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Effect of Enzymatic Treatments on the Physiochemical Properties of Different Corn Starches

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ABSTRACT

Amylose readily reassociates to form films and crystalline structures that are resistant to digestion by amylolytic enzymes and known as resistant starch type III (RS3). This study investigated the RS3 formation and cereal coating properties from enzyme-modified corn starches with varying amylose contents, including Hylon VII (70% amylose), Hylon V (50% amylose), and common corn (25% amylose). For RS3 formation, corn starches were first gelatinized and then hydrolyzed using β-amylase to varying degrees. The resultant hydrolyzed starch was debranched with isoamylase and then exposed to 3 times of temperature cycling at 135/133/133°C for 30 min and 95°C for 24 hr to promote RS3 formation. For cereal coating applications, corn starches were gelatinized and debranched, and then sprayed onto ready-to-eat breakfast cereal flakes. The proportions of amylose and amylopectin long and short chains were affected by the β-amylase treatment and varied with starch type. All three corn starches had increased RS contents after moderate β-amylolysis with Hylon V having the highest RS content at 70.7% after 4 hr of β-amylolysis. The RS content was positively correlated with amylose and amylopectin long chains, but negatively correlated with amylopectin short chains. A starch film of 50-130 μm was observed with scanning electron microscopy on the surface of the cereals coated with Hylon VII. After soaking in milk for 3 min, the peak force of the cereals coated with corn starches were higher than those of the controls. The cereals coated with Hylon VII were found to have an increase in dietary fiber content. The results suggest that RS3 formation is affected by starch composition as well as starch structure and can be increased by moderate β-amylolysis. Debranched amylose-containing corn starches could be used as cereal coatings to extend the bowl-life of ready-to-eat cereals.

This thesis is approved for recommendation
to the Graduate Council.

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CHAPTER 1

GENERAL INTRODUCTION

The starch that passes through the small intestine intact is known as resistant starch because of its ability to "resist" enzymatic hydrolysis. Resistant starch is fermented by the microflora native to the large intestine and has shown many health benefits such as lowering cholesterol, lowering glycemic index values of foods, and anti-carcinogenic effects.

Resistant starch is classified into four major categories by the mechanism in which they are resistant to digestion. Resistant starch type I (RS1) is physically inaccessible starch, type II (RS2) is native granular starch, type III (RS3) is retrograded starch, and type IV (RS4) is chemically modified starch. RS3 is primarily composed of retrograded amylose because of its strong tendency to reassociate. Therefore, amylose content is a main factor governing the formation of RS3. There are several ways to further increase RS3 formation in starch, such as debranching of starch to result in all linear glucans and temperature cycling.

Alternative cereal coatings have been sought to replace traditional sugar coatings due to waning consumer acceptance of added dietary sugar. Starch films form through the reassociation of starch molecules, a mechanism shared by the formation of RS3. One problem with starch films is that gelatinized starch dispersions tend to be viscous, thus forming thick films and trapping air bubbles, both of which are not desirable. Debranching of starch would decrease the viscosity of starch dispersion for ease of film formation and at the same time enhance reassociation and formation of crystalline structures.

CHAPTER 2

LITERATURE REVIEW

Starch Composition and Structure

Starch is a naturally abundant biopolymer made up of repeating D-anhydroglucose units (AGU) linked via glycosidic linkages that can be either α-(1-4) or α-(1-6) in nature. These different linkages give rise to two distinct types of molecules, amylose and amylopectin, and their structure and proportions vary with botanical source.

Starch is present in many staple foods across the world such as corn, rice, potatoes, and wheat. Furthermore, starch is the main ingredient in some of the most widely consumed prepared foods such as pasta, bread, and tortillas. Starch is also widely used in the food industry to provide binding, thickening, gelling, and other properties. Starch is a diverse ingredient that can be modified in many ways to give a multitude of functions leading to use in a variety of applications.

Besides the branch density, the number of glucose monomers that make up a starch molecule is also important to its properties. The size of starch molecules is often measured in degrees of polymerization (DP), in reference to the number of glucose monomers in an individual starch molecule. In nature, starch molecules, i.e. amylose and amylopectin, do not exist in loose agglomeration, but are packed into a very organized structure known as granule.

