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INFLUENCE OF ADDITION OF POLYOLS AND FOOD EMULSIFIERS ON THE RETROGRADATION RATE OF STARCH

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Abstract

The influence of polyols and emulsifiers on hardening of non-glutinous rice starch gels has been investigated. The polyols and emulsifiers were added at concentrations of 6% (w/w) and 0.2% (w/w), respectively, to starch gels (starch content, 30%). The hardening rate and the initial viscoelastic values of gels were computed by analyzing static linear viscoelastic parameters (creep compliance) of the gels stored at 0 °C for up to 3,000 minutes.

Hardening of gels, i.e., retrogradation of starch, could be described by a first order kinetic equation. Polyols prevented the retrogradation of starch, and formed rigid and stable gel matrices. Emulsifiers also retarded starch retrogradation and stabilized the gel network.

The use of the two food additives was successful in retarding the retrogradation of starch. It follows that these effects might be attributed to the water-structure formation effect of polyols and the complex-formation of starch chains with emulsifier molecules.

Key Words: Rice, starch, gelatinization, retrogradation, gel, polyols, saccharides, emulsifiers, viscoelasticity, creep, kinetics.

Introduction

Starch is valued as a food material because of its contribution to texture (Ring, 1985). Starch-containing foods are usually processed by heating in the presence of water. Above a characteristic temperature, starch granules irreversibly swell. During the swelling, the viscosity of the suspension increases until, a viscoelastic paste is formed at high concentration. Hydration and gelatinization of starch have been studied using microscopy (Schoch and Maywald, 1956), wide-angle X-ray diffraction, Photographic light scattering (Kato and Matsuo, 1983), viscometry (Oosten, 1980; Deffenbaugh and Walker, 1989), and calorimetry (Nakazawa *et al.*, 1984a; Biliaderis *et al.*, 1986). More recently, studies of starch gelatinization at the molecular level have been undertaken using rheometry (Cheer and Lelievre, 1983; Hansen *et al.*, 1990) and nuclear magnetic resonance (NMR), i.e., pulsed NMR (Chinachoti and Steinberg, 1989), ¹H-NMR (Tanner *et al.*, 1991), ²H-NMR (Richardson *et al.*, 1987), ¹³C-NMR (Chinachoti *et al.*, 1991a), and ¹⁷O-NMR (Chinachoti *et al.*, 1991a, 1991b). Electron spin resonance (ESR) has been also used to characterize gelatinization (Biliaderis and Vaughan, 1987).

After gelatinization or pasting of starch, the amylose and amylopectin are considered to be preferentially hydrated. The entangled amylose has a strong tendency to associate with adjacent amylose chains through the formation of hydrogen bonds, when the sol is cooled or allowed to stand for long periods of time. This phenomenon is known as retrogradation and manifests itself through the formation of precipitates or gels. The hardness of the gel increases with time over several weeks (Lineback and Rasper, 1988). The retrogradation of starch is of considerable commercial importance, as it impairs the texture of starch-based food products. Much work is needed to fully understand the mechanism of this process in real food systems, and thereby to find best means for preventing retrogradation. In fact, however, the factors influencing the rheological properties of the starch gel are poorly understood. Consequently it is difficult to relate the molecular processes during retrogradation to the mechanical properties of the gel.

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The retrogradation and gelation of starch and starch components have been studied widely by using wide-angle X-ray diffraction (Wu and Sarko, 1978); differential scanning calorimetry, DSC (Nakazawa *et al.*, 1984b; Russell and Oliver, 1989); pulsed NMR (Nakazawa *et al.*, 1983); beta-amylase-pullulanase method (Kainuma *et al.*, 1981); and rheometry (Horiuchi and Sada, 1989; Russell and Oliver, 1989). Recent studies have also employed Fourier-transform infrared spectroscopy, FT-IR (Wilson *et al.*, 1987); small-angle neutron scattering (Blanshard *et al.*, 1984); and small-angle X-ray scattering (Cameron and Donald, 1991).

It is known that the molecular structure of starch affects its mechanical properties (Hwang and Kokini, 1991). Roulet *et al.* (1988) reported that rigidity of the starch gel was a good index of starch retrogradation at the macromolecular network level.

Saccharides (e.g., sucrose) increase the onset temperature of starch gelatinization, according to several studies (Bean and Yamazaki, 1978a, 1978b; Chungcharoen and Lund, 1987). The precise mechanism, by which saccharides delay starch gelatinization, is still unclear. Hansen *et al.* (1989) classified the mechanism into three types; (a) competition between the saccharides and starch for available water and associated changes in free volume (Derby *et al.*, 1975; Hoseney *et al.*, 1977); (b) the ability of saccharides in water system to retard gelatinization by inhibiting the swelling of the starch granules (Savage and Osman, 1978; Wootton and Bamunuarachchi, 1980); and (c) the ability of saccharides to penetrate the starch granule and interact in the amorphous areas, thus stabilizing this region and increasing onset temperature of gelatinization (Oosten, 1984; Johnson *et al.*, 1990). There is, however, quite a limited number of reports on the ability of saccharides to affect retrogradation (Hase *et al.*, 1981; Krusi and Neukom, 1984; Biliaderis and Zawistowski, 1990; P'anson *et al.*, 1990; Kohyama and Nishinari, 1991).

