further investigate the contributions of different gel phases, studies were also done on isolated amylose gel and granule rich fractions.

MATERIALS AND METHOD

Preparation of Hydroxypropylated Wheat Starch

Hydroxypropylation of wheat starch was accomplished as described by Kim et al. (1992). Native wheat starch (Sigma Chemical Co., St. Louis, MO) was lyophilized for 48 hrs, then kept over phosphorous pentoxide (Sigma Chemical Co., St. Louis, MO) to ensure maximum dryness. Dry sample (200 g) was suspended in 440 ml of distilled water containing 40 g Na₂SO₄, then the pH adjusted to 11.3 with 1N NaOH. The mixture was placed in 500 ml screw cap jars and maintained at 35°C in a controlled temperature water bath. Aliquots (8 ml, 16 ml, or 25 ml) of propylene oxide (Acros Organics, New Jersey) were added to the jars. The reaction proceeded for 24 hrs, and was terminated by adjusting the pH to 5.5 using 1 N HCl. The suspension was washed with distilled water and centrifuged several times for 15 minutes at 3200g. Control wheat starch was prepared with the same reagents and procedure, but without addition of propylene oxide. This was to ensure that any measured changes were due to hydroxypropylation and not from effects of pH change or heating. The hydroxypropyl content was determined by the method of Johnson (1969). The molar substitution (MS) was defined as moles of substituent per mole of anhydroglucose unit (Table 1).

Gel preparation

In order to prepare homogeneous gels from highly concentrated starch suspensions, a microwave heating method was used. Starch samples (0.5 g) were weighed into glass tubes (3 cm x 1 cm diameter) fitted with screw caps. Distilled water (0.75 g, 1.0 g, and 1.5 g, respectively) was added to the tube to make a suspension (40, 33, and 25% starch by weight), then vortexed for 20 s. The sealed containers were placed

on a rotating turntable and heated in a microwave oven (Daewoo, KOR-630A) for 40 s at 800 W. After heating, the sample temperature was $94^{\circ}\text{C} \pm 2^{\circ}\text{C}$. Gels were formed as the samples were allowed to cool to 25°C for 1 hour. Fresh gels were immediately measured by NMR. For texture profile analysis, gels were formed in glass jars (6 cm diameter x 7 cm height). Cylindrical samples were cored from the gel (1 cm diameter x 1 cm height).

Amylose and Granule Remnants

In order to better understand the origin of ^1H NMR signals, preparations of isolated amylose and granule remnants were made. A 7% wheat starch paste was prepared at 70°C and gently stirred for 90 min. The paste was diluted with distilled water at 70°C of 200 ml and centrifuged at 2500 g for 15 min. The suspension was separated into two phases. The supernatant gave blue color after staining with 0.2% iodine solution (0.2g iodine, 2g potassium iodide in 100 ml of aqueous solution) and was clear of granules when examined by light microscopy. This was considered as the soluble starch fraction. The precipitate consisted of gelatinized starch granules when observed at 40X, and gave a purple color when reacted with iodine solution. This was considered as the insoluble starch fraction. To adjust the concentration of the soluble starch solution, water was allowed to evaporate from the sample while gently stirring at 72°C . The moisture contents of adjusted soluble solution and insoluble starch were determined by drying *in vacuo* at 75°C for 48 h. Gels were prepared from the amylose preparations and from the granule-rich sediment as described previously.

Texture profile analysis (TPA)

Cylindrical gel samples (1 cm length x 1cm diameter) were subjected to instrumental texture profile analysis (TPA) as described by Bourne (1982). The specimens were placed between parallel plates fitted to an Instron Model 5500R Universal Testing Machine (Instron Corp., Canton, MA). The gels were compressed twice at 1 mm/s to a distance 50% of the initial height. From the resulting force/deformation curves, the textural parameters of hardness, cohesiveness, and springiness were calculated. Hardness (N/m^2) was defined as the maximum force achieved during the first compression cycle, and cohesiveness as the ratio of the area under the first and second compression curves. Springiness was calculated as the ratio of the distance at the maximum force during first compression to the distance at the maximum force during second compression.

NMR measurements

Proton relaxation measurements were made using a MARAN 20 MHz NMR spectrometer (Resonance Instruments, Whitney, UK). The glass tubes with gel specimens were placed in 18 mm diameter NMR tubes, which were covered with parafilm. All measurements were made at $25^\circ\text{C} \pm 0.1^\circ\text{C}$. Transverse (T_2) relaxation curves were developed using the Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence: $90_x-(t-180_y-\tau\text{-echo})_n$ (Meiboom and Gill, 1958). Acquisition parameters were set to a 90° pulse of $4.1 \mu\text{s}$, relaxation delay of 6 s, and τ of $300 \mu\text{s}$. Relaxation curves obtained from the CPMG sequence were analyzed using a monoexponential model:

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

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. Alternately, a distributed exponential routine was used to analyze relaxation the curves. Based on the “contin” routine of Provenchar (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.

Statistical analysis

Five treatment groups were considered in this experiment including unmodified wheat starch, control wheat starch (subject to reaction conditions but without propylene oxide), and 3 levels of hydroxypropylation (M.S.= 0.05, 0.12, and 0.18). Gels were prepared at 3 levels of starch (25, 33, and 40% by weight), and all samples were prepared and analyzed in triplicate. SAS software was used for data analysis (SAS Institute, INC., 1990). The general linear model ANOVA was utilized to test for effects of treatments. The Duncan test was conducted to differentiate the treatments. Correlation coefficients between measurements were calculated and compared. Significance of difference was defined at $p < 0.05$.

RESULTS AND DISCUSSION

Hydroxypropylation of Wheat Starch

Analysis of modified starches showed that the molar substitution, defined as the moles of hydroxypropyl per anhydroglucose unit, was 0.05, 0.11, and 0.18, respectively (Table 1).

Transverse Relaxation

Relaxation time constants (T_2) of heterogeneous systems can be determined from CPMG decay curves using mono-exponential, multi-exponential, or distributed exponential fitting (Ruan and Chen, 1998). In this study, the proton relaxation decay curves were analyzed using mono-exponential (Equation 1) and distributed exponential (Equation 2) models. Mono-exponential analysis was used to assess the effects of chemical modification and moisture content on overall water mobility. Figure 1 shows T_2 for unmodified, alkaline-treated (control), and hydroxypropylated starch gels as a function of starch concentration. For all treatment groups, T_2 values decreased with increasing starch concentration, suggesting that on average water is less mobile in gels at higher starch concentration. More specifically, each water molecule has a greater chance of diffusing to and interacting with starch molecules. Consequently, proton decay is enhanced by decreased water mobility, proton exchange, or cross-relaxation mechanisms (Yakubu et al., 1993; Hills et al., 1990). Hydroxypropylation had a significant effect on relaxation behavior of water protons in starch gels. In general, T_2 values increased with the level of hydroxypropylation, although differences in the 33 and 40% gels at M.S. 0.12 and 0.18 were not significant. In general, this would argue that the volume in which water is free to move before interacting with starch is greater in the

hydroxypropylated starch gels. T_2 values for the control gel were intermediate between the unmodified and hydroxypropylated gels. This indicates that alkali treatment alone has some affect on starch apart from subsequent chemical modification. Alkali treatment has been shown to allow enhanced granule swelling and to produce changes in crystal structure in potato starch (Perara et al., 1997).

Distributed exponential fits to the relaxation data showed two distinct regions of T_2 values, one with T_2 values between 0.8 and 7 ms, one with values between 10 and 300 ms, depending on starch concentration. Figure 2 shows the distribution of T_2 values for unmodified wheat starch gels in the starch concentration range of 25% to 40%. The peak maxima of the 0.8-7 ms region is denoted by T_{2a} . Spin-spin relaxation is enhanced when, on average, water molecules have hindered rotation and proton spins can exchange energy. Thus, T_{2a} is associated with less mobile water, although contributions from chemical exchange and cross-relaxation may also be factors. The peak at longer time constants (10-300 ms) is denoted by T_{2b} and the region associated with more mobile water. The phenomenon of multi-phase behavior in water proton relaxation has been observed in other systems such as flour dough (Leung et al., 1979), hydrated elastin (Ellis and Packer, 1976), and bread (Chen et al., 1997; Engelsen et al., 2001). In addition, to changes in time constants, the integrated signal intensity of each region increased with moisture content, again reinforcing the notion that these signals emanate from water domains.

The source of the dual peaks is explored in more detail in Figure 3. Starch gelatinization results in solubilized starch, primarily amylose, and swollen starch granules and fragments. On cooling, solubilized amylose forms a continuous network that links

swollen granules (Hermansson and Svegmarm, 1996). Figure 3 shows distributed T_2 values for isolated amylose gels, as well as those from a granule-rich sediment. The amylose gel showed a single peak, with a maximum value of T_{2b} . T_{2b} varied from 212 ms (at 8.7% amylose) to 840 ms (at 1.1% amylose). Subsequent studies (data not shown) showed that these peaks did not change during 15 days storage at 5°C. The single peak indicates that fast-exchange conditions exist, that is the average diffusion time from the bulk water phase to a starch surface is short compared to T_2 of bulk water. Isolated granule remnants showed two peaks, one in a region similar to amylose, and one at times between 10 and 25 ms. Both regions shift considerably during storage (data not shown), indicative of changes seen in amylopectin during retrogradation (Choi and Kerr, 2001). This suggests that the T_{2b} region is associated with a fairly porous structure, perhaps an intergranular gel network. The T_{2a} region is associated with a more restricted environment of the granule remnant, and one that does not rapidly exchange water with the T_{2a} region. Kalichvsky and Ring (1987) reported that leached amylose molecules had higher water mobility compared with amylopectin molecules in mixed aqueous system of purified samples of amylose and amylopectin.

The effect of alkali treatment and hydroxypropylation on the peak relaxation times (T_{2a} and T_{2b}) in the starch gel systems is shown in Table 2. In addition, the amount of water associated with either region a (w_a) or region b (w_b) was calculated by:

$$w_a = \frac{I_a}{I_a + I_b} (\text{Total } g \text{ } H_2O) \quad (3)$$

$$w_b = \frac{I_b}{I_a + I_b} (\text{Total } g \text{ } H_2O) \quad (4)$$

where I_a or I_b are the total integrated signal intensity in regions a and b, respectively.

At all gel concentrations, hydroxypropylation increased the T_{2a} and I_{2a} values, as compared to equivalent gel concentrations of unmodified starch. Hydroxypropylation resulted in more water (w_a) being associated with the low time constant region. Using light microscopy, Perera et al (1997) showed that hydroxypropylation caused more highly deformed starch granules. Liu et al. (1999) reported that swelling was greater in starch granules after hydroxypropylation. Our results indicate that there is slightly greater moisture in the swollen granules after hydroxypropylation, and slightly less in the amylose gel network. For example, there was 12% more water in the swollen granules of the 0.18 MS 40% gel (0.131 g H₂O), as compared to the granules in the unmodified gel (0.117 g H₂O). Concurrently, there was a slight decrease in moisture in the amylose network of the 0.18 MS 40% gel (1.369 g H₂O) as compared to the amylose network of the unmodified gel (1.383 g H₂O).

In general, increased moisture in the gels was associated with slightly higher T_{2a} values. However, increases in T_{2a} and the distribution of T_2 values were not great, suggesting that the water in this region has greatly hindered motion. Larger increases in T_{2a} were observed due to hydroxypropylation than to changes in moisture content.

The T_{2b} and distribution of T_2 values in the high T_2 region also increased with moisture content and with degree of hydroxypropylation. Again, the increase in T_2 with moisture content may be attributed to a greater percentage of water that spends less time interacting with the biopolymer. The increase in moisture in this region is greater than that in the low-time constant (T_{2a}) region. For example, in the unmodified starch gels w_b increased from 0.657 g to 1.383 g (a 112% increase) as the starch concentration decreased from 40 to 25%. This may explain the greater increases in T_{2b} than were

observed in T_{2a} . T_{2b} values also increased with the degree of hydroxypropylation. This suggests that the water mobility is enhanced due to modification, even though there is less moisture in this region as compared to unmodified starch gels. Studies indicate that the bulky hydroxypropyl group on starch is mainly distributed on amylose molecules, and that this disturbs the aggregation of solubilized amylose in a continuous phase (Steeneken, 1984).

Mechanical Properties of Gels

Select textural attributes of unmodified, control, and hydroxypropylated starch gels in the starch concentration ranges of 25% to 40% are presented in Table 3. For all samples, gel hardness increased significantly with gel concentration ($p < 0.05$). In addition, gels formed from alkali treated (NaOH and Na₂SO₄) starch were less hard than those formed from unmodified starch, and those from hydroxypropylated starch were even less hard. No significant difference in gel hardness was observed due to degree of molar substitution. These results are consistent with those reported by Liu et al (1999), who demonstrated that hydroxypropylation decreased gel hardness. It is known that the extent of the amylose gel network and deformability of swollen granules are the main factors contributing to gel strength (Eliasson, 1986; Ring et al., 1987). Our results in Figure 2 show higher T_2 values for control or modified starch gels. Distributed analysis showed that there was higher water mobility in both T_{2a} and T_{2b} fractions, and that the amount of water associated with the less mobile state was increased with hydroxypropylation (Table 2). As control experiments showed that this less mobile state

was associated with granule remnants, it appears that enhanced hardness in the modified starches can be attributed to greater granule swelling.