Granule Structure

Starch granules are semi-crystalline because they contain alternating amorphous and crystalline regions (Gallant and others 1997). The crystalline regions are composed of radially oriented amylopectin molecules that form crystalline structures through double helix formation

of branch chains, and possibly intertwined with amylose molecules (Gidley and Bociek 1985). The amorphous regions are comprised of amylose and amylopectin branch points that do not align in an organized fashion (Robin and others 1974). These two distinct regions alternate, starting from the center, which is known as a hilum, to form the layers that make up the starch granule (Yamaguchi and others 1979). The location of amylose is not as well elucidated as amylopectin, but it is thought to be interspersed in both the crystalline and amorphous regions of the granule. Native starch has a crystallinity ranging from 15-45% (Zobel 1988). Studies have shown that the amylose content of starch increases closer to the surface of the granule and the average chain length of amylose molecules decreases towards the surface of the granule (Morrison and Gadan 1987). Starch granules have the ability to swell slightly in cold water but return to their original shape and size after drying.

Starch granule size and shape are affected by the botanical source. Tuber starches characteristically have larger, less dense granules than cereal starches. For example, corn starch granules range from 2-30 μm in diameter, while potato starches range in diameter from 5-100 μm (Robyt 1988). The size and shape of starch granules vary, even in starches from the same botanical source. Common starch granule shapes include spherical, lenticular, and dented (Tester and others 2004).

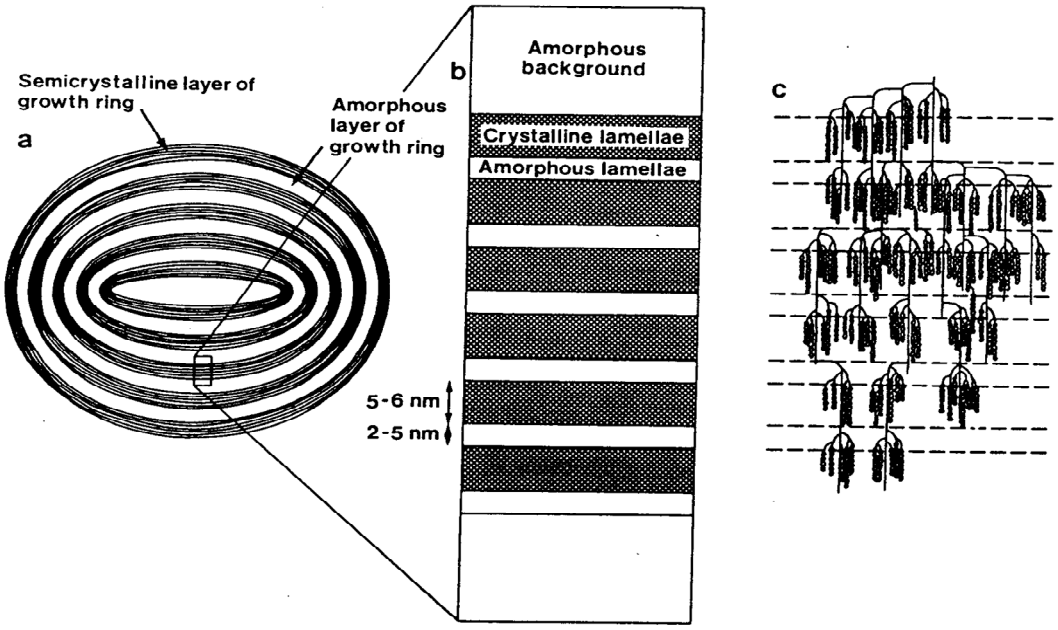

Figure 2.1. Organization and Structure of Starch Granules.

Starch granules contain trace amounts of proteins, lipids, and minerals. Tuber starches are characteristically very low in lipids. The lipid content of starch is correlated with its amylose content. High amylose corn starches have higher lipid contents than waxy corn starches, which consist exclusively of amylopectin (Tester and Morrison 1990). Because starch lipid content increases with increasing amylose content, the lipids are concentrated on the exterior of the granule where most amylose is located (Tester and Morrison 1990). Two primary types of lipids are found in starches, lysophospholipids and free fatty acids (Tester and others 2004). The type of lipid that is predominant in a starch is dependent on the botanical source.

Starch also contains varying amounts of proteins and minerals. Phosphorus is the most common mineral found in starches. In tuber starches, especially potato, phosphorus significantly impacts starch properties. Phosphorus is found in amylopectin at a much higher percentage than in amylose because of the ability of phosphorus to covalently bond to glucose units at, or near,

the branching point. These phosphorus groups can increase the viscosity and lower the gelatinization temperature of potato starch.