Amyleose forms inclusion complexes with emulsifiers (Krog, 1971; Birnbaum, 1977). Amylopectin also forms complexes with emulsifiers (Gray and Schoch, 1962; Batres and White, 1986). Emulsifiers (e.g., monoglycerides) are used in the baking industry to reduce staling of bread. Their effectiveness attributed to the formation of starch-emulsifier complexes (D'Appolonia and Morad, 1981).

Kinetics of crystal formation for wheat starch gel during aging were studied by DSC (Longton and LeGrys, 1981). Roulet *et al.* (1990) made the same type of study by simultaneously using DSC and rheology measurements. However, no kinetic analysis has been of viscoelasticity to investigate the combined influence of polyols and emulsifiers on the behavior of starch gels during storage. The term polyol refers to saccharides (e.g., D-glucose and sucrose), sugar alcohols (e.g., maltitol and maltotriitol) and linear polyols (e.g., glycerol and D-sorbitol).

The objectives of this work were to apply rheological measurements to a study on the retrogradation of

non-glutinous rice starch, the most important foodstuff in Japan, and to determine the effects of polyols (Katsuta *et al.*, 1992) and emulsifiers on hardening of starch gels during storage.

Materials and Methods

Materials

Non-glutinous rice starch was obtained from Matsutani Kagaku Kogyo Co. Ltd., Japan. The analytical data of the starch were as follows: 12.5% moisture; 86.9% carbohydrate; 0.3% protein; 0.1% lipid; 0.2% ash; and size range of granule, 3-8 μ m.

Reagent grade polyols were used without further purification. D-Xylose, D-ribose, D-glucose, D-galactose, D-fructose, maltose, maltotriose, and maltotetraose were used as saccharides. Xylitol, D-sorbitol, and galactitol were used as linear polyols. Maltitol and maltotriitol were also used as sugar alcohols.

Commercial malto-oligosaccharides were provided by Mitsubishi-Kasei Foods Corp., Japan (maltotriose syrup, maltotriose content; 47% on dry saccharide basis, dextrose equivalent value; DE 45) and Nihon Shokuhin Kako Co., Ltd. (maltotetraose syrup, maltotetraose content; 50% on dry basis, DE 31). Isomalto-oligosaccharide (isomalto-oligosaccharides content; 52%, DE 65) was provided from Syowa-Sangyo Co., Ltd. (Japan). Hydrogenated malto-oligosaccharide (maltotriitol syrup derived from the maltotriose syrup, maltotriitol content; 44% on dry basis, Mitsubishi-Kasei Foods Corp.) and hydrogenated isomalto-oligosaccharide (derived from the isomalto-oligosaccharides syrup, Nikken Chemicals Co., Ltd., Japan) were also used. All of the malto-oligosaccharides and isomalto-oligosaccharides as standards for high performance liquid chromatography (HPLC) were purchased from Nihon Shokuhin Kako Co., Ltd. (Japan) and Seikagaku Corp. (Japan), respectively.

Emulsifiers were used without further purification. Sucrose esters of fatty acids (Ryoto Sugar Ester P-1570 and S-1570) were commercial samples from Mitsubishi-Kasei Foods Corp. They were mixtures of the palmitic and stearic acid esters of sucrose; compositions of fatty acids esterified with sucrose were approximately 80% palmitic acid (C_{16:0}) and 20% stearic acid (C_{18:0}), and 30% C_{16:0} and 70% C_{18:0} for P-1570 and S-1570, respectively. Both P-1570 and S-1570 contained approximately 70% mono-ester, 24% di-ester and 6% tri- and higher esters, and had hydrophilic-lipophile balance (HLB) of approximately 15. Distilled monoglycerides, glyceryl monopalmitate (C_{16:0}, 98%) and glyceryl mono-stearate (C_{16:0}, 27%; C_{18:0}, 70%) were purchased from Tokyo Kasei Kogyo Co., Ltd. (Japan). Diacetyl tartaric acid esters of monoglycerides (POEM W-10, C_{16:0}, 30%; C_{18:0}, 70%, Riken Vitamin Co., Ltd., Japan), and sodium stearyl-2-lactylates (Musashino Chemicals Lab. Ltd., Japan) were used.

Deionized and distilled water was used in the experiments.

High performance liquid chromatography (HPLC)

Commercial oligosaccharides were separated and quantified with a LC-6A System (SHIMADZU Corp., Japan) fitted with a refractive index monitor L-3300 RI Detector (Hitachi, Ltd., Japan), a column oven CO-8010 (TOSOH Corp., Japan) and a degasser ERC-3511 (Erma Inc., Japan). Malto-oligosaccharides composition was determined by cation exchange chromatography using a column (8.0 mm I.D. x 300 mm) of MCI GEL CK-04SS (Mitsubishi Kasei Corp.). Isomalto-oligosaccharides composition analysis was accomplished by normal phase chromatography using a column (4.6 mm I.D. x 250 mm) of TSKgel Amide-80 (TOSOH Corp.).

Samples and standards were solubilized in the mobile phase at a concentration of 0.5% (w/v). Deionized and distilled water was used as the solvent system for cation exchange chromatography, and injection volumes of 20 μ l were eluted at a flow rate of 0.2 ml/min at 65 °C. Acetonitrile / water (70:30) was used for normal phase chromatography, and injection volumes of 200 μ l were eluted at a flow rate of 0.6 ml/min at 60 °C.