Cohesiveness is a measure of degree of difficulty in breaking down the gel's internal structure. The effect of moisture content in starch gels varied with the type of starch. For example, cohesiveness of unmodified and control starch gels increased with increasing moisture, whereas that of hydroxypropylated starch gels decreased. In general, hydroxypropylated starch had higher cohesiveness. Presumably, this would be due to a higher degree of networking in the leached amylose phase. Han and Hamaker (2000) observed the microstructure of waxy and normal starch gels using light microscopy and concluded that the solubilized amylose is responsible for the fibrous structure, whereas amylopectin is responsible for the cellular structure.

Springiness represents the extent of recovery of gel height and sometimes is referred to as "elasticity" (Sanderson, 1990). For unmodified and control starch gels, springiness was not significantly affected by starch concentration in the range of 25% to 40%. However, for highly substituted hydroxypropylated starch gels (0.12 and 0.18 MS), springiness decreased as moisture content increased. In the 25% gels, hydroxypropylated starch gels had lower springiness than unmodified or control gels.

Correlation Between Texture and NMR Parameters

From the TPA analysis, textural parameters such as hardness, springiness, and cohesiveness were obtained. However, hardness is considered mostly relevant to water dynamic behavior. Thus, correlation analysis between hardness and NMR parameters such as T_{2a} , I_{2a} , T_{2b} , and I_{2b} were performed using a multiple variable regression model.

The results are presented in table 4. The gel hardness had significant negative correlation with all parameters ($P < 0.05$). The level of correlation was in order of T_{2a} ($R^2=0.90$) > I_{2a} ($R^2=0.87$) > T_{2b} ($R^2=0.73$) > I_{2b} ($R^2=0.54$). The higher correlation coefficients of T_{2a} and I_{2a} than those of T_{2b} and I_{2b} may indicate that the mobility and amount of water in the less mobile state, which may be associated with starch molecules in swollen granule, is a more dominate factor in hardness of gel at high starch concentration. This interpretation is consistent with that of Evans and Lips (1992), who studied the viscoelasticity of gelatinized starch dispersions at concentrations between 5 and 25% (w/w). They proposed that the phase volume of the swollen granules and their inherent deformability were the key variables in the mechanical behaviors of concentrated gelatinized starch dispersion systems, rather than the component released from the granules.

CONCLUSION

The water mobility and textural properties of native and hydroxypropylated starch gels were investigated at different gel concentrations. Two distinct water fractions having different mobility were identified in all starch gels. Control experiments indicated that the less mobile state was associated with granule remnants, while more mobile states could be attributed to both intergranular and extragranular gel networks.

Hydroxypropylation produced gels with greater water mobility in all regions. Gel hardness and springiness were significantly decreased in hydroxypropylated samples. As cohesiveness was increased in hydroxypropylated samples, this suggests enhanced deformation of starch granule remnants. Multi-regression analysis showed that the mobility and amount of water in the less mobile state is a dominant factor for hardness of gels with starch concentration in the range of 25 to 40%.

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Table 1. The amounts of chemical used to prepare hydroxypropylated starch, the percent ratio of hydroxypropyl groups, and the molar substitution (M.S.) of hydroxypropyl groups.

Starch (200 g)	Na ₂ SO ₄ (g)	P.O. (mL)	H.P. (C ₃ H ₇ O)(%)	M.S. ^a
Control	10	0	0	0
HA	10	8	2.02	0.05
HB	10	16	4.11	0.12
HC	10	25	6.22	0.18

^aM.S.: molar substitution (moles of hydroxypropyl per anhydroglucose)

Table 2. Peak transverse relaxation times and associated water content of native, alkali-treated, and hydroxypropylated wheat starch gels

Starch	Concentration ^a (%, w/w)	Less mobile fraction		More mobile fraction	
		T _{2a} (ms)	w _a (g H ₂ O)	T _{2b} (ms)	w _b (g H ₂ O)
Native	40	2.26 ± 0.04	0.093 ± 0.001	38.83 ± 6.81	0.657±0.001
	33	2.50 ± 0.10	0.107 ± 0.005	55.1 ± 0.85	0.893±0.005
	25	2.64 ± 0.15	0.117 ± 0.002	96.04 ± 0.37	1.383±0.002
Alkali-treated	40	2.97 ± 0.01	0.093 ± 0.004	50.33 ± 3.65	0.657±0.004
	33	3.73 ± 0.17	0.105 ± 0.012	73.1 ± 1.64	0.895±0.012
	25	4.28 ± 0.16	0.111 ± 0.001	103.2 ± 1.03	1.389±0.001
Hydroxypropyl MS = 0.05	40	3.09 ± 0.11	0.010 ± 0.016	49.77 ± 2.83	0.650±0.016
	33	4.11 ± 0.18	0.117 ± 0.002	85.57 ± 3.75	0.883±0.002
	25	6.13 ± 0.02	0.122 ± 0.007	129.3 ± 0.52	1.378±0.007
Hydroxypropyl MS = 0.12	40	3.66 ± 0.27	0.115 ± 0.007	72.2 ± 0.1	0.635±0.007
	33	4.38 ± 0.35	0.122 ± 0.006	97.0 ± 1.03	0.878±0.006
	25	6.23 ± 0.17	0.125 ± 0.000	149.0 ± 0.00	1.375±0.000
Hydroxypropyl MS = 0.18	40	4.50 ± 0.15	0.121 ± 0.002	72.17 ± 0.05	0.629±0.002
	33	5.72 ± 0.31	0.129 ± 0.006	101.2 ± 4.41	0.871±0.006
	25	6.29 ± 0.27	0.131 ± 0.009	151.36 ± 1.74	1.369±0.009

^a;Starch concentrations of 40%, 33%, and 25% correspond to moisture contents of 0.75g, 1.0g,and 1.5g, respectively

Table 3. Textural properties of starch gels with different concentration

Starch	Hardness			Cohesiveness			Springiness		
	40%	33%	25%	40%	33%	25%	40%	33%	25%
Native	11.38±1.90 ^a	5.53±1.73 ^a	2.03±0.63 ^a	0.69±0.04 ^a	0.70±0.07 ^a	0.85±0.02 ^{ab}	0.81±0.02 ^a	0.83±0.03 ^a	0.83±0.03 ^a
Control	6.21±0.98 ^b	3.11±1.73 ^b	0.93±0.39 ^b	0.76±0.03 ^a	0.80±0.08 ^b	0.88±0.03 ^a	0.85±0.03 ^a	0.84±0.04 ^a	0.83±0.05 ^a
HA	1.57±0.98 ^c	0.44±0.01 ^c	0.34±0.10 ^{bc}	0.84±0.07 ^b	0.89±0.02 ^b	0.79±0.01 ^b	0.81±0.05 ^a	0.83±0.10 ^a	0.81±0.01 ^a
HB	1.25±1.29 ^c	0.39±0.06 ^c	0.21±0.09 ^c	0.87±0.03 ^b	0.87±0.03 ^b	0.82±0.06 ^{ab}	0.79±0.02 ^a	0.79±0.03 ^a	0.75±0.03 ^b
HC	0.96±0.36 ^c	0.30±0.11 ^c	0.150±0.02 ^c	0.89±0.02 ^b	0.87±0.03 ^b	0.86±0.02 ^b	0.78±0.09 ^a	0.77±0.06 ^a	0.73±0.01 ^b

Means with the same letter in low are not significant different $p < 0.05$

Table 4 . The correlation coefficient (r) and p-values between hardness and NMR parameters (T_{2a} , I_{2a} , T_{2b} , and I_{2a}) of gels.

Attribute	T_{2a}	I_{2a}	T_{2b}	I_{2b}
Hardness	- 0.9061	- 0.8774	- 0.7376	- 0.5424
	< 0.001	< 0.001	0.0017	0.0367
T_{2a}		0.88150	0.68038	0.55703
		< 0.001	0.0052	0.0310
I_{2a}			0.55505	0.21985
			0.0317	0.4311
T_{2b}				0.8421
				< 0.001
I_{2b}				

The numbers are r and p-value on the top and bottom of the cell respectively

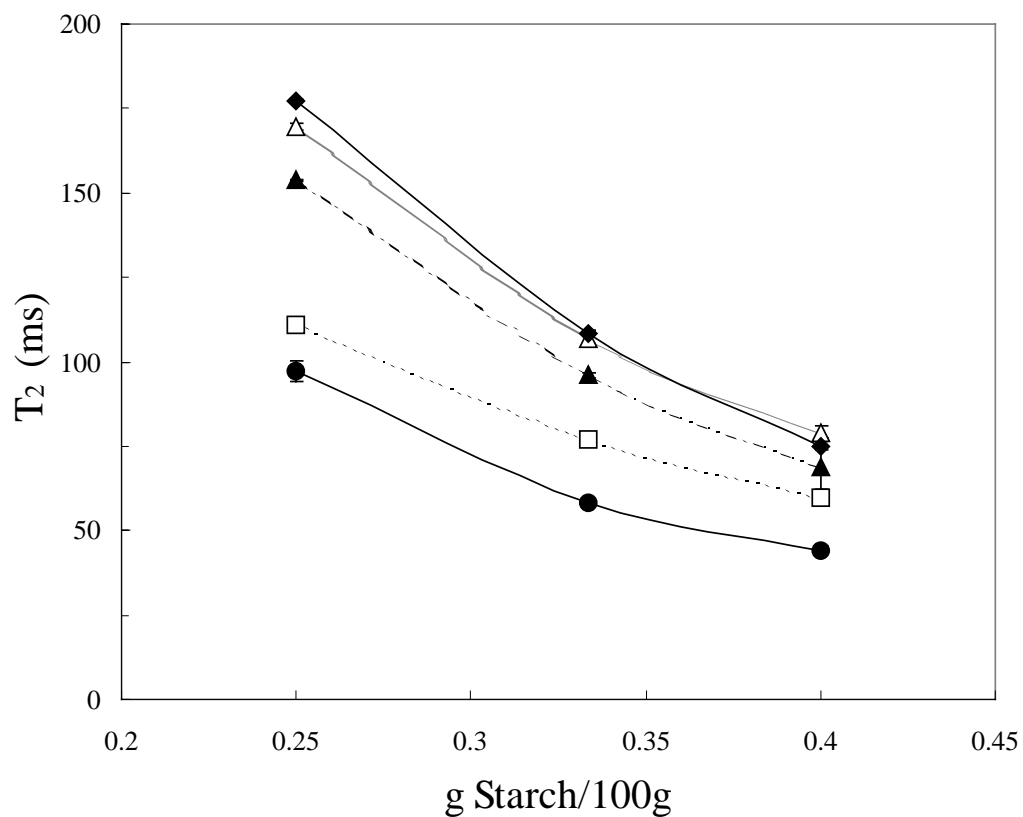


Figure 1- Single exponential transverse relaxation times (T_2) as a function of starch gel concentration for native wheat starch (●), alkali-treated wheat starch (□), and hydroxypropylated (▲ -MS 0.05, △ -MS 0.11, and ◆ -MS 0.18) wheat starch gels.

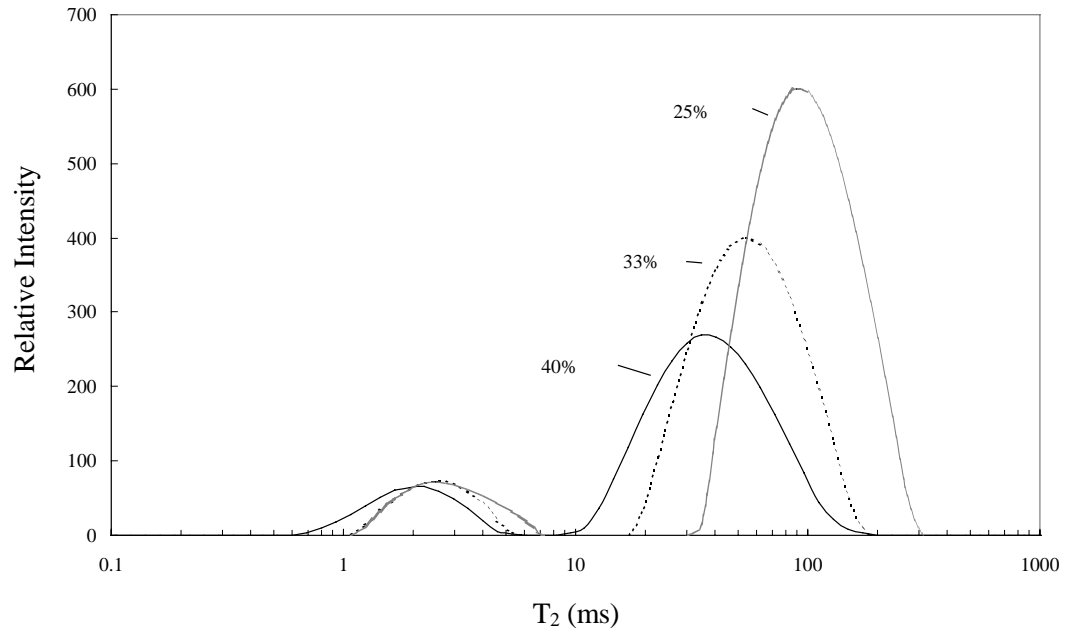


Figure 2- T_2 distribution for native wheat starch gel at 25, 33, and 40% starch concentration

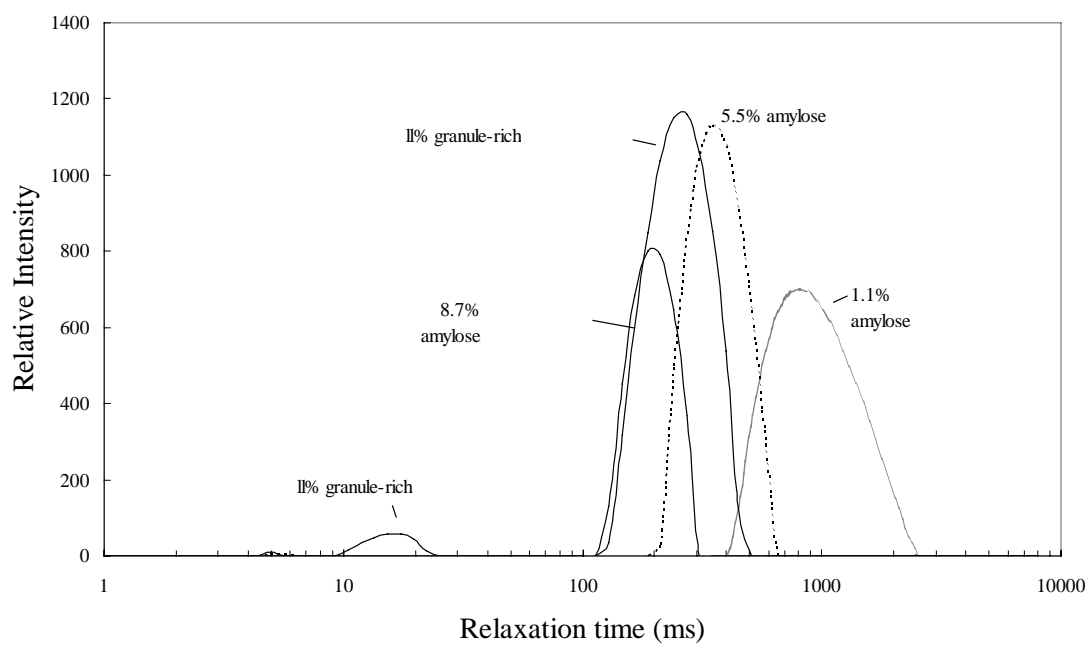


Figure 3- T₂ distribution for gels made 1.1, 5.5, and 8.7% solubilized amylose, and from 11% granule-rich sediment.