Proteins present in starch can form a variety of complexes with the starch depending on the nature of the protein. Starch-protein complexes can be formed by a variety of mechanisms such as hydrogen bonding, hydrophobic interactions, and electrostatic interactions (Damadaran and Paraf 1997). Proteins are often found bound to the surface of starch granules and have been reported to restrict granule swelling and improve the rigidity of swollen starch granules (Hamaker and Griffin 1993).

Amylose

Amylose is essentially linear and consists almost solely of α-D-(1,4) linked AGU with a very small quantity of branching points (Hizukuri and others 1981). Amylose of tuber starches, such as potato and tapioca, has a higher amount of branching points than that of cereal starches (Takeda and others 1987). Potato amylose was reported to average 9.8 chains per molecule, while corn amylose only has 2.9 chains per molecule (Takeda and others 1987).

Similar to granule size, the molecular size of amylose also varies with botanical source. Amylose from tuber starches has a DP of 1000-6000, while amylose from cereal starches is significantly smaller, having a DP of only 200-1200. Among the cereal starches, corn amylose has an average DP of 960, while wheat amylose averages DP 1290. High amylose corn starches have the smallest amylose molecules with an average DP of approximately 700 (Takeda and others 1989).

Amylose is a minor constituent in most starches making up only 20-30% of common starches. Amylose is known for its propensity to form double or single helices through hydrogen

bonding. In solution amylose is present in a random-coil state with a hydrophobic interior stabilized by hydrogen bonds. Amylose forms single helices when it wraps around another molecule of hydrophobic nature (Rundle and Edwards 1943). Hydrophobic molecules such as iodine, butyl alcohol, and fatty acids have been shown to complex with amylose (Rundle and French 1943; Bear 1944; Jane and Robyt 1984). These complexes are often referred to as V-complexes because of their unique V-type X ray diffraction pattern (Rundle and French 1943).

Amylopectin

Amylopectin is the main glucan that makes up most starches. Amylopectin is much larger than amylose and highly branched. These branching points are products of α-(1-6) linkages and are estimated to make up 4-5% of the total linkages in the molecule (Manners 1989). The size of amylopectin can reach a DP of over 2 million, making it one of the largest biomolecules in nature (Sajilata 2006). Because of its large, branching structure, amylopectin does not easily form highly organized structures after gelatinization, nevertheless amylopectin still re-associates but with a low stability.

The current model of amylopectin structure is the cluster model proposed by Hizukuri (1986). He used debranching enzymes and high performance size exclusion chromatography to well resolve amylopectin chains into a polymodal distribution with a tailing fraction, labeled as B4, B3, B2, B1, and A. Each of these chains has a distinctive chain length that confers it location within the amylopectin molecule. Of these chains, A is the shortest having a DP range of 12-16. The average chain lengths of fractions B1, B2, and B3 are DP 20-24, 42-48, and 69-75, respectively. The A and B1 fractions comprise a single cluster, whereas B2, B3, and B4 clusters span 2, 3, and 4 clusters, respectively (Hizukuri 1985).

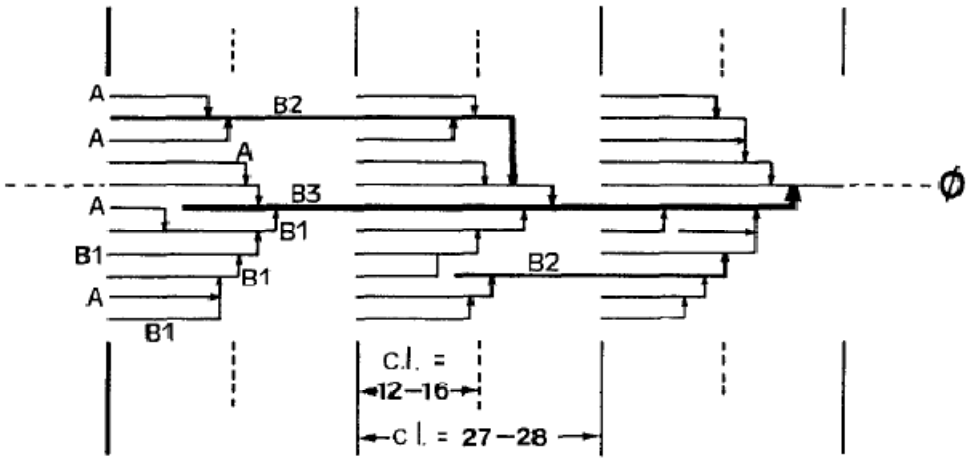

Figure 2.2. Amylopectin cluster model proposed by Hizukuri (1986).