Peak heights and areas were determined with a Chromatopac C-R3A System (SHIMADZU Corp.). The system was standardized using a series of malto-oligosaccharides and isomalto-oligosaccharides. Samples were identified and quantified using external standards.

Differential scanning calorimetry

DSC measurements were carried out to obtain gelatinization temperature of the starch for gel preparation using a DSC 100 equipped with a TA Station of SSC 5000 Series (SEIKO I&G Co., Japan).

Thirteen mg of starch were heated in silver sample pans with 40 mg distilled water, hermetically sealed, and allowed to equilibrate for 1 hour prior to analysis. A pan containing deionized/distilled water was used as a reference. Samples were heated at a rate of 2 °C/min from 20 °C to 140 °C. The calorimeter was calibrated using indium and tin metals. Enthalpy (ΔH), onset temperature (T_o), peak temperature (T_p), and completion temperature (T_c) were computed automatically.

Preparation of starch gels

Rice starch was weighed into a flask and degassed under vacuum for 15 minutes at room temperature. Then distilled water was added and the flask was degassed again while stirring for 90 minutes at room temperature. During this treatment, the starch could swell slightly and became partially hydrated. The starch dispersion was heated at 55 °C for 3 minutes to obtain the homogeneous sol state. This temperature corresponded to the onset temperature (T_o , 54.5 °C) of gelatinization for the rice starch. Other thermophysical properties of the starch were T_p , 62.7 °C; T_c , 72.6 °C; and ΔH , 1.8 MJ/mg. The starch sol was poured into glass tubes pre-coated on the inside with silicone oil, and heated at 65 °C for 5 minutes to obtain the fixed sample. After centrifugation at 700 x g for 20 minutes to remove air bubbles, the sample was heated at 95 °C for 90 minutes for complete gelatinization. Prior to heating, silicone

oil was dropped onto the surface of samples to prevent evaporation of water from sample during heating. The glass tubes filled with samples were sealed, cooled at room temperature for 15 minutes and stored at 0 °C for 180, 360, 1020, 1620, 2460, 2760 and 3000 minutes. The stored samples were carefully removed from the tubes, cut into cylindrical blocks (11 ϕ x 10 mm) using an Ultrasonic Sample-Cutter (USC-3305, YAMADEN Co., Ltd., Japan) to produce a smooth surface and submitted to creep measurements.

To obtain the self-standing gel samples, the starch concentration was 30% (w/w). Polyols and emulsifiers were added prior to gelatinization. The polyols were added to the starch dispersion at 6% (w/w), starch basis. Emulsifiers were dispersed in cold water for 30 minutes and dissolved at 65 °C prior to addition to the starch dispersion. The emulsifiers were added to the starch dispersion at 0.2% (w/w), starch basis.

Static viscoelastic measurement

A Creep Meter (Rheoner RE-3305, YAMADEN Co., Ltd.) was used for measurements of static viscoelastic properties. Gel samples were set in a temperature-controlled (25 °C) chamber. The chamber was filled with silicone oil to prevent the evaporation of water. Measurements were carried out under uniaxial compression (at 10 mm/sec of compression speed) for 300 seconds. A linear relation between stress and strain was determined by preliminary tests for deformation of less than 15%. Thus, when subsequent deformation was maintained within 10%, changes in cross-sectional area of sample were negligible and an essentially constant stress was obtained. Each experiment was performed at least three times in duplicate and data obtained were subjected to a linear regression analysis after omitting of outliers.

Results and Discussion

Retrogradation kinetics

In this study, a creep behavior which was consistent with a spontaneous elastic (Hookean) component, a retarded elastic (Voigt) component and steady-flow (Newtonian) component was observed. The creep compliance $J(t)$ of 30% (w/w) starch gel is expressed as follows:

$$J(t) = 1/E_H + \sum 1/E_{v_i} [1 - \exp(-t/\tau_{K_i})] + t/\eta_N \quad (1)$$

where E_H is the elastic modulus of Hookean component, E_{v_i} the elastic modulus of Voigt component, τ_{K_i} the retarded time, and η_N the viscosity of Newtonian component.

The Hookean component is independent of the measuring time, but both of Voigt and Newtonian components dependent on the measuring time. The creep compliance of the steady (Newtonian) flow increases linearly with time. Therefore, $J(t) - t/\eta_N$ was computed and used as the value of creep compliance (J) in this

study. Our measuring time was 300 seconds. The value of J was defined as follows:

$$J = J_{(300)} - t/\eta_N \quad (2)$$

Figure 1 shows J during storage at 0 °C. The curvilinear plot of creep compliance (J) of rice starch gels decreased with length of storage period, and was asymptotic to equilibrium creep compliance.

