EFFECT OF POLYHYDROXY COMPOUNDS ON THE THERMAL AND MECHANICAL PROPERTIES OF AGEING STARCH GELS

A Thesis

Submitted to the Faculty

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by

Dale John Prokopowich

In Partial Fulfillment of the

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of

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TABLE OF CONTENTS

LIST OF TABLES

PAGE

LIST OF FIGURES

PAGE

ABSTRACT

Starch based products, such as cakes and cookies, are formulated with relatively high amounts of sugar. Relatively little research has been done on the effects of sugars upon starch retrogradation and the anti-retrogradation mechanism of sugars is not yet understood. This study was conducted to provide further information on the effect of polyols (polyhydroxy compounds) on the thermal and mechanical properties of concentrated starch gels and provide further insight into the anti-retrogradation mechanism(s) of polyols. The development of ordered structures in ageing starch, in the presence of polyols, was probed by small strain dynamic rheometry and differential scanning calorimetry (DSC). Polyols were added at a ratio of 1:0.5:1.5 (w/w) for starch:polyol:water mixtures.

The effect of polyols on the formation of "ordered" structures within ageing amylopectin networks was studied by DSC. The addition of glucose oligosaccharides of DP 1 to 3 retarded retrogradation with increasing DP, oligomers with a DP 4 to 7 exerted little effect, while maltooctaose promoted this process. The effect of glucose-based disaccharides with different glucosidic linkages on the reorganization of amylopectin short DP chains was also examined. Disaccharides with more extended rigid structures (i.e. cellobiose, β (1→4) glucosidic linkage) retarded retrogradation of amylopectin to a greater extent than disaccharides with more flexible glucosidic linkages (i.e. maltose, α (1→4) linkage); however, differences among the disaccharides were rather minor over longer storage periods. Minor differences were found when the anti-retrogradation behaviour of sugars and their respective sugar alcohols was compared.

However, the addition of glyceraldehyde completely inhibited the formation of "ordered" structures in the ageing waxy maize starch network over the storage period examined. The effect of pentoses and hexoses on the retrogradation of waxy maize starch gels was studied. Pentoses (i.e. ribose and xylose) were found to retard the reorganization of amylopectin chains more effectively than hexoses (i.e. fructose and glucose). In fact, fructose was shown to accelerate the rate of retrogradation as compared to the control (starch-water). Finally, it was shown that retrogradation of amylopectin was strongly influenced by the concentration of polyol added. Ribose continuously retarded the formation of "ordered" structures within ageing waxy maize starch gels with increasing concentration (0-37.5% w/w), whereas fructose promoted this process at concentrations greater than 7.5% (w/w).

A comparative study was undertaken to examine the effect of polyols on the thermal and mechanical properties of waxy maize, wheat, potato and pea starch gels. The addition of polyols, at a ratio of 1:0.5:1.5 (w/w) for starch:polyol:water mixtures inhibited chain reorganization of starch gels, as followed by DSC and dynamic rheology, in the following order: ribose > sucrose > maltotriose > water alone, glucose > fructose. The effects of polyols on the development of the retrogradation endotherm (ΔH) and gel rigidity (increase in G') were found to be less pronounced for potato and pea starches than waxy maize or wheat starches.

Significant correlations ($p \leq 0.05$) were observed between the effect of monosaccharides on the retrogradation endotherm (ΔH) and their hydration properties (n_h and n_{DHN}). An anti-retrogradation mechanism of polyols was proposed on the basis of their effect on the three-dimensional hydrogen-bonded structure of water.

1.INTRODUCTION

Foods such as bread, cakes and other baked products, which contain gelatinized starch are prone to undesirable textural changes associated with increase in firmness of crumb upon storage (staling). This problem is of considerable economic importance since it is estimated that 3-5% of all baked products produced in the United States in 1990 were discarded due to the loss of freshness (approximately $1 billion) (Hebeda *et al*, 1990). Starch retrogradation has been traditionally considered to play a major role in bread staling (Kulp and Ponte, 1981).

Starch-based products such as cakes contain relatively large amounts of sugar. A typical high-ratio cake formulation may contain upwards of 140% (w/w) sugar on flour basis (Kim and Walker, 1992). Until recently, little research has been done on the effects of sugars upon the starch retrogradation. Maxwell and Zobel (1978) studied the effect of fructose, glucose (dextrose) and sucrose on the firming of concentrated wheat starch/sugar/water system, and Germani *et al*. (1983) have compared the effects of glucose and sucrose on the firming of maize starch gels. More recently Slade and Levine (1987) have investigated the effect of a series of sugars on the retrogradation of concentrated wheat starch/sugar/water (1:1:1 w/w) systems by differential scanning calorimetry. They suggested that the anti-retrogradation behaviour observed with several sugars is due to their anti-plasticizing effect on the amorphous starch matrix which raises the T_g, thus reducing the growth of starch crystallites in the gelatinized starch matrix. In contrast, I'Anson *et al*. (1990) suggested that the addition of a third component, such as sugar, to starch gels may cause a phase separation of polymer and water, thus affecting the

retrogradation of starch chains. Miura and coworkers (1992) extensively studied the effect of monosaccharides, disaccharides and oligosaccharides (2.7% w/w) on retrogradation of 30% w/w rice starch gels by creep compliance measurements. They suggested that the retarding effect these sugars had on retrogradation of starch in gels is caused by the sugar-mediated changes in structure of water surrounding the starch chains. However, controversy still exists as to the exact mechanism by which sugars affect the retrogradation of starch.

Therefore, the objectives of this study were:

1) To study systematically the effect of polyols on the thermal and mechanical properties of concentrated starch gels.

2) To provide further insight into the anti-retrogradation mechanism(s) of polyols.

2. REVIEW OF LITERATURE

2.1 Starch Structure

The structure of starch has been extensively discussed in several reports (Banks and Greenwood, 1975; Hood, 1982; French, 1984; Lineback, 1984; Galliard, 1987; Lineback and Rasper, 1988; Zobel, 1988a, 1988b; Biliaderis, 1991; Zobel, 1992) and has been identified as an α-D-glucan that has two structurally distinct components; amylose and amylopectin. These two fractions are arranged into both crystalline and amorphous phases within supermolecular aggregates called granules. The ratio of amylose and amylopectin and their structural organization within the granule can greatly affect its functionality. Therefore the granular structure of starch needs to be reviewed at two distinct levels: (i) a molecular level (structure of amylose and amylopectin) and (ii) a supermolecular level (organization of amylose and amylopectin within the granule and in concentrated starch gels).

2.1.1 Major Starch Components

In all starch molecules the basic building block is anhydro-α-D glucopyranose in the chair ($^4C_1$) conformation. Glucose is polymerized into either amylose, mainly a linear polymer, or amylopectin, a branched polymer. For some starches, such as amylomaize, a third component exists which is referred to as the intermediate fraction (Banks and Greenwood, 1975). The intermediate fraction is considered to be heterogenous in structure consisting of: (i) linear chains

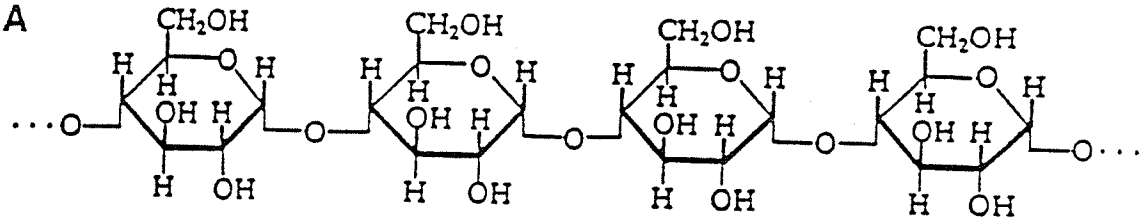
($50 < DP < 200$) and (ii) lightly-branched molecules of low molecular weight ($< 10^6$) and greater chain length than normal amylopectin. Most common starches contain 20-35% amylose and 65-80% amylopectin depending on the botanical source. Certain mutant varieties have no amylose (waxy type) or high amylose content (50-80% for amylomaize). In addition to these glucan polymers, starch also contains small amounts of non-carbohydrate constituents, particularly lipids, proteins, and phosphorous that can also affect the functional properties of starch (Galliard and Bowler, 1987).

2.1.1.1 Amylose. Amylose is essentially a linear polymer of glucopyranose units linked through α-D-(1→4) linkages (Figure 1a). Some branching has been shown to occur via α-D-(1→6) linkages (9-20 branch points per molecule) (Hizukuri *et al.*, 1981). Amylose has a molecular weight ranging from 10^5-10^6 and a degree of polymerization (DP) ranging from 200 to 5000 depending on its botanical source (Swinkels, 1985).

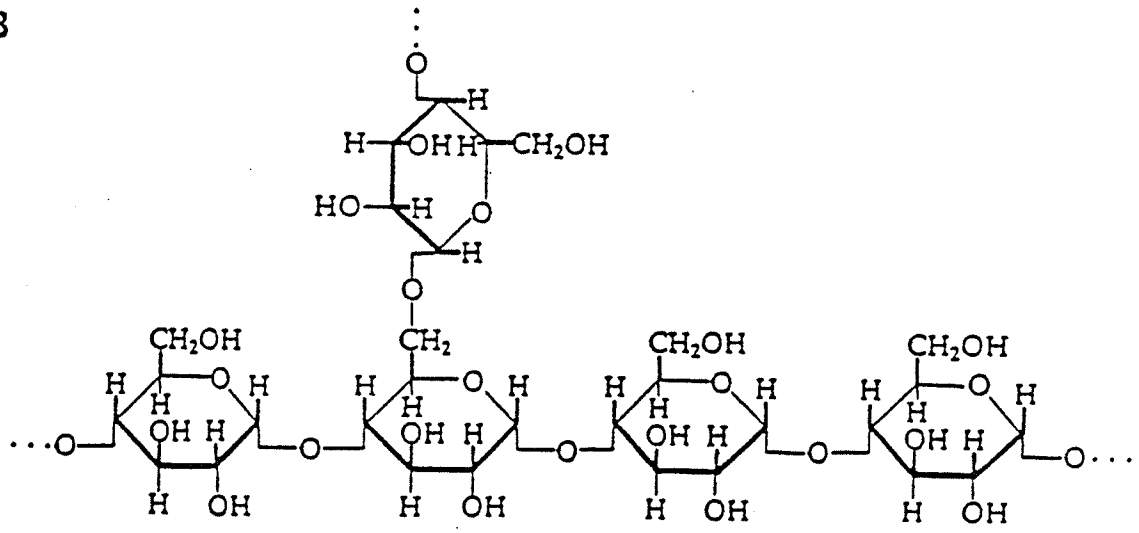
Amylose can be found in several different structural orders depending on the environment present. In neutral solution, amylose exists as a random coil (Banks and Greenwood, 1975) and in the presence of complexing agents, such as iodine and monoacyl lipids, amylose forms helical complexes (Galliard and Bowler, 1987). In aqueous systems, amylose readily forms a three-dimensional gel network through formation of double helical junction zones (Morris, 1990). Upon retrogradation of the gel, the double helices further aggregate into crystalline structures. In native starch granules, amylose may be involved in hybrid amylose/amylopectin helices and further packed into crystalline structures (Zobel, 1988b). Internal granular monoacyl lipids (lysophospholipids, free fatty acids) may also be complexed with amylose in native cereals (Galliard and Bowler, 1987).

Figure 1. Chemical structure of a) amylose and b) amylopectin.

A



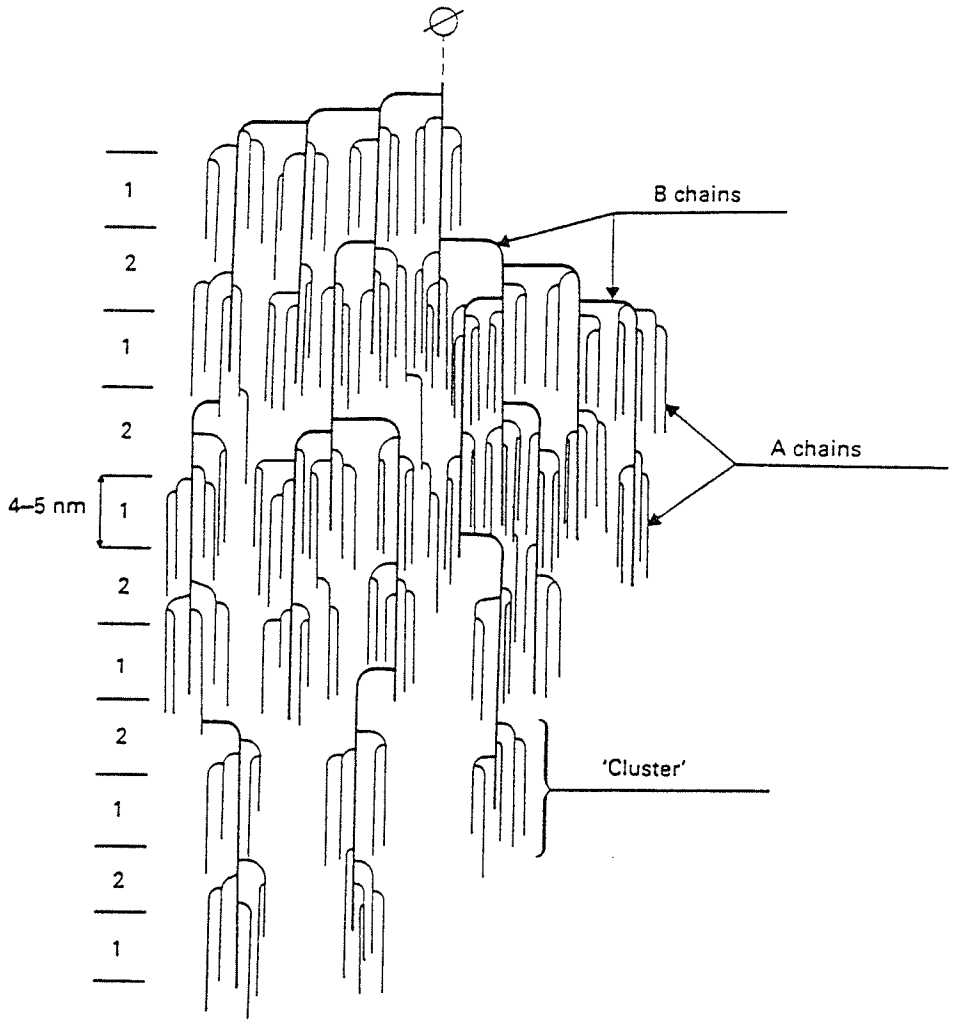
B



2.1.1.2 Amylopectin. Amylopectin is a highly branched polymer containing short chains of α-D-(1→4) glucopyranose residues linked via α-D-(1→6) linkages (Figure 1b). This polymer is one of the largest molecules in nature with a molecular weight in the range of 10^7-10^9 and DP approximately 10^4 - 10^5 (Biliaderis, 1991). The branched nature of this polymer dictates greatly its physicochemical properties.

The most widely accepted model of amylopectin structure is the "cluster" model proposed by French (1972) and Robin *et al.* (1975) (Figure 2). In this model chain segments and clustering of outer molecular chains are designated A,B and C. The A-chains are joined to the remainder of the molecule with single (1→6) bonds. The B-chains are joined through a (1→6) bond but may carry one or more A and/or B-chains on primary hydroxyl groups. The single C-chain carries the sole reducing group. The ratio of A:B chains varies (4:1 to 9:1) depending on the source of the amylopectin. When amylopectin is treated with debranching enzymes (ie. pullulanase or isoamylase) and the enzyme digest is separated by gel permeation chromatography, a bimodal chain distribution is obtained. The longer chains consist with a chains of DP ~ 45 (A chains) and the smaller chains have a DP ~ 15 (B chains). Improvements in resolution of gel permeation chromatographic techniques have revealed trimodal (MacGregor and Morgan, 1984) and polymodal (Hizukuri, 1986) chain distribution profiles. According to the "cluster" model, the structure of amylopectin has alternating crystalline and amorphous regions (French, 1984). The crystalline regions are comprised of double helices formed from the outer branches (12-18 DP) of both A and B chains, whereas the amorphous regions within the molecule contain most of the branching points.

Figure 2. Proposed cluster model for the molecular structure of amylopectin according to Robin *et al.*, 1975 (source: Blanshard, 1987).


B chains
A chains
'Cluster'
4–5 nm
1
2
1
2
1
2
1
2
1
2
1

2.1.2 The Starch Granule

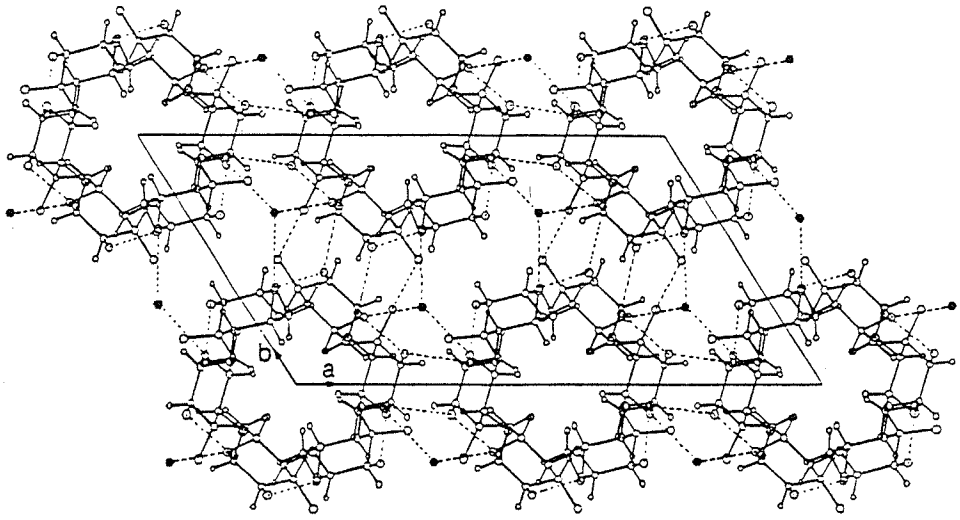
In nature, amylose and amylopectin are organized into dense, water insoluble, semi-crystalline granules. Starch granules range in size between 1 and 100 μm and have a variety of shapes (round, oval, irregular) depending on the botanical source (Lineback, 1984). Despite the differences in granular morphology, all starch granules have a similar supermolecular structure.

Within the native starch granule, amylopectin double helices are organized into crystal lattices (Zobel, 1988a). Depending on the packing arrangement of the double helices, different X-ray diffraction patterns are observed. Generally, cereal starches (rice, wheat and corn) yield A patterns; tuber, fruit, high amylose corn starches and retrograded starch yield B patterns; and certain root and legume seed starches yield C patterns. The level of crystallinity ranges from 15 to 45% depending on the source of starch (Zobel, 1988b). The outer branched clusters of amylopectin appears to be the principle crystalline component of starch. X-ray diffraction pattern of waxy maize starch (no amylose) resembles that of normal maize starch, while high amylose maize starches exhibit weaker and more dispersed patterns. Zobel (1988b) has suggested that amylose may also be associated with the outer branches of amylopectin and subsequently packed into crystalline arrays. Although the exact cause for the appearance of these different structures is not clear, studies by Hizukuri *et al.* (1983) suggest that the average chain length of amylopectin is the major determinant of crystal type among native starches; amylopectins of B-type starches have longer average chain lengths than those of A-type.

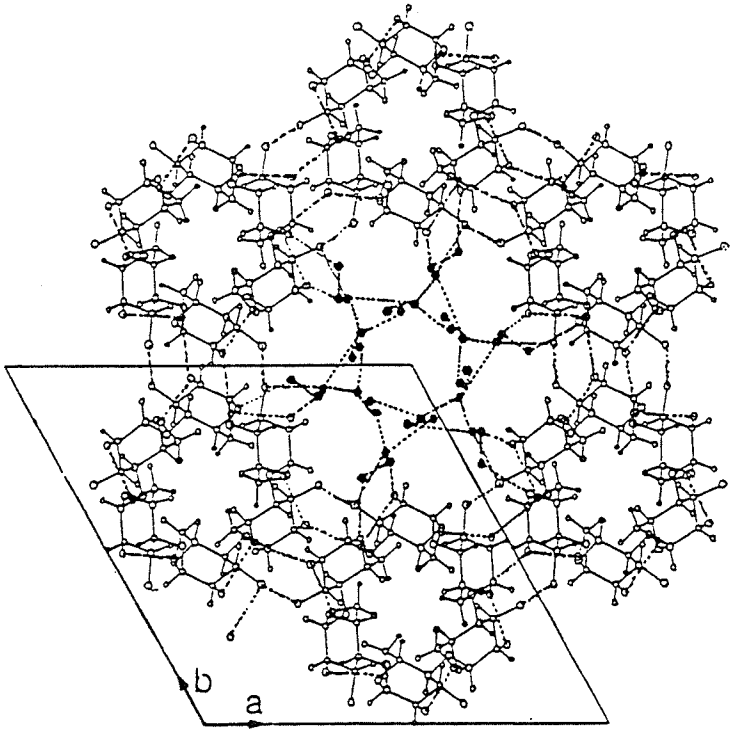
Through modelling studies of oriented amylose films Wu and Sarko (1978 a,b) proposed that the structure of B- and A-type starch crystals was due to the packing of double helices. Building on models proposed by Wu and Sarko (1978 a,b), Imberty and coworkers (1988) presented a revised three-dimensional structure of both crystalline polymorphs (Figure 3). The

Figure 3. (TOP) Projection of the A-type starch crystalline structure onto the (a,b) plane. Hydrogen bonds are indicated as broken lines; (•) indicate water molecules (Imberty *et al.*, 1988).
(BOTTOM) Projection of the B-type starch crystalline structure onto the (a,b) plane. The unit cell content and some neighbouring double helices are represented in order to show the localization of the water molecules (•) in a channel. Hydrogen bonds are indicated as dashed lines (Imberty and Perez, 1988).

A-Type



B-Type

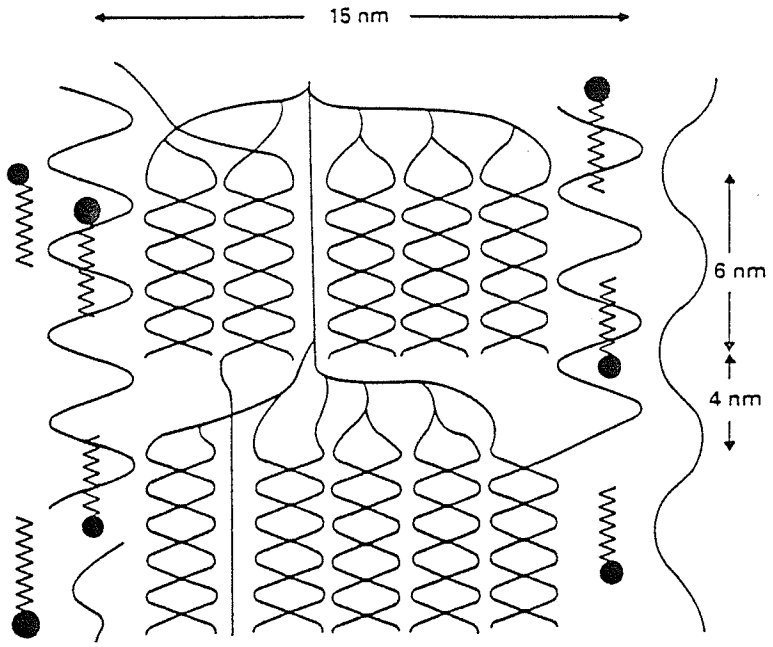


unit cell for the B type crystalline structure has two left-handed, parallel-stranded double helices that are arranged in parallel (Imberty and Perez, 1988). The double helices are hexagonally packed around a channel occupied by 36 water molecules. Half of the water molecules are tightly bound to the double helices and the other half centered around the channel. The A-unit cell similarly contains two left-handed, parallel stranded double helices packed in a parallel fashion but there are only four water molecules between helices (Imberty *et al.*, 1988). The C-type of X-ray diffraction pattern may is most likely be a mixture of A and B crystallites (Zobel, 1988a).

The molecular organization of starch constituents in the granule is shown in Figure 4 (Blanshard, 1987). Amylopectin and amylose are radially arranged toward the granular surface. The outer chains of amylopectin and possibly amylose form double helices which are packed into A and/or B-type crystalline structures depending on the source of the starch. The amorphous phase of the granule is considered to consist of amylose and branched regions of amylopectin. Complexes between monoacyl lipids and amylose may also exist in the native starch granule.

The supermolecular organization of starch molecules can be described by a series of "growth rings" (French, 1984). Concentric layers surrounding the centre of the granule can be observed through light microscopy on large hydrated granules (French, 1984). Each ring is comprised of concentrically oriented, alternating crystalline and amorphous layers. In the crystalline regions, starch double helices are hydrogen bonded to one another forming micelles which hold the granular structure together. One growth ring is considered to be composed of a single amylopectin molecule with dimensions from 1200-1400 Å and containing 20 to 80 clusters (Lineback, 1984). The molecular and supermolecular organization of the starch molecules have an impact on the granular structure.

Figure 4. Model of a starch crystallite showing the possible positioning and interactions of various components (Blanshard, 1987).



Key:

Amylopectin helix

Hybrid amylose/amylopectin helix

V-amylose helix

Free lipid

Free amylose

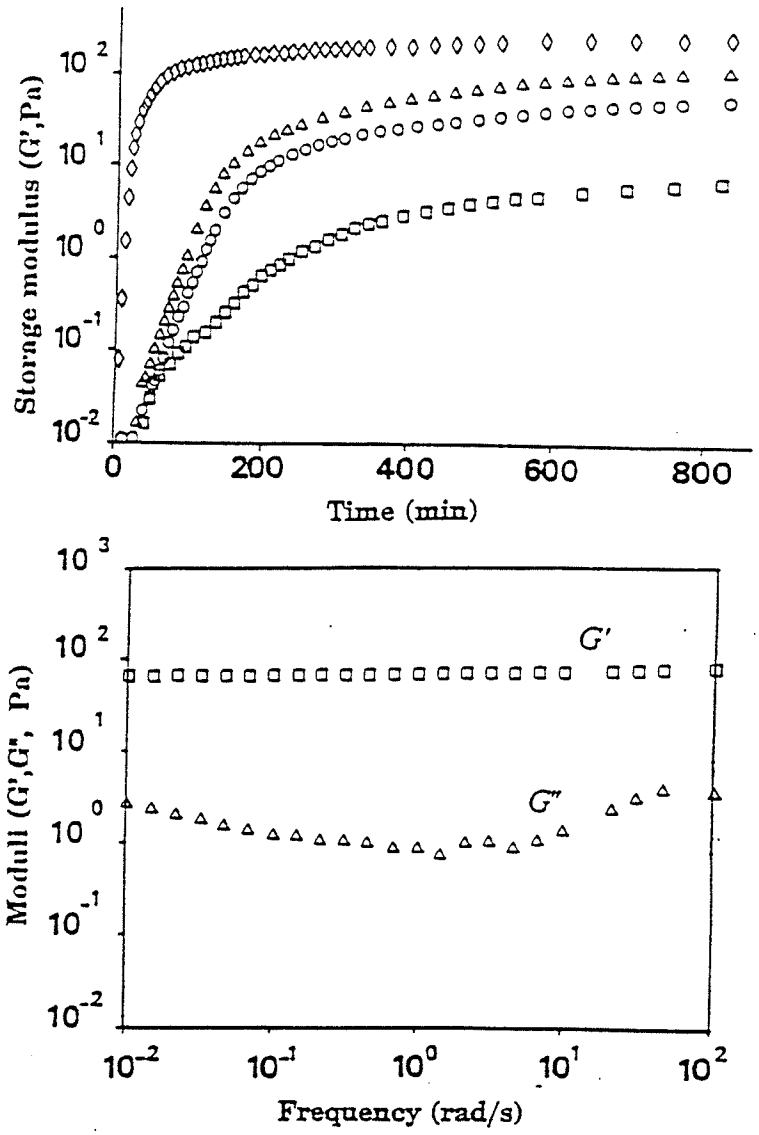
2.2 Retrogradation of Starch

At temperatures greater than the gelatinization temperature and in excess water, starch granules irreversibly swell and amylose is leached out into solution leaving amylopectin-filled granular structures (Biliaderis, 1991). Upon cooling, a viscoelastic gel forms in which swollen granules of amylopectin are embedded into an amylose cross-linked gel matrix. With time, amylopectin recrystallizes reinforcing the gel matrix and thereby increasing gel rigidity (Morris, 1990). The above physicochemical changes are referred to as "starch retrogradation". The molecular reorganization of starch chains involves the processes of gelation and recrystallization of amylose and amylopectin. The mechanisms underlying these two processes are considered below in some detail.