Starch Properties

X-ray diffraction pattern

Because of the semi-crystalline structure, starch granules exhibit distinct X-ray diffraction patterns. Cereal starches give the A-type, and tuber starches give the B-type patterns (Gallant and others 1992). The C-type pattern can be seen in certain legume starches and is a combination of the A- and B-type patterns. The V-type pattern is seen in complexes of amylose and organic molecules such as linear alcohols, fatty acids, and iodine. B-type starches have been shown to have long amylopectin branch chains, while A-type starches have shorter branch chains (Hanashiro and others 1996). Starches with a B-type pattern have a higher percentage of long chains than A-type starches (Hizukuri 1985). Amylose double helices formed from temperature cycling have been shown to produce A-type or B-type patterns depending on the temperature used during processing (Eerlingen and others 1993; Zabar and others 2008). A-type and B-type patterns reflect the presence of double helices, and their difference lies in the packing of the

double helices. A-type starches contain more closely packed double helices than B-type starches (Ratnayake and others 2001).

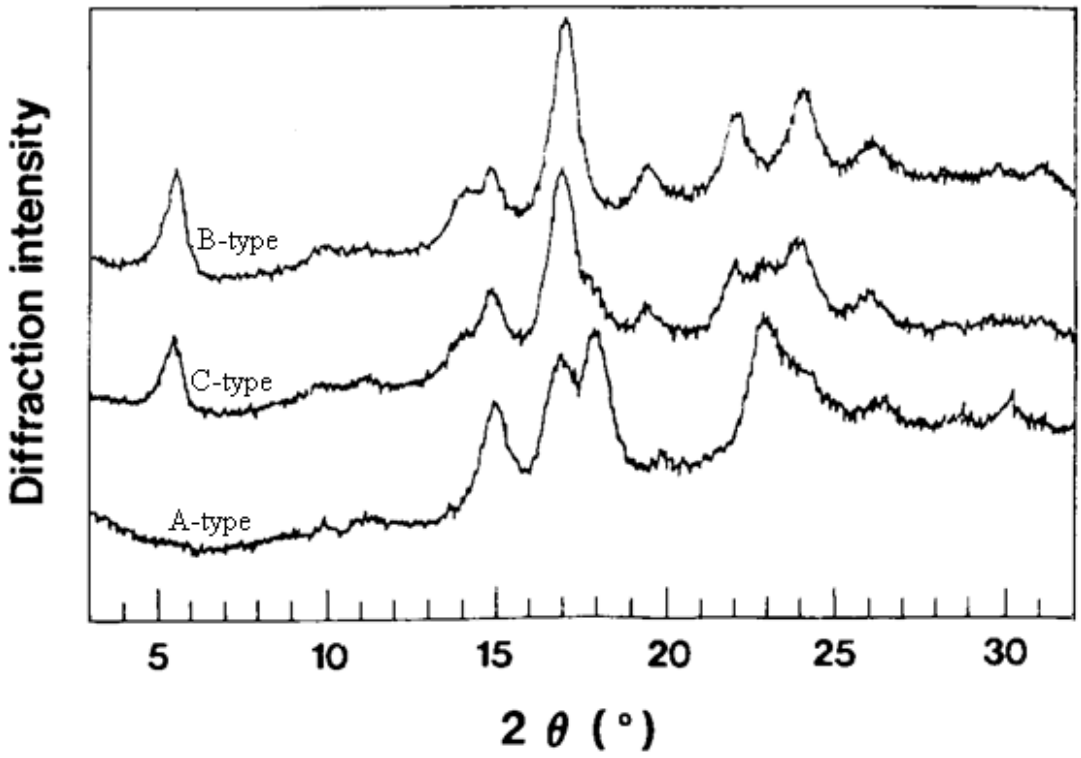

Figure 2.3. X-ray diffraction patterns of different types of starches.

Gelatinization

In its native granular form, starch is not soluble in cold water but reversibly swells to a limited extent. When heated in excess water, starch swells irreversibly and eventually loses its crystalline structure, which is known as gelatinization. Gelatinization of starch occurs in the preparation of almost all starchy foods, and gelatinized starch becomes more susceptible to hydrolysis by enzymes.