Considering the first-order reaction $A \rightarrow B$, and denoting the weight fraction of reactant and the product as X_1 and X_2 , respectively, an equation was obtained in terms of the reactant.

$$\ln X_1 = -kt \quad (3)$$

The time dependence of $J_{(t)}$ must be in accordance with that of X_1 and X_2 , and $J_{(t)}$ consists of the contribution of the reactant and the product. In the retrogradation process, we can use the following blending law.

$$J_{(t)} = X_1 \cdot J_r + X_2 \cdot J_p \quad (4)$$

The compliances for both reactant (J_r) and product (J_p) correspond to those of the gel phase (J_{gel}) and the aggregates (J_{agg}) in the rice starch gel. In the initial stage of retrogradation (crystallization) process, the weight fraction of the aggregates X_2 is small and practically negligible (Ninomiya and Ferry, 1967), so the contribution of the product could be disregarded. Hence, equation (4) can be expressed as the following equation:

$$J_{agg(t)} = X_1 \cdot J_{gel} \quad (5)$$

By inserting equation (5) into equation (3), $J_{(t)}$ is expressed as follows:

$$\ln[J_{agg(t)}/J_0] = -kt \quad (J_{gel} = J_0) \quad (6)$$

where, J_0 is the initial creep compliance extrapolated to t equal zero. In this study, J corresponds to J_{agg} . The equation (6) gives:

$$J = J_0 \cdot \exp(-kt) \quad (7)$$

In this case, the logarithmic plot of J against the storage period should be linear, illustrated in Figure 2. The linear relationship between the storage period at 0 °C and the logarithm of J of gels was obtained. The retrogradation process of non-glutinous rice starch gels in the initial stage could be kinetically interpreted and the process was expressed as a first-order reaction.

Effects of saccharides

All of the gels containing saccharides showed linear relationships between storage period and logarithmic creep compliance. Hardening of rice starch gels can be expressed by a first-order equation, irrespective of the presence of saccharides. However the slopes of the linear regression lines became less steep when saccharides

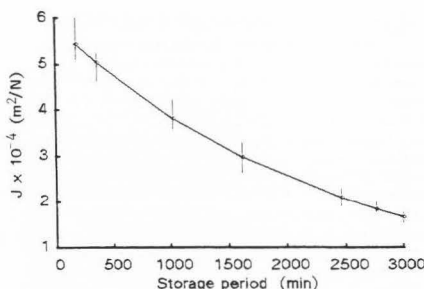


Figure 1. Changes in the creep compliance of non-glutinous rice starch gels during storage at 0 °C. Rice starch concentration is 30% (w/w).

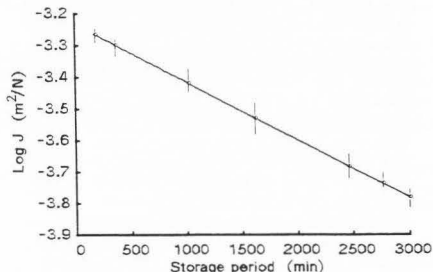


Figure 2. Linear relationship between creep compliance and length of storage of starch gel. The data are recalculated for Figure 1.

were added. The rate constants of saccharide containing gels were smaller in magnitude than that of the control system. Thus, it is concluded that the hardening of starch gel is retarded by saccharides.

Table 1 shows the rate constant, k , of these gels. Among the monosaccharides, D-glucose (G_1) and D-fructose were more effective in impeding the retrogradation than D-galactose and the pentoses (D-xylose and D-ribose). The disaccharides (sucrose and maltose; G_2) were more effective than monosaccharides in stabilizing the gels. The saccharide most effective in preventing starch retrogradation was maltotriose (G_3). Kohyama and Nishinari (1991) also reported that sucrose more effectively retarded starch retrogradation than D-glucose and D-fructose. On the other hand, Hase *et al.* (1981) reported little difference in retardation of retrogradation of potato and corn starches by several saccharides.

Retrogradation rate of starch

Saccharides (except for D-galactose) reduced the initial creep compliances (J_0) as shown in Table 1. These results indicate that saccharides stabilize the starch gels.

Effects of linear polyols and sugar alcohols

Table 2 shows the rate constants (k) for starch gels containing linear polyols or sugar alcohols. Among the linear polyols, anti-retrogradation capabilities of a pentahydric alcohol (xylitol) and a hexahydric alcohols (galactitol) were almost the same, but D-sorbitol (G₁OH) most effectively retarded starch retrogradation. These polyols were more effective than pentoses (D-xylose and D-ribose) and hexoses (D-glucose, D-galactose and D-fructose) in reducing the rate of retrogradation. From the value of J_0 , all the linear polyols and sugar alcohols (except for hydrogenated isomalto-oligosaccharides) had stabilized the starch gels as shown in Table 2.

The results suggest that interactions between polyols and starch chain, as well as the effects of polyols on water structure, differed among saccharides which have cyclic molecular structures, among linear polyols with linear structures, and among sugar alcohols with cyclic as well as linear structures.

Relationships between the structure of polyols and anti-retrogradation ability

Saccharides are believed to penetrate the melted crystalline region of starch. Hence, "sugar-bridges" (a term coined by Spies and Hosney, 1982) are formed in both crystalline and amorphous regions during gelatinization. When starch gels are cooled, the starch chains become less energetic and the hydrogen bonds become stronger. Retrogradation involves, among others, recrystallization of starch chain in the gels (Hosney, 1986). In starch-water-polyol systems, polyols interfere with the hydrogen bonds between starch chains. Thus, stabilized starch gels retard retrogradation, i.e., the rate of retrogradation is reduced by polyols.

With regard to the influence of saccharides on starch gelatinization, monosaccharides delay gelatinization less than disaccharides (Spies and Hosney, 1982). Savage and Osman (1978) studied the effects of saccharides on the swelling power of a starch-water-saccharide system and reported that maltose acted like monosaccharides. In this study, however, maltose was more effective in stabilizing the starch gels than monosaccharides and sucrose. The results suggest that the effects of saccharides on starch retrogradation are different from those on gelatinization.

