PART 5

Plant Biotechnology

9
Starch Retrogradation

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Introduction

Starch is a storage polysaccharide found in plant tissues in the form of granules. It forms a very important part of the human diet all over the world. The two main components of starch are the polysaccharides amylose and amylopectin, the ratio of which varies according to the botanical source of the starch (e.g. Fredriksson *et al.*, 1998) and can significantly alter the functional properties of the starch, as will be discussed later.

AMYLOSE

Amylose, the smaller in terms of molecular weight of the main two starch polysaccharides, is largely linear in configuration and is mainly made up of 1→4 linked α-D-glucopyranosyl units. It is now well known that this molecule also contains some α(1→6) bonds, making the molecule slightly branched. This was concluded from the incomplete hydrolysis of the amylose fraction by β-amylase (Takeda *et al.*, 1987). The number of chains found per amylose molecule depends on the botanical source, with wheat starch having ~2 and potato starch ~7 chains on average (Takeda *et al.*, 1984). The degree of polymerization (DP) of amylose will also vary depending on its botanical source, where values of 570 have been reported for wheat amylose, and much larger values of 4920 for potato amylose (Takeda *et al.*, 1984). In work carried out by Roger and Colonna (1996) on corn starch, it was concluded that the presence of the branches in the amylose did not significantly alter the solution behaviour, which was similar to that of linear chains.

An interesting feature of amylose is its ability to form molecular complexes with

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Abbreviations: CTAB, cetyltrimethylammonium bromide; DP, degree of polymerization; DSC, Differential Scanning Calorimetry; ESR, Electron Spin Resonance; FTIR, Fourier Transform Infrared; NMR, Nuclear Magnetic Resonance; RS, resistant starch; T_g, glass transition temperature; T_m, melting temperature; WAXS, Wide Angle X-ray Scattering; w/v, weight for volume; w/w, weight for weight.

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a variety of compounds, such as aliphatic alcohols, lipids, and iodine. This is the result of the ability of the amylose molecule to adopt a single helical conformation, creating an internal helical space where hydrophobic molecules, or hydrophobic side chains of molecules, can be located as ligands.

AMYLOPECTIN

Amylopectin is a much larger molecule than amylose, with a DP ranging between 3×10^5–3×10^6 (Zobel, 1988). It is also made up of α(1→4)-linked D-glucopyranosyl units; however, it additionally contains a significant amount of α(1→6) bonds, ~5–6% (Buleon *et al.*, 1998), making it a highly branched molecule.

The poly-α(1→4)glucopyranosyl chains in the amylopectin molecule are usually classified into three different chains: these are the A, B and C chains (Peat *et al.*, 1952). For every amylopectin molecule, there is only one C chain, the backbone of the molecule. This is the only chain in the molecule that has a reducing end. To the C chain are connected the B chains by an α(1→6)-linkage. The B chains are classed into short and long B chains, the latter having an average DP of ~48–73, depending on botanical source (Hizukuri, 1985). The B chains form the link between the A and C chains. Some inner B chains and the A chains are classified as short chains, with an average DP of ~16–20 (Hizukuri, 1985). Both short and long chain fractions vary in length, depending on the botanical source (Hizukuri, 1985, 1986).

In 1972, French proposed the 'cluster' model to describe the way in which the branched structure of amylopectin was found inside native starch granules, consisting of alternating crystalline and amorphous regions. He proposed that the clusters consisted of aggregates of double helices. A few years later, Robin *et al.* (1974, 1975) found that the A chains, with an average DP of 15, were more acid resistant and formed the crystalline part of the granules. They were organized into double helices, which had a length of 6 nm along the chain axis.

Starch retrogradation

The amorphous material obtained after gelatinization of starch and cooling is not in a state of equilibrium. Upon ageing of the sample, a process of reassociation and recrystallization of the polysaccharide chains occurs, known as retrogradation. This was first discovered in 1852 (Boussingault, 1852). During the retrogradation process, the two polysaccharides amylose and amylopectin play different roles.

AMYLOSE RETROGRADATION

Amylose retrogradation was found to be a fairly fast event relative to amylopectin retrogradation. Amylose solubles have been found to rapidly decrease as a result of retrogradation during the first 24 h of baking, and thereafter show hardly any change (Zobel and Kulp, 1996). Van Soest *et al.* (1994), who followed the retrogradation of potato starch–water systems (10% w/w), obtained similar insights. They observed a multi-stage retrogradation process, where the first stage involved the formation of helices and rapid formation of crystalline amylose regions. Amylopectin retrogradation was a much slower event.