2.2.1 Gelation and Crystallization of Amylose

Amylose forms opaque, partially crystalline, thermo-irreversible gels at relatively low concentrations (0.8-1.1% w/w for monodispersed amylose) (Clark *et al.*, 1989). The mechanical properties of amylose gels are strongly dependent on concentration (Doublier and Choplin, 1989; Biliaderis and Zawistowski, 1990) and molecular size of this polymer (Clark *et al.*, 1989). The effect of amylose concentration on amylose gels, as measured by small strain oscillatory (dynamic) rheometry, is shown in Figure 5. In dynamic rheological measurements, the parameter G' (storage modulus) is indicative of the rigidity of the system, whereas G'' (loss modulus) is indicative of the fluidity of the system (Biliaderis, 1992). The G'-time profiles are typical for

Figure 5. Top, evolution of shear storage modulus for amylose solutions of varying polymer concentration in 0.2 M KCL at 25°C (□, 1.03%; ○, 1.33%; △, 1.48%; ◇, 1.78%). Bottom, frequency dependence of dynamic moduli (G′,G″) for 1.33% amylose gel (0.2 M KCL; gel curing condition 25°C-15 h). Adapted from Doublier and Choplin (1989), source Biliaderis (1992).


Storage modulus (G',Pa)
10 2
10 1
10 0
10-1
10-2
0
200
400
600
800
Time (min)
Modull (G',G", Pa)
10 3
10 2
10 1
10 0
10-1
10-2
G'
G"
10-2
10-1
100
101
102
Frequency (rad/s)

amylose gelation, where an initial rapid rise in G′ is followed by a phase of much slower increases in modulus (plateau region). With increasing amylose content, the initial rise in modulus is faster and the plateau values are obtained in shorter times. Once the moduli have reached a certain constant value, G′ and the G″ become independent of frequency (Figure 5). This is a typical spectrum of a solid-like gel network (Doublier and Choplin, 1989). Clark *et al.* (1989) have also shown that the chain length of amylose can affect the gelation behaviour of amylose. With decreasing amylose chain length (DP 1100 → 250) plateau G′ values are attained earlier and the plateau values are lower.

The gelation and retrogradation of amylose have been studied by several techniques. On the basis of turbidity measurements, dilatometry, dynamic rheometry and X-ray diffraction on 2-7% polydispersed pea starch, Miles *et al.* (1985b) suggested that the gelation of amylose was initiated by phase separation of amylose into polymer rich and polymer deficient regions. Upon cooling of an amorphous sol, it was observed that the development in turbidity preceded network formation. Similarly, Doublier and Choplin (1989) observed concurrent rise in modulus and turbidity for amylose solutions. Further studies with small-angle X-ray diffraction scattering indicated that crystallization within the polymer rich regions occurred via nucleation followed by growth (I'Anson *et al.*, 1988). The growth of polymer rich regions accompanies a sharp increase in the firmness of amylose gels. The slow region of gel development (the plateau region of the G′-time curve) represented the formation of B-type crystallites within the polymer rich regions in the gel.

In contrast, work with monodispersed synthesized amylose has shown that the rate and extent of network development is dependent on the molecular size of amylose (Clark *et al.*, 1989). For shorter chains the development of turbidity preceded network formation, whereas for

longer chains, network formation preceded the development of turbidity. Morris (1990) suggested that network formation within the gel may differ depending on the molecular size of amylose. Amylose of short chain length may align better to form aggregates, which are then linked to form a network. For amylose of long chain lengths the initial formation of a fine network which then coarsens may be favoured due to poorer chain matching of short segments of the polymer. Due to polydispersity of native amyloses both processes can be considered concurrent events during the initial and later stages of amylose gelation.

The nature of crosslinks within the polymer rich regions was further probed by ^{13}C CP/MAS NMR spectrometry (Gidley, 1989). The spectrum of precipitated DP=40 amylose chains exhibited doublets for C-1 ($\sim$ 100 ppm) which reflects the possibility of two glucose residues in an asymmetric unit. Such would be the case if double-helical structures were formed. Gidley (1989) concluded that amylose gels contained rigid double-helical "junction zones" that were interconnected by more amorphous single chain segments. The length of these crosslinks (junction zones) in polydispersed amylose gels has been previously shown to occur over chain length of 50-60 anhydro glucose units (Ring *et al.*, 1987; Russell, 1987). Due to the relatively long length of the ordered regions within the gel, these structures tend to be stable to α-amylolysis and heating (melting at temperatures $>$ 120°C). There is presently interest in retrograded amylose, currently termed as "resistant starch", due to the relatively slow rate of starch hydrolysis in the gastrointestinal tract of man (Englyst and Macfarlane, 1986).

By measuring the development of short range order (ie. double helix formation) by fourier transformed infrared spectroscopy (FTIRS) and results from previous researchers (Miles *et al.*, 1985b; Gidley, 1989), the following mechanism of amylose gelation and retrogradation was proposed by Goodfellow and Wilson (1990). From an amorphous sol, double helices (50-60

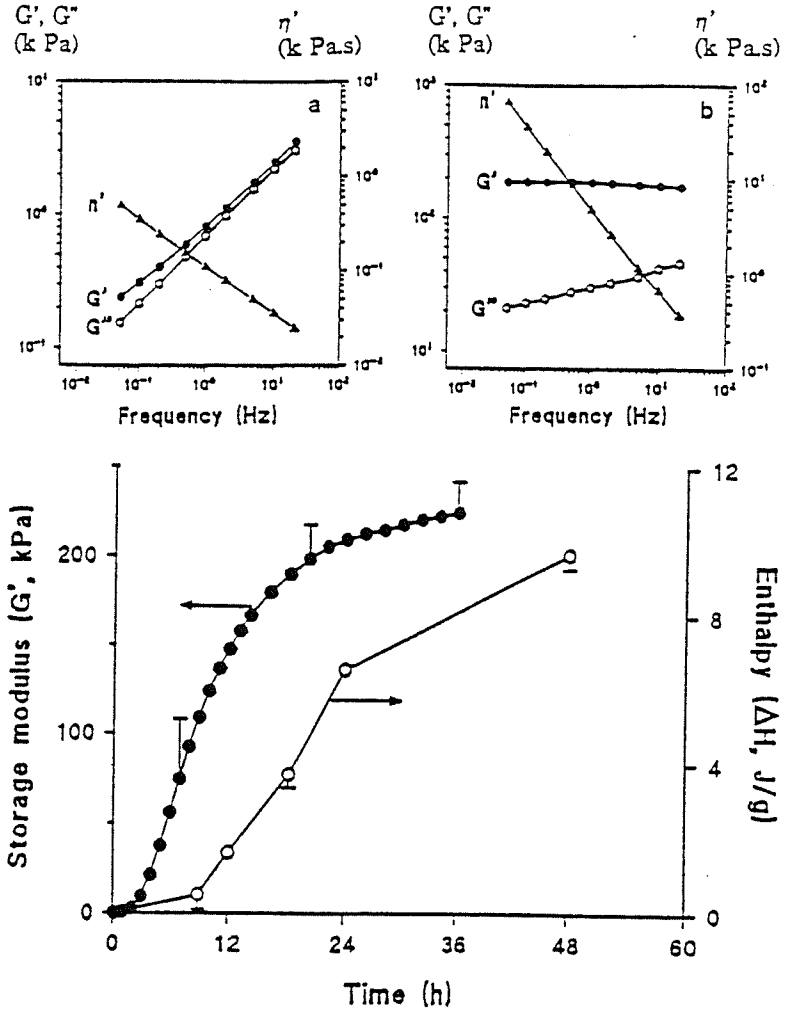
DP) form before or at the same time as phase separation occurs. This coil to helix transition is an intermolecular process which leads directly to the creation of a three-dimensional gel network in a short period of time (rapid rise in G′, Figure 5). In a longer period of time, double helices laterally associate into B-type crystalline structures (plateau G′ values, Figure 5).

2.2.2 Gelation and Crystallization of Amylopectin

In contrast to amylose gelation, amylopectin, the branched component of starch, requires a much higher polymer concentration to gel. Gels of 10% take several weeks to reach limiting values in the rheological properties (Ring *et al.*, 1987). The gelation kinetics of a 40% amylopectin gel is shown in Figure 6 (Biliaderis, 1992). Immediately after gelatinization, the G′ and G″ exhibit strong frequency dependence, indicative of a fluid nature for the material (Figure 6, inset a). This is in contrast to amylose which quickly exhibits a solid-like rheological response. The time dependent changes in modulus follows a sigmoidal curve that reaches plateau values in a much longer period of time (~ 36 hours). Upon reaching the limiting modulus values, G′ and G″ become independent of frequency, indicative of a solid-like structure (Figure 6, inset b).

Ring and coworkers (1987) studied the gelation and retrogradation of 20 % (w/w) amylopectin gels by several techniques. They observed that turbidity reached limiting values after 4-5 days, whereas the rigidity of a gel network as measured by dynamic rheometry exhibited only small increases. This indicated that the precursor of gelation are aggregates of amylopectin molecules. The formation of amylopectin gel networks was also followed by dialatometry, dynamic rheometry, DSC and X-ray diffraction. The limiting values obtained for the gels by these four techniques were attained after 30-40 days, which suggested that the intermolecular association of the chains is a crystallization process. Also, the association of amylopectin chains

Figure 6. Storage modulus (G') and melting enthalpy (ΔH) of aging waxy maize (amylopectin) starch gels at 40% (w/w). Measurements of G' at 0.2 Hz, strain $< 2.0\%$, and ΔH following storage at 6°C. Insets: (a) frequency dependence of G', G'', and η' at the start: (b) frequency dependence following storage at 8°C for 36 h (Biliaderis, 1992).


G', G"
(k Pa)
η'
(k Pa.s)
a
η'
G'
G"
Frequency (Hz)
G', G"
(k Pa)
η'
(k Pa.s)
b
η'
G'
G"
Frequency (Hz)
Storage modulus (G', kPa)
200
100
0
12
8
4
0
0
12
24
36
48
60
Time (h)
Enthalpy (ΔH, J/g)

in the gel is thermoreversible at temperatures below 100°C. Heterogenous acid hydrolysis of the gel followed by examination of the residue by gel-permeation chromatography indicated that the associated regions contain branched fragments of a DP ~ 15. Ring *et al.* (1987) suggested that the increase in firmnes of an amylopectin gel network occurs via crystallization of the outer short chains of the molecule into a B-type structure. This mechanism of gelation and retrogradation of amylopectin was reinforced by the studies of Biliaderis and Zawistowski (1990). They observed a strong temperature dependence of the rate of G´ development of 40% (w/w) amylopectin gels as probed by dynamic rheometry. The lower the temperature in which the gels were stored the greater the rate of G´ development. This implies that gelation and retrogradation of amylopectin follows nucleation kinetics, typical of a polymer crystallizing in the presence of a diluent.

A similar mechanism for the gelation and retrogradation of amylopectin was proposed by Goodfellow and Wilson (1990). The outer branches of amylopectin, once gelatinized, exist as random coils. Upon cooling, a fast formation of a short range order (formation of double helices) occurs as measured by FTIRS. This is followed by a slow lateral aggregation of double helices into crystalline B-type structures. The coil to helix transition within the side chains would not increase the development of the gel network to any extent, as it is an intramolecular process. However, over longer periods of time, crystallization of the helical side chains produces rigid sections within the molecules thereby increasing the strength of the gel network.

2.2.3 Starch Gelation and Retrogradation

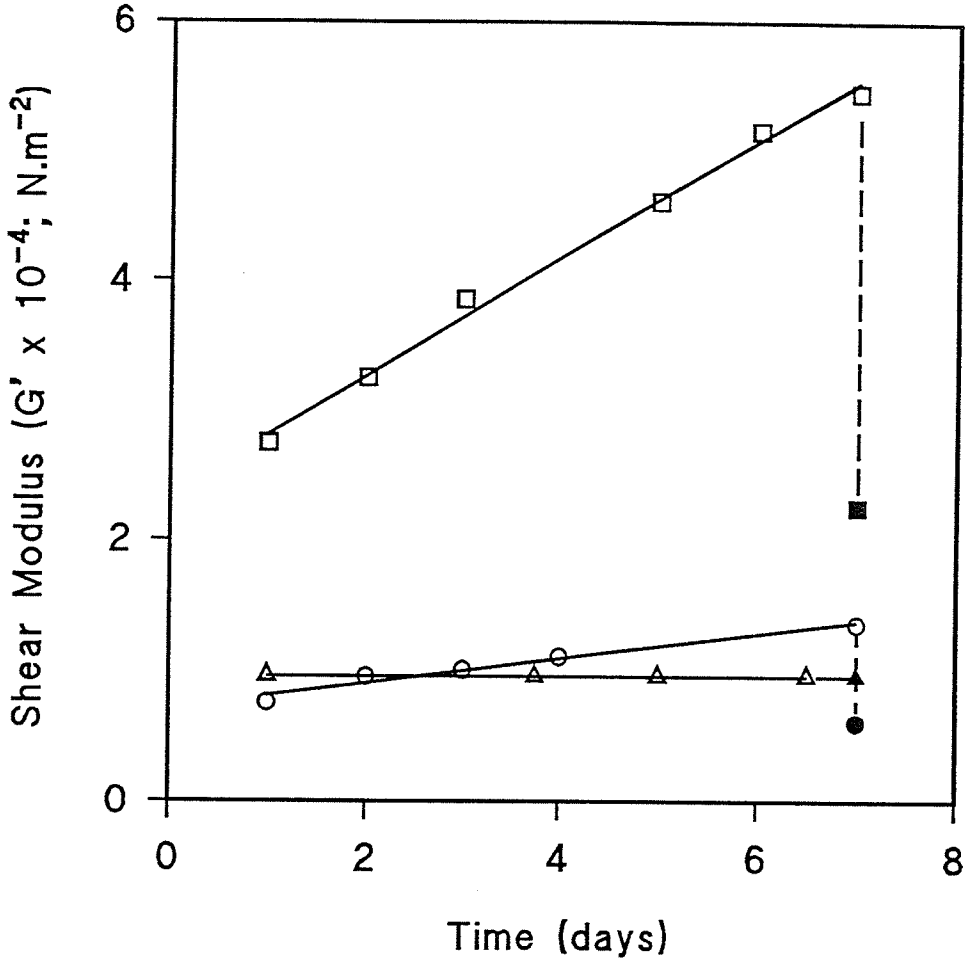
When starch is heated above a certain characteristic temperature (its gelatinization temperature) the granules irreversibly swell, amylose is solubilized and leached out of the granule

and the crystalline order of the material is lost (Biliaderis, 1991). As a result of heating, a viscous fluid is created which is composed of porous granules with an amylopectin skeleton ("ghost granules") suspended in a hot amylose solution. Upon cooling of the fluid a viscoelastic paste forms and at high starch concentrations (> 6%, w/w) an opaque elastic gel is obtained. Starch gels are thus considered as composites consisting of swollen amylopectin-enriched granules, filling an interpenetrating amylose gel matrix (Miles *et al.*, 1985a). The rheological properties of normal starches (eg. wheat starch, ~ 20% amylose) are attributed to both gelation of amylose and recrystallization of amylopectin.

The long term development of shear modulus of concentrated wheat starch and amylose gels is shown in Figure 7. The amylose gels exhibit little changes in stiffness with time, whereas the stiffness of a 20% wheat starch gel increases slowly. The slow increase in stiffness was accompanied by the formation of B-type crystals as measured by X-ray diffraction (Miles *et al.*, 1985a). Upon heating the starch gel to 90°C, the stiffness of the gel returned to the original value and the crystallinity which had developed was lost. Similar "thermo-reversible" behaviour was observed in stored swollen granules from which amylose was leached out, implying amylopectin recrystallization (Miles *et al.*, 1985a). In contrast, the amylose gels were not shown to be thermo-reversible upon heating to 90°C. The ordered regions within the amylose gel, double helical junction zones and crystalline arrays, tend to associate over much longer chain lengths (50-60 DP vs. 12-18 DP for the amylopectin) and therefore require much higher temperature to melt (> 120°C vs. ~ 60°C). Amylopectin-amylose co-crystallization may also occur, improving the binding of the granules within the amylose gel matrix (Morris, 1990).

Therefore, the biphasic gelation behaviour of composite starch gel networks (i.e. initially rapid rise in gel firmness followed by slower period of gel development) is generally attributed

Figure 7. Long-term development of the shear modulus (G´) for 10% (○) and 20% (□) starch gels, and a 3.2% (△) amylose gel at 26°C. The dotted lines indicates change after heating to 90°C and cooling. Adapted from Miles *et al.* (1985a).


Shear Modulus (G' x 10^{-4}; $N.m^{-2}$)
6
4
2
0
0
2
4
6
8
Time (days)

to an initial rapid development of amylose network followed by the slow recrystallization of amylopectin. The recrystallization of amylopectin increases the rigidity within the swollen granules which in turn reinforces the amylose gel matrix. Crystallization of amylose also occurs, but to a much lesser extent and in a shorter period of time than amylopectin. The latter phase of starch gel development (ie. recrystallization of amylopectin) is considered to cause undesirable textural changes (staling) in starch based foods (Kulp and Ponte, 1981). Further understanding of the molecular processes underlying the gelation and recrystallization of the starch molecules is needed in order to provide more effective means of controlling the staling events in baked starch based products.

2.3 Retrogradation Kinetics

The kinetics of starch retrogradation been frequently described using the Avrami theory. Initially developed by Avrami (1939,1940,1941) and later simplified by Evans (1945) and Morgan (1955), this theory was used to describe kinetics of crystallization of synthetic polymers. According to this model, crystallization results from the growth around randomly distributed nuclei. This model describes the amount of uncrystallized material (θ) with time (t) according to the following equation:

$$\theta = \exp(-kt^n) \qquad (1)$$

where k is the rate constant and n, the Avrami exponent (integer ranging from 1-4), is a parameter that is related to the type of nucleation and crystalline morphology.

Cornford *et al.* (1964) modified the Avrami equation to fit the kinetics of bread staling. This theoretical framework has been subsequently used to describe the retrogradation of starch

based on rheological measurements (Kim and D'Appolonia, 1977a; Germani *et al.*, 1983; Roulet *et al.*, 1988, 1990; Biliaderis and Tonogai, 1991; Mita, 1992), DSC (Longton and LeGrys, 1981; Russell, 1983a, 1987; Roulet *et al.*, 1988, 1990) and X-ray diffraction data (Marsh and Blanshard, 1988; Roulet *et al.*, 1988). The equation used to fit the data is:

$$\theta = (A_L - A_t)/(A_L - A_o) = \exp(-kt^n) \qquad (2)$$

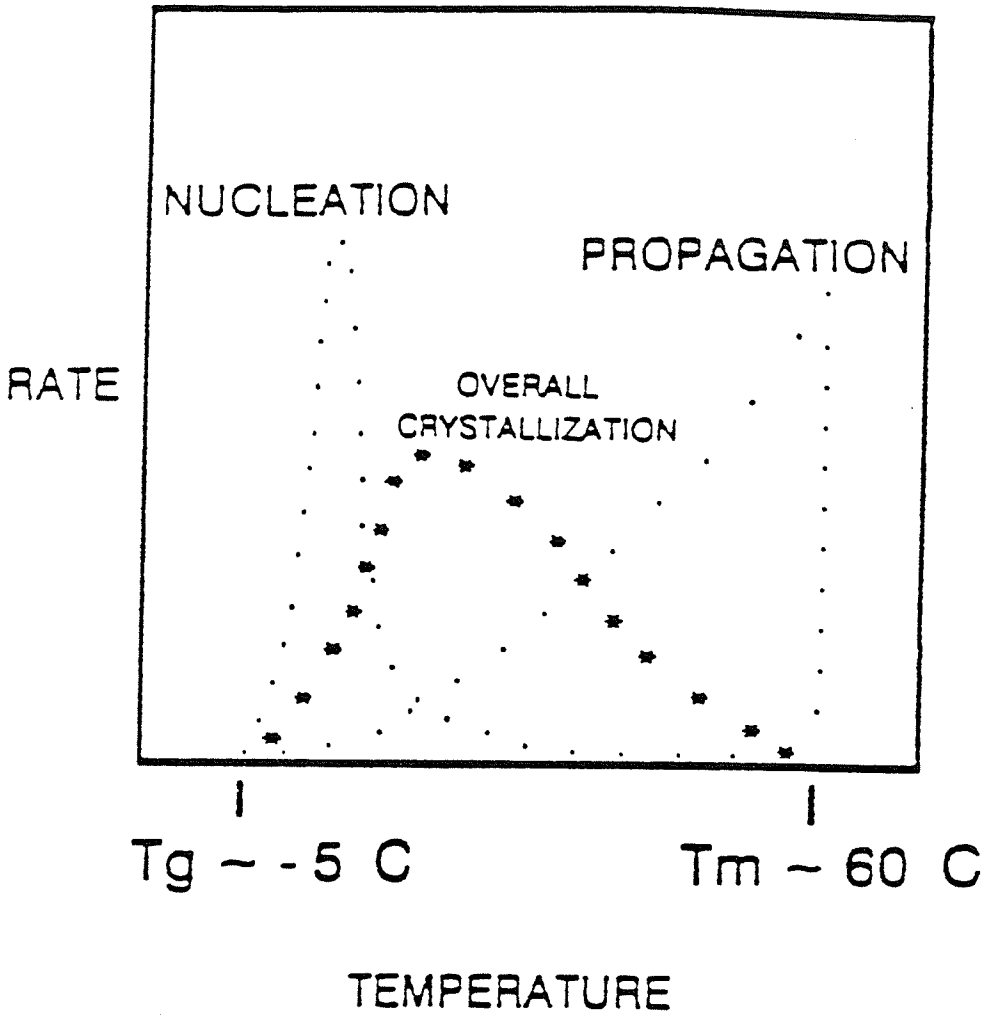
where θ is the fraction of the total change in the measured property (e.g. increase in enthalpy as measured by DSC) at time, t, and A_o, A_t and A_L are the property values obtained at time 0, time t and infinity, respectively. The experimental values may represent data collected from rheological, DSC and X-ray diffraction measurements on retrograded starch. However, there has been criticism in using the Avrami analysis to describe the gelation and retrogradation of starch (Slade and Levine, 1987; Biliaderis, 1990). Its theoretical development is based on the assumption of a single macromolecular species present in a crystallization medium, which is not the case in an aqueous starch system. The Avrami parameters were also derived to define the mechanism of crystallization under conditions of thermodynamic equilibrium. Starch recrystallization is a non-equilibrium process, occurring through a three-step mechanism (nucleation→growth→crystal perfection) (Slade and Levine, 1987). Nevertheless, the Avarmi equation does provide a convenient basis for comparing kinetic results from retrogradation/staling studies obtained by various techniques.

Recently, the kinetics of starch retrogradation have been related to the crystallization kinetics of partially crystalline polymers within the physicochemical boundaries defined by the glass transition (T_g) and gelatinization temperatures (T_m) (Slade and Levine, 1987; Biliaderis 1990; Morris, 1990; Biliaderis, 1991). Starch is a partially crystalline material and as such it undergoes two typical transitions (Biliaderis, 1991): (i) glass-rubber transition at T_g (second order

transition), and (ii) melting of crystallites at their T_m (first order transition). As reviewed by Biliaderis (1991), at temperatures below T_g, the material is glassy, and the molecular motions are reduced to such a state that crystallization does not occur (i.e. the system is kinetically stable). At $T_g < T < T_m$, the material changes to a rubbery state, and thus crystallization can occur since sufficient mobility is introduced into the system.

Slade and Levine (1987) described the kinetics of starch recrystallization through the following process: (i) nucleation - formation of critical nuclei by initiation (formation of double helices); (ii) propagation - growth of crystals from nuclei by intermolecular aggregation of ordered chain segments (lateral association of double helices into crystalline structures); (iii) maturation - crystal perfection (by annealing of metastable crystals). The kinetics of starch recrystallization between T_g and T_m is represented in Figure 8 (Slade and Levine, 1991). The nucleation rate increases exponentially with decreasing temperature, close to the T_g (-5°C, 27% w/w potato starch). The propagation rate increases exponentially with increasing temperature up to T_m (60°C, 27% w/w potato starch). The maximal rate of recrystallization at a single temperature was observed to be approximately 4°C. Nucleation is the rate limiting step that occurs during retrogradation of starch (Slade and Levine, 1987). Without a nucleated system, development of ordered structures within the gel cannot occur. This kinetic representation of starch recrystallization is important in understanding the effects of temperature on promoting or retarding starch retrogradation.

Figure 8. Crystallization kinetics of B-type starch as a partially crystalline polymer system, expressed in terms of nucleation, propagation, and overall crystallization a function of temperature (Slade and Levine, 1991).


NUCLEATION
PROPAGATION
RATE
OVERALL
CRYSTALLIZATION
Tg ~ -5 C
Tm ~ 60 C
TEMPERATURE

2.4 Factors Affecting the Retrogradation of Starch

Starch retrogradation can be controlled by several means. The simplest is by controlling the storage temperature and moisture content of the starch based food product. The addition of pentosans, lipids, and sugars can also have a significant impact on the rate of the molecular reorganization events associated with starch retrogradation. The mechanisms by which these factors affect starch retrogradation are considered below in some detail.