An explanation of the retrogradation process must consider the behavior and/or structure of water. According to our hypothesis, saccharides make an important contribution to the structural stability of water surrounding the starch chains and/or molecules.

Judging from the "Specific-hydration model", hydration of saccharides depends on the compatibility of their conformation with the "lattice" structural component of water (Tait *et al.*, 1972; Sugget, 1976). Therefore, the hydration of saccharide hydroxyl groups

Table 1. Effects of saccharides on the rate constants of retrogradation and on the initial creep compliance of starch gels.

Polyol	k ($10^{-4}/\text{min}$)	$\log J_0$ (m^2/N)
Without	4.20	-3.23
D-Ribose	3.65	-3.26
D-Xylose	3.77	-3.25
D-Glucose	3.13	-3.31
D-Galactose	3.70	-3.24
D-Fructose	3.18	-3.30
Sucrose	2.37	-3.33
Maltose	2.11	-3.37
Maltotriose	1.69	-3.41
Maltotriose syrup	1.90	-3.36
Maltotetraose	2.25	-3.39
Maltotetraose syrup	2.28	-3.36
Isomalto-oligosaccharide syrup	3.39	-3.31

Table 2. Effects of linear polyols and sugar alcohols on the rate constants of retrogradation and on the initial creep compliance of starch gels.

Polyol	k ($10^{-4}/\text{min}$)	$\log J_0$ (m^2/N)
Without	4.20	-3.23
Xylitol	2.45	-3.33
Sorbitol	2.02	-3.41
Galactitol	2.38	-3.31
Maltitol	3.14	-3.28
Maltotriitol	3.61	-3.26
Hydrogenated maltotriose syrup	2.01	-3.42
Hydrogenated isomalto-oligosaccharide syrup	3.42	-3.23

is stereospecific in nature. Yano and Janado (1990) postulated that hydration of saccharides depends on (a) their conformation (number of equatorial OH groups); (b) the concentration (intermolecular hydration cosphere-cosphere interactions); and (c) the type of glycosidic linkages (intramolecular hydration cosphere-cosphere interactions). Thus, properties that depend on the saccharide hydration, correlate well with the mean number of equatorial hydroxyl groups, $n(\text{e-OH})$, in saccharide molecules (Uedaira and Uedaira, 1985a; 1985b).

The saccharides have more $n(\text{e-OH})$ in their molecules, giving rise to a stronger stabilizing effect on water structure. On the other hand, axial hydroxyl groups (a-OH) destabilize the water structure as a consequence of the competition of two interactions: (a) between a-OH groups of the saccharide molecule and the water molecule and (b) among water molecules. The

destabilizing effect by α -OH groups depends on their numbers. Therefore, $n(\text{e-OH})$ calculated by Angyal and Pickles (1972), Uedaira and Uedaira (1985a, 1985b) and Uedaira *et al.* (1989, 1990) were used in this study.

The retrogradation rates (k) were plotted against $n(\text{e-OH})$ and the number of OH groups ($n\text{OH}$) in Figures 3 and 4, respectively. The retrogradation rate had a minimum value when a saccharide G_3 was added to the starch gel (Figure 3). However, the effect to inhibit retrogradation of G_4 was lower than of G_3 . Miyajima *et al.* (1983) reported that the contribution to water structure was $G_1 < G_2 < G_3$ and that the incompatibility of orientation of hydrogen bonds between a saccharide molecule and water molecules of an ice-like structure increased with the number of glucosidic linkages, i.e., DP. The α -(1-4)-linkage conformation in the maltodextrins (malto-oligosaccharides) of DP > 7 and amylose is essentially the same (Rees, 1970). Therefore, the effects of malto-oligosaccharides on retrogradation of starch relate to not only to $n(\text{e-OH})$ in the molecule but also to the conformation of the saccharides.

The minimum value for k was reached for $n\text{OH}$ = 6 for the polyols added to the gels (Figure 4). Hence, their gel-stabilizing effects would be dependent on both $n(\text{e-OH})$ and $n\text{OH}$ and the ratio of these in polyol molecules.

Water in relation to biopolymers (Sugget, 1975; Berendsen, 1975) has been classified to be present in three forms; (a) unfreezable (immobilized or constitutional), surrounding the nearest biopolymer molecules, and consisting of a single layer of water molecules, (b) bound (interfacial, vicinal, multilayer, entrapped) water that is freezable, and (c) free (bulk) water that is distant from biopolymer molecules. The postulated interpretations of mechanism of starch retrogradation and the role of polyols are as follows: In the starch-water system, water molecules are attached to the starch chains by hydrogen bonds and a single layer of water molecules surrounding the starch chain is restricted in thermal motion. The unfreezable water is surrounded by the bound water, and the thermal motion of the unfreezable water is influenced by the bound water. The thermal motion of free water is vigorous, since the free water dilutes the layer of bound water. Consequently, the starch chain is flexible and forms junction zones and/or rearrangements by hydrogen bonding among the chains (Figure 5a).

Water surrounding starch chains is stabilized by polyols. Thus, the flexibility of the starch chains might be reduced through strengthening of the hydrophobic interactions of the starch chains in the same manner as in protein-polyol-water systems (Gekko, 1989; Miura *et al.*, 1991). Therefore, rearrangement of starch chains, namely retrogradation, is retarded (Figure 5b).