CHAPTER VI

**THE EFFECT OF HYDROXYPROPYLATION ON RETROGRADATION AND
WATER MOBILITY IN WHEAT STARCH GELS USING¹H NMR¹**

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ABSTRACT

The water dynamics in gels made from native wheat starch, control (alkali-treated) starch, and hydroxypropylated starch were studied using ^1H NMR relaxometry. Changes in T_2 were related to water mobility. Single exponential studies showed that greatest decreases with time in T_2 occurred for native wheat starch, indicating decreased water mobility as starch retrograded. Alkali-treated starch showed less change over time, while hydroxypropylated starch showed even less. Distributed exponential studies showed at least three domains of water. Isolated amylose gels had a single, relatively mobile water domain, with T_2 dependent upon gel concentration. This fraction exhibited only minor changes during storage at 5°C , and was not reversed by heating. Granule-rich gels showed two water domains, one with a T_2 range similar to that for amylose gels, but which varied over time and were thermally reversible. Studies on alkali treated starch showed that decreases in T_2 for each region were less than for native starch, but greater than for hydroxypropylated starch. Most significant changes occurred in the region of low water mobility associated with granule remnants. These studies show that, in addition to changes in starch during retrogradation, water mobility is also affected by recrystallization and chemical modification of starch molecules.

INTRODUCTION

Recrystallization is associated with changes in the physical properties of starch gels including hardness, water absorptive capacity, opacity, degree of hydrolysis, and soluble starch content, and contributes to defects in quality of starch-containing foods (Hoover, 1995, Keetels et al., 1996a,b, and c). Based on x-ray diffraction studies, Miles et al. (1985) proposed a mechanism in which amylose was responsible for short-term irreversible development of gel structure and crystallinity, while amylopectin was involved in long-term thermoreversible crystallization during storage.

The influence of starch source, starch concentration, storage temperature, salts and sugars, lipids, and chemical modifications on starch retrogradation has been studied (Orford et al., 1987; Biliaderis and Tonogari, 1991; Teo and Seow, 1992; Wu and Seib, 1990; Hoover and Sosulski, 1986; Morikawa and Nishinari, 2000). Less understood is how water interacts with starch in the process of retrogradation. It is commonly believed that the molecular motion of water plays an important role in starch retrogradation, and a few studies have focused on the dynamic state of water in starch-based products. 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. Whistler and Daniel (1985) 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. Based on T_1 or T_2 relaxation studies, pulsed ^1H NMR has been useful for quantifying the relative amounts of water in regions of differing water mobility, or in particular, of water with differing degrees of rotational freedom. From ^1H NMR studies, it has been observed that water mobility and the

fractions of water in different environments is highly correlated with the degree of starch retrogradation (Ruan et al., 1996; Farhat et al., 2000; Wynne-Jones and Blanshard, 1986). In addition, Le Botlan and Desbois (1995) and Teo and Seow (1992) have shown that ^1H NMR is useful for determining the degree of starch mobility, based on measurements of the signal intensity from the “solid-like” component in starch gels.

Chemical modification of starch, as by acetylation, cross-linking, or hydroxypropylation, has been used to improve the functional properties and storage stability of starch-based foods. Among them, hydroxypropylation has been particularly effective at retarding retrogradation, increasing water holding capacity, and improving freeze-thaw or cold-storage stability (Kim et al., 1993; Perera and Hoover, 1999). This has been attributed to steric effects imposed by bulky hydroxypropyl groups, which prevent alignment of starch chains leading to aggregation and crystallization. The influence of hydroxypropylation on starch retrogradation has been measured by the amount of water exuded during freezing and thawing, turbidity, x-ray diffraction, and DSC. However, little research has been published on the influence of hydroxypropylation on the changes in water relations during storage.

The objective of this research was to investigate the effect of starch concentration and degree of hydroxypropylation on the dynamic states of water in starch gels during storage, to compare the rate of such changes with that of retrogradation, and to increase further understanding of how hydroxypropylation retards starch retrogradation.

MATERIALS AND METHODS

Preparation of Hydroxypropylated Wheat Starch

Hydroxypropylation of wheat starch was accomplished as described by Kim et al. (1992). Native wheat starch (Sigma Chemical Co., St. Louis, MO) was lyophilized for 48 hrs, then kept over phosphorous pentoxide (Sigma Chemical Co., St. Louis, MO) to ensure maximum dryness. Dry sample (200 g) was suspended in 440 ml of distilled water containing 40 g Na_2SO_4 , then the pH adjusted to 11.3 with 1N NaOH (Table 1). The mixture was placed in 500 ml screw cap jars and maintained at 35°C in a controlled temperature water bath. Aliquots (8 ml, 16 ml, or 25 ml) propylene oxide (Sigma Chemical Co., St. Louis, MO) were added to the jars. The reaction proceeded for 24 hrs, and was terminated by adjusting the pH to 5.5 using 1 N HCl. The suspension was washed with distilled water and centrifuged several times for 15 minutes at 3200 g. The hydroxypropyl content was determined by the spectrophotometric method of Johnson (1969). The molar substitution (MS) was defined as moles of substituent per mole of anhydroglucose unit.

Control wheat starch was prepared with the same reagents and procedure, but without addition of propylene oxide. This was to ensure that any measured changes were due to hydroxypropylation and not from effects of pH change or heating.

Gel Preparation

In order to prepare homogeneous gels from highly concentrated starch suspensions, a microwave heating method was used. Starch samples (0.5 g) were weighed into glass tubes (3 cm x 1 cm diameter) and fitted with screw caps. Distilled water (0.75 g, 1.0 g, and 1.5 g,

respectively) was added to the tube to make a suspension (40, 33, and 25% starch by weight), and vortexed for 20 s. The sealed containers were placed on a rotating turntable and heated in the microwave oven (Daewoo, Seoul, South Korea) for 40 s at 800 W. After heating, the sample temperature was $94^{\circ}\text{C} \pm 2^{\circ}\text{C}$. Gels were formed as the samples were allowed to cool to 25°C for 1 hour. Fresh gels were immediately measured by NMR. For storage studies, gels were kept at $5^{\circ}\text{C} \pm 2^{\circ}\text{C}$ in a laboratory refrigerator and removed periodically for NMR analysis. All samples were equilibrated to 25°C for 1 hour prior to measurement.

Amylose and Granule Remnants

A 7% wheat starch paste was prepared at 70°C and gently stirred for 90 min. The paste was diluted with distilled water at 70°C to 200 ml and centrifuged at 2500 g for 15 min. The suspension was separated into two phases. The supernatant gave blue color after staining with 0.2% iodine solution (0.2g iodine, 2g potassium iodide in 100 ml of aqueous solution) and was clear of granules when examined by light microscopy. This was considered as the soluble starch fraction. The precipitate consisted of gelatinized starch granules when observed at 40X, and gave a purple color when reacted with iodine solution. This was considered as the insoluble starch fraction. To adjust the concentration of the soluble starch solution, water was allowed to evaporate from the sample while gently stirring at 72°C . The moisture contents of adjusted soluble solution and insoluble starch were determined by drying *in vacuo* at 75°C for 48 h. Gels were prepared from the amylose preparations and from the granule-rich sediment as described previously.

NMR Measurement

Proton relaxation measurements were made using a MARAN 20 MHz NMR spectrometer (Resonance Instruments, Whitney, UK). The glass tubes with gel specimens were placed in 18 mm diameter NMR tubes, which were covered with parafilm. All measurements were made at $25^{\circ}\text{C} \pm 0.1^{\circ}\text{C}$. Transverse (T_2) relaxation curves were developed using the Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence: $90_x-(t-180_y-\tau\text{-echo})_n$ (Meiboom and Gill, 1958). Acquisition parameters were set to a 90° pulse of $4.1\ \mu\text{s}$, relaxation delay of 6 s, and t of $300\ \mu\text{s}$. Relaxation curves obtained from the CPMG sequence were analyzed using a single exponential fitting model:

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

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. Alternately, a distributed exponential routine was used to analyze relaxation curves. Based on the “contin” routine 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 regions, 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 untreated, control, and hydroxypropylated starch at different concentrations, normalized changes in T_2 values and signal intensity were made:

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

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

where $T_{2,o}$ and $T_{2,t}$ are the time constants at time 0 and time t, respectively, and S_o and S_t are the pre-exponential factors at time 0 and time t, respectively.

Statistical Analysis

Five different treatment groups were developed: no treatment, control (all reagents and processing except propylene oxide), and three levels of hydroxypropylation (0.05, 0.11, and 0.18 MS). The modification procedure was repeated three times, and from each batch 3 samples were analyzed. Samples were analyzed over time at 1 and 12 hr, as well as at 1, 2, 3, 5, 7, 10, and 15 days. Analysis of variance and the Duncan test (SAS Institute, Cary, NC) were used to test for differences amongst samples at a given time and for differences over time. Non-linear regression techniques were used to fit the Avrami equation to plots of normalized T_2 and signal intensity versus time.

RESULTS AND DISCUSSION

Hydroxypropylation of Wheat Starch

Analysis of modified starches showed that the molar substitution, defined as the moles of hydroxypropyl per anhydroglucose unit, was 0.05, 0.11, and 0.18, respectively (Table 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 (Luan 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.

Mono-Exponential Model

Figures 1a-c shows changes in T_2 during storage of untreated, control, and hydroxypropylated wheat starch gels with starch concentrations of 25, 33, and 40%. In general, T_2 decreased most during the first 5 days, then changed more gradually up until 10 days. No significant differences in T_2 were measured between days 10 through 15. Similar changes over time have been observed for general measures of retrogradation including those of compression modulus, x-ray diffraction patterns, and dynamic mechanical moduli (Roulet et al., 1988). The decrease in T_2 over time indicates that water molecules *in toto* become less

mobile during storage. Using cross-relaxation NMR, Wu and Eads (1993) showed that a component of 25-40% starch gels became immobilized during storage. Reasons for changes in water mobility have been postulated. 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. We suggest that an additional cause of more rapid transverse decay (shorter T_2) over time can be interpreted in terms of a two-site rapid exchange model. Clearly, water in any particular domain is involved in rapid exchange processes, as we do not observe a relatively large T_2 indicative of bulk water. According to the rapid exchange model, the apparent T_2 is given by:

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

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. One interpretation is that fraction B represents “bulk” water, while fraction A represents water that is “bound”, immobilized, or engaging in chemical exchange with the polymer. As segments of the starch chains become less mobile with retrogradation, water near the polymer also has reduced motion. This reduces T_{2A} and therefore reduces the effective T_2 .

In general, the extent and rate of change in T_2 over time depended on starch concentration; the greater the starch concentration, the greater the extent and rate of change. Changes in T_2 for the control wheat starch were less than that of the untreated starch. This suggests that the alkaline treatment with NaOH and Na₂SO₄ retards starch retrogradation to

some extent. Similar conclusions were reached by Perara and Hoover (1999) using DSC and turbidity measurements. Based on x-ray diffraction studies, Perara et al. (1997) showed that alkaline reagents disrupt the double helices within amorphous regions of potato starch, and alter the crystalline structure of other regions. They concluded that the alkaline modified starch granules exhibit decreased retrogradation due to decreased chain aggregation.

Hydroxypropylated starch showed the least change in ^1H transverse relaxation over time, presumably due to a lower extent of aggregation with time. The extent of change in T_2 over time decreased as the degree of molar substitution of hydroxypropyl groups increased. Others have found that retrogradation is decreased as degree of hydroxypropylation is increased (Kim and Eliasson, 1993; Liu et al., 1998; Yook et al., 1993).