2.4.1 Storage Temperature

The temperature at which baked products are stored can significantly affect the extent of staling and therefore the shelf life of the product. The effect of temperature on retrogradation of concentrated starch gels has been extensively studied by DSC (Longton and LeGrys, 1981; Nakazawa *et al.*, 1985; Jankowski and Rha, 1986; Slade and Levine, 1987; Marsh and Blanshard, 1988). A negative relationship was observed between the extent of retrogradation of starch, as measured by the increase in the enthalpy (ΔH) of the retrogradation endotherm, and storage temperature from 2 to 50°C. Similar observations were observed by large deformation rheological tests (Jankowski and Rha, 1986) and small strain oscillatory rheometry (Biliaderis and Zawistowski, 1990) on concentrated starch gels. Zeleznak and Hoseney (1987) also observed that bread stored at 4°C staled faster, as measured by DSC, than at 25°C or 40°C.

The effect of storage temperature on starch retrogradation can be schematically represented by the recrystallization kinetics of B-type starch in Figure 8. At temperatures below T_g recrystallization of starch is inhibited because nucleation and propagation requires segmental mobility of the outer branched chains of amylopectin for the formation and aggregation of double

helices (Slade and Levine, 1987). The mobility that is required for such molecular reorganization is disallowed in such highly viscous systems such as glasses, where $\eta > 10^{12}$ Pa·s. At temperatures above T_m, crystallization also goes to zero because crystals can neither nucleate or grow at temperatures at which they are melted. Therefore, storage of starch based food products below their respective T_g can effectively retard starch retrogradation.

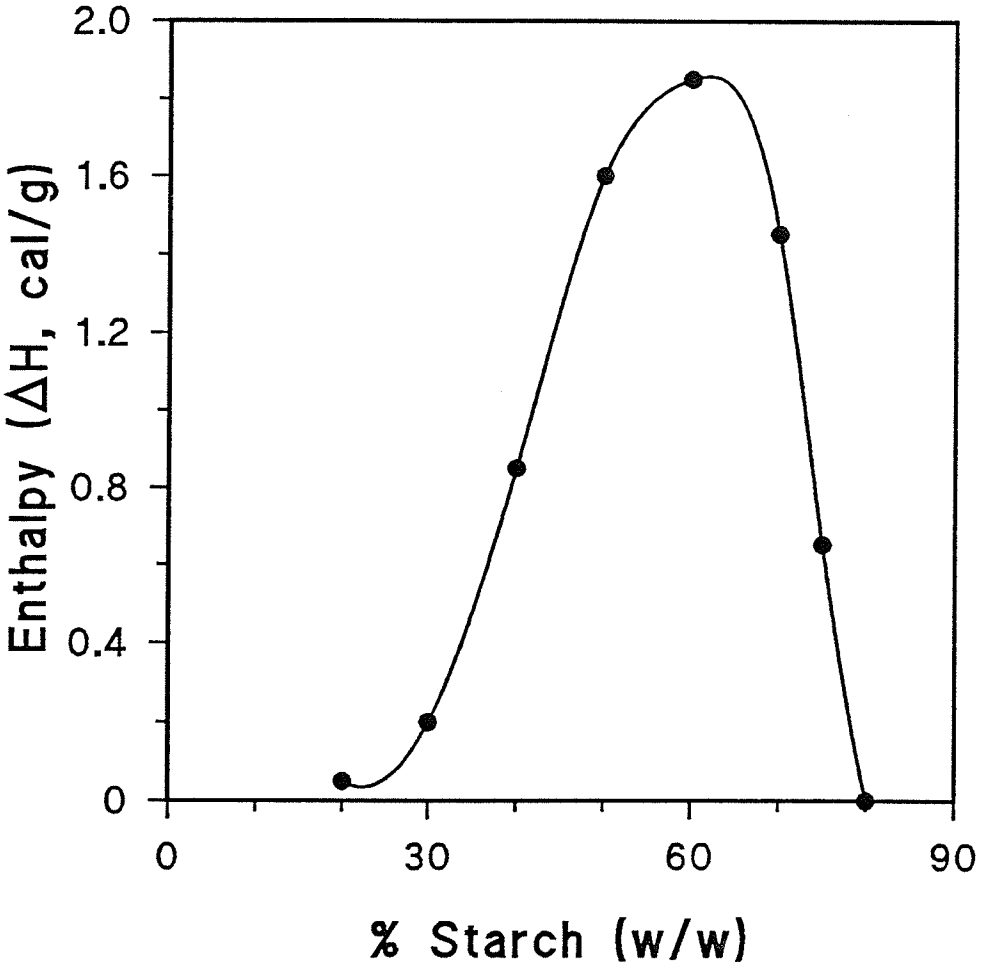
In some instances, the promotion of starch retrogradation is desirable as in the case of staled bread for stuffing mixes. Slade and Levine (1987) accelerated the staling of bread by cooling it close to T_g to promote crystal nucleation and then heating close to T_m to maximize crystal growth. This achieves a similar level of staling in hours to that achieved by isothermal storage at room temperature for days.

2.4.2 Moisture Content

The amount of moisture, or the concentration of an aqueous starch system, plays an important role in influencing the rate of retrogradation. Essentially, the reorganization of starch polymers does not occur below 10% or above 80% solids as was shown by calorimetry data (Longton and LeGrys, 1981; Zeleznak and Hoseney, 1986; Slade and Levine, 1987). The overall response to starch retrogradation, as measured by DSC, follows a bell shaped curve (Figure 9). The maximal rate of retrogradation of wheat starch was observed to occur approximately at 60% solids. The rate of starch retrogradation (increase in ΔH) increased from 80% to 60% solids due to increasing plasticization of the starch system by water, then decreased with further increases in moisture up to 20% solids due to a dilution effect.

Slade and Levine (1987) explained the effect of moisture on starch retrogradation from the viewpoint of water acting as a plasticizer in a starch/water system. Water is considered to

Figure 9. Effect of moisture present during aging on the enthalpy (ΔH in calories per gram of starch) of retrograded starch in starch gels. Adapted from Zeleznak and Hoseney (1986).


2.0
1.6
1.2
0.8
0.4
0
Enthalpy (ΔH, cal/g)
0
30
60
90
% Starch (w/w)

be preferentially absorbed in the amorphous regions of partially crystalline materials (ie. branched zones of amylopectin) (Slade and Levine, 1987). Low MW plasticizing diluents, such as water, are considered to increase the free volume. The increase in free volume allows for an increase in segmental chain mobility in the amorphous regions which decreases their T_g. As the volume fraction of water in the system decreases, T_g increases. As a result, for low moisture foods, such as a crackers, the T_g is approximately equal to room temperature and these products exist in the glassy state. Therefore, the ability of double helices of the outer branches of amylopectin to associate is severely restricted and starch retrogradation is inhibited (Slade and Levine, 1987). By manipulating the moisture content of starch based foods, the extent and rate of staling can be significantly reduced or inhibited.

2.4.3 Additives

2.4.3.1 Sugars. Starch based products such as cakes and cookies are formulated with relatively high amounts of sugar. Until recently little research has been done on the effects of small molecular weight carbohydrates upon starch retrogradation (Maxwell and Zobel, 1978; Germani *et al.*, 1983; Slade and Levine, 1987; I'Anson *et al.*, 1990; Carins *et al.*, 1991a,b; Kohyama and Nishinari, 1991; Katsuta *et al.*, 1992a,b,c; Miura *et al.*, 1992). Understanding these effects is important for improving the texture and shelf life of high sugar content starch products.

Initial work on the effect of sugars on retrogradation of starch gels was undertaken by measuring the increase in firmness during storage using large deformation testing, such as the Instron Universal Testing Instrument. Maxwell and Zobel (1978) observed that by incorporating sweeteners such as dextrose and sucrose (1:1:1 w/w sweetener:starch:water), the development

of gel rigidity was significantly reduced, with sucrose having a greater anti-retrogradation effect than dextrose. In contrast, Germani *et al.* (1983) observed that sugars (18 and 36% w/w) increased the retrogradation rate of concentrated corn starch gels (50% w/w) as analyzed by the Avrami equation. In general, maltose and sucrose were observed to be more effective in increasing the velocity of retrogradation than glucose.

The first systematic study on the effect of sugars on starch retrogradation was carried out by Slade and Levine (1987). The retrogradation of wheat starch:sugar:water (1:1:1 w/w) gels was measured by DSC after being stored at 25°C for 8 days. They observed that the extent of recrystallization decreases in the order: fructose > mannose > water alone > galactose > glucose > maltose > sucrose > maltotriose > xylose > lactose > malto-oligosaccharides (enzyme-hydrolysed, DP>3). Slade and Levine (1987) concluded that for the oligomers within this series the molecular weight and the resultant T_g are the primary determinants of their anti-staling activity. As the molecular weight of the glucose-oligomers increases the greater the anti-plasticizing effect in the amorphous gels matrix. By addition of these sugars, the ability to promote mobility is reduced, increasing the effective T_g, which reduces the rate of aggregation of double helices into crystalline arrays. However, for the other sugars in the series, it was suggested that coplasticizer (sugar) mobility as determined by free volume and local viscosity may play a role in their anti-staling effect. Slade and Levine (1987) could not explain why fructose/water accelerated retrogradation compared to water alone.

Further work by I'Anson *et al.* (1990) and Carins *et al.* (1991 a,b) questioned the anti-staling mechanism of sugars proposed by Slade and Levine (1987). In model starch/sugar/water (1:1:1 w/w) systems ribose, a low molecular weight sugar, inhibited the reorganization of wheat starch gels as measured by large deformation testing and X-ray diffraction (I'Anson *et al.*, 1990).

Similarly, Carins *et al.* (1991 a,b) observed a strong retardation of starch retrogradation by ribose and xylose as observed through X-ray diffraction measurements. These effects were accentuated by increasing sugar concentration from 0 to 32% (w/w). In contrast, the addition of fructose with increasing concentration promoted the level of crystallinity within the wheat starch gel. Alternatively, I'Anson *et al.* (1990) suggested that sugars may affect the phase separation of starch polymers in an aqueous medium which may in turn affect the rate of starch retrogradation. Kohyama and Nishinari (1991) have reported that sugars may affect the extent of reorganization within a starch gel by interacting with starch molecular chains to stabilize the amorphous and entangled matrix of gelatinized starch.

Miura and coworkers (1992) also systematically studied the influence of polyols (saccharides and sugar alcohols, 2.7% w/w) on the hardening of rice starch gels (30% w/w). They proposed that the anti-retrogradation effect is based on the polyol's effect on the structural stability of water surrounding the starch polymer chains. A negative correlation was observed between the rate of increase in the gel firmness to the mean number of equatorial OH groups (n(e-OH)) in the saccharide molecule. The saccharides which have more n(e-OH) (i.e. maltose vs glucose) in their molecular structure in solution, exhibit greater stabilizing effect on water structure. According to these researchers, if the structure of the water surrounding the starch chains is increased, the flexibility and thus ability for interassociations is reduced, therefore retarding the retrogradation process. However, controversy still exists as to the mechanism by which sugars affect the retrogradation of starch. Considerably more work has to be done to come up with a unified model to explain the interactions in the ternary system starch-sugar-water and how this may influence the reorganization of the polysaccharide chains.

2.4.3.2 Lipids. For many years lipids and emulsifiers have been added to baked products

as texture modifiers and anti-staling agents. Commonly used surfactants such as glycerol monostearate (GMS) and sodium stearoyl lactylate (SSL) are well known to form helical inclusion complexes with amylose (Kulp and Ponte, 1981). This complex formation was initially used to explain the anti-staling effects of lipids and emulsifiers on baked products (Kulp and Ponte, 1981). However, since amylopectin and not amylose is responsible for the retrogradation of starch, it is still not clear how lipids or emulsifier delay or retard the retrogradation of starch. Nevertheless, it is clear that complexation between monoacyl lipids and amylopectin does take place (Evans, 1986).

The effect of saturated monoglycerides, such as GMS, on improving the shelf life of bread has been well documented in the literature (Kulp and Ponte, 1981; Russell, 1983b; Krog *et al.*, 1989; Huang and White, 1993). To further investigate the anti-staling properties of lipids and emulsifiers model starch/lipid/water systems have been studied. Surfactants such as SSL and cetyltrimethylammonium bromide (CTAB) were observed to significantly reduce the amount of retrogradation in wheat and waxy maize starch gels as measured by DSC (Eliasson, 1983; Eliasson and Ljunger, 1988). The effects of surfactants were observed to be greater for 100% amylopectin as opposed to blends of amylopectin/amylose of 50-90% amylopectin (Gudmundsson and Eliasson, 1990). The type of lipid or emulsifier also plays an important role in its effect on the retrogradation of starch (Whittam *et al.*, 1986). Monoglycerides of varying chain length, C_{10} to C_{18}, exhibited different effects on the rigidity of pea starch gels. The addition of glycerol monomyristin (C_{14}) resulted in the greatest reduction in gel rigidity. Biliaderis and Tonogai (1991) studied the influence of lipids, endogenous and added, on the viscoelastic properties of concentrated starch gels by dynamic rheometry. L-α-lysophatidylcholine (LPC), an endogenous wheat granular lipid, was observed initially to strengthen pea and rice starch gels whereas during

the later stages of network development (ie. recrystallization of amylopectin) LPC was observed to reduce this process. The question then arises how lipids frequently reported to complex only with amylose can affect starch retrogradation, primarily an amylopectin dominated process.

It was hypothesised that lipids can also complex with the outer 12-18 DP branches of amylopectin. Indirect evidence of amylopectin-surfactant complexes was observed. Evans (1986) reported a decrease in the melting enthalpy value of waxy maize starch in the presence of sodium dodecyl sulphate (SDS) and CTAB with increasing surfactant concentration, thus implying some type of association between the surfactant and the amylopectin. Batres and White (1986) also reported that the iodine affinity of amylopectin was significantly reduced in the presence of glycerol monomyristate (GMM) and glycerol monopalmitate (GMP). The reduction in iodine affinity was attributed to the complexation of amylopectin with GMP and GMM.

Direct evidence of amylopectin-surfactant complexing was reported by Slade and Levine (1987) and Gudmundsson and Eliasson (1990). Slade and Levine (1987) observed complexation between waxy maize starch and SSL when comelted at low moisture (< 10% w/w) and then rescanned by DSC. Gudumundsson and Eliasson (1990) further studied this interaction with blends of amylopectin and amylose when a series of surfactants were added. Thermograms of 100% amylopectin gels (50% w/w) in presence of CTAB (1.9 mg/100 mg mixture) revealed a transition at ~110°C, temperatures normally associated with amylose-lipid complexes. X-ray diffraction of CTAB/amylopectin gels supported the presence of an amylopectin-surfactant complex. At lower concentrations of CTAB a mixture of B-and V-patterns was observed, but at higher concentrations the V-pattern was stronger and the B-pattern was much weaker. These findings provided direct evidence of interactions between amylopectin and emulsifiers/surfactants, probably in the form of helical inclusion complexes such as in the case of amylose-lipid

complexes.

Lipids/surfactants are known to significantly reduce the amount of starch retrogradation. Through indirect and direct evidence lipids/surfactants seem to complex with amylopectin in the form of helical inclusion complexes with the outer short chains of amylopectin (Gudmundsson and Eliasson, 1990). Amylopectin-surfactant complexes would prevent the formation of double helices by the outer 12-18 DP branches of amylopectin and their subsequent aggregation into crystalline structures. This would have a retarding effect on the retrogradation of starch.

2.4.3.3 Pentosans. The starchy endosperm of cereals contains primarily starch and protein, but significant amounts of cellulose fibres and other cell wall components are also present. In wheat, and particularly in rye, these constituents are mainly composed of pentosans, including arabinoxylans and arabinogalactan-peptides (Gudmundsson *et al.*, 1991). Aqueous solutions of pentosans exhibit properties typical of gums; they are viscous at room temperature, thin out during heating and are highly hydrophillic (Kulp and Ponte, 1981). The role of pentosans on starch retrogradation has been examined. It was shown that pentosans decreased staling and firmness of bread (Kim and D'Appolonia, 1977c; Jankiewicz and Michniewicz, 1987), but promoted starch retrogradation as analyzed by DSC (Gudmundsson *et al.*, 1991; Biliaderis and Izydorczyk, 1992). The increase in starch retrogradation was attributed to the hydrophillic properties of arabinoxylans, the main component of pentosans, which caused localized increases in starch concentration. Starch retrogradation increases with increasing starch concentration towards a maximum of ~ 60% starch (Zeleznak and Hoseney, 1986).

The work of Rattan (1992) on the effect of low and high MW arabinoxylans on the mechanical properties of white pan bread has given further insights on the anti-staling properties of pentosans. There was an observed decrease in crumb firmness with addition of both the low

and high MW arabinoxylans. The decrease was accentuated with increasing concentration of arabinoxylan added. Pentosans have been suggested to influence the texture of bread crumb by interacting with gluten to form composite hydrated film networks and also by increasing the water absorption of dough which in turn contributes to the texture of the crumb (Rattan, 1992). Additional water was required for the arabinoxylan fortified doughs to develop similar dough consistency levels. Water acting as a plasticizer of the gluten-starch matrix lowered the modulus of the composite network (Levine and Slade, 1990). The lower firmness values observed for bread crumbs fortified with arabinoxylans, as compared to the control samples, is likely due to the higher moisture content (Rattan, 1992). Although arabinoxylans promoted starch retrogradation as measured by DSC, there was a decrease in the extent of staling as assessed by firmness measurements, in "complex" systems such as bread.

2.5 The Role of Starch in the Staling of Bakery Products

The staling process generally is described as a loss of freshness characterized by a toughening of the crumb or firming of texture and all associated losses in flavour and aroma constituents (Reineccius, 1992). The economic losses to the baking industry due to staling (loss of perceived freshness by consumers) is enormous. Hebeda and coworkers (1990) reported that 3-5% of all baked products produced in the United States in 1990 is discarded due to staling (a loss of about $ 1 Billion). Significant amount of research in the causes of staling and methods of controlling this physicochemical process have been done as reviewed by Kulp and Ponte (1981) and recently by Lineback (1991). Many factors have been identified as causing or being involved in the staling of baked products: flour type, protein, pentosans, lipids, sugars, moisture migration

between starch and gluten, the ratio of starch to protein and starch retrogradation (Lineback, 1991). Starch retrogradation has traditionally been considered to play a major role in staling and will be discussed herewithin.

Work by Schoch and French (1947), continued by Kim and D'Appolonia (1977 a,b) resulted in a widely accepted explanation for the role of starch in firming (staling). The percentage of soluble starch extracted from the crumb of bread decreased with increasing storage time. For the soluble starch there was only a little decrease in the amount of amylose fraction but considerable decrease in the amount of extracted amylopectin. The same behaviour was also observed for 50% wheat starch gels. These changes in the starch fraction were shown to occur concurrently with an increase in firmness of both bread crumbs and starch gels. It was therefore concluded that within the continuous gluten matrix of bread crumb, amylose quickly forms a three-dimensional gel network upon cooling, thus becoming insoluble. Within the amylose continuous gel network, amylopectin-enriched swollen granules are embedded (Morris, 1990). With time, amylopectin recrystallizes increasing the rigidity of the swollen granules which in turn reinforces the gel matrix. This increase in rigidity of the dispersed phase of the crumb is reflected by the increases in firmness of the whole crumb.

Recently, the role of starch retrogradation in staling has been debated. The work by Martin and coworkers (1991) suggests that the cause of firming within the bread crumb is due to formation of cross-links (hydrogen bonds) between the continuous protein matrix of the crumb and the discontinuous remains of the granules. The exact mechanism of staling still remains controversial. The staling of baked products most likely reflects both the retrogradation of starch and physicochemical changes with the gluten matrix. Depending on the type of product, processing and formulation the relative role of these processes will differ.

3. MATERIALS AND METHODS

3.1 Materials

Commercial samples of waxy maize (amioca), wheat, potato and pea starches were obtained from National Starch and Chemical Corp. (Bridgewater, NJ), Ogilvie Mills (Midland, ON), Sigma Chemical Co. (St. Louis, MO) and Protein Technologies International (St. Louis, MO), respectively. The starches were vacuum dried (60°C) and kept in a desiccator until used. All polyols (sugars and sugar alcohols) were of analytical grade. Oligomers of glucose, G4 - G7, were purchased from Boehringer Mannheim Canada Ltd. (Laval, PQ); maltooctaose (G8) isolated by gel permeation chromatography of a starch hydrolysis product from American-Maize Products Co. (Amaizo, Hammond, IN); fructose and glucose from Mallinkrodt (Paris, KY); sucrose from Fisher Scientific (Montreal, PQ); and all other polyols (glyceraldehyde, glycerol, ribose, xylose, methyl β-D-xylopyranoside, arabinose, talose, galactose, 3-O-methyl-D-glucopyranose, sorbitol, lactose, maltose, trehalose, cellobiose, gentiobiose, isomaltose, maltitol, lactitol and maltotriose) were obtained from Sigma Chemical Co. (St. Louis, MO). All other reagents were of analytical grade. Distilled water was used in all experiments.

3.2 Methods

3.2.1 Composition and Physical Properties of Starch

3.2.1.1 Starch Content. Starch was assayed by a dual enzyme method using the α-amylase (Tenase, Miles Laboratories, Elkart, IN) and glucoamylase (Diazyme L-200, Miles Laboratories, Elkhart, IN) enzyme system as described by Banks *et al.* (1970). Glucose was assayed by the glucose-oxidase-peroxidase-4 aminoantipyrine (dye) (Sigma Chemical Co., St. Louis, Mo) enzyme system (Biliaderis and Grant, 1979).

3.2.1.2 Amylose Content. The starch samples (3-4 g) were defatted by Soxhlet extraction with 85% methanol (16 h) then dried under vacuum (60°C) overnight before amylose determination. Amylose content was then determined, based on its iodine affinity value, according to Schoch (1964).

3.2.1.3 Lipid Content. Crude fat content of starch samples (3-4 g, dry weight) was determined by Soxhlet extraction (16 h) with 85% methanol as described by the AACC method 30-25 (1983).

3.2.1.4 Thermal Characteristics. Differential scanning calorimetry (DSC) of granular starch was carried out using a DuPont 9900 thermal analyzer equipped with an ambient DSC pressure cell (200 kPa N_2 was used to flush the cell). Starch samples were suspended in distilled water to obtain a concentration of 40% (w/w) and then hermetically sealed in DuPont DSC pans. The samples were allowed to equilibrate for one hour before measurements. The starch suspensions were scanned from 20 to 135°C at 10°C/min. The DSC was calibrated using indium and an empty pan was used as the reference. All other conditions for the operation of the calorimeter were as described previously by Biliaderis *et al.* (1985).

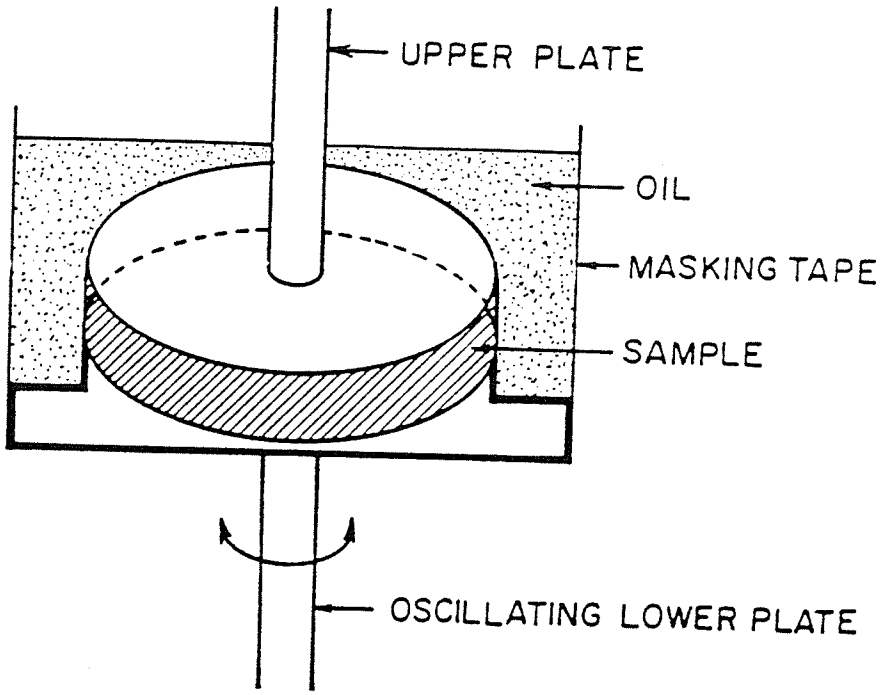
3.2.2 Preparation of Starch Gels

3.2.2.1 Waxy Maize Starch. The waxy maize starch gels were prepared by heating starch/polyol slurries in hermetically sealed stainless tubes (30 mm i.d. x 65 mm height). Polyol solutions were added to the starch (2g) to obtain a ratio of 1:0.5:1.5 (w/w) starch:polyol:water mixture. The tubes were immersed in a boiling water bath (98°C) for 15 min and then subsequently cooled in a water bath at 25°C for 15 min. Immediately after cooling, the gels were transferred to the lower precooled (8°C) plate of the parallel plate geometry (30 mm diameter) of the rheometer. The upper plate was then lowered onto the gel to a gap of 1.0 mm and excess material was trimmed from the periphery with a blade. Light mineral oil (Mallinckrodt, Paris, KY) was added to a level just covering the upper plate and held by a layer of masking tape surrounding the base of the apparatus, as shown in Figure 10. Using this method, evaporation of the sample was prevented during kinetic rheological measurements. The samples were allowed to relax for 10 min before rheological measurements were made.

3.2.2.2 Wheat, Potato and Pea Starch. Starch gels (containing amylose) were prepared by heating starch/polyol slurries in a specially designed hermetically sealed container (disk dimensions 80 mm i.d. x 1 mm thickness; Biliaderis and Tonogai, 1991). Using this apparatus, 1 mm thick gels were cast without the loss or addition of water and mechanical damage of the network. Polyol solutions were added to the starch (3 g) to obtain a ratio of 1:0.5:1.5 (w/w) starch:polyol:water mixture and stirred in a closed sample container for 5 min to ensure that a homogenous slurry was formed. The slurry was then transferred to the stainless steel container and heated. The following gel curing procedures were carried out to prepare the starch gels:

(i) wheat starch - 15 min boiling water (98°C); 15 min water bath at 25°C
(ii) potato starch - 15 min swell at 60°C; 15 min oil bath at 105°C; 15 min water bath at 25°C

Figure 10. Fixture geometry configuration of the measuring system indicating the positioning of the sample and the covering thin layer of mineral oil (Biliaderis and Zawistowski, 1990).


UPPER PLATE
OIL
MASKING TAPE
SAMPLE
OSCILLATING LOWER PLATE

(iii) pea starch - 15 min oil bath at 120°C; 15 min water bath at 25°C.

The gel disks with a diameter of 30 mm were then cut from the gel and immediately placed on the bottom plate of the parallel plate geometry and the top plate was lowered onto the gel. Paraffin oil was added to prevent the loss of moisture as described in section 3.2.2.1.