Effects of emulsifiers

Table 3 shows the k for starch gels containing emulsifiers. The abilities to reduce the retrogradation decreased in the following order: glyceryl monopalmitate (GMP) > glyceryl monostearate (GMS), sucrose esters of palmitic acid ($C_{16:0}\text{SE}$) and diacetyl tartaric acid

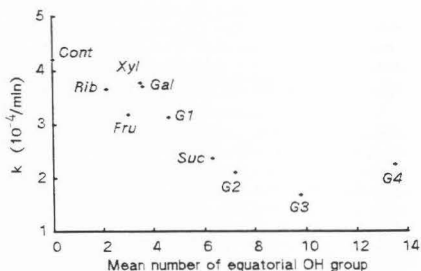


Figure 3. Relationship between the rate constants of retrogradation and the mean number of equatorial OH groups in saccharide molecules. Cont: control; Rib: ribose; Xyl: xylose; G_1 : glucose; Gal: galactose; Fru: fructose; Suc: sucrose; G_2 : maltose; G_3 : maltotriose; G_4 : maltotetraose; TRI: maltotriose syrup; TET: maltotetraose syrup; ISO: isomalto-oligosaccharide syrup.

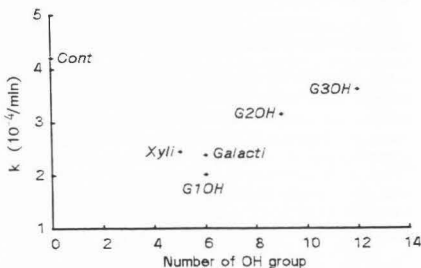


Figure 4. Relationship between rate constants of retrogradation and the number of OH groups in linear polyol and sugar alcohol molecules. Cont: control; Xyli: xylitol; $G_1\text{OH}$: sorbitol; Galacti: galactitol; $G_2\text{OH}$: maltitol; G_3 : maltotriitol.

esters of monoglycerides (DATEM) > sucrose esters of stearic acid ($C_{18:0}\text{SE}$) and sodium stearyl-2-lactylate (SSL). However, the differences in the effects of emulsifiers on the retrogradation rate were small compared to those of polyols. The mono-ester contents of GMP and GMS were high (above 90%), compared to only about 70% in SE. Consequently, no in-depth evaluation of the functionality of the emulsifiers can be presented. Among the sucrose esters of fatty acids, $C_{16:0}\text{SE}$ more effectively reduced the retrogradation rate than $C_{18:0}\text{SE}$.

Retrogradation rate of starch

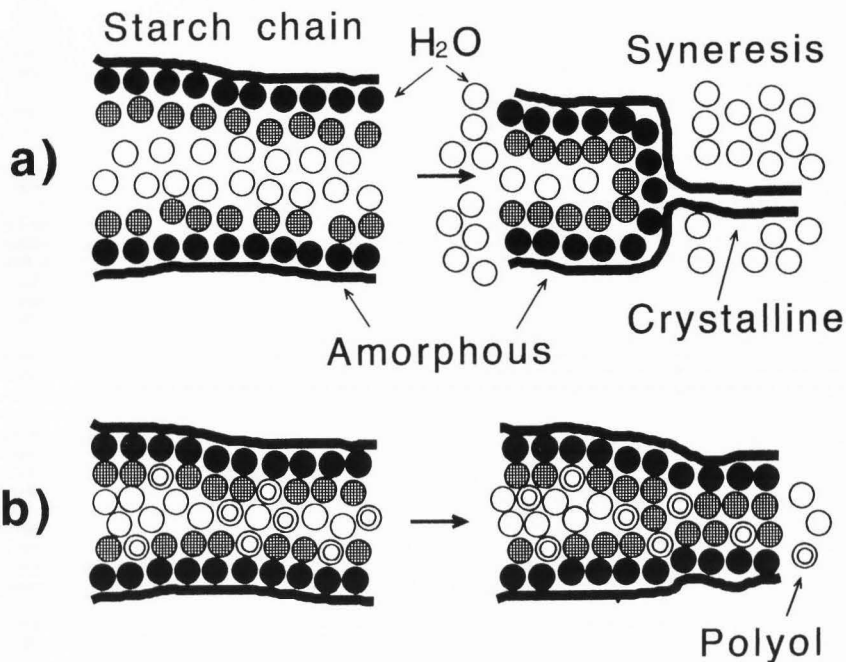


Figure 5. Model of a mechanism of starch retrogradation and the role of polyol (left figures show gelatinized starch chains and the right figures show retrograded starch chains): **a)** no polyol addition system; **b)** polyol-containing system. Polyol molecules (\odot), which are compatible of conformation with the "lattice" structural component of water, enter the water structure and stabilize the water structure surrounding starch chains. Water is classified in three categories; unfreezable (solid circles), bound (hatched circles), and free (hollow circles).

Table 3. Effects of emulsifiers on the rate constants of retrogradation and on the initial creep compliance of starch gels.