During the initial swelling some amylose begins to leach out of granules (Miller and others 1973). In cases of high heat and shear, the granules can be completely destroyed (Fannon and BeMiller 1992). In most food systems this level of granular disruption is never reached, and

the granule remnants, or granule ghosts, remain in the system (Hoseney and others 1977; Fannon and BeMiller 1992). The ability of starch to swell and produce a viscous paste when heated in water is very important for its functionality in food systems.

Retrogradation

When a starch paste is allowed to cool, the molecules begin to re-associate to a lower energy state (Atwell and others 1988), which is termed retrogradation. Retrogradation was first reported by Katz (1928) during the investigation into the cause of bread staling. The retrogradation of starch is often called crystallization, and the increase in crystallinity is often used to measure the extent of retrogradation of a starch paste or gel. The cooling of a starch paste has been reported to slowly form a B-type X-ray diffraction pattern, typical of native tuber starch (Rundel and others 1944). The rate of retrogradation depends on amylose to amylopectin ratio, chain length, starch concentration, temperature, and other substances present in the system. In general amylose retrogrades much faster than amylopectin (Jane and Robyst 1984). An optimum chain length of approximately 80-100 is reported to be ideal for retrogradation (Pfannemuller and Burchard 1969; Gidley 1989; Eerlingen and others 1993). Starch solutions containing 10-30% (w/v) have shown good retrogradation characteristics (Berry 1986). Temperature cycling has been shown to be the most efficient way of promoting retrogradation in starch solutions (Berry 1986). The presence of sugars has been shown to slow the pace of retrogradation because of its ability to interrupt the hydrogen bonds that stabilize amylose double helices (Kohyama and Nishinari 1991). Lipids are also known to retard retrogradation by forming a complex with amylose.

Resistant Starch

Resistant starch was first reported as a source of error in calculating total dietary fiber in foods (Englyst and others 1987). It was later shown that resistant starch functions nutritionally similar to dietary fiber (Asp 1992). Resistant starch is defined as the fraction of starch that escapes digestion in the stomach and small intestine (Englyst and others 1992). When resistant starch reaches the large intestine, it is fermented by microorganisms into an array of short chain fatty acids. Resistant starch is presently classified into four types according to the mechanism of digestive resistance. A fifth type of resistant starch, known as amylose-lipid complex, has been recently proposed.

Resistant Starch Type I (RS1)

RS1 refers to starch that escapes digestion because of physical inaccessibility. These starches are typically found in whole grains, seeds, and vegetables (Sajilata and others 2006). RS1 is often reduced in food products by milling and further mastication once consumed. The amount of RS1 is calculated by the difference between the glucose released by enzymatic depolymerization of the whole food and the glucose released from the homogenized whole food (Sajilata and others 2006).

Resistant Starch Type II (RS2)

RS2 refers to granular starch that is not susceptible to enzymatic attack because of its tightly packed crystalline structure. Native B-type starches are more resistant to enzymatic degradation than A-type or C-type starches. It has been reported that B-type starches contain a larger amount of branching points and short branch chains in their amorphous region than A-type

points and short branch chains in their crystalline regions (Jane and others 1997). The higher proportion of branching points and short branch chains in the crystalline layers of A-type granules makes the granule more susceptible to enzyme digestion. Evans and Thompson (2004) showed that native potato starch of B-type pattern had over 70% resistant starch. However, upon cooking the resistant starch content of the potato starch decreased to less than 1%. High amylose corn starch is also highly resistant to enzymatic degradation and has become the basis for the successful commercial resistant starch product, e.g. Novelose 240 (National Starch LLC., Bridgewater, N. J., U.S.A.). In the creation of Novelose 240 high amylose corn starch is heated in limited water in the presence of swelling inhibitor, usually in the form of an inorganic salt (Chiu and others 1999). This treatment keeps the constituents of starch granules from leaching out and allows the starch to retain its granular structure. The amylose and amylopectin re-associate upon cooling, thus increasing the resistant starch content of the starch.

Resistant Starch Type III (RS3)

RS3 is formed as a result of retrogradation. Retrograded starch is the most common resistant starch type in processed foods, and therefore is the most important from a nutritional and technological viewpoint (Garcia-Alonso and others 1999). Both amylopectin and amylose retrograde, but RS3 mainly consists of retrograded amylose because the branched structure of amylopectin is not ideal for reassociation. The reassociation of branch chains is sterically hindered by the branching points and shorter than the optimum length for resistant starch with high thermal stability. Conversely, amylose retrogrades easily because of its linear structure. Retrograded amylose is often easily characterized by its thermal properties. It has been widely reported that retrograded amylose has a distinct B-type pattern and an endotherm at 130 – 170^{o}C

(Eberstein 1980). Debranching of high-amylose corn starch has been used to create some of the first commercially available RS3 products (Henley and Chiu, 1995).