3.2.3 Measurements of the Thermal and Mechanical Properties of Ageing Concentrated Starch Gels

3.2.3.1 Dynamic Rheometry. The mechanical properties of the starch gel were probed by small amplitude oscillatory testing using a Bohlin VOR Rheometer (Bohlin Reologi, Edison, NJ) operated with a parallel plate geometry (30 mm diameter) and a torque element of 93.2 g.cm. The kinetic aspects of gel structure development were probed at 0.2 Hz, 10% amplitude and 8°C; data were collected for 36 hours at 15 min intervals. Preliminary work indicated that all dynamic measurements were performed at lower than 2% strain, a range within the linear viscoelastic region of the gels (Appendix I). Further details of the experimental procedures for the rheological tests are described elsewhere (Biliaderis and Zawistowski, 1990; Biliaderis and Tonogai, 1991).

3.2.3.2 DSC. Calorimetric measurements of ageing starch/polyol gels were carried out using a DuPont thermal analyzer equipped with a 910 DSC high pressure cell. Starch samples (3.0-3.3 mg) were suspended in aqueous polyol solutions to obtain a ratio 1:0.5:1.5 (w/w) starch:polyol:water mixtures and then hermetically sealed in DuPont DSC pans. The starch suspensions were first heated from 25 to 135°C (10°C/min) to gelatinize the granules under pressure (1400 kPa, with N_2) to eliminate the problem of pan failure due to moisture loss at temperatures above 120°C. The samples were cooled to room temperature and stored for a designated period of time (9, 12, 18, 24, 48, 72, 96, 144 h) at 6°C. Analysis of the

retrogradation endotherm was carried out using the ambient DSC pressure cell as described in section 3.2.1.4.

3.2.4 Viscosity Measurements

Viscosity of the polyol solutions (0.5:1.5 w/w, polyol:water) was measured by a Ubbelohde viscometer (International Glassware, Kenilworth, NJ; No. 098) at 8°C. The resulting kinematic viscosity was converted to absolute viscosity by the following equation:

$$\eta = \rho \times \nu \qquad (3)$$

where ν is the kinematic viscosity (centistokes), ρ is the density at 8°C (g/ml) and η is the absolute viscosity at 8°C (centipoise) of the polyol solutions. The density of the polyol solutions was determined using a pycnometer (25 mL; Fisher Scientific, Montreal, PQ) at 8°C as described by Joslyn (1970). The kinematic viscosity, density and calculated absolute viscosity values of the various polyol solutions are shown in Appendix II.

3.3 Statistical Analysis

Analysis of variance was carried out on the thermal and rheological data and differences among samples were determined by the Duncan's Multiple Range Test using the NCSS statistical software (ver 5.0, Kaysville, UT). Differences in the effect of polyols on retrogradation between saccharides and their alcohol derivatives, were determined by paired T-tests. Data presented are the means of at least triplicate runs unless otherwise stated. Three dimensional plots were generated using the Systats software package (Evanston, IL), and a distance weighted least squares (DWLS) smoothing function was applied to fit a surface through the data set points. The

levels chosen for the two independent variables were: the polyol level (X) in the starch:polyol:water (1:X:1.5) mixture which was adjusted to 0, 0.1, 0.3, 0.5, 0.7, 0.9 and the storage periods for the composite gels which were 12 h, 1, 2, 3, 4, 6, 8, 10 days.

An attempt was also made to relate the effect of polyols on starch retrogradation (as measured by DSC) with the hydration characteristics and other physicochemical properties of the polyols in aqueous solutions. The values for these properties were taken or calculated from published literature reports (Slade and Levine, 1988; Uedaira *et al.*, 1989, 1990; Galema and Hoiland, 1991). More specifically, the following physicochemical parameters of the aqueous polyol solutions were used in this analysis:

1) Viscosity of 25% w/w polyol solutions:

As determined in section 3.2.4.

2) Hydration Number (n_h) (Galema and Hoiland, 1991):

The hydration number is a measure of the number of water molecules located in the first hydration sphere surrounding a solute. The n_h values of the polyols used in the present study were reported by Galema and Hoiland (1991) based on the compressibility measurements of the aqueous solutions using pulsating ultrasonic radiation. The n_h gives an indication of the number of water molecules that are disturbed from the regular network structure in the presence of polyols. The better the compatibility of a polyol with the three-dimensional hydrogen bonded structure of water is, the smaller the number of water molecules which are disturbed by the presence of the polyol.

3. Isentropic Partial Molar Compressibilities ($k_2°$; $cm^3mol^{-1}bar^{-1}$) (Galema and Hoiland, 1991):

The isentropic partial molar compressibility reflects the compressibility of the hydration layer surrounding a solute. These measurements are made by passing ultrasonic radiation through a solution and measurement of the frequency of radiation. Pure water has an isentropic partial molar compressibility of $+8.17 \times 10^{-4}$ $cm^3mol^{-1}bar^{-1}$. In the case of solute hydration, water molecules in the hydration sphere will form stronger bonds to each other and therefore the hydration layer will be less compressible than pure water.

4. Rotation Correlation Times (τ_c^h/τ_c°) (Uedaira *et al.*, 1989; Uedaira *et al.*, 1990):

The ratio of τ_c^h/τ_c° represents the rotational correlation times of water molecules in the solution over that of pure water. The rotational correlation times represents the average time (ps) the water molecules require for a molecular rotation. Spin lattice relaxation times of aqueous solutions of polyols were determined by natural abundance O^{17} NMR experiments (Uedaira *et al.*, 1989; Uedaira *et al.*, 1990). The higher the ratio of τ_c^h/τ_c° the less mobile the water molecules are in the hydration cosphere surrounding the solute.

5a. Dynamic Hydration Number (n_{DHN}) (Uedaira *et al.*, 1989, Uedaira *et al.*, 1990):

The n_{DHN} is defined as:

$$n_{DHN} = n_h \, (\tau_c^h/\tau_c^\circ - 1) \qquad (4)$$

where n_h is the coordination number (the maximum number of OH groups which may be engaged in hydrogen bonding with the surrounding water molecules) and τ_c^h/τ_c° is the rotational correlation times of water molecules in the polyol solution (τ_c^h) over that of pure water (τ_c°). The value of n_{DHN} is an indicator of the dynamic state of water molecules in the first hydration sphere of the solute (polyol) relative to the bulk water formed by the same number of water molecules as the cosphere. Values of the n_{DHN} for some of the polyols used in the present study are given in Appendix III.

5b. In order to more accurately describe the dynamic hydration state of water molecules surrounding a polyol, the coordination number in equation (4) was replaced with the hydration number estimated for a series of polyols by Galema and Hoiland (1991) on the basis of molar compressibility value (see section 3, above). The values for the dynamic hydration number derived this way are summarized in Appendix III. This parameter has been denoted n_{DHN}^*.

6) "Relative Mobility" (Slade and Levine, 1988)

Another parameter related to the rotational diffusion times of solutes, and thereby reflecting the inherent mobility of an aqueous polyol system, is the "relative mobility" as introduced by Slade and Levine (1988). The "relative mobility" of a polyol represents the distance (in units of temperature) between the experimental temperature (T) and the temperature at which the maximally freeze concentrated polyol solution undergoes the glass-rubber transition (T_g'). These parameters are normalized with respect to the difference of melting and glass transition temperatures of the dry polyol (T_m and T_g, respectively). The relative mobility is thus calculated by the equation:

$$\text{relative mobility} = (T - T_g')/(T_m - T_g) \qquad (5)$$

The calculated values for the relative mobility of a series of polyols are presented shown in Appendix IV.

4. RESULTS

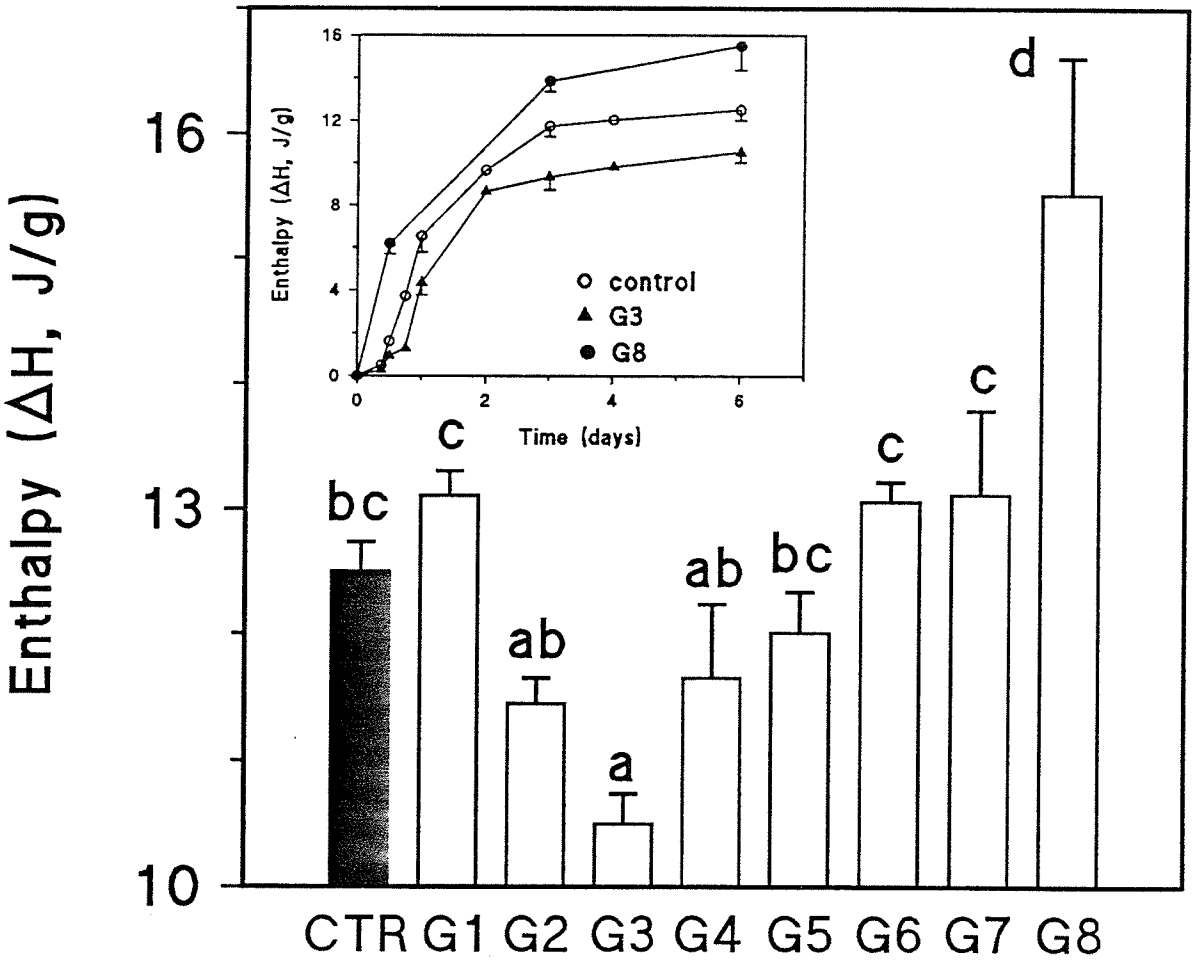
This chapter presents the experimental results of the effect of polyols on the retrogradation of concentrated starch gels. The first section (4.1) describes a study in which the effect of polyols on the retrogradation of concentrated waxy maize starch gels, as measured by DSC, was explored. The second section (4.2) describes a comparative study of selected polyols (from those employed in section 4.1) on the ageing of waxy maize, wheat, potato and pea starch gel networks by small strain dynamic rheometry and DSC. Throughout this chapter and the following discussion, the term "polyol" will refer to saccharides (e.g. glucose and sucrose), sugar alcohols (e.g., maltitol and lactitol), oligosaccharides (e.g., maltoheptaose) and linear polyhydroxy compounds (e.g. glycerol and glyceraldehyde). The interpretation and comparison of the results is discussed in the next chapter.

4.1 The Effect of Polyols on the Retrogradation of Concentrated Waxy Maize Starch Gels.

4.1.1 Oligosaccharides

The effect of added glucose oligomers on the retrogradation of waxy maize starch was studied by DSC (Figure 11). The addition of oligomers of glucose, G1-G2 and G4-G7, did not significantly retard the development of the retrogradation endotherm (ΔH) after six days storage at 6°C. In contrast, maltotriose significantly retarded starch retrogradation, while maltooctaose significantly promoted this process (Figure 11, inset).

Figure 11. Effect of added glucose oligosaccharides on the retrogradation endotherm (ΔH) of waxy maize starch gels stored at 6°C for 6 days. Inset: effect of maltotriose (G3) and maltooctaose (G8) on the retrogradation endotherm (ΔH) of waxy maize starch stored at 6°C. Oligomers were incorporated at a ratio of 1:0.5:1.5 w/w for starch:oligomer:water mixtures. The control gel had a weight ratio of starch:water 1:1.5. Means $\pm$ SD (n=3); bars followed by the same letter are not significantly different ($P \leq 0.05$).

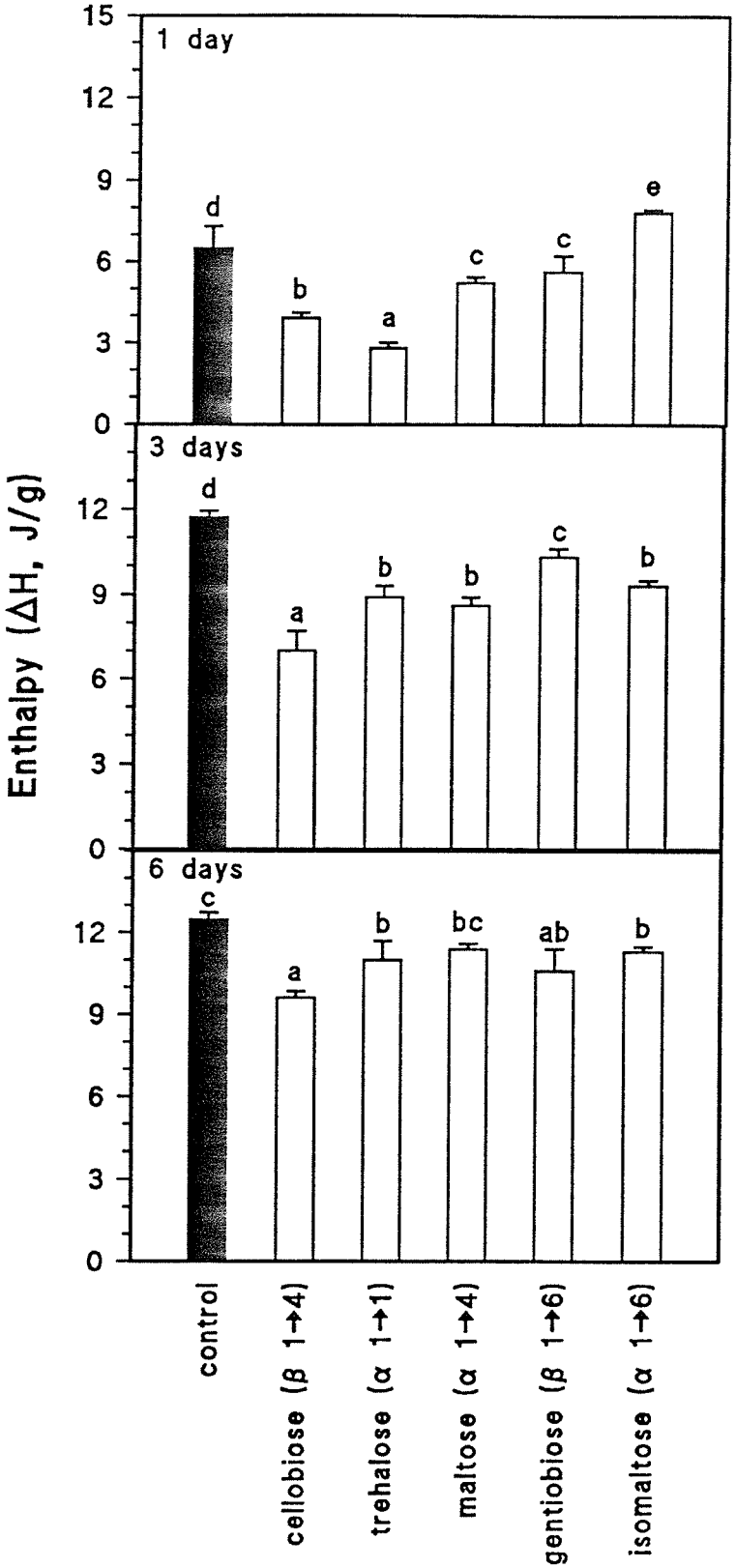

Enthalpy (ΔH, J/g)
16
13
10
CTR G1 G2 G3 G4 G5 G6 G7 G8
bc
c
ab
a
ab
bc
c
c
d
Enthalpy (ΔH, J/g)
16
12
8
4
0
0
2
4
6
Time (days)
control
G3
G8

The relative ranking of glucose oligomers for their effect on the development of the retrogradation endotherm (ΔH) of waxy maize starch gels was observed to vary depending on the designated period of storage (i.e. 9, 12, 18, 24, 96 or 144 hours) (Appendix V). At 12 hours of storage the oligomers G2-G5 did not significantly affect the retrogradation of waxy maize starch gels whereas G1, G6-G8 promoted the reorganization of branched starch molecules. In contrast, at 72 hours of storage, G2-G5 were found to significantly retard the retrogradation, G1, G6, G8 exerted no significant effect, and G8 promoted the process. Overall, it appears that oligomers of lower DP (1 to 3) retarded retrogradation with increasing DP, oligomers with a DP 4 to 7 exerted little effect, and maltooctaose (DP 8) promoted the development of "ordered" structures within the ageing waxy maize starch gel network.

4.1.2 Disaccharides

The effect of glucose-based disaccharides with different glucosidic linkages on the reorganization of amylopectin short DP chains in ageing waxy maize starch gels (40%, w/w), upon storage, is shown in Figure 12. Cellobiose (β 1→4) and trehalose (α 1→1), after storage for 24 hours, were found to retard retrogradation of amylopectin to a significantly greater extent than maltose (α 1→4), gentiobiose (β 1→6) and isomaltose (α 1→6). The differences in the effect of these disaccharides on retrogradation of amylopectin diminished over longer storage periods. Generally, the addition of glucose disaccharides inhibited the retrogradation of waxy maize starch gels in the following order: cellobiose > trehalose > gentiobiose > maltose > isomaltose > water alone (Appendix VI).

Figure 12. Effect of glucose disaccharides on the retrogradation endotherm (ΔH) of waxy maize starch gels stored at 6°C. Sugars were incorporated at a ratio of 1:0.5:1.5 w/w for starch:sugar:water mixtures. The control gel had a weight ratio of starch:water 1:1.5. Means $\pm$ SD (n=3); bars followed by the same letter are not significantly different ($P \leq 0.05$).


Enthalpy (ΔH, J/g)
1 day
3 days
6 days
control
cellobiose (β 1→4)
trehalose (α 1→1)
maltose (α 1→4)
gentiobiose (β 1→6)
isomaltose (α 1→6)

4.1.3 Sugars and their Alcohol Derivatives

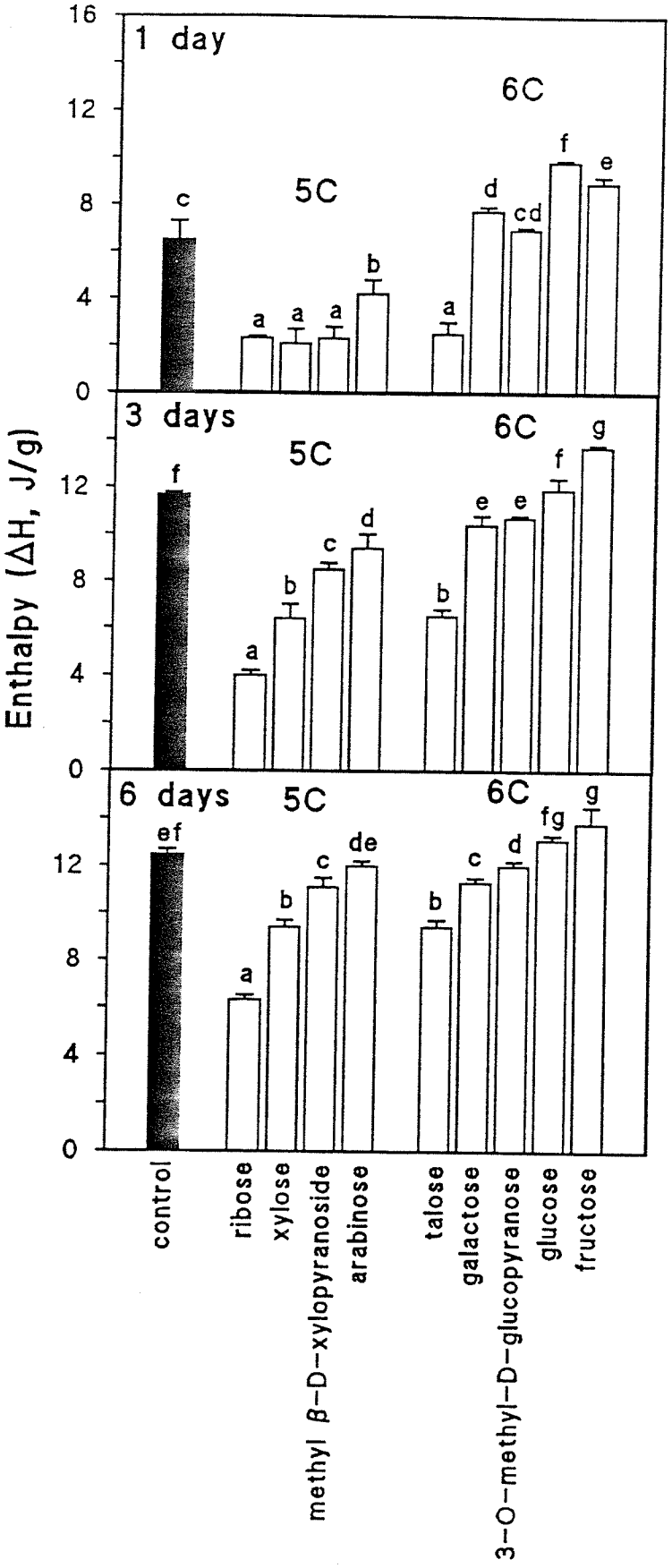
A comparison between the effect of sugars and their alcohol derivatives on the retrogradation of waxy maize starch gels was also made (Figure 13). Sorbitol was found to retard the development of the retrogradation endotherm (ΔH) more than glucose upon storage for 1, 3 and 6 days. However, there were no significant differences in the development of retrogradation endotherm (ΔH) between the disaccharides, maltose and lactose, and their respective sugar alcohols. Finally the addition of glyceraldehyde completely inhibited the formation of "ordered" structures in the ageing waxy maize starch gel networks over the storage period examined. In fact, there was no detectable retrogradation endotherm developing upon storage of starch/glyceraldehyde/water gels for 30 days at 6°C. In contrast, glycerol was not found to exert such a strong anti-retrogradation effect. These observations were consistent at all storage times in which measurements were taken (ie. 9, 12, 18, 48 and 96 hours) (Appendix VII).

4.1.4 Pentoses and Hexoses

The effect of added pentoses and hexoses on the retrogradation of waxy maize starch gels was also studied by DSC (Figure 14). Pentoses were shown to significantly retard the formation of "ordered" structures in ageing waxy maize starch gels, with ribose exhibiting the strongest effect. Xylose, methyl β-D xylopyranoside and arabinose were less effective in reducing the retrogradation rate than ribose. Hexoses, such as talose, galactose, 3-O-methyl-D-glucopyranose significantly retarded the retrogradation of starch, whereas fructose promoted the reorganization of amylopectin short DP chains. Addition of glucose first promoted retrogradation at short storage periods, but upon longer storage ($>$ 2 days; Appendix VIII) it did not significantly affect the development of the retrogradation endotherm (ΔH). Generally, it was observed that the anti-

Figure 13. A comparison between sugars and sugar alcohol derivatives on their effect on the retrogradation endotherm (ΔH) of waxy maize starch gels stored at 6°C. Polyols were incorporated at a ratio of 1:0.5:1.5 w/w for starch:polyol:water mixtures. The control gel had a weight ratio of starch:water 1:1.5. Means $\pm$ SD (n=3); bars followed by the same letter are not significantly different ($P \leq 0.05$).

Figure 14. Effect of added pentoses and hexoses on the retrogradation endotherm (ΔH) of waxy maize starch gels stored at 6°C. Polyols were incorporated at a ratio of 1:0.5:1.5 w/w for starch:polyol:water mixtures. The control gel had a weight ratio of starch:water 1:1.5. Means $\pm$ SD (n=3); bars followed by the same letter are not significantly different ($P \leq 0.05$).


1 day
3 days
6 days
5C
6C
Enthalpy (ΔH, J/g)
0
4
8
12
16
control
ribose
xylose
methyl β-D-xylopyranoside
arabinose
talose
galactose
3-O-methyl-D-glucopyranose
glucose
fructose

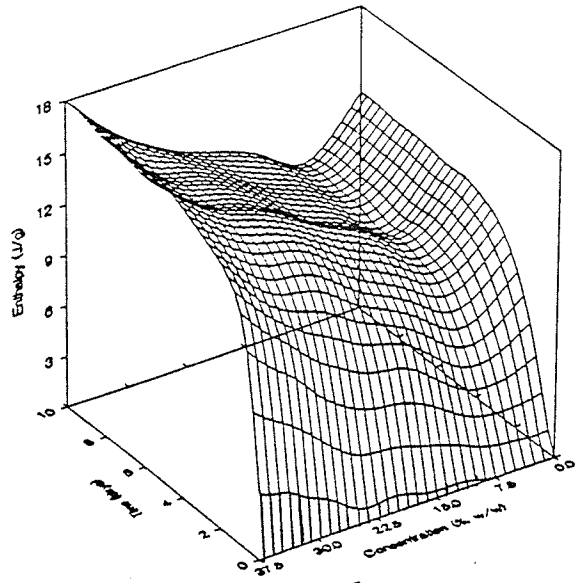
retrogradation effect of pentoses was more pronounced than that of hexoses. However, arabinose appeared to behave more like a hexose and talose and galactose appeared to behave more like pentoses in their anti-retrogradation behaviour. Generally, the addition of monosaccharides (pentoses and hexoses) inhibited chain ordering in waxy maize starch gels, as probed by DSC, in the following order: ribose > xylose, talose > methyl β-D-xylopyranoside > arabinose > galactose > 3-O-methyl-D-glucopyranose > water alone, glucose > fructose (Appendix VIII).