Polyol	k ($10^{-4}/\text{min}$)	$\log J_0$ (m^2/N)
Without	3.73	-3.28
Sucrose esters of palmitic acid	2.72	-3.48
Sucrose esters of stearic acid	2.91	-3.42
Glyceryl monopalmitate	2.22	-3.66
Glyceryl monostearate	2.67	-3.60
Diacetyl tartaric acid esters of monoglycerides	2.78	-3.74
Sodium stearoyl-2-lactylates	2.96	-3.60

Among the monoglycerides palmitate was more effective than stearate. The J_0 of starch gels containing emulsifiers was lower than that of the control gel (Table 3). This suggests that emulsifiers tend to stabilize starch gels.

The results suggest that interaction between emulsifiers and starch chains and the effects of emulsifiers on starch gel structure differ among emulsifiers that vary in hydrophobic (alkyl groups) or hydrophilic parts of the molecule.

To compare the ability of emulsifiers to react with amylose, a "Complexing Index" was calculated by Krog (1971). The index increased in the order GMP/GMS (mono-ester content minimum 90%) > SSL > DATEM > C_{18:0}SE (commercial monostearate sample) > C_{18:0}SE (commercial distearate sample). The maximum

complex formation was found for glyceryl monomyristate (GMM) when 1-monoglycerides with different chain lengths were compared on the same weight basis. Lagendijk and Pennings (1970) reported that GMP exhibited the highest complex-forming capacity. It has been postulated that emulsifier-amylose complex-formation prevents the starch retrogradation. However, the decreasing order for k did not completely conform to the increasing order for the amylose complexing index. Thus, the abilities of emulsifiers to reduce hardening of starch gels may be attributed not only to the complexation of emulsifiers with starch, but also to other factors, such as effects on water structure and intermolecular interactions. Consequently, additional research is needed to elucidate the relationships between structure and functionality of emulsifiers. We are, therefore, currently investigating the anti-hardening effect of emulsifiers in a series of experiments that involve: (a) the addition to starch gels of several types of emulsifier (non-ionic, anionic and cationic), (b) addition of emulsifiers produced from pure fatty acid, and (c) the addition of mono-esters in combination with a variety of emulsifiers.

Conclusions

Retrogradation of non-glutinous rice starch gels could be described by a first-order kinetic equation. Polyols and emulsifiers retarded the retrogradation and resulted in stable gel matrices. Based on our results, we propose that polyols retarded starch retrogradation by their effects on the water structures, and emulsifiers decreased the rate of retrogradation mainly through emulsifier-starch complex-formation.

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Discussion with Reviewers

M. Wootton: The authors have compared the effects of different polyols and sugar alcohols on inhibiting retrogradation. Could they also comment on the likely effect of inositol in this context?

Authors: In this study, we examined only linear polyols. Therefore, we cannot comment on the likely effect

of inositol. In order to clarify the effects of polyols totally, we must examine a series of linear polyols (glycerol, erythritol, xylitol, arabitol, ribitol, galactitol, sorbitol, mannitol etc.), cyclic polyol (inositol), sugar alcohols derived from homo oligosaccharides with various glycosidic linkage (maltitol, isomaltitol etc.) and sugar alcohols derived from hetero-oligosaccharides (lactitol etc.).

M. Wootton: Did the authors consider any possible relationship between the HLB number of the emulsifiers and their effect on inhibiting retrogradation?

Authors: No. The HLB value can only provide useful information concerning the use of emulsifiers in simple systems. We believe the structure is important for use of emulsifiers.

M. Wootton: The authors use the term "water structure" in this paper. It seems to me that they really mean "water-starch gel structure" or similar term.

A.C. Eliasson: The nomenclature with unfreezable, bound and free water might be misleading. The use of "bound" and "free" water should be avoided.

Reviewer V: It is confusing what is the fundamental difference between "unfreezable" water versus "bound" water? Can you have bound water that is freezable? The authors postulate that "In the starch-water system, water molecules are attached to the starch chains by hydrogen bonds and a single layer of water molecules surrounding the starch chain is restricted in thermal motion. The unfreezable water is surrounded by the bound water, and the thermal motion of the unfreezable water is influenced by the bound water. The thermal motion of free water is vigorous, since the free water dilutes the layer of bound water. Consequently, the starch chain is flexible and forms junction zones and/or rearrangements by hydrogen bonding among the chains." These statements have no scientific date supported either by the authors or by any reference!

Authors: We frequently find the terms "water structure", "bound water" and "free water" used in the literature, e.g., "Water: A Comprehensive Treatise Vol. 1-7, Franks F. (ed.), Plenum Press, New York (1972-1982); "Properties of Water in Foods", Simatos D and Multon JL. (ed.), Martinus Nijhoff Publishers, Dordrecht (1985); and "Water Science Reviews" Vol. 1-5, Franks F. (ed.), Cambridge University Press, Cambridge (1985-1990). We, therefore, believe these terms are commonly and widely used in the water science, and have accordingly employed them in our paper.

The "bound water" is freezable. As implied in the text, the model of mechanism of starch retrogradation is indeed our speculation!

Reviewer V: I am not convinced that water structure in a starch-polyol-water system affect starch chain flexibility in the same way as protein-polyol-water systems!

Authors: Our statements in text are: "Water surrounding starch chains is stabilized by polyols. Thus, the

flexibility of the starch chains might be reduced through strengthening of the hydrophobic interactions of the starch chains in the same manner as in protein-polyol-water systems (Gekko, 1989; Miura *et al.*, 1991). Therefore, rearrangement of starch chains, namely retrogradation, is retarded (Figure 5b)." As implied, we only speculated.