Processing methods, such as extrusion, have also been used to create RS3 (Faraj and others 2004; Hasjim and Jane 2009). Extrusion has benefits over batch cooking methods of creating RS3, such as less energy, time, and moisture needed (Guy 2001). Hasjim and Jane (2009) produced products of 29.8 % RS by combining acid hydrolysis with low-shear extrusion.

Resistant Starch Type IV (RS4)

Resistant starch IV is formed by chemical modifications, such as crosslinking and dextrinization (Seib and others 2001). Crosslinking raises the gelatinization temperature, allowing the granule to stay intact at higher temperatures. Dextrinization creates new linkages between starch molecules that are unable to be degraded by mammalian digestive enzymes. These modifications render the starch more resistant to amylolytic degradation because the modified starch molecule can no longer fit properly in the active site of the amylase (Woo 2010).

Resistant Starch Type V (RS5)

Recently amylose-lipid complex has been proposed as a new type of resistant starch. When lipids are present they can form complexes with the available amylose (Schoch and Williams 1944; Mikus and others 1946). These complexes are resistant to amylase attack, and therefore can be considered resistant starch (Larsson and Miezis 1979, Mercier and others 1980, Eliasson and Krog 1985, Guraya, and others 1997).

Mechanism of Amylose Retrogradation

Amylose forms double helices that give RS3 a very stable structure from extensive hydrogen bonding. The high thermal stability of retrograded amylose allows it to endure the thermal processing of most foods, which is of particular interest in food applications.

Unlike amylose V-complexes, the details of amylose crystalline structures and the mechanism in which they form are not well known. There are two proposed mechanisms for amylose aggregation: micelle formation and lamellae formation (Eerlingen and Delcour 1995). Both structures contain a resistant portion formed by amylose double helices and an amorphous region. The micelle structure is thought to be similar to amylose V-complexes, which are made of crystalline fibrils approximately 100 Å in length with amorphous regions interdispersed between them, and the amorphous region is composed of non-aggregated amylose (Jane and Robyt 1984). In the lamellar structure, the amylose double helices are folded on top of each other with a lamellar thickness of approximately 100 Å (Pfannemuller and Bauer-Carnap 1977), and the amorphous region is present at the folds of each amylose chain. Upon further retrogradation, amylose double helices are packed into hexagonal unit cell (Haralampu 2000).

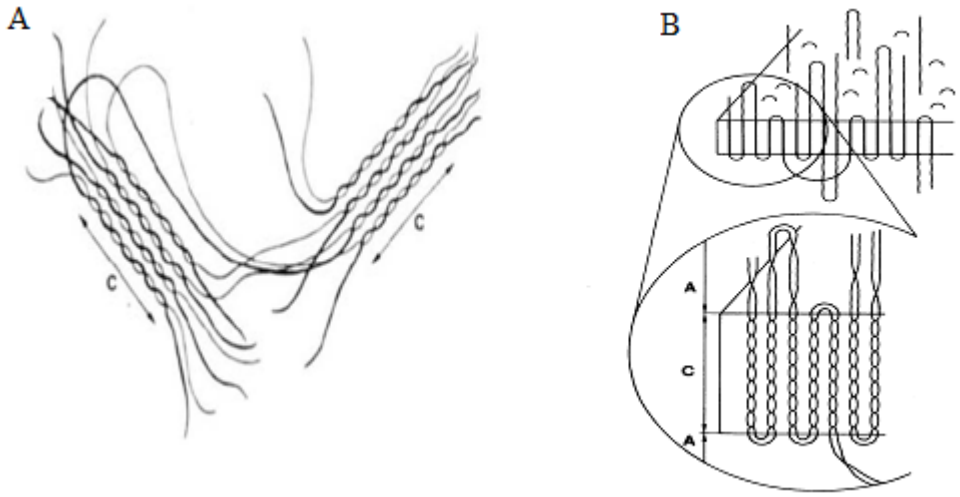

Figure 2.4. Proposed structures of retrograded amylose micelle (A) and lamella (B) (Eerlingen and others 1993).