Miles *et al.* (1985b), using a range of techniques, including viscometry, turbidity and x-ray diffraction, to study dilute amylose solutions (<10% starch w/w), found that if polymer–polymer entanglements occur at the C* concentration, at <C* precipitation was observed, whereas at >C* a gel was formed. The gel formation was linked to a phase-separated system, where the crystallinity gradually developed in the polymer rich phase.

Sarko and Wu (1978) on the other hand, proposed that amylose gelation may occur due to double helical 'gel junction zones' forming between molecules. This proposal was in agreement with results obtained by Gidley (1989) using NMR. He found that gelation was possible from 'dilute non-entangled' amylose solutions, and that phase separation did not need to precede it, therefore suggesting that a specific molecular interaction occurred during gelation. At very low concentrations (~<1% w/v), precipitation and crystalline B-type packing was observed, whereas in more concentrated amylose gels (10% w/v), the presence of B-type double helices and mobile amorphous single chains was observed. He proposed a model for the concentrated amylose gels to contain double-helical junction zones that were connected by amorphous single chains, the latter he described as being 'elastically active'.

Goodfellow and Wilson (1990) prepared a 10% w/w amylose gel, and followed its retrogradation with FTIR. They proposed a model for the processes occurring during the retrogradation of amylose. Directly after gelatinization, the amylose would be in the random coil formation. However, fairly rapidly, reordering would occur and double helices would form, creating a gel network, and this would occur simultaneously or before phase separation occurred. These helices could then aggregate together to form crystalline structures. They stated, however, that the slower process of crystallization could not be observed by FTIR with their amylose gels due to there still remaining disordered amorphous regions connected to the aggregated regions, and hence there would be no significant change to the range of bond energies.

Gidley and Bulpin (1989) showed that short chain lengths and low concentrations favoured precipitation of amylose, whereas for long chain lengths, extensive cross-linking occurred, which would lead to gelation. If, as Gidley (1989) proposed, the cross-linking mechanism was through double-helical junction zones then, if the length of a junction zone was significantly shorter than the total chain length, a single chain could take part in several separate junction zones to form a cross-linked network (Gidley and Bulpin, 1989). However, if the junction zone length was not significantly shorter than the total chain length, chain alignment would dominate, and if lateral aggregation followed, precipitation would eventually be the result (Gidley and Bulpin, 1989).

Gidley (1989) also showed, using x-ray diffraction, that longer chain length precipitated amyloses had much broader and less intense diffraction peaks than shorter chain length amyloses. This was possibly an indication of less extensive crystallization and/or smaller crystalline domains in the former samples (Gidley, 1989).

AMYLOPECTIN RETROGRADATION

Many studies have shown that the retrogradation of amylopectin occurs over a longer time period than that of amylose (Miles *et al.*, 1985a; Orford *et al.*, 1987; van

Soest *et al.*, 1994), probably due to the essentially linear structure of amylose, as opposed to amylopectin. Work carried out by Ring *et al.* (1987) suggested that, as in the native starch granule, the exterior amylopectin branched chains crystallized during retrogradation.

As opposed to amylose crystallization, Goodfellow and Wilson (1990) found that it was possible to monitor amylopectin crystallization with FTIR. They stated that this was due to its branched structure, as once the helices had formed, they were still connected to the main polymer chain through $\alpha(1\rightarrow6)$ branch points and, on aggregating to form crystals, the range of bond energies for the main chain and the segments at the branching points would decrease and hence be observed with FTIR. It was believed that, as with amylose retrogradation, there were two distinct stages involved in amylopectin retrogradation (Goodfellow and Wilson, 1990). The main difference between the retrogradation of the two polymers was that for amylopectin, due to its highly branched nature, the double helices that had formed were all connected to the same backbone of the polymer, and were in closer proximity. Therefore, even in concentrated systems, they would aggregate together to form crystals, unlike for amylose, as explained in the previous section.