4.1.6 Polyol Concentration

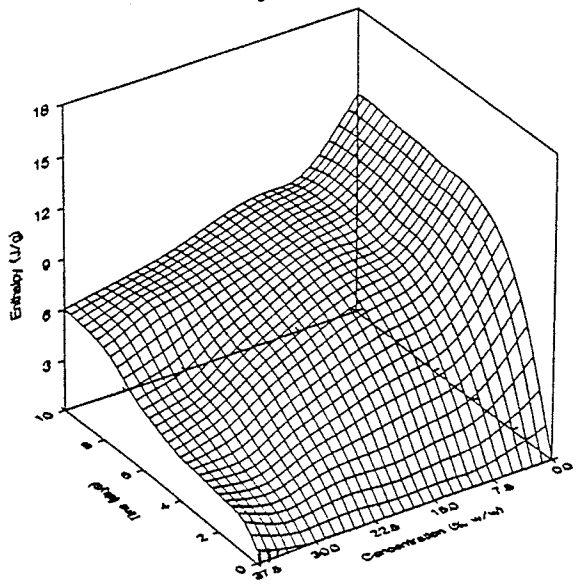
The effect of sugars on retrogradation of starch is highly dependent on the concentration used (Carins *et al.*, 1991a,b). In this study, the effect of varying concentration of fructose, ribose and sucrose on the development of the retrogradation endotherm (ΔH) was studied (Figure 15). The development of "ordered" structures in waxy maize starch gels, as measured by DSC, increased rapidly during the first few days and then slowed down reaching to a plateau value. The addition of fructose in waxy maize starch gels at low concentration (< 7.5%) resulted in decreasing retrogradation. However, higher concentrations of fructose (>7.5%) accelerated the formation of "ordered" structures within the ageing starch gel networks. Ribose exerted the opposite effect; with increasing concentration of this sugar, the development of retrogradation endotherm (ΔH) decreased. For sucrose, at concentrations below 7.5% (w/w), there was a reduction in the retrogradation endotherm compared to the control gels (starch:water 1:1.5 w/w). Above this concentration, the magnitude of the retrogradation endotherm was found to be independent of the weight fraction of sucrose in the composite gels.

Figure 15. The effect of sugar concentration on the retrogradation endotherm (ΔH) of waxy maize starch gels stored at 6°C. Sugars were incorporated at a ratio of 1:X:1.5 (w/w) for starch:sugar:water mixtures (X varied between 0.1 and 0.9). The concentration of sugar solutions were expressed as %, w/w. The surface-response plots were generated form data corresponding to 5 different sugar concentrations (0, 6.3, 16.7, 25.0, 31.8 and 37.5% w/w) and 8 storage times (12h, 1, 2, 3, 4, 6, 8 and 10 days).

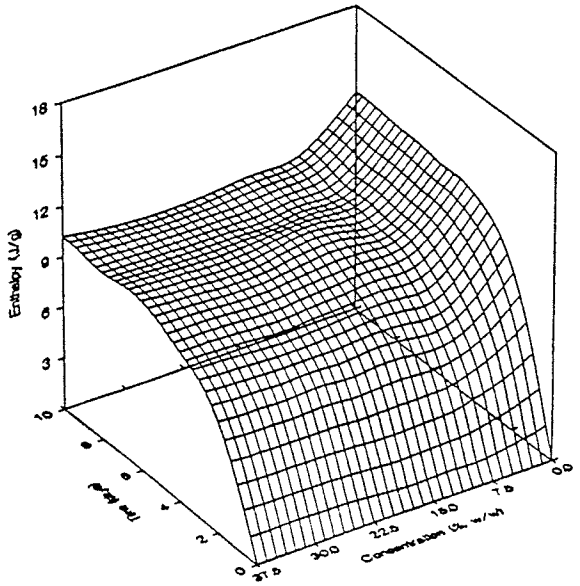
Fructose



Ribose



Sucrose



4.1.7 Relationships Between the Effect of Polyols on the Retrogradation of Waxy Maize Starch Gels and the Hydration Characteristics and Physicochemical Properties of Aqueous Polyol Solutions.

The viscosity of various polyol solutions (25% w/w) at 8°C were determined with a Ubbelohde viscometer (Appendix II). Among the polyol solutions measured, ribose had the lowest viscosity, whereas maltotriose and trehalose were observed to have the highest viscosity. The relationship between viscosity of polyol solutions and the effect the polyols had on the retrogradation endotherm (ΔH) after 12 hours storage at 6°C is shown in Figure 16. With the exception of pentoses, glyceraldehyde and glycerol, as the viscosity of the crystallization medium increases, the development of the retrogradation endotherm decreases. A highly significant correlation ($p \leq 0.001$) between the viscosity of the polyol solutions and their effects on retrogradation was found. The correlation coefficient was observed to decrease as the period of storage was increased from 12 hours to 6 days (Table 1).

Galema and Hoiland (1991) studied the hydration of carbohydrates in aqueous solutions and their effect on water structure using ultrasound measurements. These authors calculated the hydration numbers (n_h) and partial molar compressibilities of various polyols and related these parameters to their effect on water structure. In view of their findings, a possible relationship between the compatibility of the polyols with the structure of water and their effects on retrogradation of waxy maize starch gels was explored. The relationships between the effect of polyols on the development of the retrogradation endotherm (ΔH) at various storage periods and both n_h and isentropic molar compressibilities are summarized in Table 1. Highly significant correlations ($p \leq 0.01$) were observed for the monosaccharides, taken as a separate group, for both parameters (Table 1, Figures 17 and 18). The highest correlation coefficients ($r = 0.91$ and 0.90) were achieved for the 2-days stored samples (Figures 17 and 18).

Figure 16. Relationship between the effect of added polyols on the retrogradation endotherm (ΔH) of waxy maize starch gels (12 hours, 6°C) and absolute viscosity (8°C). Polyols were incorporated at a ratio of 1:0.5:1.5 (w/w) for starch:polyol:water mixtures. Viscosity of sugars solutions were measured at a ratio of 0.5:1.5 (w/w) polyol:water. (gly=glyceraldehyde; glyol=glycerol; rib-ribose; xyl=xylose; ara=arabinose; gal=galactose; fru=fructose; G1=glucose; G2=maltose; G3=maltotriose; suc=sucrose; lac=lactose; cel=cellobiose; tre=trehalose). Glyceraldehyde, glycerol, arabinose, xylose, ribose were not used in the regression analysis (O symbols).

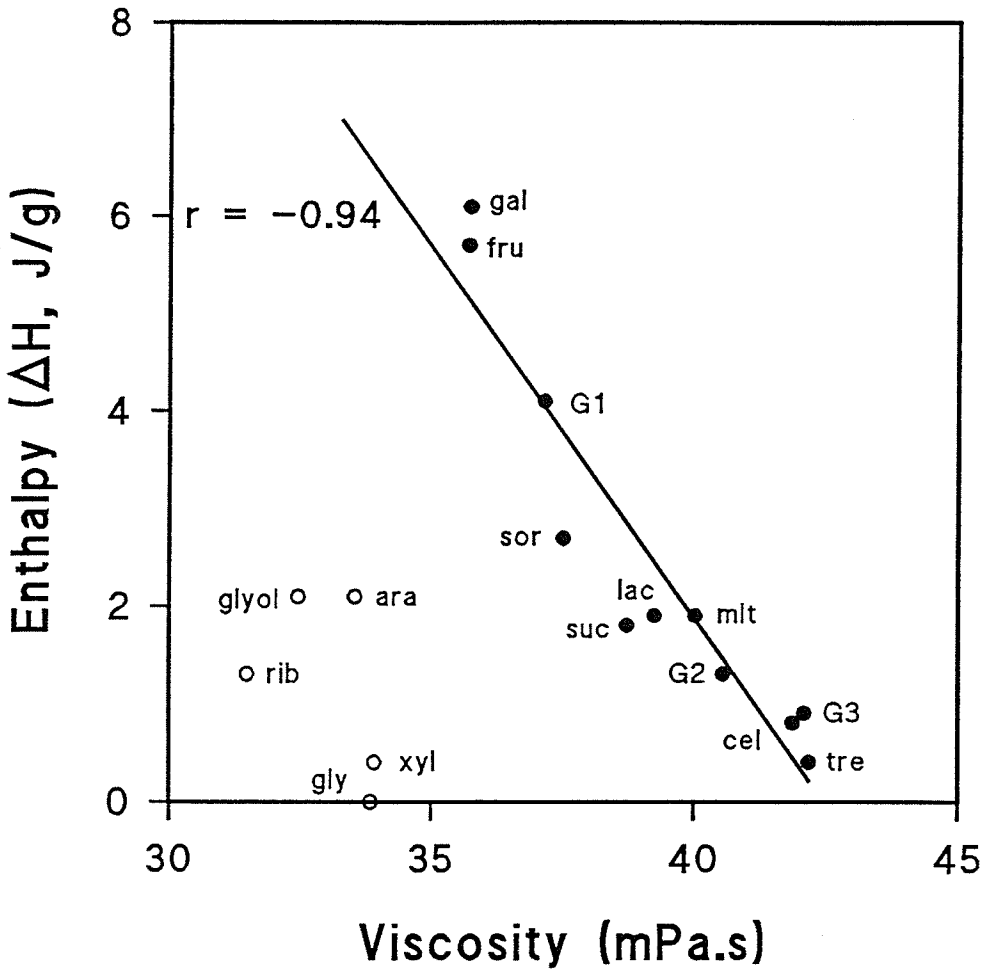

8
6
4
2
0
r = −0.94
gal
fru
G1
sor
glyol
ara
lac
suc
mlt
rib
G2
G3
cel
tre
gly
xyl
30
35
40
45
Enthalpy (ΔH, J/g)
Viscosity (mPa.s)

Table 1. Relationships between the effect of polyols on the retrogradation of waxy maize starch gels (ΔH at specified storage times) and the selected physicochemical properties of aqueous polyol solutions.

Parameter	Reference	D.F.	Correlation Coefficient (r)				
			12 hours	1 day	2 days	3 days	5 days
Polyol Viscosity (mPa.s) (25 % w/w solutions)	determined in section 3.2.4	10	-0.94^{++++}	-0.83^{++++}	-0.80^{+++}	-0.72^{+++}	-0.61^{++}
Hydration Number (n_h)	Galema and Hoiland, 1991						
monosaccharides		8	0.85^{+++}	0.90^{++++}	0.91^{++++}	0.89^{++++}	0.80^{+++}
disaccharides		5	-0.05	0.32	-0.03	0.66	0.22
both		14	-0.18	-0.02	-0.12	0.02	0.05
Isentropic Partial Molar Compressibilities ($cm^3mol^{-1}bar^{-1}$)	Galema and Hoiland, 1991						
monosaccharides		6	-0.82^{++}	-0.80^{++}	-0.90^{+++}	-0.89^{+++}	-0.84^{+++}
disaccharides		4	-0.39	-0.09	0.64	-0.62	-0.22
both		11	-0.16	-0.19	-0.17	-0.37	-0.11
Rotational Correlation Times (τ_c^h/τ_c^o)	Uedaira *et al.*, 1989 Uedaira *et al.*, 1990						
monosaccharides		5	0.60	0.80^{++}	0.80^{++}	0.80^{++}	0.81^{++}
di/trisaccharides		3	-0.01	0.62	-0.65	-0.10	0.68
both		9	0.49	0.74^{+++}	0.69^{++}	0.76^{+++}	0.80^{++}
Dynamic Hydration Number (n_{DHN})	Uedaira *et al.*, 1989						
monosaccharides	Uedaira *et al.*, 1990	5	0.60	0.80^{++}	0.80^{++}	0.80^{++}	0.81^{++}
di/trisaccharides		3	0.13	0.41	0.79	0.68	0.06
both		9	-0.32	-0.09	0.05	0.12	0.14
Dynamic Hydration Number (n_{DHN}^*)	Determined in section 3.3						
monosaccharides		5	0.82^{++}	0.94^{+++}	0.93^{+++}	0.91^{+++}	0.87^{++}
di/trisaccharides		2	-0.69	0.35	-0.94^{+}	0.95^{++}	0.99^{+++}
both		8	-0.15	0.07	0.06	0.24	0.32
Relative Mobility $(T_{exp}-T_g')/(T_m-T_g)$	Slade and Levine, 1988	13	0.64^{++}	0.56^{++}	0.62^{++}	0.64^{++}	0.52^{++}

$^{+}$ significant at $p \leq 0.1$; $^{++}$ significant at $p \leq 0.05$; $^{+++}$ significant at $p \leq 0.01$; $^{++++}$ significant at $p \leq 0.001$

Figure 17. Relationship between the effect of added polyols on the retrogradation endotherm (ΔH) of waxy maize starch gels (2 days, 6°C) and their hydration number as determined by Galema and Hoiland (1991). Polyols were incorporated at a ratio of 1:0.5:1.5 (w/w) for starch:polyol:water mixtures. (rib=ribose; xyl=xylose; ara=arabinose; me-β-xylp=methyl β-D-xylopyranoside; 3-o-me-glu=3-O-methyl D-glucopyranose; tal=talose; gal=galactose; fru=fructose; G1=glucose; G2=maltose; suc=sucrose; lac=lactose; gen=gentiobiose; tre=trehalose; cel=cellobiose).

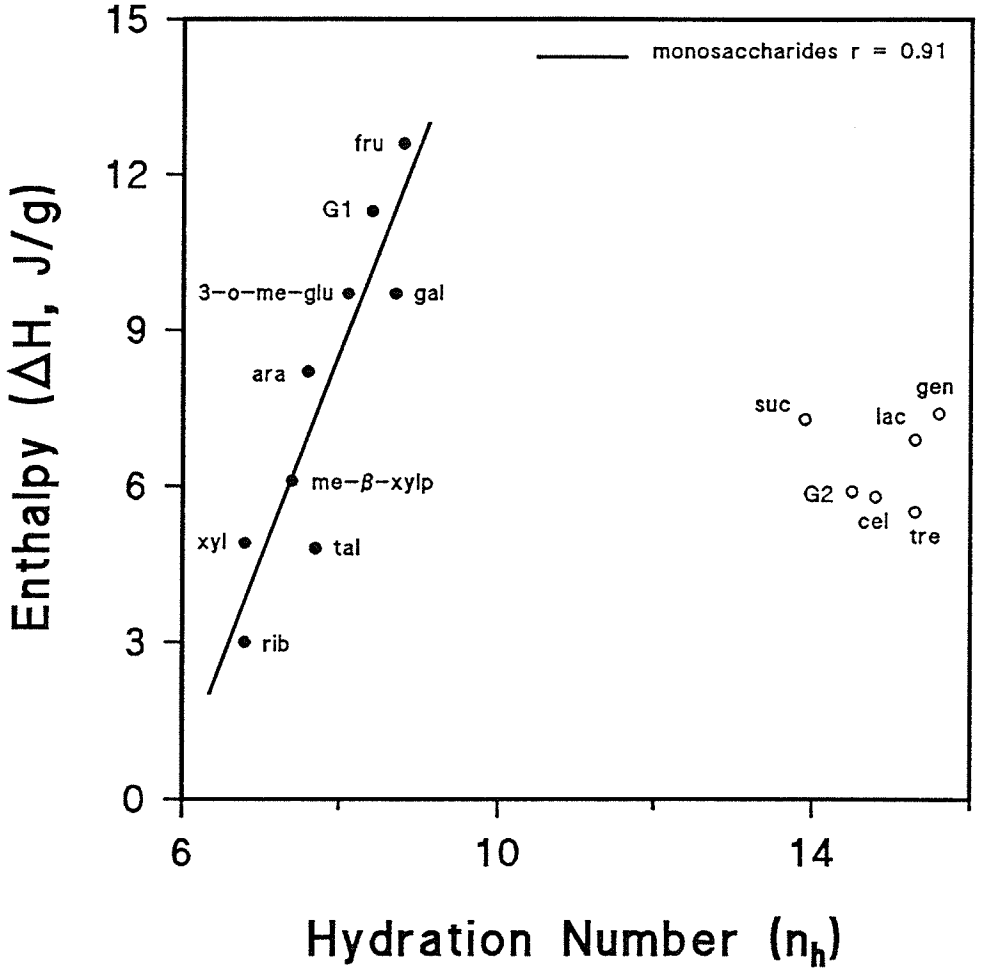
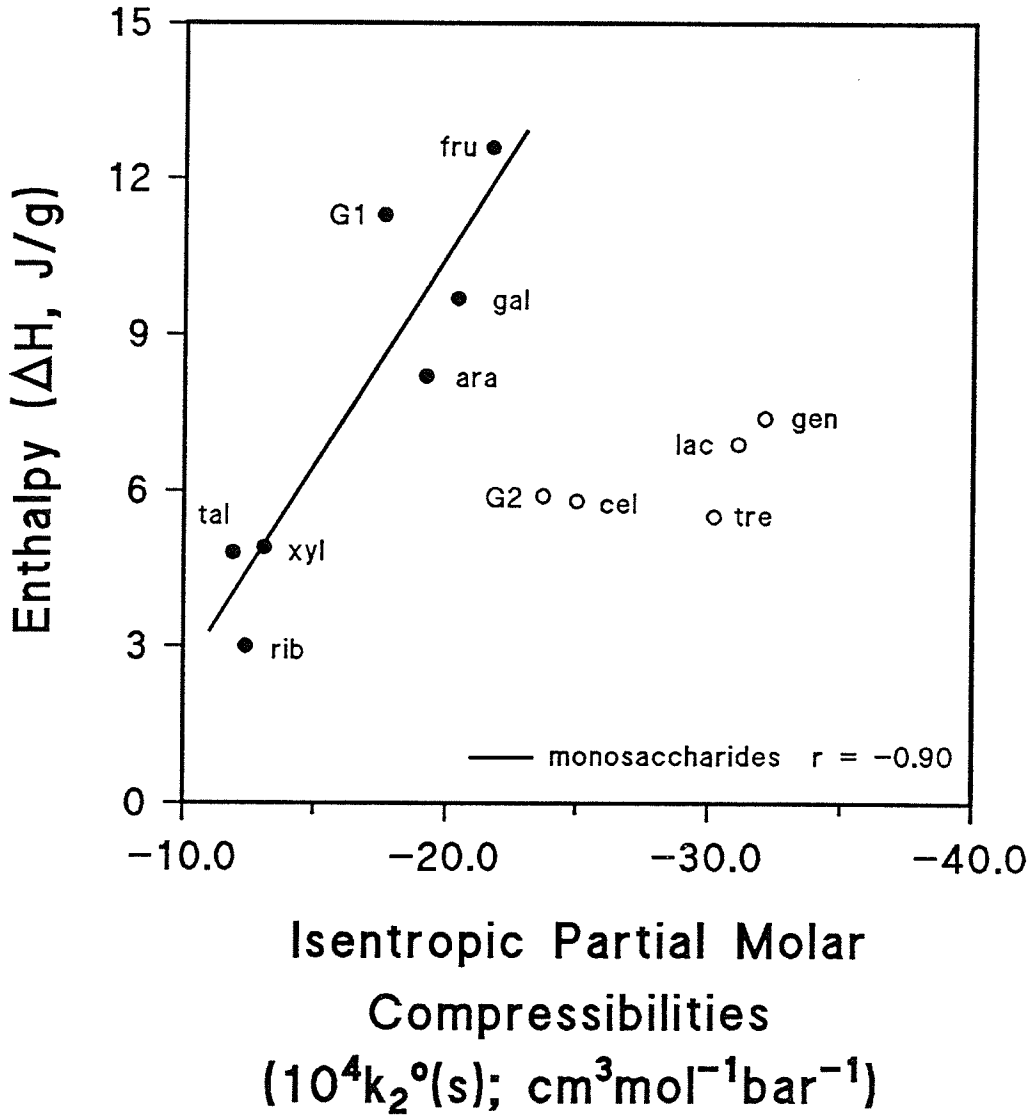

monosaccharides r = 0.91
fru
G1
3-o-me-glu
gal
ara
me-β-xylp
xyl
tal
rib
suc
gen
lac
G2
cel
tre
15
12
9
6
3
0
6
10
14
Enthalpy (ΔH, J/g)
Hydration Number (nh)

Figure 18. Relationship between the effect of added polyols on the retrogradation endotherm (ΔH) of waxy maize starch gels (2 days, 6°C) and their isentropic partial molar compressibilities as determined by Galema and Hoiland (1991). Polyols were incorporated at a ratio of 1:0.5:1.5 (w/w) for starch:polyol:water mixtures. (rib=ribose; xyl=xylose; ara=arabinose; tal=talose; gal=galactose; fru=fructose; G1=glucose; G2=maltose; suc=sucrose; lac=lactose; gen=gentiobiose; tre=trehalose; cel=cellobiose).


15
12
9
6
3
0
−10.0
−20.0
−30.0
−40.0
Enthalpy (ΔH, J/g)
fru
G1
gal
ara
gen
lac
G2
cel
tre
tal
xyl
rib
monosaccharides r = −0.90
Isentropic Partial Molar Compressibilities
(10⁴k₂°(s); cm³mol⁻¹bar⁻¹)

Uedaira *et al.* (1989, 1990) have reported on the hydration properties of a series of polyols. They have related the structure of polyols, with respect to the mean number of equatorial OH groups (n(e-OH)), to the hydration properties of these solutes, as described by their rotational correlation times (τ_c^h/τ_c^o) and dynamic hydration numbers (n_{DHN}). The relationships between the effect of polyols on the development of retrogradation endotherm (ΔH) and the respective rotational correlation times and n_{DHN} are summarized in Table 1. Significant relationships ($p \leq 0.05$) were observed between the rotational correlation times and the retrogradation endotherm (ΔH) for the 1-day to 6-day stored samples. The correlation coefficient was greatly improved when only data for the monosaccharides were used (Figure 19). Significant relationships ($p \leq 0.05$) were also found between the effect of monosaccharides on the development of the retrogradation endotherm (ΔH), stored for 1 up to 6 days, and their n_{DHN} (Table 1). In contrast, relatively poor relationships were shown when only di/trisaccharides or all the polyols (mono/di/trisaccharides) were used for these plots).

The coordination number (n_h) used in the calculation of n_{DHN} by Uedaira *et al.* (1989, 1990), reflects the maximal number of OH groups of the polyol which may be engaged in hydrogen bonding with water. Another description of hydration number (denoted as n_h) was also given by Galema and Hoiland (1991), based on molar compressibility measurements. Incorporating these hydration numbers into equation 4 (section 3.3), slightly different values of n_{DHN} for the polyols were derived (denoted n_{DHN}^*). As with the other hydration parameters, significant relationships ($p \leq 0.01$) were found between ΔH and n_{DHN}^* (Table 1, Figure 20). In fact, higher correlation coefficients were obtained using the n_{DHN}^* numbers than the dynamic hydration numbers reported by Uedaira *et al.* (1989, 1990).

Figure 19. Relationship between the effect of added polyols on the retrogradation endotherm (ΔH) of waxy maize starch gels (1 day, 6°C) and their rotational correlation times (τ_c^h/τ_c^o) as determined by Uedaira *et al.*(1989) and Uedaira *et al.* (1990). Polyols were incorporated at a ratio of 1:0.5:1.5 (w/w) for starch:polyol:water mixtures. (rib= ribose; xyl=xylose; ara=arabinose; gal=galactose; fru=fructose; G1=glucose; G2=maltose; G3=maltotriose; suc=sucrose; tre=trehalose; cel=cellobiose).

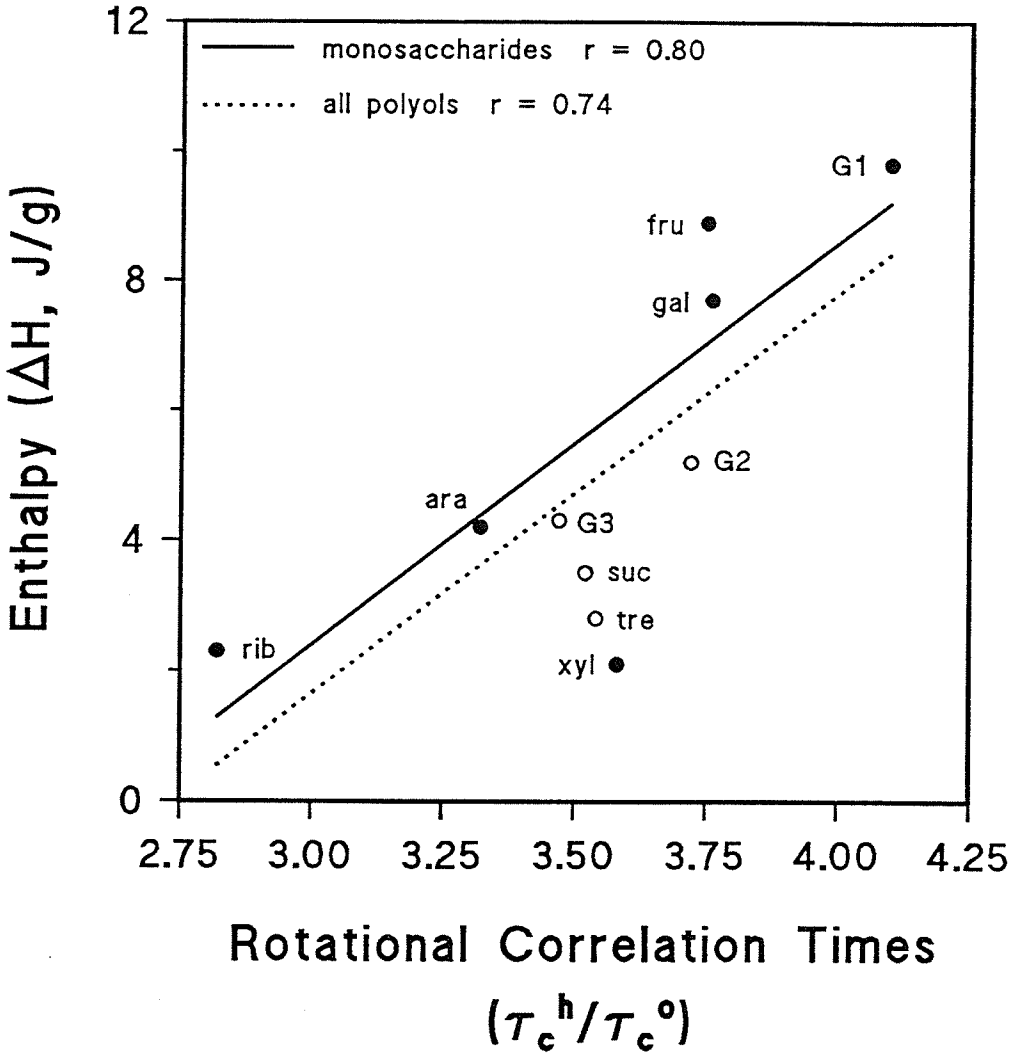
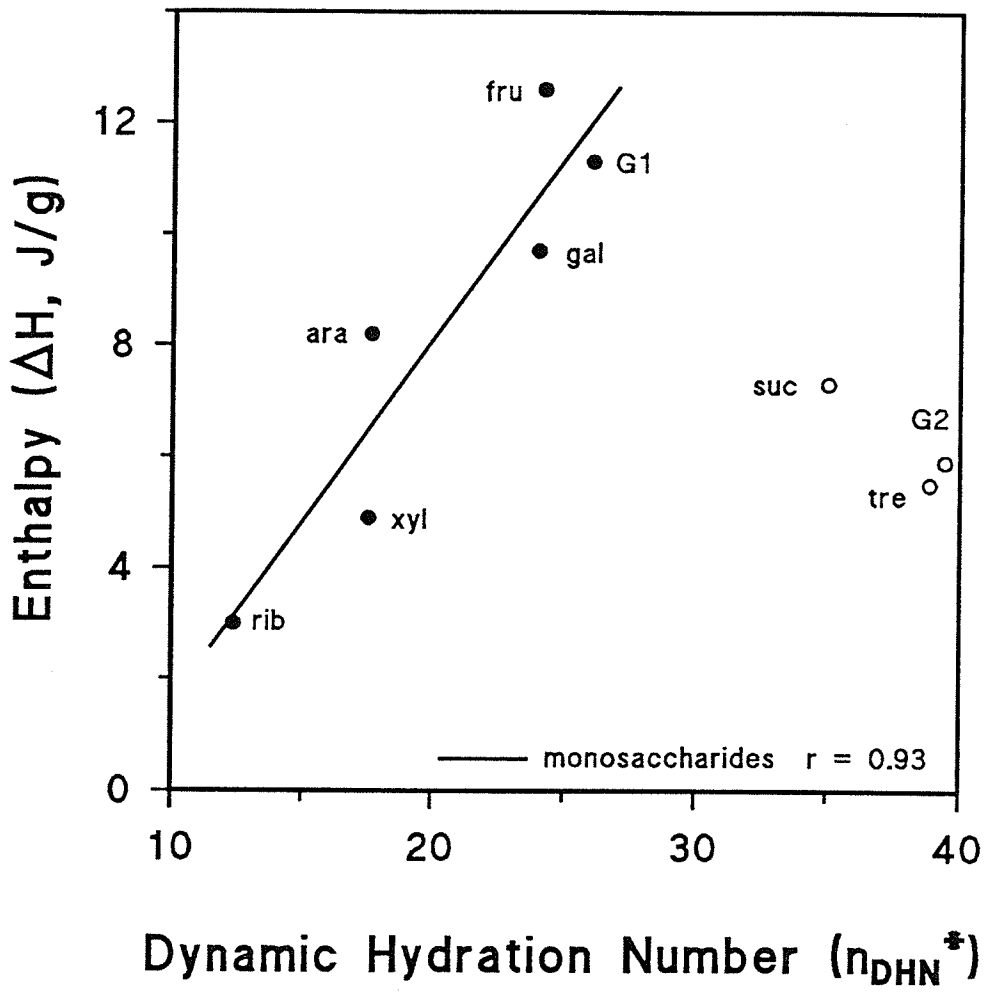

12
8
4
0
monosaccharides r = 0.80
all polyols r = 0.74
G1
fru
gal
G2
ara
G3
suc
tre
rib
xyl
2.75
3.00
3.25
3.50
3.75
4.00
4.25
Enthalpy (ΔH, J/g)
Rotational Correlation Times
(τ_c^h/τ_c^o)

Figure 20. Relationship between the effect of added polyols on the retrogradation endotherm (ΔH) of waxy maize starch gels (2 days, 6°C) and their dynamic hydration number (n_{DHN}^{*}) as determined in section 3.3 (Appendix III). Polyols were incorporated at a ratio of 1:0.5:1.5 (w/w) for starch:polyol:water mixtures. (rib=ribose; xyl=xylose; ara=arabinose; gal=galactose; fru=fructose; G1=glucose; G2=maltose; suc=sucrose; tre=trehalose).