Factors affecting RS3 Formation

Starch Source

Starches from a variety of backgrounds have been used to produce RS3, with high amylose corn starches showing the best results. Amylose, with its fundamentally linear structure, is much more prone to the formation of double helices. Amylopectin does not easily form resistant starch and is often enzymatically debranched to enhance the formation of resistant starch (Berry 1986). Waxy starches, after debranching, become essentially linear chains that are lower in molecular weight than the amylose chains from high amylose corn starches. These shorter amylose chains have also been used as a successful starting material for producing RS3 (Berry 1986; Cai and Shi 2010). Berry (1986) reported that waxy corn starch showed an increase in resistant starch content from 0.2% to 33.6% after debranching. For purified potato amylopectin, debranching increased resistant starch from 1.3% to 46.8%. Nevertheless, RS3 products from debranched amylopectin have a significantly lower thermal stability than RS3 products from longer chain glucans. For example, it has been reported that retrograded products from waxy corn have a melting temperature of 136^{o}C, while retrograded products form high amylose corn starches have a melting temperature above 150^{o}C (Ozturk and others 2009; Cai and Shi 2009).

Debranching Treatment

In the preparation of resistant starch, the significance of the branching α-(1-6) linkages is twofold. Primarily, the branch points create a steric hindrance to the reassociation of starch molecules. The second is the slower speed at which amylolytic brush border enzymes hydrolyze α-(1-6) bonds compared with α-(1-4) bonds (Pazur and others 1960, Pazur and Kleppe 1962).

Debranching is a valuable technique for the formation of RS3 and is usually accomplished with two main enzymes, isoamylase and pullulanase (Berry 1986, Ozturk and others 2009). The ability of debranched starch to form higher amounts of resistant starch than native starches lies within the lack of branching points to physically inhibit crystallization. The products from the debranching of various starches have unique chain lengths depending on the botanical source of the original molecule. Waxy starches produce short linear chains that have a narrow distribution of chain lengths. Other starches, such as high amylose corn starch, will produce a wide array of molecular-weight starch chains after debranching.

Amylose Chain length

There has been much debate on which chain lengths are ideal for the promotion of RS3 formation. Early studies found that amylose double helix formation was independent of the amylose chain length, provided the minimum length (DP 10) for double helix formation was met (Eerlingen 1993). Eerlingen and others (1993) stated that the optimum chain length for resistant starch formation was DP 100, while earlier amylose aggregation was shown to be maximized at a DP of approximately 80 (Pfannemuller and Bruchard 1969). Gidley and others (1995) showed that amylose aggregated into crystalline double helices when DP was between 10 and 100 glucose units. Guraya and others (2001) have hypothesized that shorter chain amyloses are more prone than longer chain amyloses to retrograde due to the propensity of large amylose molecules to form crosslinked networks that are not resistant to enzymatic degradation.

To obtain glucan chains of a specific length, various enzymatic treatments have been explored. The debranching of waxy starches has been used extensively to create short-chain amylose molecules. Robin and others (2008) reported longer chains of debranched potato

amylopectin produced more resistant starch than shorter chains fractionated from the same potato amylopectin. Recently, Cai and Shi (2010) reported that the longer chains produced from waxy potato starch, DP = 32.1, were more resistant to enzymatic degradation than the amylose chains produced from waxy corn and waxy wheat with an average DP of 24.1 and 21.8, respectively (Cai and Shi 2010). Conversely enzymes such as amylomaltase can be used to synthesize linear glucans chains of 20-35 glucose units capable of forming products upon retrogradation of up to 94% RS (Schmiedl 2000). The discrepancies regarding the role of chain length on resistant starch formation could be due to the amylose concentration in the systems being studied. The amylose concentration was 30% in Schmiedl (2000) in contrast to 0.5 – 1.0 % concentration in Gidley and others (1995). These results suggest that the optimum chain length for amylose aggregation may also be affected by amylose concentration.

Temperature

Temperature cycling has emerged as the predominant method of promoting RS3 formation. Sievert and Pomeranz (1990) showed that the temperature cycling of high amylose starch increases the resistant starch by approximately 15%. During temperature cycling a starch solution is heated, usually in an autoclave, to a very high temperature (>120^{o}C). After the autoclaving period the solution is stored at a specified temperature to promote retrogradation. In the autoclaving, less stable structures are melted, and the subsequent storage cycle would promote the formation of more stable structures.