Klucinec and Thompson (1999) suggested that the $\alpha(1\rightarrow6)$ branch point may aid in keeping two amylopectin chains close together and align them to form a double helix. Additionally, Eliasson *et al.* (1987) found that the length of the linkage formed by the $\alpha(1\rightarrow6)$ branch point was very similar to the van der Waals distance formed between double helices. Imberty and Perez (1989) have also shown, by using the α-D-glucosylmaltotriose molecule as a model, that the stereochemistry of the $\alpha(1\rightarrow6)$ branch point in amylopectin and the two chains connected by it is such that it can coincide with the stereochemistry of a stable double helix, where the distortion to the molecule is nominal.

The resulting partially crystalline structure can best be conceptualized using the 'fringed micelle' model, used by Levine and Slade (1988), which they adapted from partially crystalline synthetic polymer science. The model shows that amorphous starch regions surround the starch crystals; the former part of the system contains the water that acts as a plasticizer. Within the 'fringed micelle' model, it is possible for one polymer chain to have numerous helical and random coil regions situated in various crystallite and amorphous regions respectively.

Another approach that is often used to describe retrograded starch is the chain-folded polymer model, where the theory to describe their crystallization kinetics was developed for synthetic polymers by Lauritzen and Hoffman (1973). In chain-folded polymers, the crystalline regions are made up of lamellae, where the chains are placed in adjacent positions, perpendicular to the surface of the lamellae, and they then fold back on themselves. Although retrograded starch is not strictly a chain-folded polymer, the chain-folded model can be used to describe it, where the double helices will form the crystallites and the branching regions make up the 'folds' (Farhat *et al.*, 2000b).

Miles *et al.* (1985a) found that in dilute gels the amylopectin crystallinity was reversible <100°C, whereas this was not the case for the amylose where the temperature was >100°C. They suggested that the smaller amylopectin chains were attached as clusters onto the longer chains; however, inter-chain association could only occur

over DP 15 chains before being interrupted by a branch point, as opposed to amylose. Amylose is essentially linear, and therefore it could form longer, and thus more stable, inter-chain associations. This was later confirmed by the work of Ring *et al.* (1987), who believed that the inter-chain association in amylopectin involved DP ~15 chain segments, whereas in amylose these segments were much longer with DP ~50. However, even longer chain segments of <100 residues have also been suggested for amylose (Gidley and Bulpin, 1989).

AMYLOSE AND AMYLOPECTIN CO-CRYSTALLIZATION

Several researchers (Miles *et al.*, 1985a; Russell, 1987; Leloup *et al.*, 1991) proposed that there could be co-crystallization of amylose with amylopectin during retrogradation. This has already been suggested by Blanshard (1987) when looking at the native starch granule. It was confirmed with DSC results obtained by Gudmundsson and Eliasson (1990) when studying amylopectin retrogradation, although they observed an interaction when the amylopectin content was below 50% (w/w). Above 50%, there was no significant affect by the amylose on the retrogradation melting enthalpy.

Upon comparing the retrogradation rates of potato and waxy maize starch using FTIR, van Soest *et al.* (1994) suggested that one of the reasons for potato starch retrograding more rapidly than waxy maize in their study was due to potato starch containing amylose, which could form an ordered matrix and act as seed nuclei for the amylopectin.

Studies of maltodextrin/amylose and amylose/amylopectin mixtures using low resolution ^{1}H NMR and DSC led Schierbaum *et al.* (1992) to propose that the outer chains of amylopectin molecules could interact with amylose to form a network.

There would be two possible ways in which mixed junction zones could form between the two different polysaccharides (Klucinec and Thompson, 1999). The first type of junction zone is where a double helix is composed of a chain from each polysaccharide, although this type of junction zone would be disadvantageous from a kinetic standpoint, as both chains must come together, and this is not as easy as if they were both linked by an $\alpha(1\rightarrow6)$ link, as in amylopectin (Klucinec and Thompson, 1999). The second type of junction zone is where the crystal unit cell is composed of both amylose and amylopectin double helices, and would depend on the proximity of the double helices from the two polysaccharides, and also the flexibility with which the double helices are connected to their respective molecules (Klucinec and Thompson, 1999). These theories brought forward by Klucinec and Thompson could explain why Gudmundsson and Eliasson (1990) only observed an interaction when the amylopectin content was below 50% w/w, as this could increase the probability of the two polysaccharides coming into proximity.