Enthalpy (ΔH, J/g)
12
8
4
0
10
20
30
40
Dynamic Hydration Number (n_{DHN}^*)
fru
G1
gal
ara
suc
G2
tre
xyl
rib
monosaccharides r = 0.93

Another molecular approach to study the mobility and dynamics of aqueous polyol solutions has been proposed by Slade and Levine (1988) and is based on calorimetric measurements. These investigators reported a strong negative relationship between rotational diffusion time (τ_{rot}) for concentrated aqueous sugar solutions and a "relative mobility" parameter calculated from the ratio $(T_{exp} - T_g')/(T_m - T_g)$, as described in section 3.3. The relative mobility values for the series of polyols used in the present study were calculated from data published by Slade and Levine (1988) and are summarized in Appendix IV. A possible relationship between relative mobility of the polyols and their effect on retrogradation was also explored. Indeed, significant correlation coefficients ($p \leq 0.05$) were shown between these two parameters, at all storage periods, when the entire group of polyols was considered. A typical relationship between ΔH and relative mobility is shown in Figure 21. It is clear from this figure, however, that despite the relatively high r values obtained, there is quite a substantial scattering of the data. This suggests that a polyol-specific effect might be also important in determining the rate of retrogradation for some of these solutes.

4.2 A Comparative Study of the Effect of Polyols on the Thermal and Mechanical Properties of Concentrated Waxy Maize, Wheat, Potato and Pea Starch Gels.

4.2.1 Composition and Physical Properties of Starches

The composition and properties of granular starches used in this study are given in Table 2. A broad range of physical and chemical properties was found among these starches. Starch content assays gave values greater than 96.4% in all cases. With respect to factors known to affect starch rheology, pea starch was observed to have the highest amylose content of 32.5%

Figure 21. Relationship between the effect of added polyols on the retrogradation endotherm (ΔH) of waxy maize starch gels (1 day, 6°C) and their relative mobility as determined by Slade and Levine (1988). Polyols were incorporated at a ratio of 1:0.5:1.5 (w/w) for starch:polyol:water mixtures. (rib=robose; xyl =xylose; gal=galactose; fru=fructose; tal=talose; sor=sorbitol; glyl=glycerol; G1=glucose; G2=maltose; G3=maltotriose; suc=sucrose; tre=trehalose; cel=cellobiose).

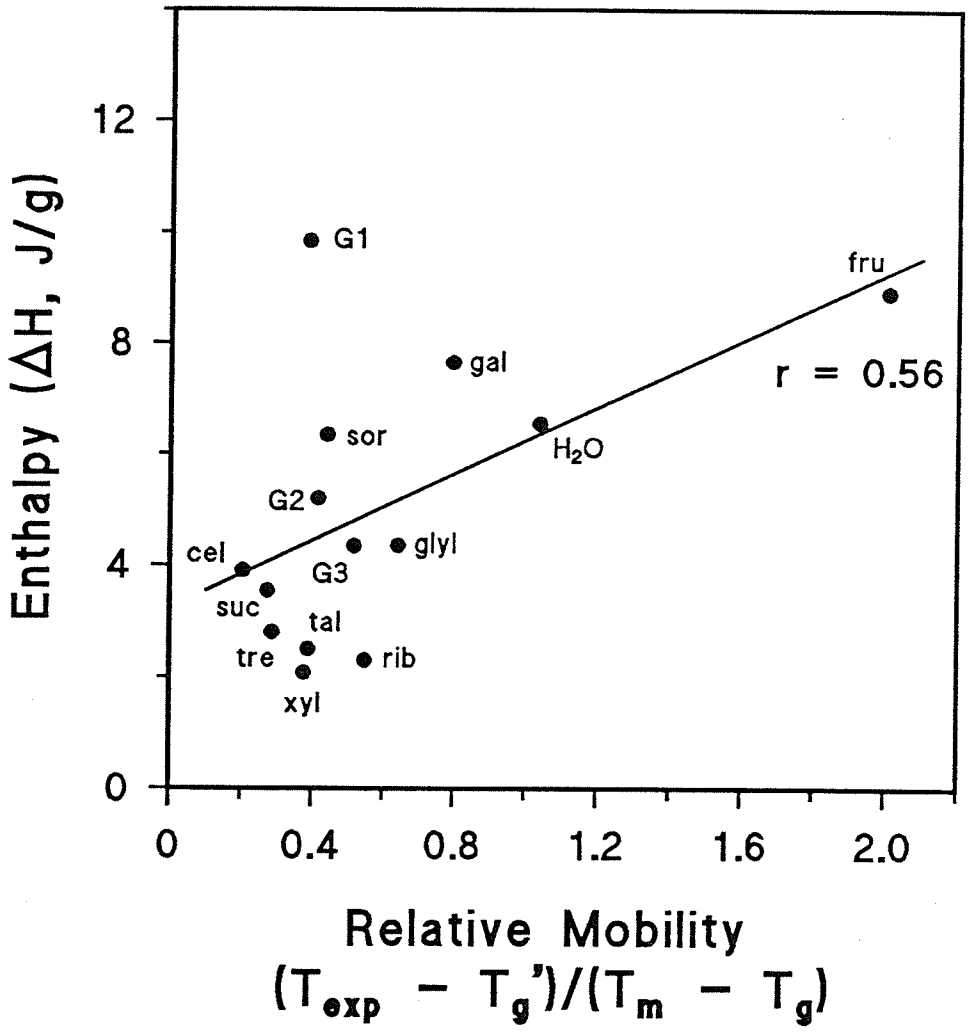

Enthalpy (ΔH, J/g)
12
8
4
0
0
0.4
0.8
1.2
1.6
2.0
Relative Mobility
(T_exp − T_g')/(T_m − T_g)
r = 0.56
G1
fru
gal
sor
H2O
G2
glyl
cel
G3
suc
tal
tre
rib
xyl

Table 2. Composition and physical properties of starches

	Starch Content (%)[1]	Amylose Content (%)[2]	Lipids (%)[3]	DSC Characteristics[4]			
				Starch Gelatinization		Amylose-Lipid Complex	
				T_p (°C)	ΔH (J/g)	T_p (°C)	ΔH (J/g)
Waxy Maize	98.5±0.2	< 2.0	0.50	70.0±0.1	14.1±0.2		
Wheat	98.9±0.1	21.1±0.4	0.68	59.2±0.1	10.1±0.3	105.0±0.3	1.8±0.1
Potato	98.1±0.2	25.0±0.6	0.01	57.5±0.1	15.5±0.3		
Pea	96.4±0.1	32.5±0.6	0.01	60.4±0.2	11.1±0.1		

[1] Means ± SD (n=3).
[2] Based on starch content.
[3] SEM < 5%
[4] DSC data on 40% (w/w) starch dispersions.

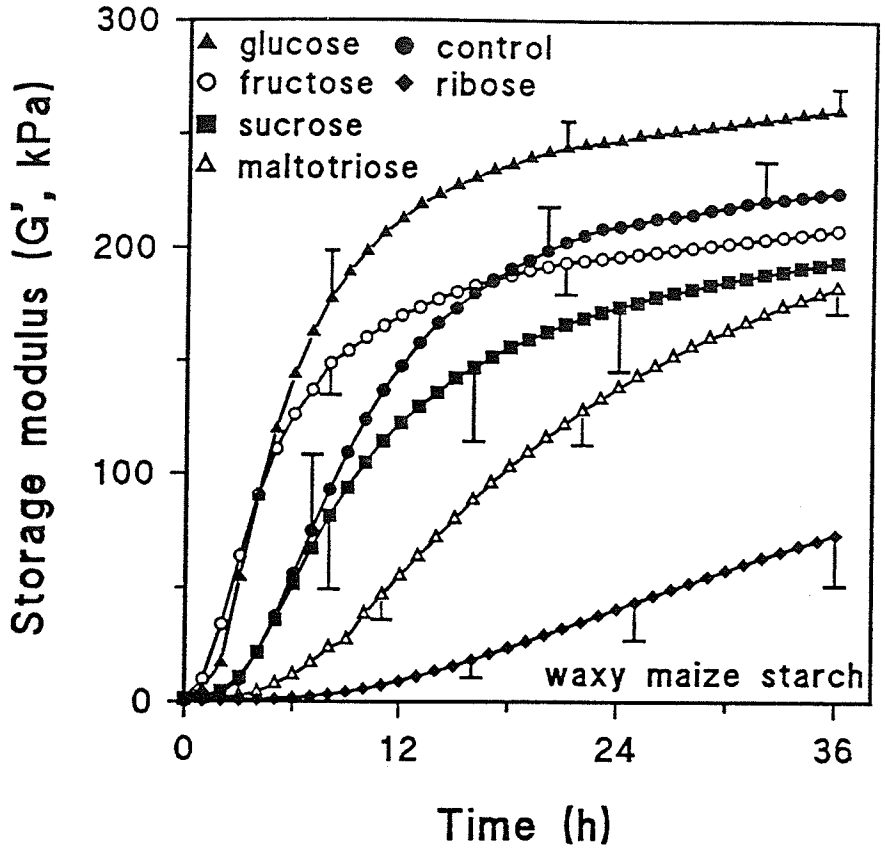
followed by potato and wheat starch. The amylose content of waxy maize starch was determined to be less than 2%. The lipid content of waxy maize starch and wheat starches was 0.50 and 0.68%, respectively. Only traces of granular lipids were found in both pea and potato starch. All starches exhibited a relatively high gelatinization enthalpy, with potato starches having the highest enthalpy value (15.5 J/g) and wheat starch the lowest (10.1 J/g). This implies that no major structural alteration occurred in the granules of these starches during isolation. Wheat, potato and pea starch were found to have similar gelatinization temperatures ($T_p \sim 60°C$) whereas waxy maize starch (40% w/w) exhibited a much higher value for T_p (70.0°C). The thermal curve of wheat starch exhibited an additional melting endotherm at $T_p = 105.0°C$ associated with the melting of amylose-lipid complexes.

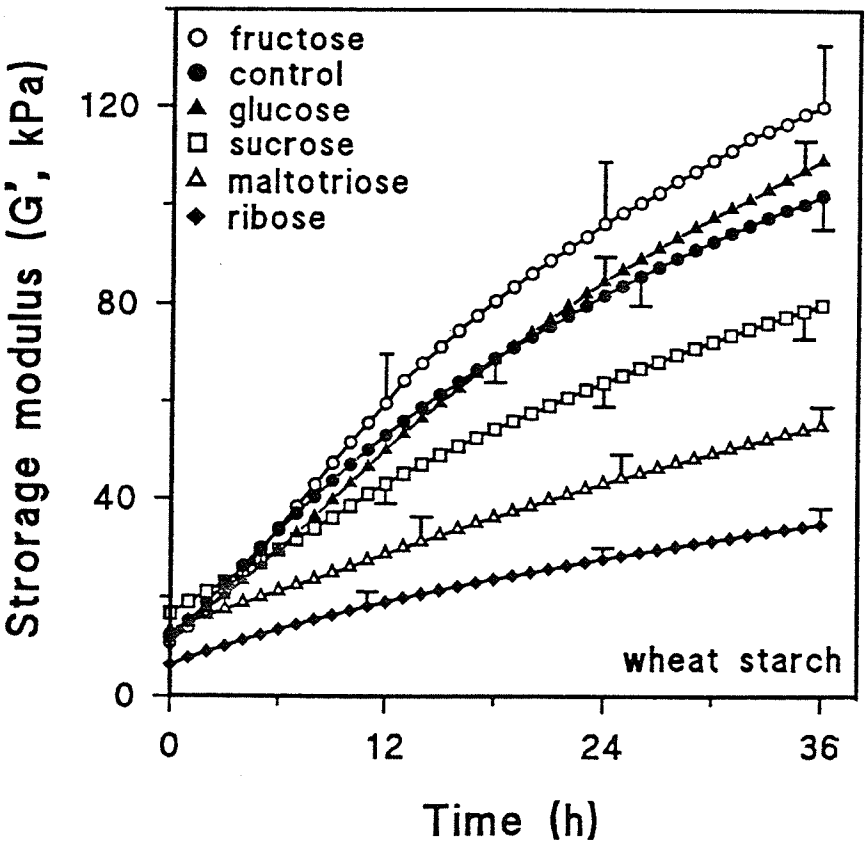
4.2.2 Dynamic Rheological Studies

Dynamic rheological testing is a particularly useful physical tool to monitor the kinetics of network development provided that measurements are taken within the linear viscoelastic regime of the starch gel specimen. Under these conditions, rheological measurements are taken without disturbing any molecular structures which may form during the gelation or retrogradation of starch gel networks. The present section describes the influence of polyols (fructose, glucose, sucrose, maltotriose and ribose) on the viscoelastic properties of concentrated waxy maize, wheat, potato and pea starches (40% w/w) upon storage at 8°C.

The effect of added polyols on the time dependent mechanical changes of concentrated waxy maize starch gels is shown in Figure 22 (Top). The overall kinetics of the gelation process, generally followed a sigmoidal G´-time curve. The rigidity of the pure starch gel reached almost plateau values after 24 hours of storage. The addition of polyols dramatically influenced

Figure 22. Effect of added polyols on the storage modulus (G´) of ageing waxy maize (TOP) and wheat (BOTTOM) starch gels stored at 8°C. Dynamic rheological measurements were made at 0.2 Hz and strain < 2%. Polyols were incorporated at a ratio of 1:0.5:1.5 (w/w) for starch:polyol:starch:water mixtures.


300
200
100
0
Storage modulus (G', kPa)
glucose
control
fructose
ribose
sucrose
maltotriose
waxy maize starch
0
12
24
36
Time (h)

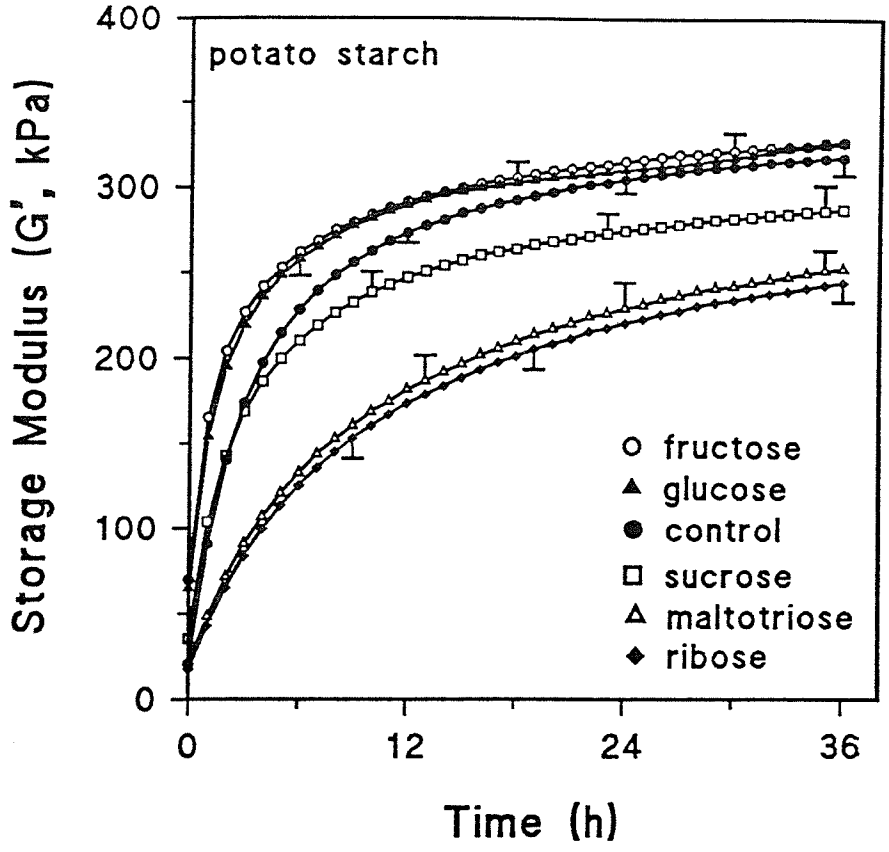

120
80
40
0
Strorage modulus (G', kPa)
fructose
control
glucose
sucrose
maltotriose
ribose
wheat starch
0
12
24
36
Time (h)

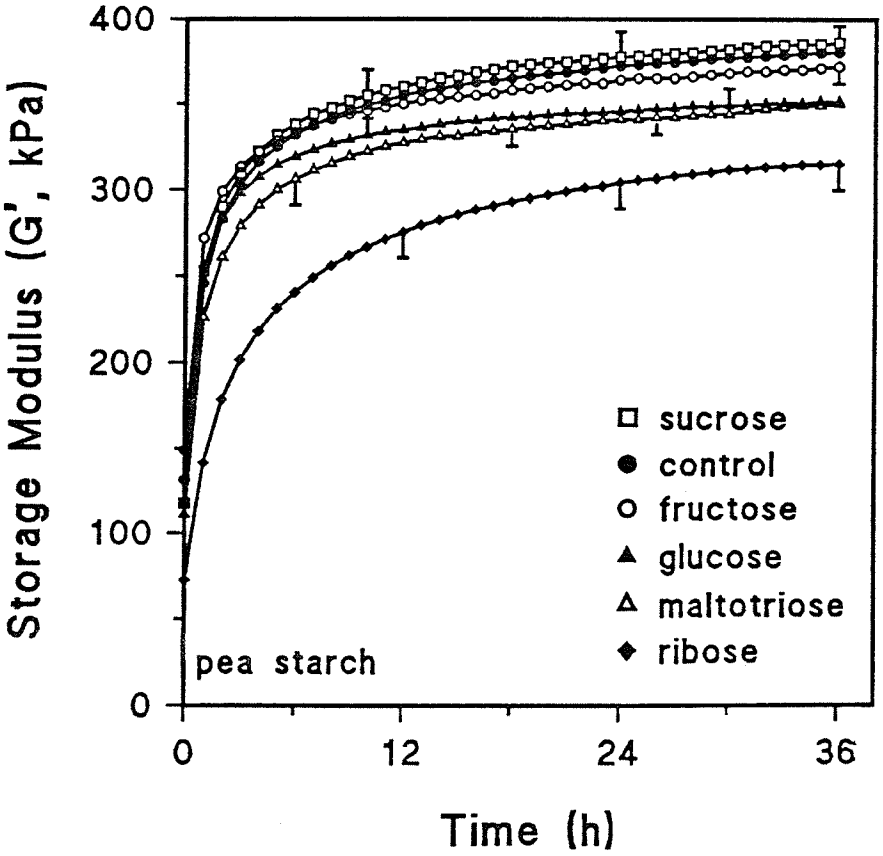
the kinetics of structure development within the gel network. Among the sugars used, glucose promoted rigidity development of the starch gel throughout the entire storage period whereas fructose initially (first 12 hours) promoted G´ development, as compared to the control, and later slightly retarded this process. Sucrose, maltotriose and ribose were observed to significantly retard the development of G´ throughout the storage period; ribose exhibited the strongest anti-retrogradation effect.

Figure 22 (Bottom) shows the influence of polyols on the evolution of G´ with time for 40% (w/w) wheat starch gels upon storage at 8°C. In general, the G´-time profiles displayed a biphasic gelation process: an initial rapid rise in the modulus value followed by a phase of slower G´ development. The initial phase of gel development (often rapidly accomplished) was not significantly affected by the addition of fructose, glucose and maltotriose. The addition of polyols, however, greatly affected the rate of rigidity development during the first 36 h of storage. This phase of G´ development was retarded by the addition of sugars, at a ratio of starch:sugar:water 1:0.5:1.5 w/w, in the following order: ribose > maltotriose > sucrose > water alone, glucose > fructose.

The influence of polyols on gelation and retrogradation of potato starch gels (40% w/w) was also studied by small strain dynamic rheometry (Figure 23, Top). A similar biphasic G´-time development was observed. Fructose, glucose and sucrose promoted the formation of gel network in the initial phase of the gelation process. During this phase (up to 24 h) G´ development was accelerated by the addition of both glucose and fructose. During the second phase of structure development, the added polyols (glucose, fructose, sucrose, maltotriose) seemed to have little effect on the rate of G´ increase. Sucrose also exerted a retarding effect on G´ development after 3 h storage. Maltotriose and ribose decreased considerably the rate of

Figure 23. Effect of added polyols on the storage modulus (G´) of ageing potato (TOP) and pea (BOTTOM) starch gels stored at 8°C. Dynamic rheological measurements were made at 0.2 Hz and strain < 2%. Polyols were incorporated at a ratio of 1:0.5:1.5 (w/w) for starch:polyol:starch:water mixtures.


potato starch
Storage Modulus (G', kPa)
Time (h)
0
100
200
300
400
0
12
24
36
fructose
glucose
control
sucrose
maltotriose
ribose


pea starch
Storage Modulus (G', kPa)
Time (h)
0
100
200
300
400
0
12
24
36
sucrose
control
fructose
glucose
maltotriose
ribose

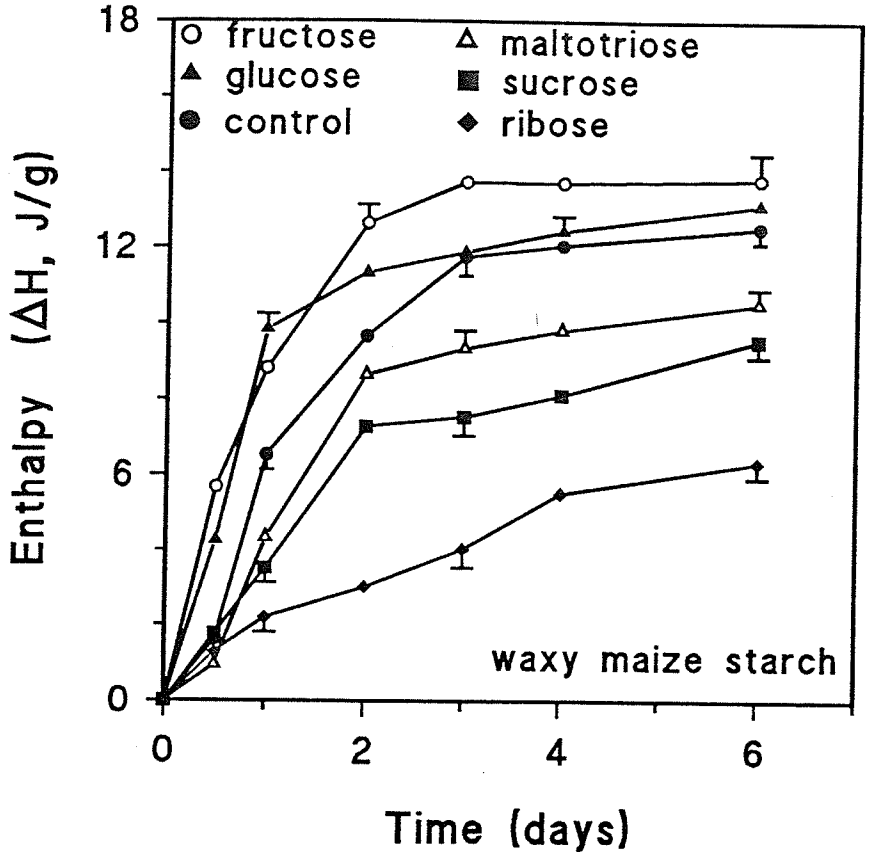
network development over the entire storage period.