Distributed Exponential Model

Figure 2 a-c shows plots of continuous distributions of T_2 for native wheat starch at concentrations of 25, 33, and 40% held at 5°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 0.5-8 ms) and one at relatively longer times (8-200 ms). It should be noted that both regions represent relatively short T_2 values in comparison with freely mobile water ($T_2 \sim 2$ secs). The two distributions likely originate from distinct structural regions in the starch gels. Current theories on composite starch gel microstructure suggest that the continuous gel matrix is formed from amylose solubilized from the granule during cooking (Ring and Stainsby, 1982; Eliasson and Bohlin, 1982). Embedded within this matrix are gelatinized granule remnants, composed primarily of branched amylopectin (Han et al., 2000; Hibi, 1998). Gelation of the amylose-rich network occurs through formation of

junction zones, which are crystalline in nature (Zobel and Stephen, 1995). There is some evidence that amylose-amylopectin binding ties the granules within the gel network (Miller et al., 1973). In any event, the presence of the granules increases the stiffness of the gels (Miles et al., 1985). During storage, “staling” and increasing stiffness occurs and this has been attributed to formation of additional B-type crystalline regions (Miles et al., 1985). This appears to involve amylopectin-rich granules as the increased stiffness is thermoreversible. Amylose gels are not reversible at temperatures up to 100°C, while gelatinized granules which have been leached of amylose are both thermoreversible and show increasing stiffness during storage. However, changes which do occur with amylopectin seem to involve the outermost short branches of amylopectin which have lower melting points.

To better identify the peaks, studies on isolated amylose gels and granule remnants were conducted. Figure 3a shows distributed fits of T_2 values for amylose gels. In all cases, only a single peak was observed. T_2 values shifted to lower values, and distributions became more narrow, as amylose concentration was increased. The peak T_2 values were 840, 350, and 212 ms, for 1.1%, 5.5%, and 8.7% gels, respectively. This can be attributed to the increased cross-link density with increasing amylose concentration. In essence, any given water molecule has a shorter distance to diffuse to and interact with neighboring starch chains. Amylose gels showed only small changes in T_2 distributions during storage at 5°C (Figure 3b). For example, the maximum T_2 was 350, 400, and 390 ms at 0, 1, and 10 days of storage. Miles et al (1985) showed that the stiffness of amylose gels did not change during storage over several days.

In contrast, the granule rich sediment (11%) showed two distinct T_2 distributions (Figure 3c). The larger peak had a peak T_2 value of 240 ms, while the smaller peak had a peak T_2

value of 15 ms. The longer T_2 region has values on the same order of magnitude as that for the amylose gel. This suggests water in a fairly open structure, perhaps in an intergranular gel network. In contrast, the smaller T_2 region is likely due to water in a much more restricted environment. This region is similar to that found in the native starch gel, although at a slightly higher T_2 . However, the fractionated granule-rich material had more extensive heating than gels made from native starch. Thermal history has been shown to be a factor in influencing granule swelling and deformability (Keetels et al., 1996a; Liu et al., 1998; Hansen et al., 1991). A longer T_2 would indicate a more swollen environment.

Figure 3d shows that there is significant decrease in T_2 values during aging of the granule-rich material. For example, peak T_2 varies from 240, 187, and 100 ms for the long component, and 15, 9, and 6 ms for the short component, at storage times of 0, 1, and 10 days, respectively. Studies on isolated granules have shown that changes in stiffness occur during storage (Miles, 1985), and can be traced to an increase in B-type crystal regions. These crystalline regions are thermoreversible, and likely involve outermost short branches of amylopectin. Figure 3d shows that T_2 distributions were mostly restored with reheating. The peak T_2 for the long component was the same as that for the fresh gel. However, there was a broadening of the T_2 distribution. This suggests that there was an increase in the size distribution of interchain spaces in the amylopectin gel.

Figure 2 d-f shows plots of continuous distributions of T_2 for hydroxypropylated wheat starch (MS=0.18) gels at concentrations of 25, 33, and 40%, held at 5°C over 15 days. In general, there was no decrease in the average T_2 values for the more mobile (longer T_2) population. The change in integrated signal intensity during storage for both populations is shown in Figure 4 a-f, which includes curves for the untreated, control, and

hydroxypropylated starch gels. Changes in intensity for the more mobile fraction were significant but not large. However, there were no significant differences amongst the untreated, control, or different-MS hydroxypropylated starch gels. For the less mobile component, however, changes with storage were much more dramatic. The greatest change over time was observed for the untreated starch. The control starch showed slightly lower changes in signal intensity, but by days 7-15, was not significantly different than the untreated starch. Hydroxypropylated starch showed the least change in integrated signal intensity for the less mobile water component. By day 15, the least change was observed for the high MS hydroxypropylated starches at all starch concentrations. The decrease in signal intensity can be attributed to a decrease in water in the less mobile population. This is in agreement with results reported by Ishiguro et al. (2000) and Czuchajowska et al. (1998), who determined water loss in different starch gel types during storage. Miyazaki et al. (2000) suggested that water loss could be used as an index of retrogradation.

These results indicate that changes in the less mobile fraction of water are most closely associated with retrogradation. Steeneken (1984) reported that at the molecular level, hydroxypropyl groups were mainly distributed in whole amylose molecules and amorphous regions of amylopectin rich in α -1,6. Substitutions occur in about 33% of the amylopectin backbone, but no hydroxypropyl groups were found in the side chains, which constitute the crystalline regions of amylopectin. Based on x-ray diffraction and birefringence of starch granules, Hoover and Sosulski (1985) reported that all of the hydroxypropyl groups were located in the amorphous regions of the granule. They suggested that the altered structure influences starch retrogradation by decreasing chain aggregation between amylose and parts of amylopectin. As the short T_2 region has been associated with water in granule remnants,

the decrease in signal intensity indicates that water is expelled from swollen granule remnants. Similar observations were reported by Morikawa and Nishinari (2000) using a particle size analyzer. They demonstrated that the average particle size of swollen granules decreased as starch retrogradation proceeded.

Retrogradation Kinetics

The Avrami equation has been used to study the kinetics of starch recrystallization in terms of the increase in nucleation and growth of crystallites (Avrami, 1940). The fraction of uncrystallized material (θ) at time t is given by:

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

where k is the crystal growth rate constant and n is the Avrami exponent that depends on the type of nucleation. In general, the degree of crystallization is determined by x-ray diffraction or differential scanning calorimetry. Below, we have modified Equation 5 to test whether the change in T_2 of the less mobile water fraction is described by Avrami kinetics. Thus,

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

where $T_{2,o}$ is the initial average value, $T_{2,\infty}$ the average value at long storage time, and $T_{2,t}$ the value at time t . Data for all gels was fit to Equation 6 using a non-linear regression routine. In most all cases, the data was well fit with R^2 values greater than 0.92. Values for k and n were calculated from the fitted curves and are shown in Table 2. Values of n for untreated starch gels ranged from 0.629 to 0.816, depending on starch concentration, and is in good agreement with values reported by Teo and Seow (1992). Values of n for control and hydroxypropylated starches were less (0.324 to 0.689 for control; 0.271 to 0.479 for all hydroxypropylated

samples), implying that these treatments influence the nature of nucleation during recrystallization. In general, the rate of retrogradation for starch gels as measured by T_2 decreased in the order: untreated, control, hydroxypropylated (0.05 MS), hydroxypropylated (0.11 MS), and hydroxypropylated (0.18 MS). In addition, as the starch concentration increased, retrogradation proceeded at a faster rate.

CONCLUSION

The effects of hydroxypropylation and starch concentration on retrogradation and water mobility in starch gels was investigated. Measurements of transverse proton relaxation as measured by T_2 show that changes in water mobility accompany retrogradation. Greatest changes were observed with native wheat starch, less with alkali treated starch, and even less with hydroxypropylated starch. More detailed studies indicate that at least two domains of water exist in the starch gels. Measurements on isolated amylose gels indicate that a fairly mobile water state is associated with the gel, does not change over time nor is thermoreversible. Studies on granule rich fractions show a fairly less water state that changes with time, and a more restricted state that also changes with time. Greatest changes were observed for water in restricted regions of granule remnants.

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Table 1: Molar substitution (MS) of hydroxypropyl groups on wheat starch modified with propylene oxide

Starch (g)	Na ₂ SO ₄ (g)	Propylene Oxide (mL)	Hydroxypropyl (C ₃ H ₇ O)(%)	MS
200	10	0	0	0
200	10	8	2.02	0.05
200	10	16	4.11	0.12
200	10	25	6.22	0.18

Table 2-Avarami constants (Equation 6) for starch gels held up to 15 days

Sample	%Starch	k	n	R ²
Native	25	0.384	0.629	0.96
	33	0.712	0.644	0.97
	40	0.873	0.816	0.98
Control	25	0.187	0.324	0.98
	33	0.188	0.479	0.98
	40	0.385	0.689	0.95
0.05 MS HP	25	0.067	0.361	0.97
	33	0.098	0.337	0.98
	40	0.150	0.380	0.96
0.11 MS HP	25	0.038	0.372	0.98
	33	0.045	0.461	0.99
	40	0.089	0.461	0.93
0.18 MS HP	25	0.013	0.479	0.99
	33	0.017	0.378	0.92
	40	0.037	0.271	0.76

Unit for k: day⁻¹

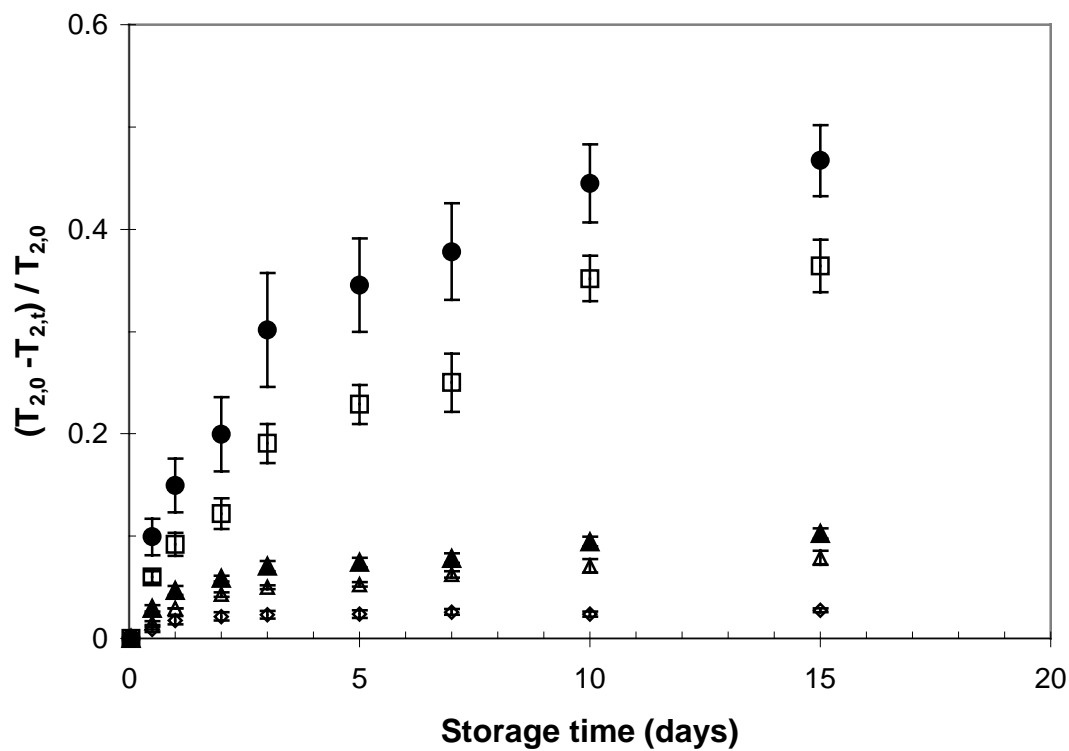


Figure 1a-Normalized change in single transverse relaxation time (T_2) for native wheat starch (●), alkali-treated wheat starch (□), and hydroxypropylated (▲ -MS 0.05, △ -MS 0.12, and ◆- MS 0.18) wheat starch gels at starch concentrations of 25% by weight.