Klucinec and Thompson (1999) believed that data collected by small-angle dynamic oscillatory rheometry and DSC of high amylose starch gels indicated the existence of amylose–amylopectin interactions. Further evidence for the formation of junction zones between the two polysaccharides has been shown, and the importance of the nature of the amylopectin molecule, e.g. size and external chain length, as well as the ratio of amylose to amylopectin in a gel, was emphasized (Klucinec and Thompson, 2002).

STARCH CRYSTALLINE STRUCTURE

There are two different types of crystals that can form when the amylose or amylopectin double helices aggregate together. These are known as the A- and B-type crystal polymorphs. The key differences between these two polymorphs are the way in which their double helices are arranged together, and the amount of water held inside the crystals. This has been studied in depth by many researchers but the most recent models by Imberty *et al.* (Imberty and Perez, 1988; Imberty *et al.*, 1988, 1991) have been widely accepted. Both crystal unit cells contain left-handed, parallel stranded, double helices made up of 12 glucose units. However, the A-type crystal is arranged into a monoclinic unit cell containing 4 water molecules, whereas the B-type crystal is arranged into a hexagonal unit cell containing 36 water molecules. Generally, native cereal starches contain the A-type crystal polymorph, whereas native tuber starches contain the B-type polymorph. It is also possible to find the C-type polymorph, now acknowledged to be a mixture of the A- and B-polymorphs (Sarko and Wu, 1978), as is often the case for native legume starches and was demonstrated for pea starch (Bogracheva *et al.*, 1998).

STARCH–LIPID INTERACTIONS

Amylose–lipid complex formation

Starches will contain a certain amount of lipid. In cereal starches, this is mainly in the form of free fatty acids and lysophospholipids (Buleon *et al.*, 1998). The lipid content will vary significantly depending on the botanical source of the starch (Swinkels, 1985). These lipids have the ability to form an amylose–lipid complex. The complex of amylose and monoglyceride was studied by Carlson *et al.* (1979) and involved the hydrophobic 'tail' of a lipid molecule entering within the internal helical space of a single amylose helix and the hydrophilic 'head' of the lipid remaining outside the helix. The complex formed with amylose and lipids usually contains three turns of the amylose helix, each composed of six glucose units. However, if bulkier complexing agents are present, seven or eight glucose units may be present per helix turn, as reviewed by French and Murphy (1977).

Godet *et al.* (1993) have shown, using molecular modelling, that for amylose–fatty acid complexes the polar 'head' was located near the entrance to the amylose helix; however, it could not be placed inside as a result of steric and electrostatic repulsions. Therefore, in longer amylose chains, when more than two fatty acid molecules can complex with a single amylose molecule, the polar 'heads' of the fatty acid molecules are likely to introduce distortions into the amylose chain (Godet *et al.*, 1993).

It has been difficult to determine whether the amylose–lipid complex exists in native starches or whether they are formed upon heating above the gelatinization temperature (Kugimiya *et al.*, 1980). Evans (1986) and Blanshard (1987) suggested that the amylose–lipid complex already existed within the native granule. This has now been shown in the case of several native starches (Morrison *et al.*, 1993a,b); however, the complex was not believed to exist in crystalline packing, as the V-type diffraction pattern could not be observed for native starches (French, 1984).

Le Bail *et al.* (1999) monitored the crystallization of amylose–lipid complexes with synchrotron x-ray diffraction, showing that it formed upon heating of the samples. They also found that in low moisture content maize starch samples, crystallization of amylose–lipid complex occurred before complete melting of the native crystalline A-type structure. They suggested that during heating some of the individual complexes already present in the native starch would aggregate together to form crystals.

Amylopectin–lipid complex formation

The interaction of amylose with lipids is well established; however, the possibility of amylopectin forming a complex was only discovered more recently, and from earlier work carried out, it was often deduced that amylopectin could not form complexes at all (Kugimiya *et al.*, 1980). Indirect evidence of the presence of the amylopectin–lipid complex was found (Evans, 1986; Hahn and Hood, 1987), and Biliaderis and Vaughan (1987) suggested the possibility of complex formation, using electron spin resonance (ESR), of amylopectin with labelled fatty acids.

Eliasson and Ljunger (1988) have examined the interactions between amylopectin, in the form of waxy maize starch and that obtained from potato starch, with lipid additives. The results indicated that the interactions between amylopectin and lipids were similar to those of amylose and lipids, which was also found in later work carried out by Gudmundsson and Eliasson (1990).