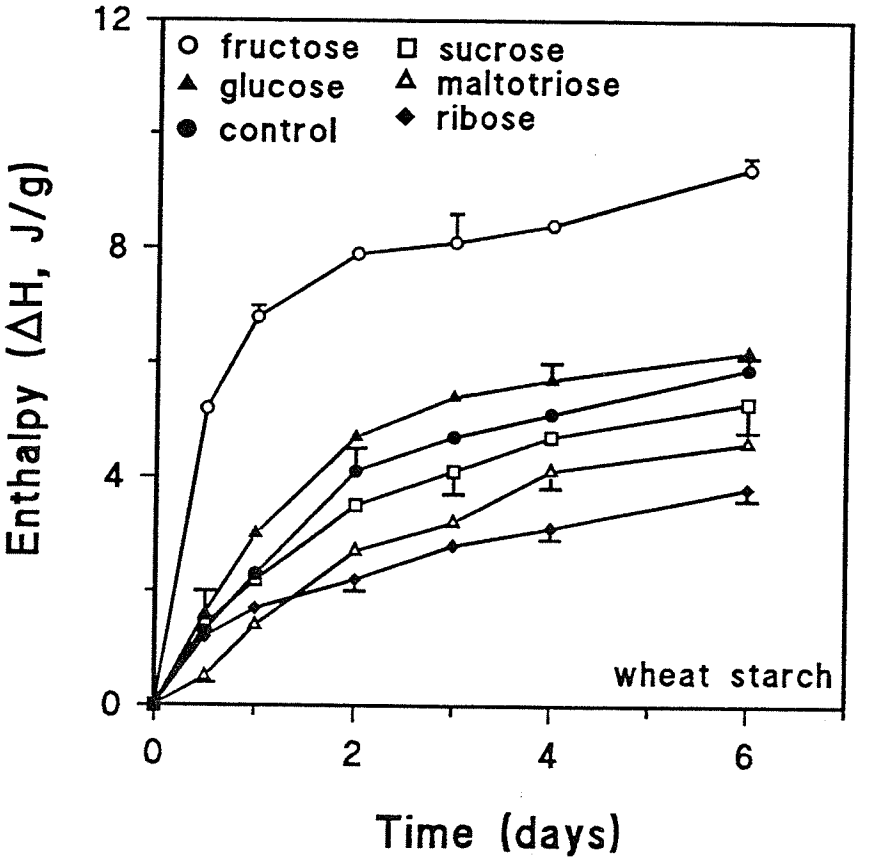
Finally, the influence of polyols on the time dependent mechanical changes of concentrated pea starch gels (40% w/w) was studied (Figure 23, Bottom). Similar biphasic gelation patterns were observed as with wheat and potato starch gels. The initial phase of G´ development was retarded by ribose, maltotriose and sucrose, and promoted by fructose. The addition of glucose did not cause any significant change in the rate of gel rigidity development. Throughout the latter phase of G´ development the polyols exerted little influence on the viscoelastic properties of the aging pea starch gels (essentially constant G´ values were attained after 12 h storage), except ribose. Sucrose slightly promoted the formation of structure, whereas fructose, glucose and maltotriose slightly retarded this process. In contrast the addition of ribose, as with all other starch gels studied, exhibited a strong anti-retrogradation effect as monitored by dynamic rheometry.

4.2.3 Differential Scanning Calorimetry Studies

Complementary to the dynamic rheological testing, DSC was employed to follow the development of "ordered" structures in ageing waxy maize, wheat, potato and pea starch gels as influenced by the addition of fructose, glucose, sucrose, maltotriose and ribose. Calorimetry is more sensitive to detect changes in structure at a molecular level while the mechanical tests mostly monitor the associated structural changes at a macro-level. The influence of polyols on the development of the retrogradation endotherm for waxy maize starch gels (40% w/w) is shown in Figure 24 (Top). The addition of polyols, at a ratio of 1:0.5:1.5 (w/w) for starch:sugar:water mixtures inhibited chain reorganization of amylopectin gels (40% w/w), as followed by DSC in the following order: ribose > sucrose > maltotriose > water alone, glucose > fructose.

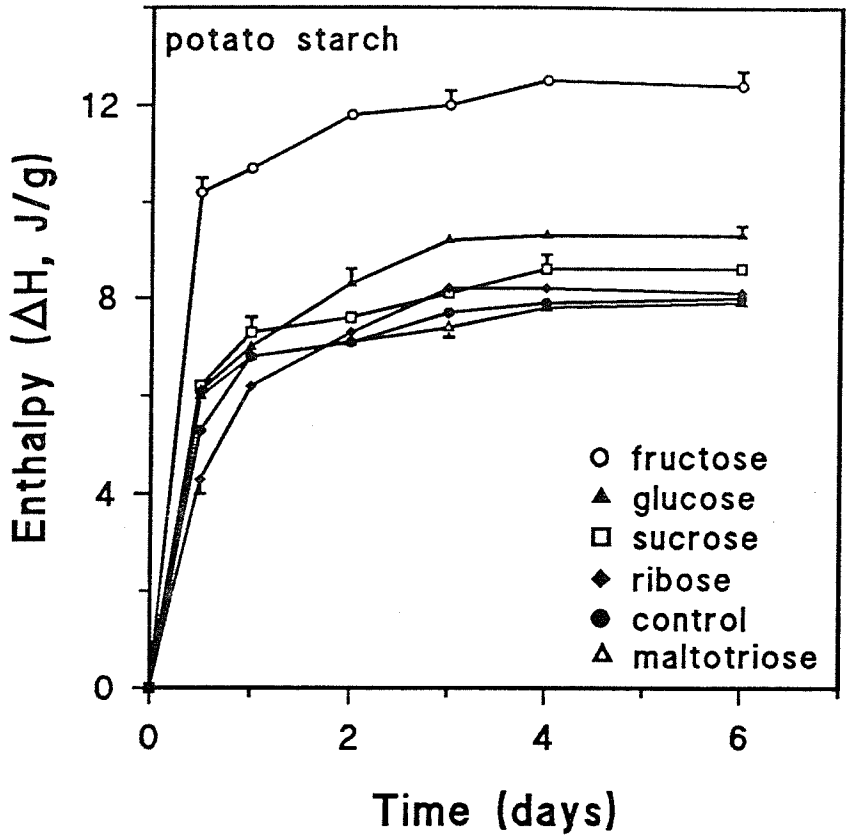
Figure 24. Effect of added polyols on the retrogradation endotherm (ΔH) of ageing waxy maize (TOP) and wheat (BOTTOM) starch gels stored at 6°C. Polyols were incorporated at a ratio of 1:0.5:1.5 (w/w) for starch:polyol:water mixtures.

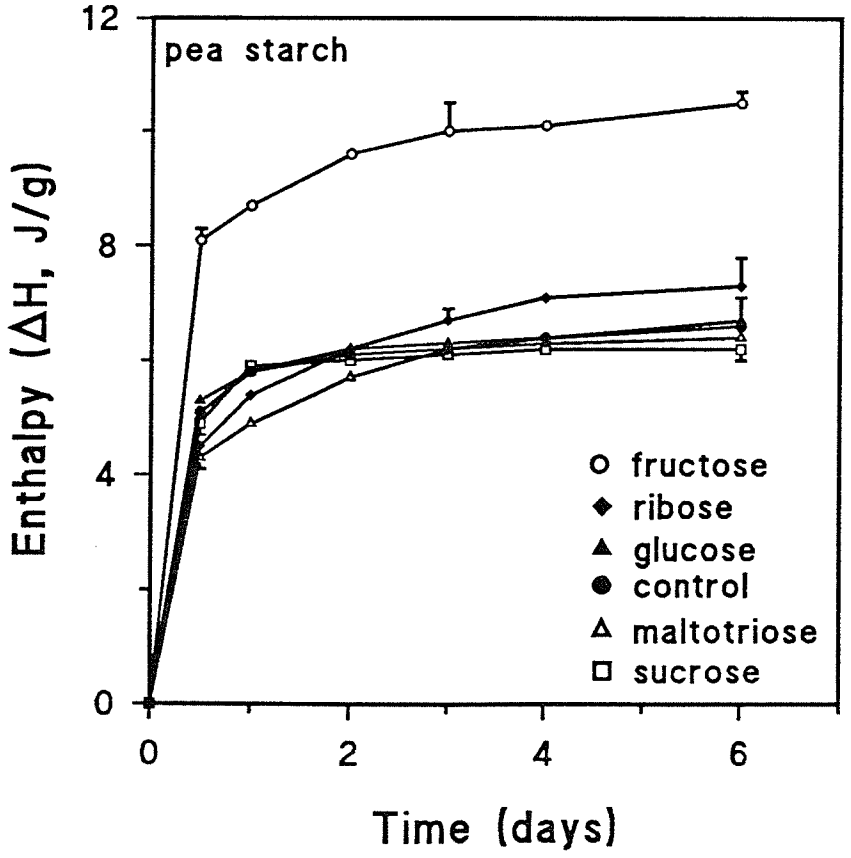

18
12
6
0
Enthalpy (ΔH, J/g)
fructose
glucose
control
maltotriose
sucrose
ribose
waxy maize starch
0
2
4
6
Time (days)


12
8
4
0
Enthalpy (ΔH, J/g)
fructose
glucose
control
sucrose
maltotriose
ribose
wheat starch
0
2
4
6
Time (days)

Similar effects of the polyols on the time dependent development of the retrogradation endotherm (ΔH) were also observed for 40% (w/w) wheat starch gels (Figure 24, Bottom). In contrast, the effects of these polyols on the development of retrogradation endotherm (ΔH) were less pronounced for potato and pea starch gels compared to those of waxy maize and wheat starches (Figure 26 Top and Bottom). The addition of fructose to both potato and pea starch gels strongly accelerated the retrogradation events in the ageing gel networks. The addition of glucose also promoted slightly the retrogradation of potato starch, compared to the polyol-free control gel (Figure 26, Top). On the other hand, sucrose, maltotriose and ribose were not found to significantly affect the formation of "ordered" structures in the amylopectin component for both potato and pea starch gels upon storage. Obviously, the trends seen in ΔH values for the retrogradation endotherm of ribose-containing potato and pea starch gels do not exactly corresponds with those obtained for the G'-time profiles (Figures 24, Top and Bottom). These findings clearly indicate that DSC and small deformation rheological testing are sensitive to different structural elements of the ageing gel networks.

Figure 25. Effect of added polyols on the retrogradation endotherm (ΔH) of ageing potato (TOP) and pea (BOTTOM) starch gels stored at 6°C. Polyols were incorporated at a ratio of 1:0.5:1.5 (w/w) for starch:polyol:water mixtures.


potato starch
Enthalpy (ΔH, J/g)
12
8
4
0
0
2
4
6
Time (days)
fructose
glucose
sucrose
ribose
control
maltotriose


pea starch
Enthalpy (ΔH, J/g)
12
8
4
0
0
2
4
6
Time (days)
fructose
ribose
glucose
control
maltotriose
sucrose

5. DISCUSSION

In this chapter the interpretation and comparison of the results for the effect of polyols on retrogradation of concentrated starch gels, given in chapter 4, is presented. The first section (5.1) deals with the effect of various polyols on retrogradation of amylopectin gels which is discussed in the context of current literature information in this area. The second section (5.2) refers to the effects of polyols on thermal and mechanical properties of ageing waxy maize, wheat, potato and pea starch gels. In the last section (5.3) an attempt is made to relate the effect of polyols on starch retrogradation with their hydration characteristics and other physicochemical properties of their aqueous solutions.

5.1 Retrogradation of Amylopectin in the Presence of Polyols

5.1.1 Oligosaccharides

Oligosaccharides are generally accepted as food ingredients which impede the hardening of starch-based foodstuffs. There are, however, relatively few reports on the effect of oligosaccharides on starch retrogradation, and the exact mechanism by which inhibition is brought about remains unclear (Slade and Levine, 1987; Carins *et al.*, 1991a; Katsuta *et al.*, 1992c). In these studies glucose was found to promote retrogradation in waxy maize starch gels at short storage periods, while glucose oligomers retarded this process more effectively with increasing DP from 1 to 3 (Appendix V). Glucose oligomers of greater chain length (DP 4-7) had little

effect on retrogradation, while maltooctaose (DP 8) strongly promoted the development of "ordered" structures within ageing amylopectin gel networks. Similarly, Slade and Levine (1987), Carins *et al.* (1991a) and Katsuta *et al.* (1992c) reported that retrogradation of starch was reduced with increasing chain length of glucose oligosaccharides from 1 to 3. Furthermore, Slade and Levine (1987) reported that higher oligosaccharides (enzyme-hydrolyzed malto-oligosaccharides with DP > 3) retarded retrogradation more than maltotriose, whereas Katsuta *et al.* (1992c) observed that maltotetraose had a lesser effect of decreasing the firmness of rice starch gels than maltotriose.

Slade and Levine (1987) have rationalized the effect of oligosaccharides on retrogradation of starch from a polymeric perspective. As the molecular weight of the oligomer increases, there is greater localized viscosity of the aqueous medium surrounding the starch chains. This reduces chain mobility due to increases in the effective T_g for the polymer-solute-water mixture which in turn results in slower rates of starch recrystallization. However, contrary to this point of view, oligomers of higher DP (> 3) were found to have little effect on the rate of starch retrogradation.

5.1.2 Disaccharides

There are no previous studies reporting how added saccharides with different chain linkages affect the rate of starch retrogradation. In the present study, glucose disaccharides with β (1→4) and α (1→1) linkages were found to retard the development of "ordered" structures in amylopectin gels to a greater extent than those with α (1→4), β (1→6) and α (1→6) linkages, especially during the early stages of storage (Figure 12).

The disaccharides used in this study have similar hydration properties (Uedaira *et al.*, 1989, 1990) but differ in the viscosity of their 25% (w/w) solutions (Appendix II). Disaccharides and oligosaccharides with β (1→4) linkages (cellobiose) have an extended, rigid conformation (Glass, 1986) and therefore occupy relatively larger hydrodynamic volumes. In contrast, glucans with α (1→4) and α (1→6) linkages are more flexible (Glass, 1986), occupying smaller hydrodynamic volumes. The larger the hydrodynamic volume, the greater the viscosity of the medium at a constant solute concentration. The results of this study indicate that glucose disaccharides with different glucosidic linkages have different viscosities in solution and this may affect the rate of starch retrogradation. However, the observed differences in the retrogradation rates observed for the various disaccharides are rather minor.

5.1.3 Sugar Alcohols

A study was also undertaken to identify whether there are differences in the effect between sugars and their alcohol derivatives on starch retrogradation. Miura *et al.* (1992) have compared the effect of such polyols at a lower concentration (2.7% w/w) by creep compliance measurements on ageing rice starch gels (30% w/w). These authors have reported that maltitol retarded the rate of retrogradation more than maltose. However, in the present studies the DSC results (Figure 13) indicated that there were no significant differences between these two solutes for their effect on the retrogradation process for waxy maize starch gels. On the other hand, sorbitol seems to retard starch retrogradation compared to glucose. It is also important to note here that Miura and coworkers (1992) added polyols to the starch/polyol/water system at a much lower concentration (2.7% w/w solution) than the level used in the present studies (25% w/w). It is well known that the effect of polyols on starch retrogradation is strongly dependent on their

concentration in the composite gel (Carins *et al.*, 1991 a,b), as was apparent from the results of this study (Figure 15).

Interestingly, when glyceraldehyde was incorporated into starch gels it inhibited the formation of "ordered" structures within the ageing amylopectin networks, as measured by DSC, even after 30 days of storage. This strong anti-retrogradation behaviour has not been previously reported. Dynamic rheological tests also indicated that the addition of glyceraldehyde prevented the formation of well-established (set) starch gel networks at high polymer concentration (40% w/w) for both waxy maize and wheat starches. For this solute, it is possible that during heating there is degradation and/or modification of the polymer, thus preventing any structural reordering upon storage.

5.1.4 Pentoses and Hexoses

The DSC results of the pentose-containing waxy maize gels clearly indicated that pentoses retard the retrogradation of starch more effectively than hexoses (Figure 14). Ribose was the most effective solute in retarding the formation of "ordered" structures within amylopectin gels, whereas fructose was shown to promote this process. Other researchers have also realized the unique anti-retrogradation properties of pentoses such as ribose and xylose (Slade and Levine, 1987; I'Anson *et al.*, 1990; Carins *et al.*, 1991 a,b). In contrast, Katsuta *et al.* (1992b) and Miura *et al.* (1992) observed the opposite effect. Hexoses (glucose and fructose) were found to reduce the rate of retrogradation more than pentoses (ribose and xylose). This apparent contradiction may reflect the much lower concentration of polyols used in studies of Miura and coworkers (1992) (2.7 vs 25% w/w) and the different techniques used to probe the structural changes in amylopectin gels (large deformation mechanical tests vs. DSC). A possible

mechanism accounting for the different effects of polyols on retrogradation at high sugar concentrations will be discussed in section 5.3.

5.1.5 Polyol Concentration

The development of "ordered" structures in ageing amylopectin gel networks was strongly influenced by the concentration of the polyol added. Ribose continuously retarded the retrogradation of starch with increasing concentration, whereas fructose promoted this process at concentrations greater than 7.5% (w/w) (Figure 15). Similar concentration effects on the crystallinity (measured by X-ray diffraction techniques) of ageing wheat starch gels were also observed by Carins *et al.* (1991 a,b) for these two sugars. The retrogradation of starch was surprisingly not affected by increasing the concentration of sucrose above 7.5% w/w. The dependence of retrogradation rate on sugar concentration does have significant implications in the baking industry, particularly for high-sugar based products (cakes, cookies, etc.). The importance of polyol concentration on the kinetics of starch retrogradation has been also recognized by other researchers. For example, Katsuta *et al.* (1992b) and Miura *et al.* (1992) have found that at low concentration (2.7% w/w) both ribose and fructose retarded the retrogradation of starch, with fructose exhibiting a greater effect. In the present studies, 25% (w/w) fructose solutions were found to promote recrystallization of amylopectin, while the addition of ribose (25% w/w) strongly retarded this process.

5.2 Thermal and Mechanical Properties of Ageing Starch Gels in the Presence of Polyols

A comparative study was undertaken to examine the effect of polyols on the thermal and mechanical properties of waxy maize, wheat, potato and pea starch gels. The polyols chosen for this study were fructose and glucose due to their ability to promote chain reorganization in ageing starch gels, maltotriose and ribose for their strong anti-retrogradation behaviour, and sucrose a common sugar used in the baking industry. The time-dependent changes in structure of the composite gels (from granular starches differing in composition and properties) were followed by both small strain dynamic rheometry and DSC.

Dynamic rheometry has proven a useful physical probe to monitor the kinetics of structure development in ageing starch gels. At small strains, it allows continuous measurements of the dynamic moduli without disturbing any molecular structures formed in the sample upon ageing, provided that testing is carried out in the linear viscoelastic region for the specimen. There are relatively few reports on dynamic rheological testing of starch networks, particularly for concentrated systems (i.e. > 30% solids) (Biliaderis, 1992). In fact, there have been no rheological studies of the effect of polyols on starch gelation and retrogradation precesses at high starch solids levels. A high concentration of starch (40% solids) was chosen in the present study to simulate the low moisture environment of most baked products.

The botanical source of starch significantly influences the mechanical properties of its aqueous pastes and gels (Orford *et al.*, 1987; Roulet *et al.*, 1990; Biliaderis, 1992). The initial structure development in starch gels is dominated by the formation of a three-dimensional chain network involving amylose molecules (Morris, 1990). The higher amount of amylose solubilized during gelatinization, the greater is the rigidity of the gel (Orford *et al.*, 1987; Roulet *et al.*, 1990; Biliaderis, 1992). A comparison of the initial G' development in the starch gels studied

showed the following trend of initial modulus values: pea > potato > wheat > waxy maize starch; this ranking certainly follows the amylose content trend of these starches (Table 2). The relative high initial rigidity values of potato starch gels, contradicted the work by Langton and Hermansson (1989) as well as Svegmark and Hermansson (1991 a,b). These authors reported that cereal starches formed stronger gels than potato starch due to a more efficient separation of amylose and amylopectin during gelatinization. However, in their investigation much lower starch concentrations were employed (5-10% w/w) compared to those used in the present studies.

The long-term changes in the mechanical properties of wheat, potato and pea starch gels are due to recrystallization of amylopectin as seen in the case of waxy maize starch gels. Chain reorganization in the amylopectin molecule is considered as the main cause of retrogradation of starch and staling (firming) of baked products (Kulp and Ponte, 1981). The rate of long-term G´ development of native starch gels followed the order of: potato > pea > waxy maize > wheat. Similar observations were made by Roulet *et al.* (1990) who measured firmness development during ageing of 40% (w/w) starch gels from different botanical sources using large deformation mechanical testing. The greater extent of rigidity development for pea and potato starch gels may also reflect the lack of endogenous granular lipids from these starches which are known to retard amylopectin recrystallization (Orford *et al.*, 1987, Biliaderis and Tonogai, 1991).

The mechanical properties of ageing gels were strongly influenced by the addition of polyols. The extent to which polyols affected the initial phase of G´ development (associated mostly with amylose gelation) varied among wheat, potato and pea starch gels. It is not clear if the influence of polyols on the initial structure development was caused by a direct effect on the interassociations between amylose chains or indirectly by affecting the amount of amylose which was leached out of the granules during gelatinization. The latter phase of G´ development

(mainly due to amylopectin recrystallization) was also found to be strongly influenced by the polyols. The waxy maize and wheat starch gels were more sensitive to changes in rigidity in the presence of polyols than those of potato and pea starches. This may be related to the higher amylopectin content of these gels. The results of this study clearly indicated that the higher the amylopectin/amylose ratio the wider the range of G´ values for the composite gel networks.

Differential scanning calorimetry was also used to probe the development of "ordered" structures within ageing waxy maize, wheat, potato and pea starch gels in the presence of selected polyols (fructose, glucose, sucrose, maltotriose and ribose). Similarly to the rheological data, the modification of gel structure, as measured by DSC, decreased in the order: waxy maize > wheat > potato > pea starch. In all starch gels, fructose strongly promoted the development of the retrogradation endotherm (ΔH). Among the other polyols studied the differences seen in ΔH development were minimized with increasing amylose content of starch.

A comparison between the DSC and rheological data for pea and potato starch gels also revealed some differences in the sensitivity between the two techniques to monitor gel structure development. For example, while the DSC data indicated greater differences between control and fructose-containing gels, the corresponding rheological responses were smaller (Figures 23 and 25). Similar observation can be made for the control and ribose-containing gels when comparing the DSC and rheological data (Figures 23 and 25). These findings may reflect the different time scales over which DSC and rheological measurements were made and/or different structural elements of the ageing gels to which the two techniques respond to.

5.3 Starch Retrogradation Kinetics in the Context of Physicochemical Properties of Polyol Solutions

In order to further elucidate the underlying molecular mechanisms by which polyols could influence the retrogradation of starch, the hydration characteristics and other physicochemical properties of aqueous sugar solutions were also examined. The estimated values for these properties, derived from literature data, were related to the effects polyols had on the retrogradation endotherm (ΔH) of waxy maize starch gels (section 4.1.7). The starch gels used in these studies contained a large amount of water (60% w/w). For hydrated amorphous starch gels water is considered as a plasticizer which facilitates chain mobility and thereby recrystallization of starch molecules. If the ability of the starch chains to move is impeded, the rate of formation of "ordered" structures is reduced.

The viscosities of 25% w/w polyol solutions significantly correlated with their effect on starch retrogradation. As the viscosity of the polyol solution increased, the rate of starch retrogradation decreased. An increase in the viscosity of the medium surrounding the starch chains may have an anti-plasticizing effect on the amorphous gel matrix. According to Slade and Levine (1987) this increase in localized viscosity will increase the T_g and thereby retard the recrystallization of amylopectin. However, this theory does not explain the large anti-retrogradation effect observed for pentoses. These polyols do not fit the relationship between viscosity and rate of retrogradation seen in Figure 16.

Another approach to explain the influence of polyols on starch retrogradation kinetics is to consider their effect on the three-dimensional hydrogen-bonded structure of water. It is clear from the findings of several studies that the influence of polyols on water structure is specific to each particular solute (i.e. monosaccharides may affect water structure differently than

disaccharides or oligosaccharides). It is also apparent from the relationships presented in Figures 17-20 that the effects of monosaccharides on starch retrogradation are influenced by their hydration properties. Both Uedaira and coworkers (1989, 1990), and Galema and Hoiland (1991), using ^{17}O-NMR and ultrasound measurements, respectively, indicated that the greater the hydration number (n_h or n_{DHN}) the greater the number of water molecules which are structured around a polyol molecule in solution. The highly structured water cosphere around the polyol molecules also exhibits less compressibility, and this is reflected by lower isentropic molar compressibility values for such polyol solutions. Therefore, there is a greater number of water molecules which are disturbed in the presence of a polyol which has poor compatibility with the three-dimensional hydrogen-bonded structure of water. Uedaira *et al.* (1989) have found a direct relationship between the structure of monosaccharides and their hydration properties. Polyols with a large number of equatorial OH groups (n(e-OH)) have relatively large dynamic hydration numbers (n_{DHN}). Thus polyols with high n(e-OH) are generally considered to have a larger disturbing effect on the "normal" water structure; i.e. they are less compatible with water structure, thus creating a more disturbed hydration layer around them.

In view of the above considerations, two different effects can be envisioned to explain the different starch retrogradation rates observed with various polyols in ternary starch-polyol-water gel systems. First, polyols would tend to reduce the effective water concentration of the gel matrix due to the formation of a hydration layer around these solutes. This would result in increased retrogradation rates as long as the weight ratio of starch/water remains within a range of 0.1 to 1.5. In fact, for model wheat starch gels and bread systems Zeleznak and Hoseney (1986) as well as Longton and LeGrys (1981) have shown by DSC that starch retrogradation increases with increasing weight ratios of starch/water between 0.1 to 1.5; at starch to water

ratios above 1.5 the reverse trend was observed. Using published hydration numbers for the various polyols (Uedaira *et al.*, 1989, 1990; Galema and Hoiland, 1991), the amount of available "mobile" water in composite gels at a specified starch-polyol-water composition can be calculated. For the control gels (starch-water) used in the present studies the ratio of starch/water was about 0.67; i.e. this value is within the range of 0.1 - 1.5 where acceleration of retrogradation events would be expected with a reduction in water content. Using fructose (promoting retrogradation) and ribose (retarding retrogradation) as the two extreme polyol solutes, the ratio of starch to remaining "available" water in the ternary starch/polyol/water (1:0.5:1.5 w/w) gel systems can be calculated with the assumption of a monolayer-type of hydration cosphere around these solutes. Thus, using the hydration numbers published by Galema and Hoiland (1991) for fructose (n_h=8.8) and ribose (n_h=6.8), the ratios of starch/available water for the two polyols would be 0.99 and 0.91, respectively. These numbers are still within the range of 0.1 -1.5. Similarly, if the n_{DHN} values for these sugars are used (Uedaira *et al.*, 1989, 1990) in the calculations (fructose n_{DHN}=16.5 and ribose n_{DHN}=10.9), the corresponding ratios become 1.47 and 1.19 for fructose and ribose, respectively. It would appear from these assumptions that both sugars must exhibit a promoting effect on starch retrogradation if only the increase in effective polymer concentration (higher starch/available water ratios) in the composite gel is considered. Furthermore, the relative change in starch/available water ratios is similar between the two sugars and thus it cannot account for the large differences in starch retrogradation kinetics observed between ribose and fructose (Figure 14). An increase in effective polymer concentration can not also explain why ribose retards the retrogradation events compared to the control gels (starch-water only).