Reviewer V: There is much emphasis on the molecular interpretation of results without any supporting data or reference. Rheological properties may be influenced by a number of factors. Inhibition or retardation of "retrogradation" by polyols could be simply physical hindrance or increased local viscosity rather than water structure concept.

Authors: We also understand that starch retrogradation is attributable to rearrangement of starch chains involving crystallization. In the present study, we have been discussing the roles of polyols on the inhibition of hardening of starch gel from the stand point of rearrangement of starch chains. We intend to confirm our hypothesis with additional research using NMR etc.

M. Wootton: The term "acylglycerols" has largely superseded "glycerides".

Authors: You are correct, but we use the term "glycerides" because it is more familiar to scientists and technologists dealing with emulsifiers.

C.E. Walker: Can you please better identify the number of replicates and their reproducibility?

A.C. Eliasson: What is the experimental error in the measurements? Are the differences in the J_0 values significant?

Authors: As stated in the text, each experiment was performed at least three times in duplicate. First of all, we calculated skewness and kurtosis of these data in order to omit outliers. Then a linear regression analysis was performed between creep compliance and storage period, and we obtained k and J_0 . The statistical variation in creep compliance in triplicate was rather smaller than that between samples containing various polyols.

C.E. Walker: Under the sub-heading "Preparation of starch gels", it is stated: "Rice starch was weighed into a flask and degassed under vacuum for 15 minutes at room temperature. Then distilled water was added and the flask was degassed again while stirring for 90 minutes at room temperature. During this treatment, the starch could swell slightly and became partially hydrated." What is meant by "starch could swell slightly"? Was there any evidence that granules had actually swollen at that time?

Authors: Native starch granules are known to be insoluble in cold water, but they can swell slightly and become partially hydrated [Waniska RD, Gomez MH. (1992). Dispersion behavior of starch. *Food Technol.* 46(6), 110-123]. We did not ascertain that the granules had actually swollen, but we believe that after hydration

granules are in equilibrium. After stirring for 90 minutes, the starch dispersion had a certain endothermic peak on DSC.

C.E. Walker: Can you explain why galactose appears to act more like a pentose than hexose?

Authors: We do not think D-galactose appears to act more like a pentose than hexose, but D-xylose appears to act more like a hexose than pentose. Solutions of reducing sugars are known to consist of equilibrium mixtures of anomers. Angyal and Pickles reported the proportions of pyranose (α - and β -anomers) and furanose (α - and β -anomers) forms of aldopentoses and aldohexoses at equilibrium in D_2O solutions [Angyal SJ, Pickles VA. (1972). Equilibria between pyranoses and furanoses II. *Aldoses.* *Aust. J. Chem.* 25, 1695-1710]. The mean numbers of e-OH of D-xylose and D-galactose are 3.5 and 3.6, respectively. The number of D-xylose is higher than those of other pentoses because of rather lower proportion of furanose form.

C.E. Walker: Can the failure of xylose, galactose and glucose to follow the main trend line be explained by a dynamic balance in the chair-boat configuration equilibrium, whereby some of the hydroxyls are partially hidden?

Authors: We think the failure of those saccharides to follow the main trend line is attributed to a proportion of pyranose and furanose forms at equilibrium. D-xylose and D-ribose have lower amounts of pyranose forms than other pentoses, D-xylose has the lowest furanose content in pentoses, and D-galactose has furanose forms but D-glucose does not have them. Then each saccharide has its own n(e-OH) through configuration and conformation equilibrium of saccharide molecule.

C.E. Walker: Within both the SE and GM pairs of emulsifiers, the effectiveness appears to be inversely related to molecular weight. Can this be related to the emulsifiers ability to penetrate the starch helix?

Authors: We cannot state clearly the influence of molecular weight of emulsifier in the ability to penetrate the starch helix form only those data. We expect the effectiveness of emulsifier can be related to molecular structure, i.e., geometrical structure and construction containing hydrophilic and hydrophobic areas.

A.C. Eliasson: How were the levels of polyols (6%) and emulsifiers (0.2%) chosen?

Authors: We chose the values as dosage level in market products.

A.C. Eliasson: What would the expected effect be if both polyols and emulsifiers were added to the starch?

Authors: We expect they can synergistically inhibit starch retrogradation. In bread system, we obtained the results suggesting the synergistic effect of them.

A.C. Eliasson: Could the effects of sugars be discussed

in relation to the glass transition temperature of starch?

Authors: This is an interesting idea for another research undertaking. We could not obtain T_g at 30% starch dispersion by DSC using our apparatus.

A.C. Eliasson: Is equation (4) justified or could there be interactions between phases?

Authors: Yes. This equation deals with the mechanical properties of the total system in terms of those of the amorphous solution and the crystallites.

Reviewer V: Throughout the text, the authors refer to the data from creep compliance to mean starch retrogradation, which to many people, involves starch crystallization and a decrease in starch solubility. In order to avoid confusion, the term retrogradation should be replaced with a more appropriate term unless some true measurement on starch retrogradation accompanies the rheological data.

Authors: As pointed out in the Introduction, Roulet *et al.* (1988) did a comparative study of retrogradation kinetics of starch using X-ray diffraction, DSC, and dynamic mechanical analysis. They reported that elastic modulus of starch gel was a good index of starch retrogradation. We, therefore, believe that we can use the term "retrogradation".