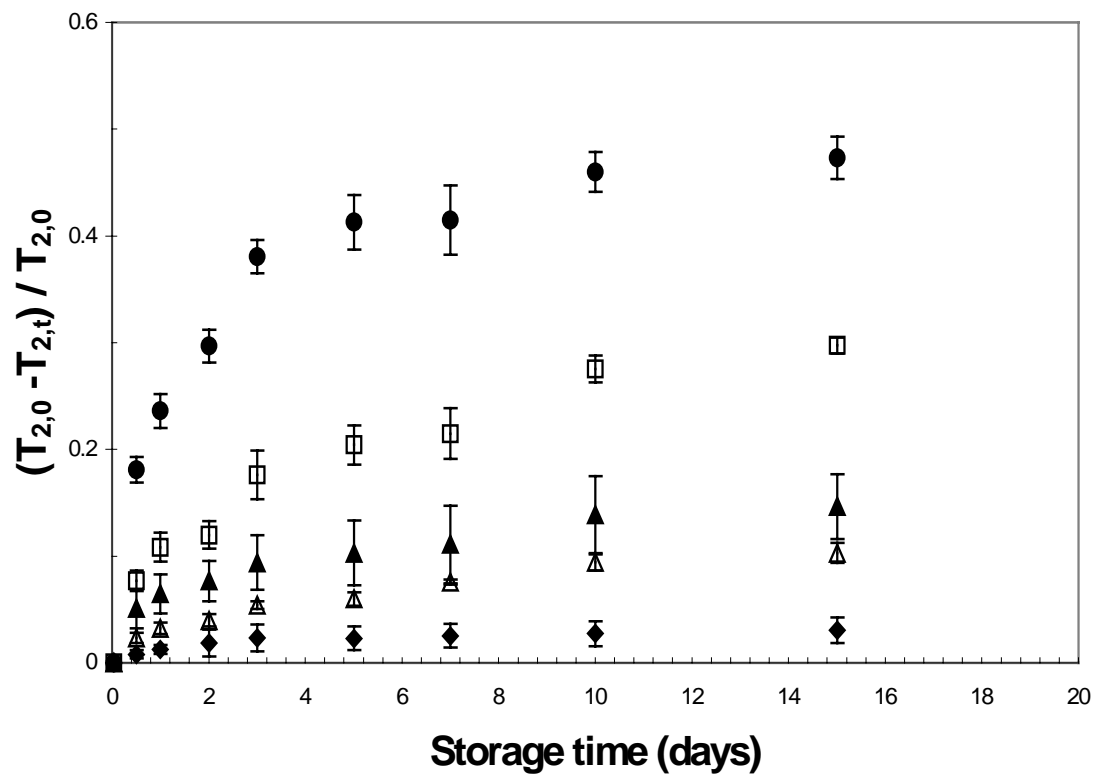


Figure 1b-Normalized change in single transverse relaxation time (T_2) for native wheat starch (●), alkali-treated wheat starch (□), and hydroxypropylated (▲ -MS 0.05, △ -MS 0.12, and ◆- MS 0.18) wheat starch gels at starch concentrations of 33%.

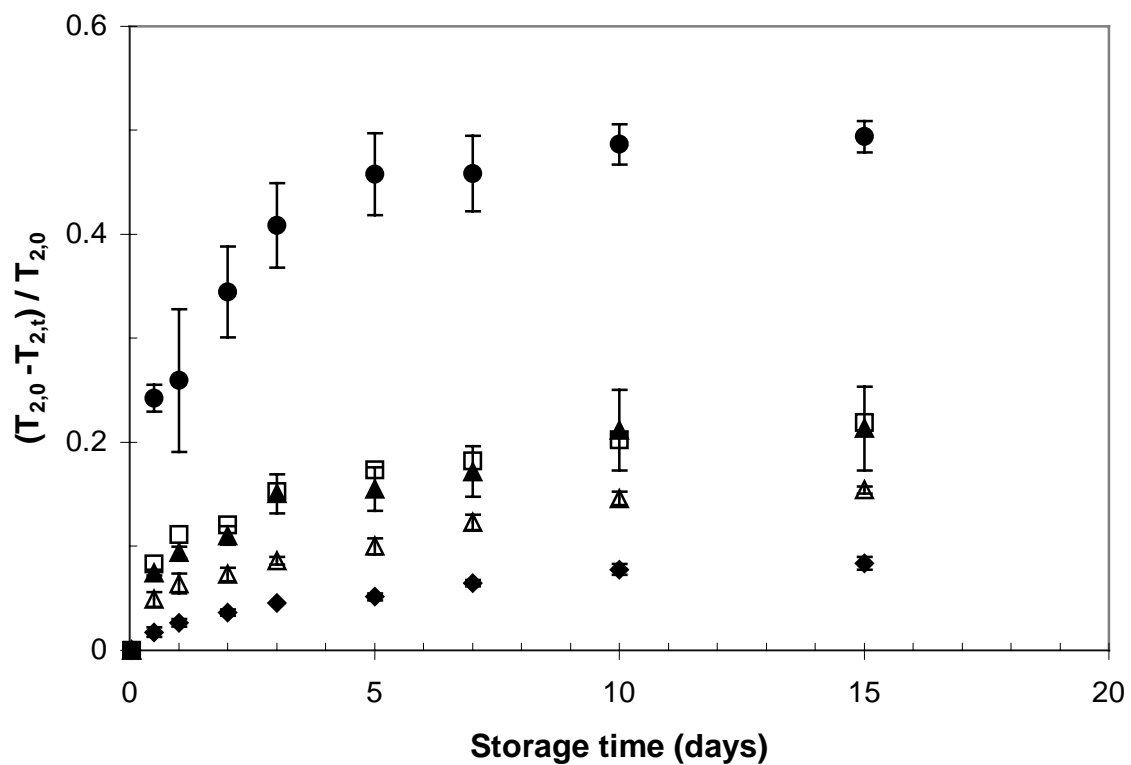


Figure 1c-Normalized change in single transverse relaxation time (T_2) for native wheat starch (●), alkali-treated wheat starch (□), and hydroxypropylated (▲ -MS 0.05, △ -MS 0.12, and ◆- MS 0.18) wheat starch gels at starch concentrations of 40%.

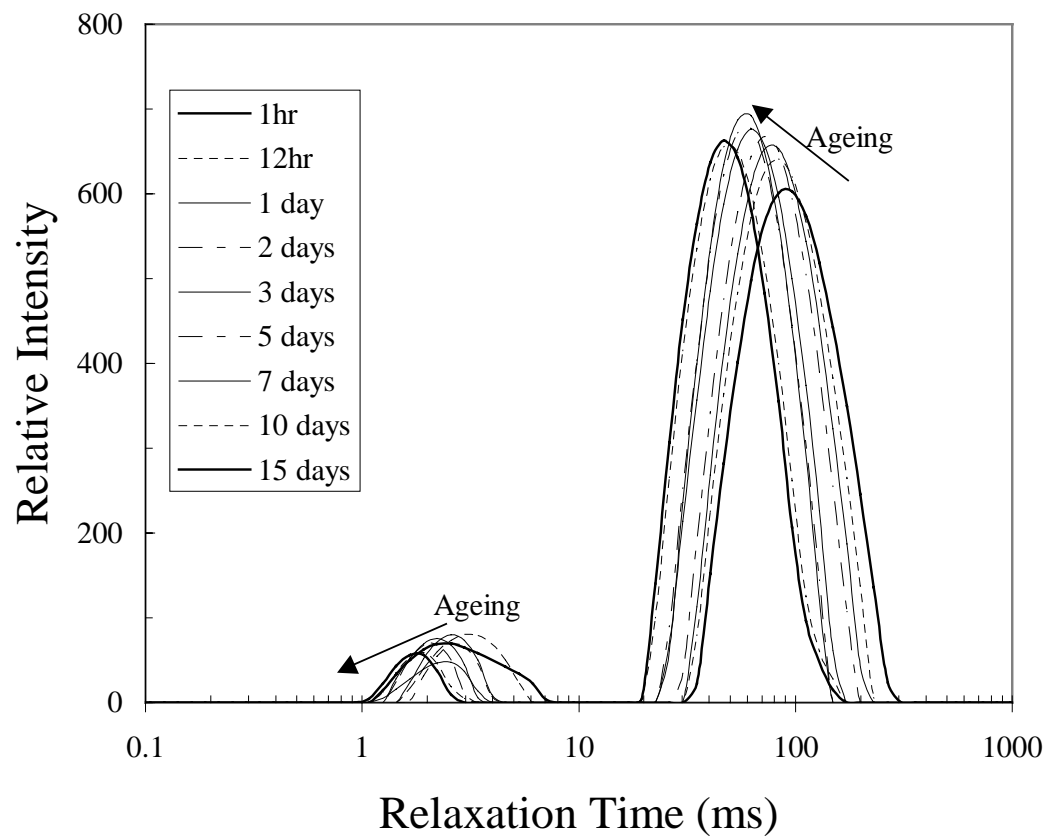


Figure 2a- Distributed exponential fits of T_2 over time for native wheat starch gels at 25% concentration.

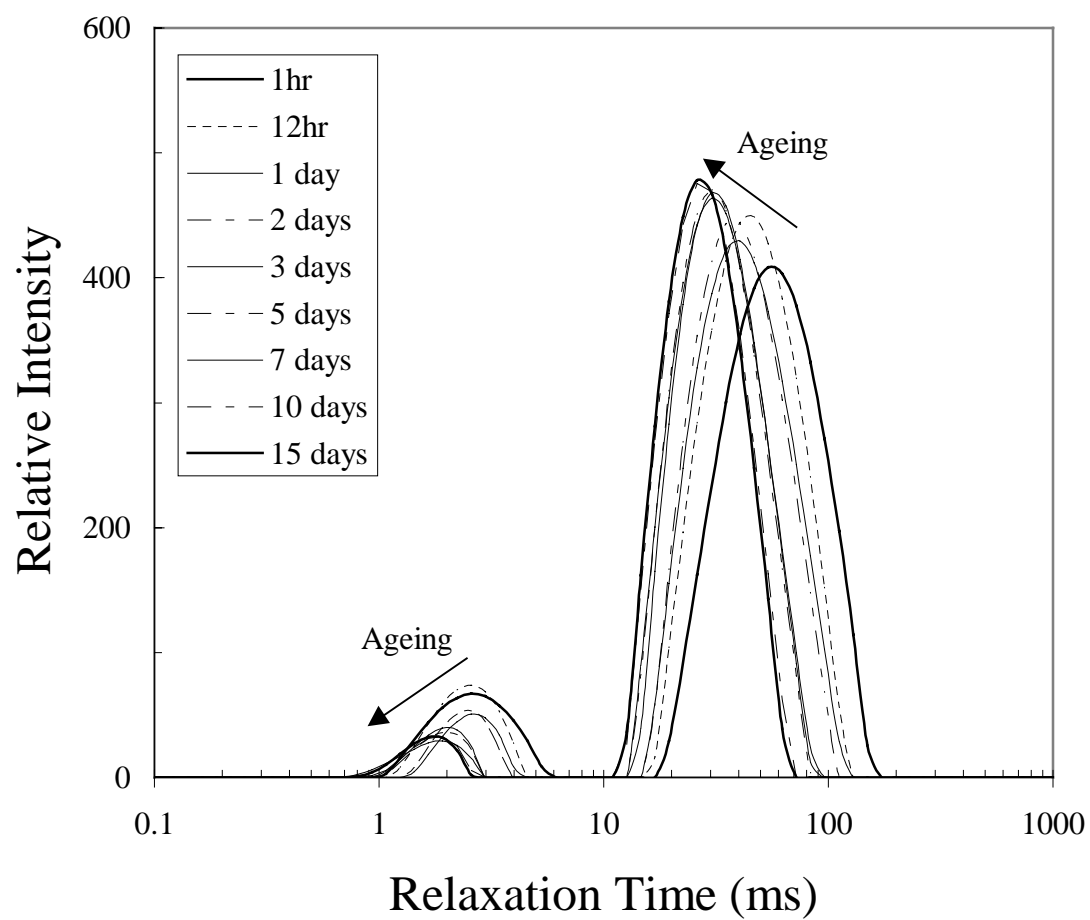


Figure 2b- Distributed exponential fits of T₂ over time for native wheat starch gels at 33% concentration.

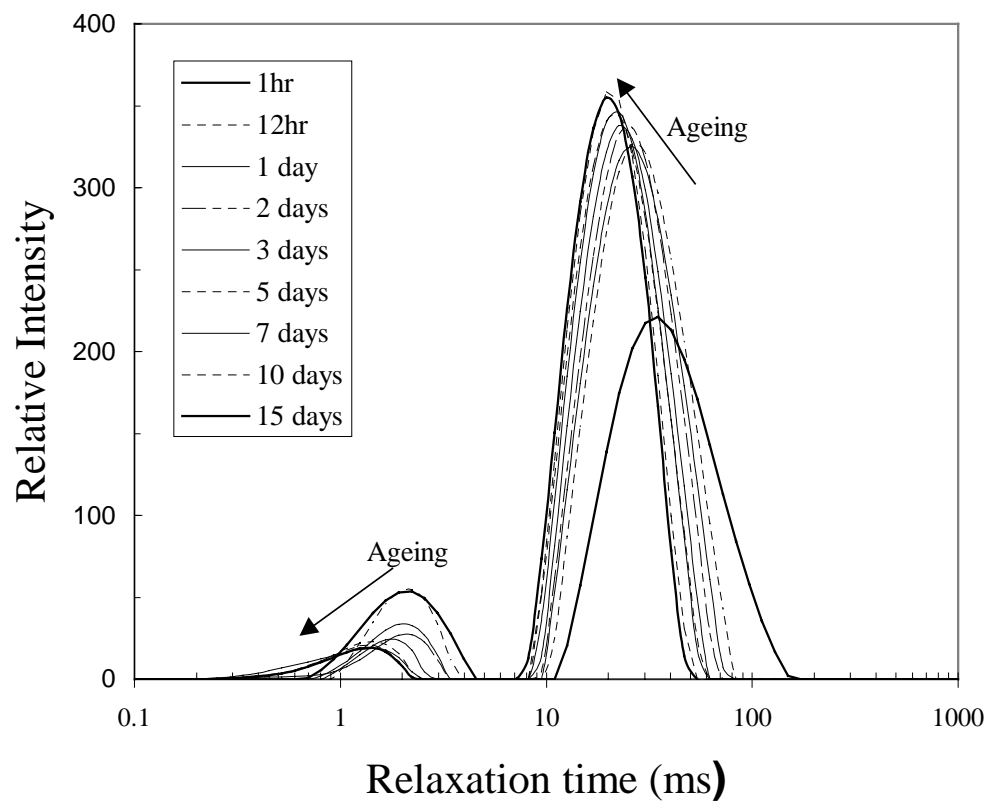


Figure 2c- Distributed exponential fits of T_2 over time for native wheat starch gels at concentration.