However, on comparing the complexing ability of amylose to that of amylopectin, it was found that amylose had the better ability and would be the first to form the complex (Gudmundsson and Eliasson, 1990). This was also suggested by Pearce *et al.* (1987), who proposed that both amylose and amylopectin could complex with stearic acid, yet the longer linear sections of amylose were more effective than the shorter sections of the amylopectin molecule.

Effect of starch–lipid complexes on starch retrogradation

Starch–lipid complex formation is a well-known occurrence in the food industry; however, its effect on starch retrogradation is less well understood. Gudmundsson and Eliasson (1990) suggested that added surfactants/emulsifiers would directly complex with amylopectin, thus decreasing the melting enthalpy measured by DSC. The amylopectin–lipid complex would obstruct the growth of the amylopectin crystal (Eliasson and Larsson, 1993), and hence result in a decrease in retrogradation.

Krog *et al.* (1989) looked at the effect of monoglycerides on the retrogradation of wheat bread. They found that the monoglycerides mainly interact with free amylose, but when adding >1%, all the free amylose will form a complex and there will be an increased interaction with the amylopectin fraction, resulting in a decrease in the retrogradation and a decrease in the firmness measured of the bread on ageing.

Gudmundsson (1992) found that on adding cetyltrimethylammonium bromide (CTAB)–amylose complex to waxy maize starch and heating them to gelatinize the starch, but leaving the CTAB–amylose complex intact, did not significantly decrease the retrogradation, which was monitored as a change in the melting enthalpy by DSC. Yet, if the same experiment was repeated but the complex was melted out,

the retrogradation of the starch was decreased. These results pointed towards a direct interaction between the CTAB and amylopectin.

Others (Conde-Petit and Escher, 1994) have shown that for high concentration starch gels gelatinized with the addition of emulsifiers in closed moulds (i.e. low shear conditions), the gelation and ageing process of the amylose fraction was changed, as compared to the uncomplexed sample. They investigated the changes with uniaxial compression tests. The amylose–lipid complex initially resulted in an accelerated gelation process of the amylose, as compared to the uncomplexed sample. However, upon storage, the retrogradation of the complexed amylose was not possible, resulting in a weakened cohesion between starch granules rich in amylopectin, and that this was the main reason for the anti-firming effect observed. From their DSC data, amylopectin–lipid interaction did not appear to be a significant factor.

Work carried out by Becker *et al.* (2001) showed that the presence of amylose–lipid complexes in granular starch particles played an important role in restricting the water penetration and starch granule swelling during thermal conversion without shear. Therefore, for samples prepared under low shear conditions, firming of the sample could be decreased due to inhibition of amylose leaching from the granules.

From these examples, it is accepted that the presence of the starch–lipid complex can affect the retrogradation of the sample. Results have shown that it could have a direct effect on the amount of retrograded amylopectin, as would be measured from the DSC melting enthalpy, or it could be affecting the network structure formed between the amylose and lipids.

EFFECT OF TEMPERATURE AND MOISTURE CONTENT ON STARCH RETROGRADATION

The effects of both moisture content and temperature on the rate of retrogradation have been reviewed in detail by Slade and Levine (1991). Many of the theories that have been developed in synthetic polymer science have also been applied to biopolymers. The theory developed to describe the growth of chain-folded synthetic polymer crystals (Lauritzen and Hoffman, 1973) was later used to describe the kinetics of starch retrogradation (Marsh and Blanshard, 1988; Farhat and Blanshard, 2001).

Effect of temperature

Temperature has a very significant impact on the retrogradation rate of starch, as is shown in *Figure 9.1*, due to its effects on the nucleation and growth steps. Below the glass transition temperature (T_g), nucleation is unlikely to occur due to the extremely high viscosity of the system (Marsh and Blanshard, 1988), making molecular mobility difficult. As the sample is heated and enters into the rubbery state, the dramatic decrease in viscosity that occurs results in a dramatic increase in the rate of nucleation. However, as the temperature increases further, the rate of nucleation decreases, due to the nuclei melting out and the critical nucleus size increasing (Marsh and Blanshard, 1988). Yet at the same time, due to the temperature increase, the mobility of the molecules increases, leading to an increase in the