The second factor that may play a role in the kinetics of retrogradation is related to solute compatibility with the structure of water and its partitioning between the bulk water phase and

the hydration layer of the starch molecules. For monosaccharides which cause very little disturbance in water structure (e.g. ribose, xylose) it is reasonable to expect greater access of these solutes to the hydration cosphere of the starch chains. Consequently, the polymer chains would experience a localized environment of increased viscosity (anti-plasticizing effect compared to water alone). This would in turn reduce molecular mobility in the hydration cosphere of the polymer chains and consequently result in slower retrogradation rates. On the other hand, polyols which greatly disturb the "normal" water structure (e.g. glucose, fructose), are expected to form a stronger hydration layer around them and thus be excluded from the vicinity of the starch chains. For these solutes, the effect from increasing the effective polymer concentration is greater than the anti-plasticizer effect of the sugar resulting in a net increase of the retrogradation rate.

Uedaira *et al.* (1990) have found that oligosaccharides do not follow the same trend as monosaccharides in their hydration properties. For monosaccharides a direct relationship was observed between rotational correlation times of their aqueous solutions (τ_c^h/τ_c^o) and n(e-OH). The rotational correlation times of water molecules in the hydration cosphere (τ_c^h) of such solutes are therefore high compared to that of bulk water (τ_c^o). For glucose oligomers, with increasing DP from 1 to 3, Uedaira and coworkers (1990) have found that the τ_c^h/τ_c^o decreases with increasing n(e-OH) (opposite effect than for monosaccharides). This implies an increased mobility of water molecules surrounding these solutes with increasing DP; i.e. maltotriose seems to have a better fit into the structure of water than maltose or glucose. It is likely therefore that maltotriose can partition more effectively into the hydration cosphere of the starch polymer chains than glucose, and act as an anti-plasticizer of the polymer gel matrix. Furthermore, because of its higher molecular weight, maltotriose would be more effective in elevating the T_g of the

hydrated polymer microphase, thus greatly reducing chain mobility.

For glucose oligosaccharides of even greater DP (>3) the reported τ_c^h/τ_c° values remained relatively constant with respect to increasing n(e-OH) (Uedaira *et al.*, 1990). This would suggest that an additional molecular parameter affects the hydration properties of these oligosaccharides (Uedaira *et al.* 1990). Addition of glucose oligomers with DP>3 to starch gels resulted in very little changes in the retrogradation kinetics (Figure 11), except for maltooctaose which seemed to accelerate the retrogradation rate. It has been suggested that glucose oligosaccharides of higher DP (>3) can form helical structures in solution (Katsuta *et al.*, 1992c), thus resembling the linear starch chains (amylose). It is also well documented that mixing of amylose with amylopectin in aqueous solutions leads to phase separation of these polymers (Kalichevsky *et al.*, 1987). In an aqueous environment the branched and linear starch molecules are thermodynamically incompatible leading to demixing and formation of amylose- and amylopectin-rich phases (Kalichevsky and Ring, 1987; German *et al.*, 1992). The addition of higher DP (>3) glucose oligomers may thus bring about phase separation between amylopectin and the added polyol, causing an increase in the polymer concentration in the amylopectin-rich microphase. This would be more pronounced with maltooctaose, leading to acceleration of the retrogradation process for the amylopectin molecules.

The above rationalization is in agreement with the findings of the present studies as well as literature data from earlier investigations (Slade and Levine, 1987; I'Anson *et al.*, 1990; Carins *et al.*, 1991 a,b). Clearly, more experimental information, particularly at a molecular level, is required to arrive to a complete description of the underlying mechanism(s) for the polyol effects in ageing starch gels. Such studies could lead to a better understanding of the interactions between water-solute-polymer and their implication to chain reorganization and

aggregation processes in hydrated starch gel matrices. This would be useful in establishing a theoretical framework to modify and control the mechanical properties, organoleptic attributes and shelf-life of starch based products.

7. CONCLUSIONS AND RECOMMENDATIONS

The present study was undertaken to systematically study the effect of polyols on the thermal and mechanical properties of concentrated starch gels, and provide further insight into the anti-retrogradation mechanism(s) of these solutes.

The effect of polyols on the formation of "ordered" structures within ageing amylopectin gel networks was studied. The addition of glucose oligosaccharides of DP 1 to 3 retarded retrogradation with increasing DP. Glucose oligomers of greater chain length (DP 4 to 7) had little effect on retrogradation, while maltooctaose promoted the reorganization of amylopectin short DP chains. The effect of added glucose disaccharides with different glucosidic linkages on the formation of "ordered" structures in ageing waxy maize starch gels was also examined. The observed differences in the retrogradation rates for the various disaccharides were rather minor. Similarly, little differences were found when the anti-retrogradation behaviour of sugars and their respective sugar alcohol were compared. However, the addition of glyceraldehyde to waxy maize starch gels completely inhibited any structural reorganization of amylopectin over the time period examined. Further studies are required to resolve why glyceraldehyde exhibits such strong anti-retrogradation behaviour. The effect of various pentoses and hexoses on amylopectin retrogradation was also studied. Pentoses (i.e. ribose and xylose) were found to retard the reordering of amylopectin more effectively than hexoses (i.e. glucose). Finally, it was shown that the retrogradation of starch was strongly influenced by the concentration of polyol added, particularly in the case of ribose.

A comparative study was undertaken to examine the effect of polyols on the thermal and mechanical properties of concentrated gels prepared from granular starches with a broad range of physical and chemical properties. Waxy maize and wheat starch gels were found to be more sensitive to the effect of polyols on the developments of structural "order" as probed by both dynamic rheological measurements and DSC than those of potato and pea starches. The higher the amylopectin/amylose ratio in the gels the wider the range of G' and ΔH responses for the composite gel networks.

A comparison between the rheological and DSC data revealed some differences in sensitivity between these two techniques for monitoring gel structure development. These observations clearly point to the fact that DSC and small strain rheological testing examine different structural elements of ageing starch gels. First, DSC follows structure development at a molecular level, namely formation of double helices involving the outer short DP chains of the amylopectin molecules. On the other hand, dynamic rheological measurements are sensitive to the development of physical-crosslinks established by entanglement of linear starch molecules (amylose) as well as recrystallization of the amylopectin short DP chains.

The anti-retrogradation mechanism of polyols was explained on the basis of their effect on the three-dimensional hydrogen-bonded structure of water. The findings of several studies clearly indicate that the influence of polyols on water structure is specific for each particular solute. It is also apparent from the data presented in this thesis that the effects of monosaccharides on starch retrogradation are influenced by their hydration properties. Monosaccharides such as fructose, having a large number of structured water molecules around the solute in solution (poor compatibility with the structure of water), deprive the starch chains of water. The increase in localized polymer concentration would accelerate the rate of

retrogradation compared to the control (starch-water only). In contrast, pentoses (i.e. ribose), bringing about very little disturbance in the water structure could have a greater access to the hydration cosphere of the starch chains. This may cause an anti-plasticizing effect on the hydrated polymer chains (increasing T_g), thereby retarding the reorganization of amylopectin chains into crystalline structures. For glucose oligosaccharides with DP 1 to 3 a better fit into the structure of water occurs with increasing DP (i.e., maltotriose fits better than maltose), allowing access to the hydration cosphere of the starch chains. The higher the molecular weight of the oligosaccharides the greater the anti-plasticizing effect, elevating the effective T_g of the hydrated polymer microphase; this would impede chain mobility, thereby reducing the rate of amylopectin recrystallization. On the other hand, oligosaccharides of higher DP (>3) can form helical structures in solution and may exhibit thermodynamic incompatibility with the branched starch molecules: this could lead to phase separation between amylopectin and the added oligosaccharide. As a result there is an increase in polymer concentration in the amylopectin-rich phase of the composite gel network, accelerating the rate of retrogradation. More experimental data, particularly at a molecular level, are required to arrive at a more complete description of the underlying mechanism(s) for the effect of polyols on ageing starch gels.

Finally, there is a need to examine the behaviour of polyols in more "complex" systems, particularly those with high sugar levels (cakes, cookies, etc.) and verify if similar effects on "staling" also occur.

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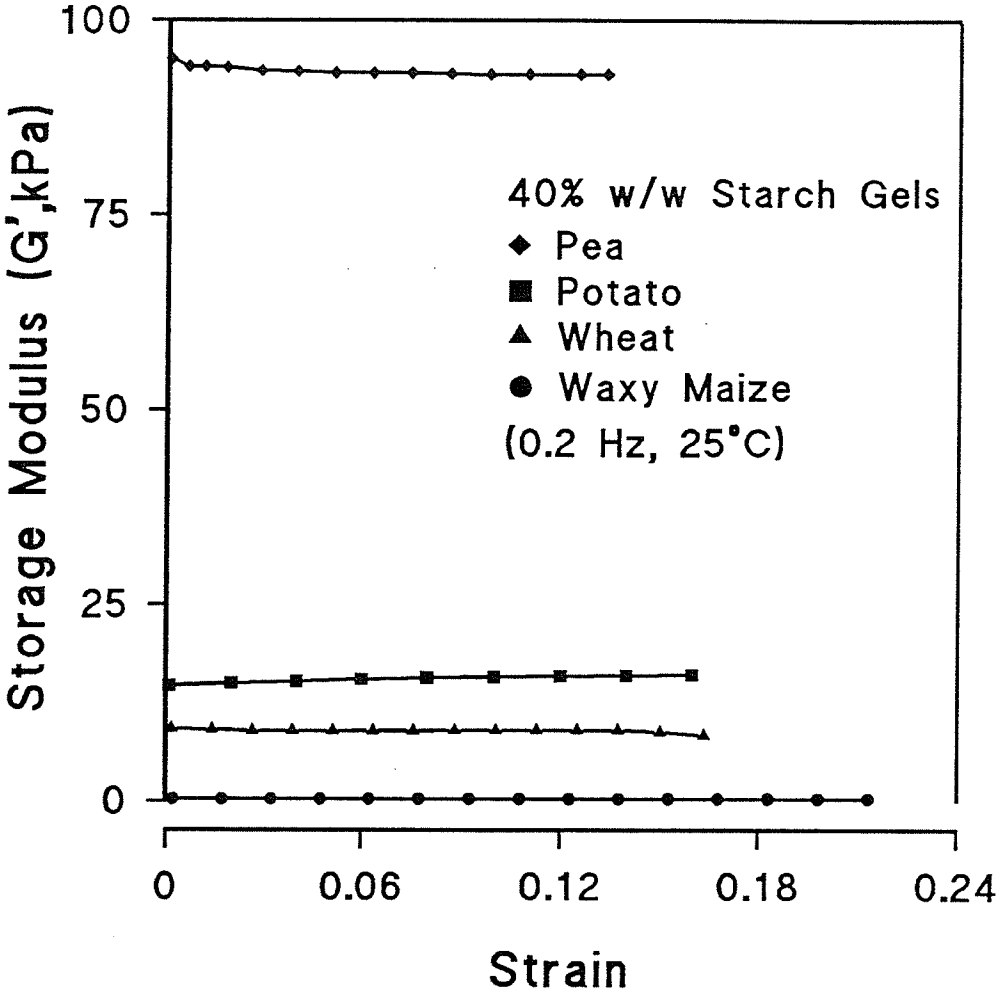
APPENDICES

Appendix I. Typical shear strain sweeps of 40% (w/w) waxy maize, wheat, potato and pea starch gels at 25°C. The values of the storage modulus (G´) were measured at 0.2 Hz as a function of increasing strain.


100
75
50
25
0
Storage Modulus (G',kPa)
40% w/w Starch Gels
Pea
Potato
Wheat
Waxy Maize
(0.2 Hz, 25°C)
0
0.06
0.12
0.18
0.24
Strain

Appendix II. Determination of absolute viscosity of 25% (w/w) polyol solutions at 8°C.

Polyol Solution (25% w/w)	Density (g/ml)	Kinematic Viscosity (cS)	Absolute Viscosity	
			(cP)	(mPa.s)[2]
Water	1.000[1]	1.471[1]	1.471	1.471
Ribose	1.100	2.862	3.148	3.148
Arabinose	1.106	3.032	3.353	3.353
Glycerol	1.068	3.038	3.245	3.245
Xylose	1.102	3.077	3.391	3.391
Glyceraldehyde	1.068	3.169	3.384	3.384
Fructose	1.109	3.219	3.570	3.570
Galactose	1.109	3.222	3.573	3.573
Glucose	1.107	3.356	3.715	3.715
Sorbitol	1.097	3.419	3.751	3.751
Sucrose	1.108	3.495	3.872	3.872
Lactose	1.100	3.568	3.925	3.925
Maltitol	1.104	3.626	4.003	4.003
Maltose	1.109	3.657	4.056	4.056
Cellobiose	1.110	3.774	4.189	4.189
Maltotriose	1.112	3.787	4.211	4.211
Trehalose	1.110	3.802	4.220	4.220

[1] n=3; SEM < 5%
[2] 1 centipoise = 1 mPa.s

Appendix III. Hydration characteristics and physicochemical properties of sugars in aqueous solutions at 25°C presented by Uedaira and coworkers (1989, 1990).

Sugar	n(e-OH)[1]	n_h[2]	τ_c^h/τ_c^o[3]	n_{DHN}[4]	n_h[5]	n_{DHN}*[6]
Ribose	2.1	6.0	2.82	10.9	6.8	12.4
Arabinose	2.6	6.0	3.32	13.9	7.6	17.6
Xylose	3.5	6.0	3.58	15.5	6.8	17.5
Fructose	3.0	6.0	3.75	16.5	8.8	24.2
Galactose	3.6	6.0	3.76	16.6	8.7	24.0
Glucose	4.6	6.0	4.10	18.6	8.4	26.0
Sucrose	6.3	10.0	3.52	25.2	13.9	35.0
Maltose	7.2	10.0	3.72	27.2	14.5	39.4
Trehalose	7.2	10.0	3.54	25.4	15.3	38.9
Maltotriose	9.8	14.0	3.47	34.6		

[1] Mean number of equatorial OH groups.
[2] Coordination numbers.
[3] Rotational correlation times.
[4] Dynamic hydration numbers calculated from the equation: $n_{DHN} = n_h(\tau_c^h/\tau_c^o - 1)$.
[5] Hydration numbers determined by Galema and Hoiland (1991).
[6] Dynamic hydration numbers calculated using the hydration numbers estimated by Galema and Hoiland (1991)(section 3.3).

Appendix IV. Determination of the relative mobility of polyols from data published by Slade and Levine (1988).

Polyol	Molecular Weight	T_g (°K)	T_g' (°K)	T_m (°K)	T_g (°K)	Relative Mobility $(T_{exp} - T_g')/ (T_m - T_g)$
Water	18.0					1.04[1]
Glycerol	92.1	279.2	208.0	291.0	180.0	0.64
Ribose	150.1	279.2	226.0	360.0	263.0	0.55
Xylose	150.1	279.2	225.0	426.0	282.5	0.38
Fructose	180.2	279.2	231.0	397.0	373.0	2.01
Glucose	180.2	279.2	230.0	431.0	304.0	0.39
Talose	180.2	279.2	229.2	413.2	284.7	0.39
Galactose	180.2	279.2	231.5	443.0	383.0	0.80
Sorbitol	182.2	279.2	229.5	384.0	271.0	0.44
Sucrose	342.3	279.2	241.0	465.0	325.0	0.27
Maltose	342.3	279.2	243.5	402.0	316.0	0.42
Trehalose	342.3	279.2	243.7	476.2	352.2	0.29
Cellobiose	342.3	279.2	244.2	522.2	350.2	0.20
Maltotriose	504.5	279.2	249.5	406.5	349.0	0.52

[1] Reported by Slade and Levine (1988).

Appendix V. The effect of glucose oligomers on retrogradation (ΔH) of waxy maize starch gels (40% w/w) stored at 6°C.

Oligomer[2]	Transition Enthalpy (J/g)[1]							
	9 h	12 h	18 h	24 h	48 h	72 h	96 h	144 h
Control	0.5 ± 0.2^{abc}	1.6 ± 0.1^{a}	3.7 ± 0.4^{cd}	6.5 ± 0.8^{cd}	9.7 ± 0.3^{bc}	11.7 ± 0.1^{c}	12.0 ± 0.1^{cde}	12.5 ± 0.2^{bc}
Glucose	1.9 ± 0.1^{e}	4.1 ± 0.6^{b}	6.5 ± 0.5^{d}	9.8 ± 0.1^{f}	11.3 ± 0.5^{a}	11.9 ± 0.5^{c}	12.4 ± 0.2^{de}	13.1 ± 0.1^{c}
Maltose	0.3 ± 0.1^{a}	1.3 ± 0.1^{a}	1.5 ± 0.1^{a}	5.2 ± 0.2^{ab}	5.9 ± 0.4^{a}	8.6 ± 0.7^{a}	10.2 ± 0.2^{ab}	11.5 ± 0.1^{ab}
Maltotriose	0.3 ± 0.2^{a}	0.9 ± 0.8^{a}	1.3 ± 0.3^{a}	4.3 ± 0.6^{a}	8.7 ± 0.5^{b}	9.3 ± 0.6^{a}	9.8 ± 0.4^{a}	10.5 ± 0.2^{a}
Maltotetraose	0.5 ± 0.2^{ab}	1.5 ± 0.1^{a}	3.4 ± 0.1^{b}	5.2 ± 0.2^{ab}	9.1 ± 0.1^{bc}	10.4 ± 0.3^{b}	10.6 ± 0.2^{b}	11.7 ± 0.6^{ab}
Maltopentaose	0.7 ± 0.1^{bc}	1.2 ± 0.6^{a}	3.3 ± 0.4^{b}	5.7 ± 0.4^{bc}	8.8 ± 0.8^{bc}	10.6 ± 0.4^{b}	11.7 ± 0.1^{cd}	12.0 ± 0.3^{bc}
Maltohexaose	0.8 ± 0.1^{c}	4.2 ± 0.2^{b}	4.8 ± 0.4^{c}	7.8 ± 0.1^{e}	9.2 ± 0.7^{e}	12.0 ± 0.1^{c}	11.6 ± 0.3^{c}	13.1 ± 0.2^{c}
Maltoheptaose	1.2 ± 0.1^{d}	3.3 ± 0.5^{b}	6.3 ± 0.7^{d}	7.5 ± 0.8^{de}	9.9 ± 0.5^{c}	12.5 ± 0.1^{c}	12.6 ± 0.1^{e}	13.1 ± 0.7^{c}
Maltooctaose		6.2 ± 0.1^{c}				13.9 ± 0.1^{d}		15.5 ± 1.1^{d}

[1] Means ± SD (n=3); values followed by the same letter (column) are not significantly different ($p \leq 0.05$).
[2] Oligomers were incorporated at a ratio of 1:0.5:1.5 (w/w) for starch:oligomer:water mixtures.

Appendix VI. The effect of glucose disaccharides (of different glucosidic linkage) on the retrogradation (ΔH) of waxy maize starch gels (40% w/w) stored at 6°C.

Sugar[2]	Transition Enthalpy (J/g)[1]							
	9 h	12 h	18 h	24 h	48 h	72 h	96 h	144 h
Control	0.5 ± 0.2^{a}	1.6 ± 0.1^{cd}	3.7 ± 0.4^{c}	6.5 ± 0.8^{d}	9.7 ± 0.3^{d}	11.7 ± 0.1^{d}	12.0 ± 0.1^{c}	12.5 ± 0.2^{c}
Cellobiose (β 1→4)	0.3 ± 0.1^{a}	0.8 ± 0.2^{b}	1.1 ± 0.1^{a}	3.9 ± 0.2^{b}	5.8 ± 0.5^{a}	7.0 ± 0.7^{a}	8.9 ± 0.4^{a}	9.6 ± 0.2^{a}
Trehalose (α 1→1)	0.2 ± 0.1^{a}	0.4 ± 0.1^{a}	0.9 ± 0.3^{a}	2.8 ± 0.2^{a}	5.5 ± 0.4^{a}	8.9 ± 0.4^{b}	9.5 ± 0.3^{ab}	11.0 ± 0.7^{b}
Maltose (α 1→4)	0.3 ± 0.1^{a}	1.3 ± 0.1^{bc}	1.5 ± 0.1^{a}	5.2 ± 0.2^{c}	5.9 ± 0.4^{a}	8.6 ± 0.3^{b}	10.1 ± 0.7^{abc}	11.5 ± 0.1^{bc}
Gentiobiose (β 1→6)	1.2 ± 0.2^{b}	1.9 ± 0.4^{d}	2.9 ± 0.5^{b}	5.6 ± 0.6^{c}	7.4 ± 0.5^{b}	10.3 ± 0.3^{c}	10.1 ± 1.9^{b}	10.6 ± 0.8^{ab}
Isomaltose (α 1→6)	2.0 ± 0.6^{c}	2.8 ± 0.3^{e}	5.5 ± 0.4^{d}	7.8 ± 0.1^{e}	8.7 ± 0.1^{c}	9.3 ± 0.2^{b}	11.2 ± 1.6^{bc}	11.3 ± 0.2^{b}

[1] Means ± SD (n=3); values followed by the same letter (column) are not significantly different ($p \leq 0.05$).
[2] Sugars were incorporated at a ratio of 1:0.5:1.5 (w/w) for starch:sugar:water mixtures.

Appendix VII. A comparison of the effect of sugars and their respective sugar alcohols on retrogradation (ΔH) of waxy maize starch gels (40% w/w) stored at 6°C.

Polyol[2]	Transition Enthalpy (J/g)[1]							
	9 h	12 h	18 h	24 h	48 h	72 h	96 h	144 h
Control	0.5±0.2	1.6±0.1	3.7±0.4	6.5±0.8	9.7±0.3	11.7±0.1	12.0±0.1	12.5±0.2
Glucose	1.9±0.1[a]	4.1±0.6[a]	6.5±0.5[a]	9.8±0.1[a]	11.3±0.5[a]	11.9±0.5[a]	12.4±0.2[a]	13.1±0.1[a]
Sorbitol	2.3±0.1[b]	2.7±0.1[a]	4.6±0.2[b]	6.3±0.1[b]	9.3±0.6[b]	10.3±0.4[b]	11.4±0.1[b]	12.0±0.4[b]
Maltose	0.3±0.1[a]	1.3±0.1[a]	1.5±0.1[a]	5.2±0.2[a]	5.9±0.4[a]	8.6±0.7[a]	10.2±0.2[a]	11.5±0.1[a]
Maltitol	1.3±0.5[a]	1.9±0.1[b]	3.8±0.1[b]	5.5±0.4[b]	7.3±0.3[a]	8.7±0.4[b]	9.6±0.6[a]	10.4±0.8[a]
Lactose	0.8±0.2[a]	1.9±0.3[a]	2.4±0.6[a]	5.2±0.4[a]	6.9±0.2[a]	8.2±0.3[a]	9.9±0.4[a]	9.6±0.5[a]
Lactitol	1.8±0.7[a]	3.1±0.2[b]	3.3±0.3[a]	6.3±0.1[a]	7.5±0.2[b]	8.1±0.3[a]	9.1±0.4[a]	9.4±0.3[a]
Glyceraldehyde[3]	0.0[a]	0.0[a]	0.0[a]	0.0[a]	0.0[a]	0.0[a]	0.0[a]	0.0[a]
Glycerol	0.6±0.1[b]	2.1±0.1[b]	2.2±0.2[b]	4.4±0.5[b]	6.3±0.6[b]	8.2±0.4[b]	9.1±0.4[b]	10.2±0.1[b]

[1] Means ± SD (n=3); values followed by the same letter (column) are not significantly different ($p \leq 0.05$).
[2] Polyols were incorporated at a ratio of 1:0.5:1.5 (w/w) for starch:polyol:water mixtures.
[3] There was no detectable retrogradation by DSC upon storage for 30 days at 6°C.

Appendix VIII. The effect of pentoses and hexoses on retrogradation (ΔH) of waxy maize starch gels (40% w/w) stored at 6°C.

Polyol[2]	Transition Enthalpy (J/g)[1]							
	9 h	12 h	18 h	24 h	48 h	72 h	96 h	144 h
Control	0.5 ± 0.2^{ab}	1.6 ± 0.1^{bc}	3.7 ± 0.4^{d}	6.5 ± 0.8^{c}	9.7 ± 0.3^{e}	11.7 ± 0.1^{f}	12.0 ± 0.1^{ef}	12.5 ± 0.2^{ef}
Ribose	0.8 ± 0.1^{b}	1.3 ± 0.1^{abc}	1.9 ± 0.1^{c}	2.3 ± 0.1^{a}	3.0 ± 0.1^{a}	4.0 ± 0.2^{a}	5.5 ± 0.2^{a}	6.3 ± 0.2^{a}
Xylose	0.3 ± 0.2^{ab}	0.4 ± 0.1^{a}	1.2 ± 0.1^{a}	2.1 ± 0.6^{a}	4.9 ± 0.1^{b}	6.4 ± 0.6^{b}	7.3 ± 0.1^{b}	9.4 ± 0.3^{b}
Me β-D-xylp[3]	0.3 ± 0.1^{a}	0.3 ± 0.1^{a}	1.3 ± 0.2^{a}	2.3 ± 0.5^{a}	6.1 ± 0.2^{c}	8.5 ± 0.3^{c}	10.2 ± 0.9^{c}	11.1 ± 0.4^{c}
Arabinose	0.8 ± 0.3^{ab}	2.1 ± 0.2^{c}	3.3 ± 0.6^{d}	4.2 ± 0.6^{b}	8.2 ± 0.6^{d}	9.4 ± 0.9^{d}	10.3 ± 0.1^{c}	12.0 ± 0.2^{de}
Talose	0.4 ± 0.1^{ab}	0.7 ± 0.1^{ab}	1.1 ± 0.2^{a}	2.5 ± 0.5^{a}	4.8 ± 0.3^{b}	6.5 ± 0.3^{b}	7.2 ± 0.6^{b}	9.4 ± 0.3^{b}
Galactose	2.4 ± 0.1^{c}	6.1 ± 0.5^{e}	7.1 ± 0.2^{e}	7.7 ± 0.2^{d}	9.7 ± 0.2^{e}	10.4 ± 0.4^{e}	10.9 ± 0.1^{cd}	11.3 ± 0.2^{c}
3-O-me-D-glcp[4]	0.4 ± 0.1^{ab}	1.5 ± 0.1^{bc}	3.7 ± 0.4^{d}	6.9 ± 0.1^{cd}	9.7 ± 0.2^{e}	10.7 ± 0.1^{e}	11.3 ± 0.1^{de}	12.0 ± 0.2^{d}
Glucose	1.9 ± 0.1^{c}	4.1 ± 0.6^{d}	6.5 ± 0.1^{e}	9.8 ± 0.1^{e}	11.3 ± 0.5^{f}	11.9 ± 0.5^{f}	12.4 ± 0.2^{f}	13.1 ± 0.1^{fg}
Fructose	3.5 ± 0.6^{d}	5.7 ± 0.5^{e}	8.2 ± 0.1^{f}	8.9 ± 0.3^{f}	12.6 ± 0.3^{g}	13.7 ± 0.1^{g}	13.8 ± 0.8^{g}	13.8 ± 0.7^{g}

[1] Means ± SD (n=3); values followed by the same letter (column) are not significantly different ($p \leq 0.05$).
[2] Polyols were incorporated at a ratio of 1:0.5:1.5 (w/w) for starch:polyol:water mixtures.
[3] methyl β-D-xylopyranoside
[4] 3-O-methyl-D-glucopyranose