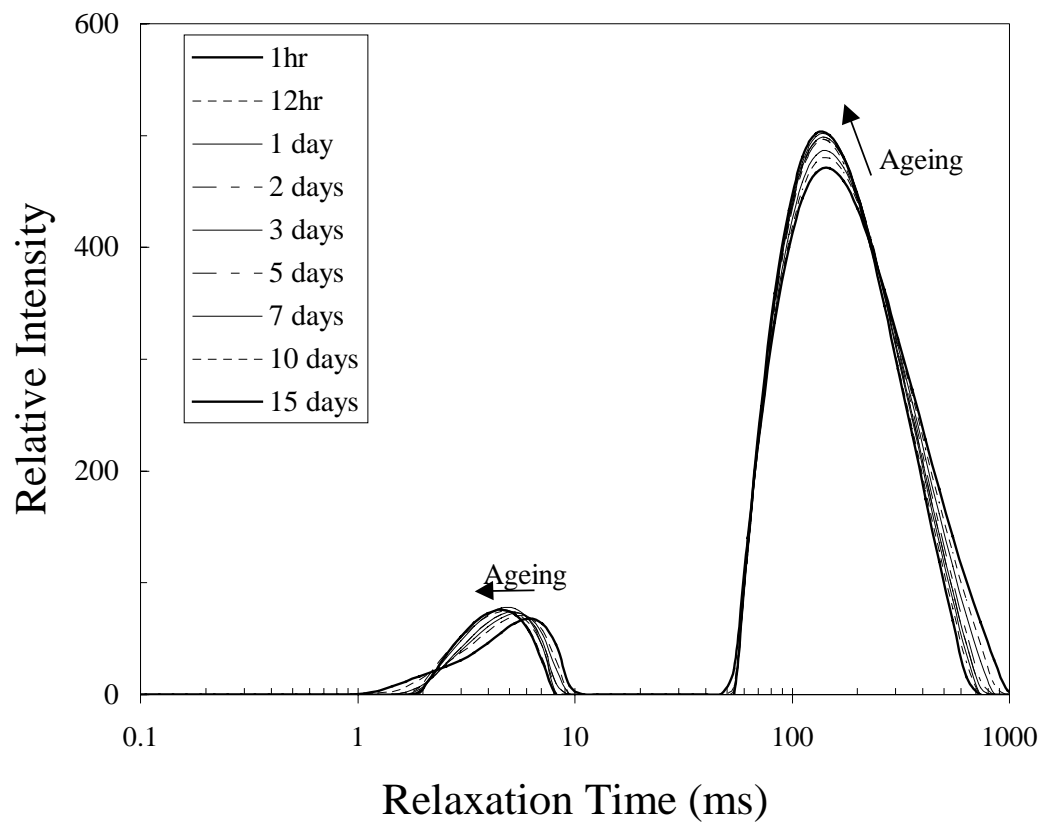


Figure 2d- Distributed exponential fits of T_2 over time for hydroxypropylated (0.18 MS) wheat starch gels at 25% concentration.

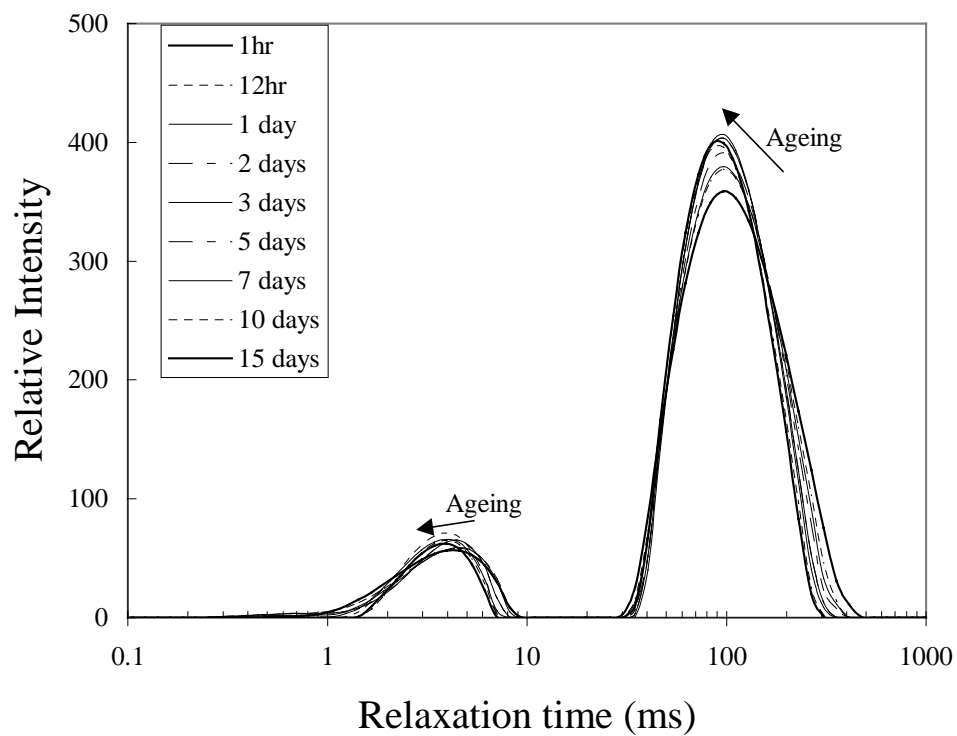


Figure 2e- Distributed exponential fits of T_2 over time for hydroxypropylated (0.18 MS) wheat starch gels 33% concentration.

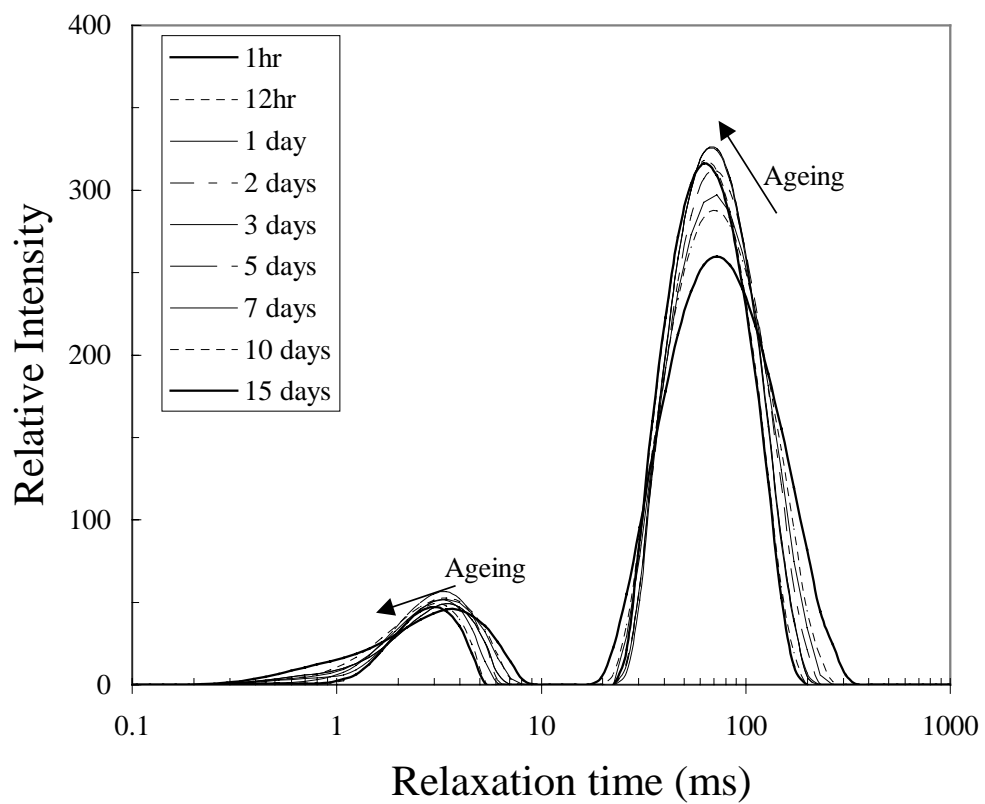


Figure 2f- Distributed exponential fits of T_2 over time for hydroxypropylated (0.18 MS) wheat starch gels at 40% concentration.

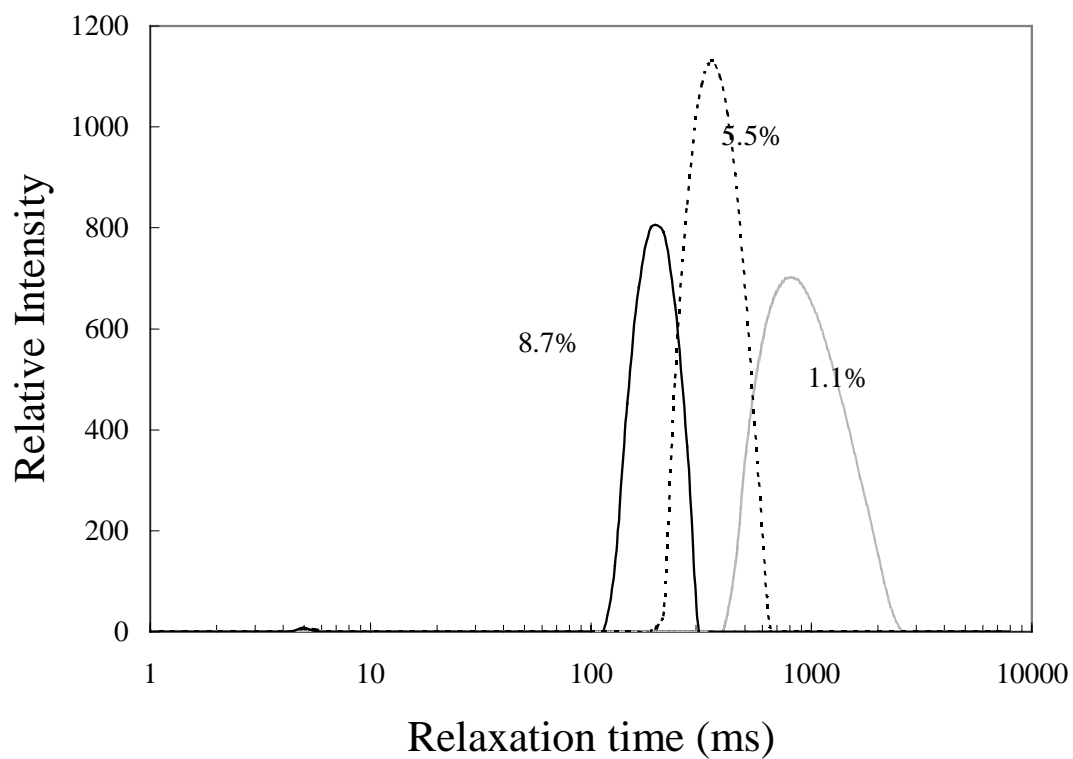


Figure 3a- Distributed exponential fits of T_2 over time for gels made from fractionated starch components: amylose gels at 1.1%, 5.5%, 8.7% starch.

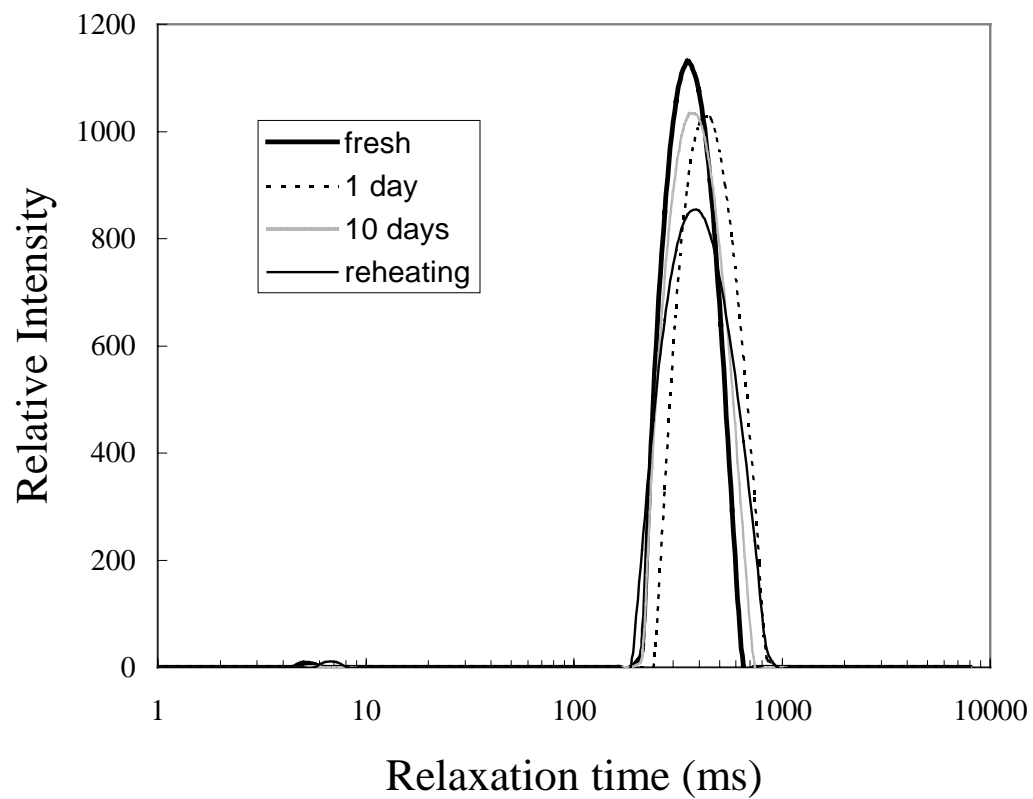


Figure 3b- Distributed exponential fits of T_2 over time for gels made from fractionated starch components: 5.5% amylose gels over time and after reheating at 90°C.