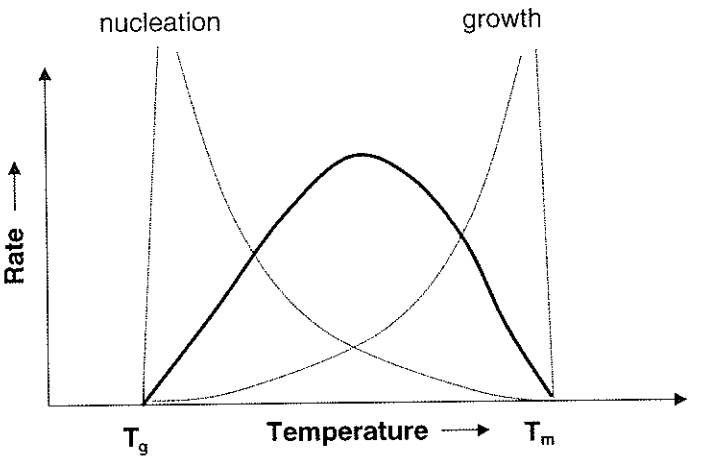

Figure 9.1. Crystallization kinetics of amorphous and partially crystalline polymers (adapted from Levine and Slade, 1989).

rate of growth. Both nucleation and growth will no longer occur above the melting temperature (T_m). The net result of both nucleation and growth on the rate of crystallization is that it has a bell-shaped dependency on temperature, where the maximum occurs approximately halfway between T_g and T_m.

Effect of moisture content

Moisture content is an important parameter for determining the rate of retrogradation. Water, being a relatively small molecule, can act as an effective plasticizer for starchy materials, as shown by several researchers (Orford *et al.*, 1989; Kalichevsky and Blanshard, 1993; Farhat *et al.*, 2000b) and reviewed by Levine and Slade (1988). The plasticizer can separate the polymer chains from each other, thus making reptation easier (Sperling, 1986). By adding water to a system, its T_g and T_m will decrease. However, whether an increase in the moisture content of the system will

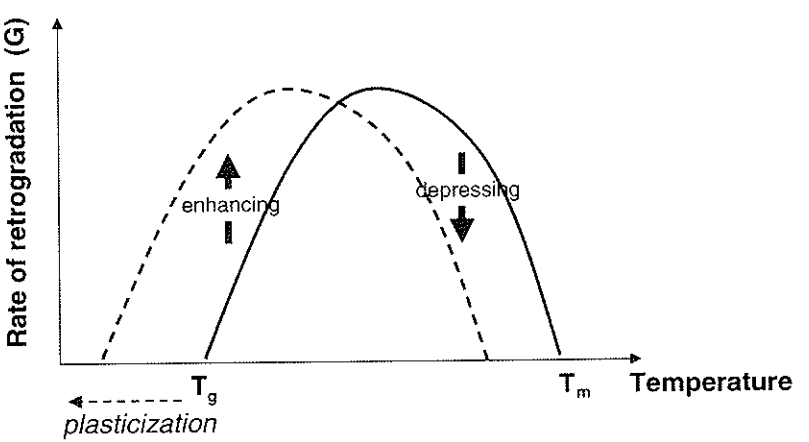

Figure 9.2. Effect of plasticizer on the crystallization kinetics of partially crystalline polymers (adapted from Farhat *et al.*, 2000a).

result in an increased rate of retrogradation will depend on where precisely the sample is located on the bell-shaped curve of crystallization rate versus temperature, as shown in *Figure 9.2*. The assumption is made that a sample was obtained that was located on the solid black curve. If the sample was located to the left of the maximum crystallization rate, then adding a small amount of plasticizer to that system would enhance the retrogradation rate, whereas if the sample had been located to the right of the maximum, the addition of plasticizer would lead to a depression of the retrogradation rate. Therefore, it is not possible, without knowing the storage temperature relative to T_g and T_m of a sample, to draw a conclusion as to what the effect of added plasticizer would be on the rate of retrogradation.