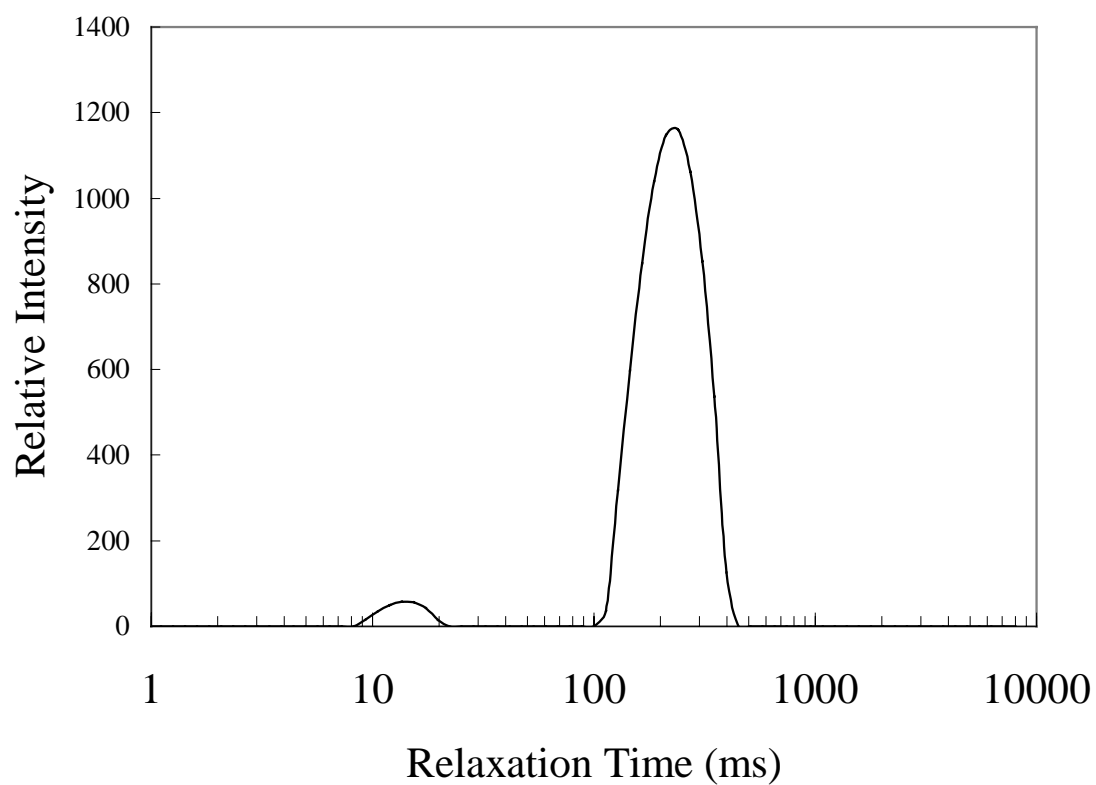


Figure 3c- Distributed exponential fits of T_2 over time for gels made from fractionated starch components: granule-rich sediment (11% solids).

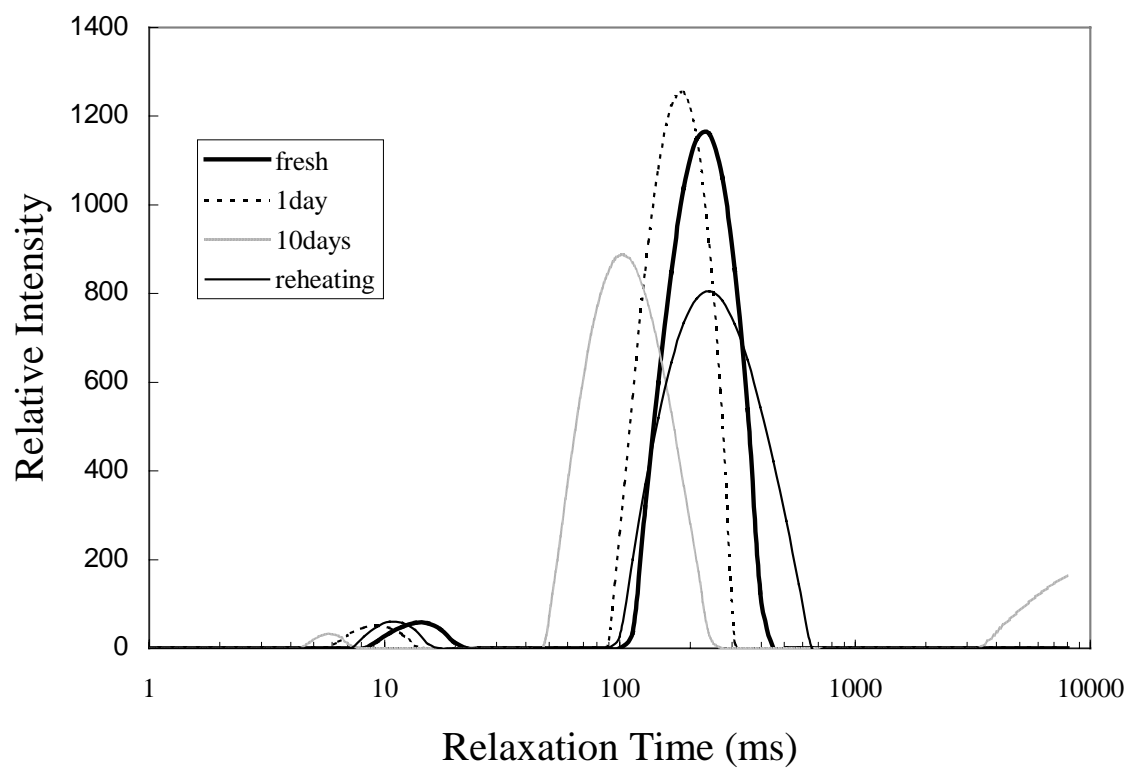


Figure 3d- Distributed exponential fits of T_2 over time for gels made from fractionated starch components: granule-rich sediment during storage and after reheating.

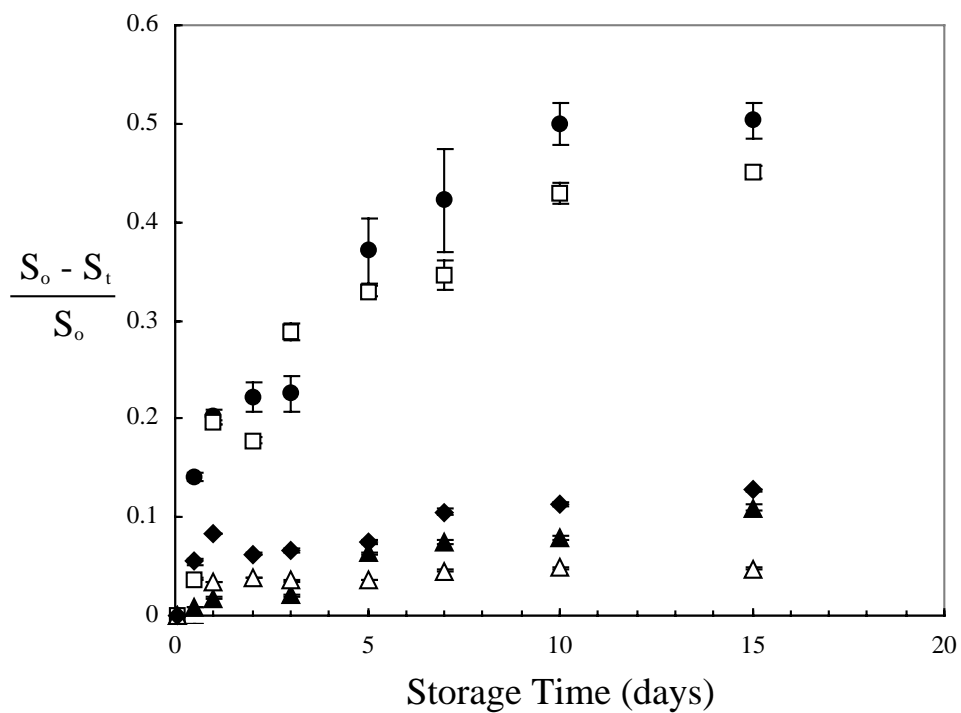


Figure 4a- Normalized signal intensities from less mobile (low T_2) regions of native wheat starch gels (●), alkali-treated wheat starch gels (□), and hydroxypropylated starch gels (▲ -0.05 MS, △-0.12 MS, ◆-0.18 MS) at 25% concentration.

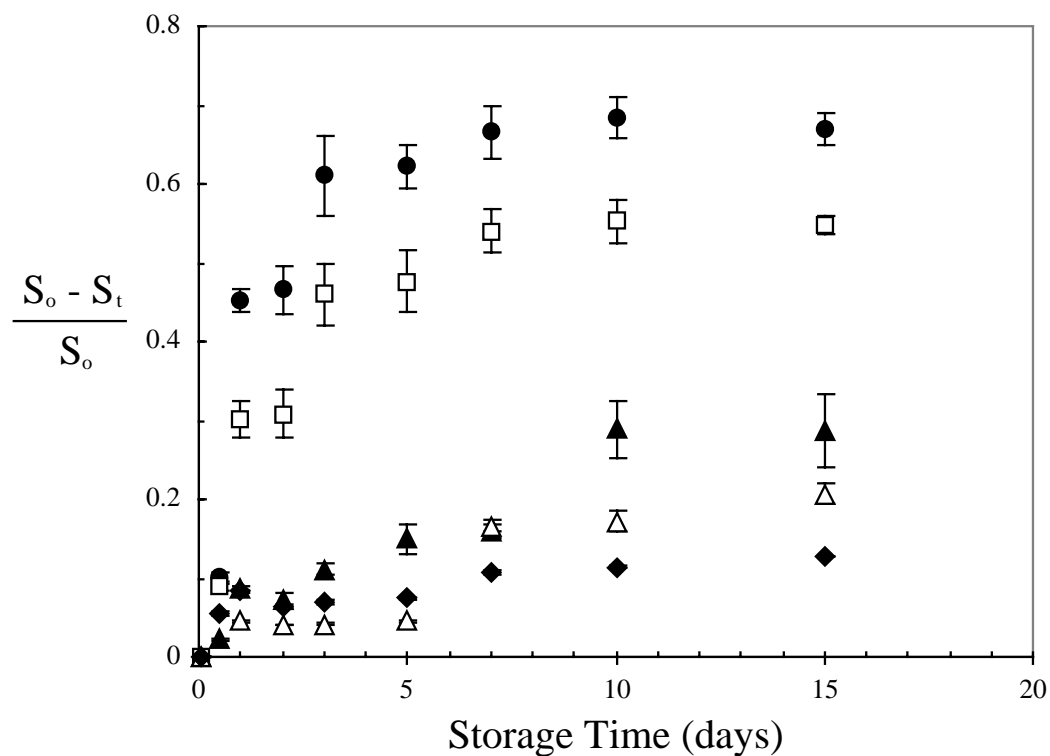


Figure 4b - Normalized signal intensities from less mobile (low T2) regions of native wheat starch gels (●), alkali-treated wheat starch gels (□), and hydroxypropylated starch gels (▲ -0.05 MS, △-0.12 MS, ◆-0.18 MS) at 33% concentration.

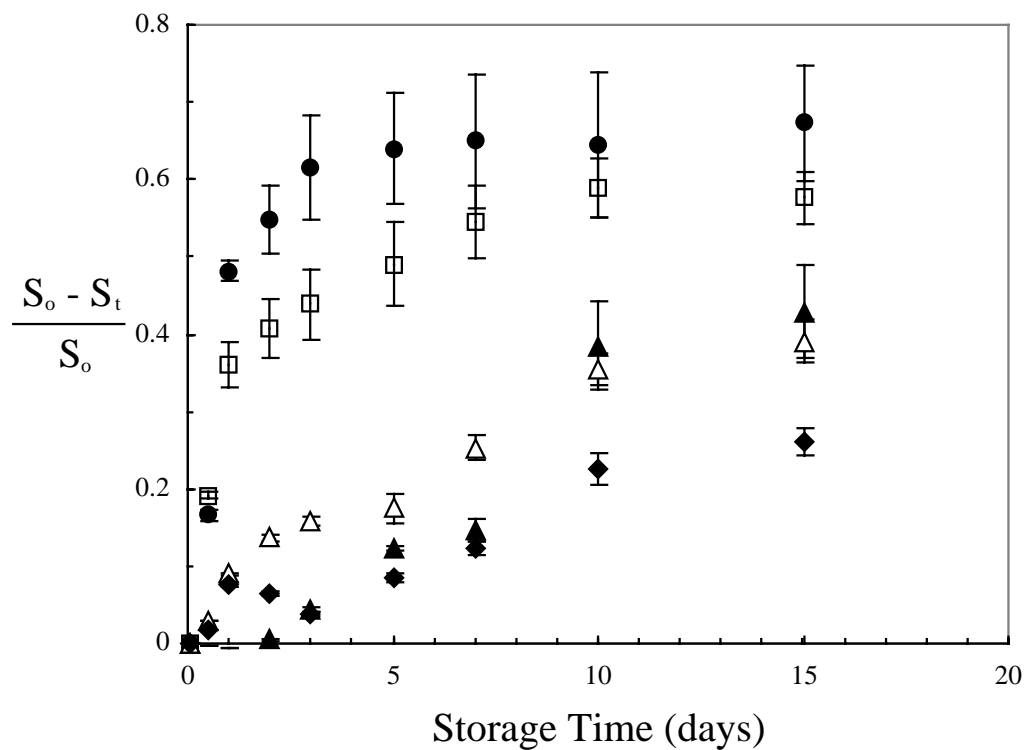


Figure 4c- Normalized signal intensities from less mobile (low T2) regions of native wheat starch gels (●), alkali-treated wheat starch gels (□), and hydroxypropylated starch gels (▲ -0.05 MS, △-0.12 MS, ◆-0.18 MS) at 40% concentration.

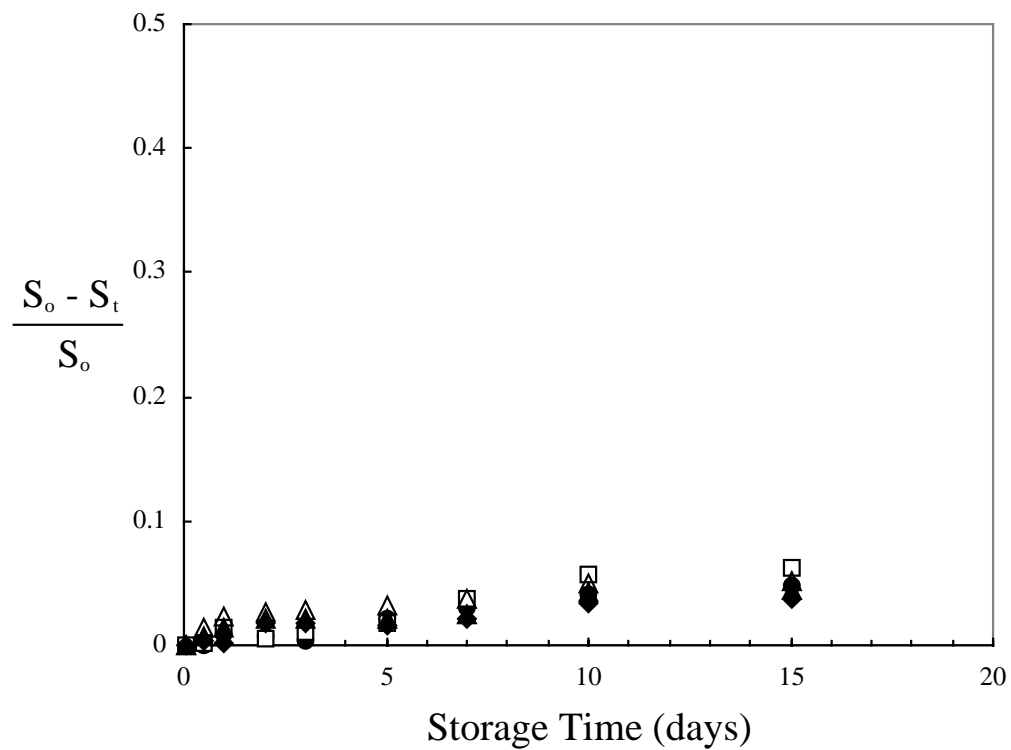


Figure 4d- Normalized signal intensities from more mobile (high T2) regions of native wheat starch gels (●), alkali-treated wheat starch gels (□), and hydroxypropylated starch gels (▲ -0.05 MS, △-0.12 MS, ◆-0.18 MS): at 25% concentration.

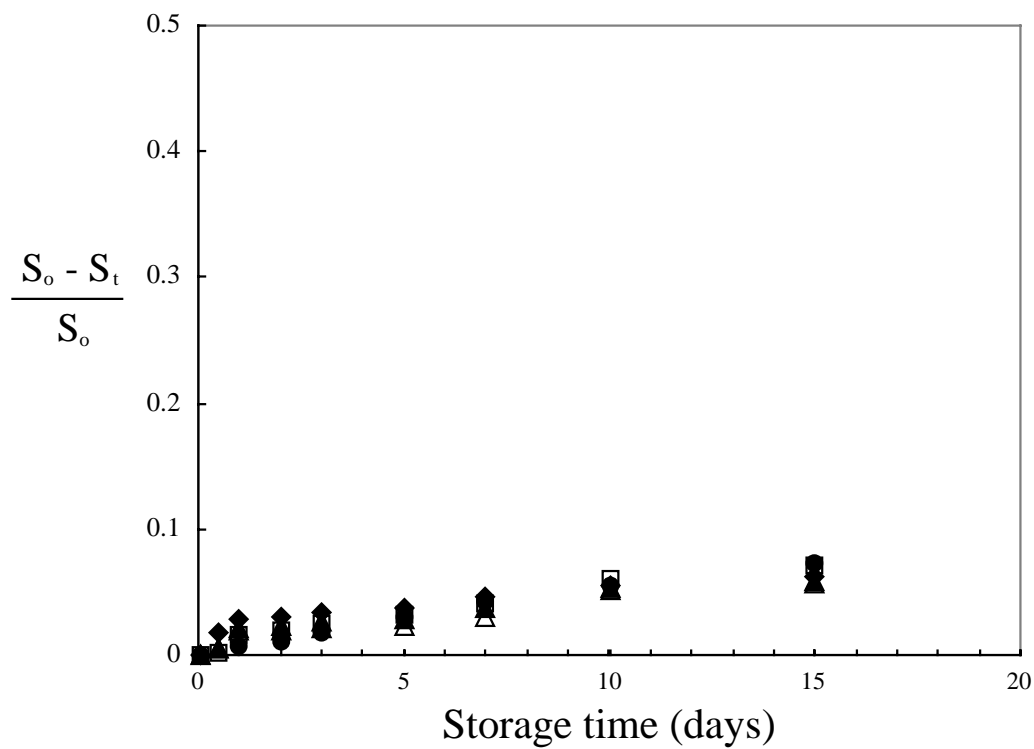


Figure 4e- Normalized signal intensities from more mobile (high T2) regions of native wheat starch gels (●), alkali-treated wheat starch gels (□), and hydroxypropylated starch gels (▲ -0.05 MS, △-0.12 MS, ◆-0.18 MS): at 33% concentration gel.

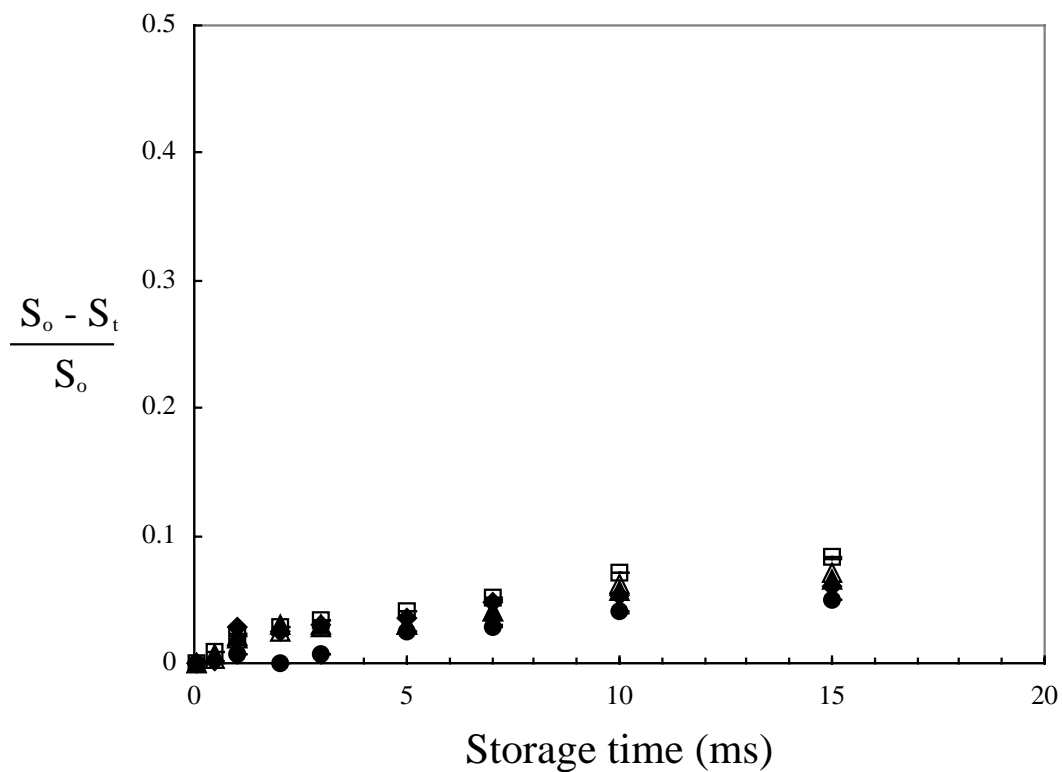


Figure 4f- Normalized signal intensities from more mobile (high T2) regions of native wheat starch gels (●), alkali-treated wheat starch gels (□), and hydroxypropylated starch gels (▲ -0.05 MS, △-0.12 MS, ◆-0.18 MS) at 40% concentration.

CHAPTER VII

SUMMARY AND CONCLUSIONS

The goals of this research were to study the effects of chemical modification on the molecular dynamics of starch-water suspensions at low moisture levels or in the gel state, and to investigate the relationship with textural properties and retrogradation.

In the first experiment, pulsed ^1H NMR techniques were developed to identify and quantify the molecular motion of starch chains and water molecules of starch suspensions at low moisture levels. In addition, the techniques were also employed to determine moisture content through analysis of FID curves. Dry samples were equilibrated to water activity values between 0 and 0.93. Transverse proton relaxation (T_2) of the systems were identified using distributed exponential analysis of one pulse and CPMG experiments. Proton relaxation associated with starch molecules increased linearly from 7.1 to 10.1 μs as a_w increased from 0 to 0.93, suggesting increased mobility due to plastisizing effects of water. The proton relaxation associated with water molecules showed a linear increase up to a_w of 0.69 and deviation from linearity above a_w of 0.81. Such deviation may be attributed to enhancement of relaxation by exchange between water molecules in different states. Results indicate there are multiple regions of hydration by water molecules, which is shown in moisture sorption isotherm curve. Moisture determination by NMR agreed well with gravimetric determinations ($R^2= 0.99$).

The effects of hydroxypropylation and cross-linking on water sorptive capacity and the molecular mobility of wheat starch suspensions at low moisture were

investigated. Wheat starch was hydroxypropylated with propylene oxide (MS of 0.05, 0.12, and 0.18) or cross-linked with phosphorus oxychloride at concentrations of 0.03%, 0.1%, and 0.2%, respectively. The water absorption capacity of hydroxypropylated starch increased with increasing degree of molar substitution of hydroxypropyl group at a_w above 0.53. Cross-linking with phosphorus oxychloride at concentrations of 0.03% and 0.1% did not alter water sorption capacity, while crosslinking at 0.2% caused a slight decrease in water absorption at $a_w > 0.53$. Proton relaxation associated with starch molecules increased with increasing molar substitution of hydroxypropyl group, indicating increased mobility of starch chains. However, mobility was not significantly affected by cross-linking. Transverse proton relaxation associated with water in control (alkali treated), hydroxypropylated and cross-linked starch showed two distinct populations in the a_w range of 0.53 to 0.93. This may suggest that treatment with alkaline reagents used in chemical modification may cause structural changes in starch granules.

In a third experiment, the effect of starch concentration and degree of hydroxypropylation on the water dynamics and textural attributes of starch gels was studied. Gels were prepared at concentration between 25 and 40%. For all gels, two distinct water fractions were identified using distributed exponential analysis of CPMG results. For the less mobile fraction, T_{2a} ranged from 2~6ms while for the more mobile state, T_{2b} ranged from 20~200ms. Values of both T_{2a} and of T_{2b} increased with increasing moisture content and hydroxypropyl M.S. Control experiments indicated that more mobile states could be associated with both the extragranular amylose gel, as well as intragranular amylopectin-rich gel. The less mobile state was associated only with granule remnants. Gel hardness and springiness were significantly decreased in

hydroxypropylated samples ($p < 0.05$). As cohesiveness was increased in hydroxypropylated samples, this suggests enhanced deformation of starch granule remnants. Multi-regression analysis showed that the mobility and amount of water in the less mobile state is a dominant factor for hardness of gels with starch concentration in the range of 25 to 40%.

Finally, the water dynamics in native, alkali-treated, and hydroxypropylated gels prepared at concentrations between 25 to 40% during storage at 5°C were investigated. Measurement of transverse proton relaxation showed that changes in water mobility accompany retrogradation, indicating a decrease in water mobility. The kinetics of retrogradation was analyzed by fitting the change in T_2 to an Avrami equation. The extent and rate of change in T_2 over time depended on starch concentration; as the starch concentration increased, retrogradation proceeded at a faster rate. In addition, the rate of retrogradation for starch gels decreased in the order: native, alkali-treated, hydroxypropylated (0.05 MS), hydroxypropylated (0.11 MS), and hydroxypropylated (0.18 MS). The results of proton transverse relaxation behavior analyzed by a distributed fitting model showed that water associated with starch molecules in granule remnants significantly decreased in mobility and was expelled from the granule as retrogradation proceeded. In addition, hydroxypropylation significantly retarded the changes.

In conclusion, chemical modification by alkali-treatment, cross-linking, and hydroxypropylation caused changes in molecular dynamics of starch molecules and its water binding properties. In addition, the molecular mobility of both starch and water was highly related to water sorptive capacity, textural attributes, and retrogradation behavior. This provides not only understanding of the altered functional properties but

also better control of the modification process for developing chemically modified starch with desired properties.