Combined effect of temperature and moisture content on crystalline structure

It is a well-known fact that the crystalline structure of the retrograded material is not necessarily the same as that found in the native granule from which the gelatinized starch was obtained. This was observed back in the early 20th century by Katz (1934), who observed that the native granule structure for wheat starch was different to that obtained upon retrograding bread. At a later stage, work on linear sweet potato amylodextrin (Hizukuri, 1961) and wheat starch (Marsh, 1986) showed that the moisture content and storage temperature conditions affect the type of starch crystal polymorph formed upon retrogradation. The results reported by Marsh (1986) for wheat starch are summarized in *Figure 9.3*. At higher storage temperature and lower moisture content conditions, the A-type starch crystal would form preferentially. However, at low temperature and high moisture content conditions, the

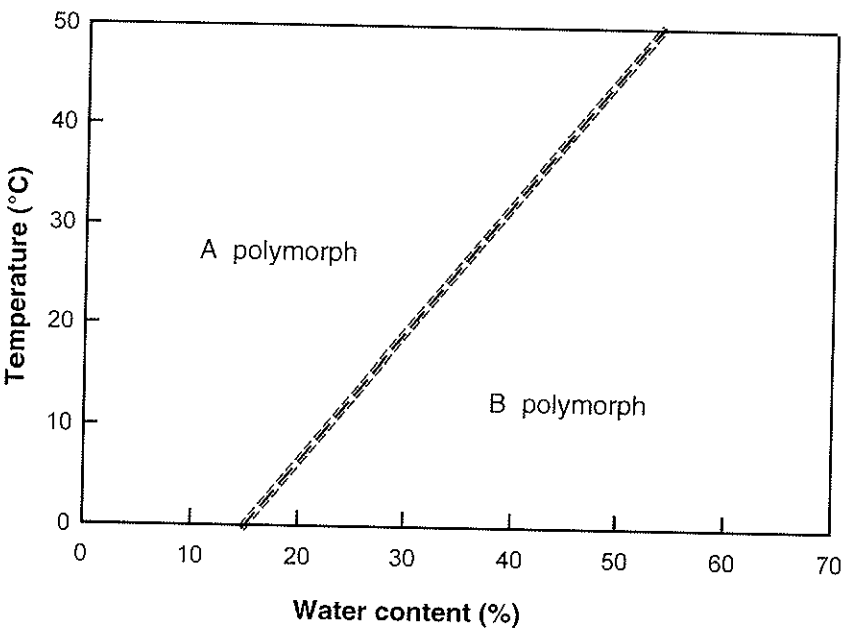

Figure 9.3. Dependence of the wheat starch crystal polymorph on the storage temperature and moisture content conditions during retrogradation (adapted from Marsh, 1986).

B-type crystal polymorph would be favoured. Towards the A/B boundary region, mixtures of A and B are usually obtained, giving WAXS diffractograms intermediate between that of A and B, sometimes referred to as the C-type.

Impact of retrogradation on scientific and commercial issues

In the early 20th century, Katz (1928) investigated the problem of retrogradation with respect to bread, as it was a problem in Holland at the time. The bakers had to carry out night work in order to avoid product loss through staling. Even nowadays, there continues to be product loss due to retrogradation, as it can lead to a dramatic increase in the hardness of baked goods, making them unattractive to consumers. Hence, for several baked products, such as baguettes, starch retrogradation is a greater problem for limiting the shelf-life of the product than microbial stability.

However, the effects of starch retrogradation are not always negative, as in some products these can be desirable, such as in the manufacture of croutons and breadcrumbs. Additionally, over the past twenty years there has been increasing interest in resistant type starches and their potential health benefits. The precise physiological effects of these starches on the body are still of research interest. It was found that, as resistant type starches are not digested by enzymes, they are fermented in the intestine, the fermentation of which results in the production of short chain fatty acids (e.g. Goni *et al.*, 2000) that are believed to be beneficial to health and decrease the risk of colonic cancer (Vanmunster and Nagengast, 1993). There are at least four groups of resistant starches. Raw ungelatinized starch is termed as resistant starch type 2 (RS2), whereas retrograded starch is termed as type 3 (RS3). It is often stated that RS3 is mainly due to amylose; however, amylopectin retrogradation can significantly increase the amount of resistant starch (Eerlingen *et al.*, 1994; Farhat *et al.*, 2001). Resistant starches are important as they can yield foods with greater nutritional quality, such as muesli bars, which contain only partially milled cereals.

For all the reasons mentioned above, it is important to gain a deeper scientific understanding of the events occurring during retrogradation, and the factors affecting it, in order to be able to control the process.

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