Because retrogradation is a crystallization process, temperature plays an important role in RS3 formation. Different storage temperatures have been examined based on crystallization principles. Amylose retrogradation, like any other crystal formations, is guided by the principles

that guide any crystallization. Wunderlich (1976) outlined the three stages that crystals go though during their production (Figure 3). The first stage is nucleation, which is the formation of a nucleus. The second stage is propagation or the expansion of the nuclei. The third phase is the maturation, which in most cases refers to the slow growth or increasing of molecular order that occurs over time (Eerlingen and others 1993).

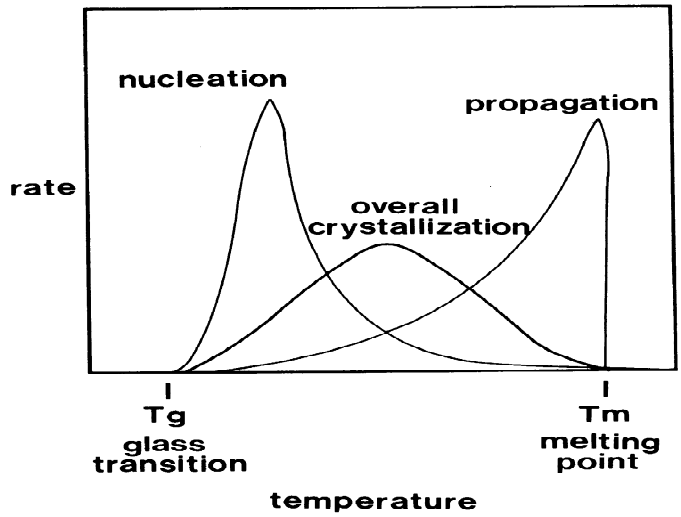

Figure 2.5. Effect of temperature on amylose recrystallization (Eerlingen and others 1993).

At lower temperatures, amylose has lower mobility than it would have at higher temperatures. This lowered mobility is the primary reason that crystal nucleation is favored at lower temperatures where amylose molecules are less mobile. Conversely, at high temperatures nucleation is not favored because of the large amount of energy in the system. Overall crystallization is favored at a central temperature between the glass transition temperature and the melting temperature. The optimum temperature for amylose reassociation has yet to be determined. Garcia-Alonso and others (1999) reported that storage at 60^{o}C produced more resistant starch than storage at 100^{o}C. However, recently Ozturk and others (2009) reported that debranched high amylose starches when stored at 95^{o}C produced 57.8% resistant starch

compared with 51.7% when stored at 4°C (2009). The starch source in Garcia-Alonso and others(1999) contained amylopectin, which provides insight into the lower optimum retrogradation temperature reported. Retrograded amylopectin melts at temperatures close to 60°C, therefore at storage temperatures above 60°C any retrograded amylopectin would have been melted. The research by Garcia-Alonso and others (1999) and Ozturk and others (2009), showing more retrogradation at middle to high temperatures, suggests that propagation may be the step of amylose reassociation that is most affected by temperature.

The autoclaving parameters have been shown to have a less pronounced effect on the final resistant starch content than the storage temperature of the starch (Sievert and Pomeranz 1989). Autoclaving at temperatures up to the melting temperature of crystalline amylose (> 140°C) have been used in the production of resistant starch. Berry (1986) observed a steady increase in resistant starch formation with an increase in autoclaving temperature from 100°C to 134°C. Sievert and Pomeranz (1989) showed that autoclaving high amylose corn starch at 134°C produced more resistant starch than autoclaving at 121°C or 148°C. These two studies show the optimum autoclaving temperature to be very close to 134°C.

The drying method also can impact the final RS content of starch. Different drying methods such as freeze drying, vacuum drying, oven drying have been used to dry resistant starch products (Berry 1986; Sievert and Pomeranz 1989; Escarpa and others 1996). Oven drying has been shown to be just as good and in some cases better than more complex drying methods in promoting the formation of resistant starch (Escarpa and others 1996).

Starch to Water Ratio

In temperature cycling starch slurry is prepared with a specified amount of water and starch. By varying the amount of each component, the retrogradation of amylose is affected.

Sievert and Pomeranz (1989) showed that the water content directly affects the ability of amylose to re-associate. A starch to water ratio of 1:3.5 (w/v) was shown to promote more resistant starch formation in high amylose corn starch products than a 1:10 (w/v) starch to water ratio. The low water content in 1:3.5 (w/v) starch slurry is often hard to manage because of the viscosity of the starch gel at this high of solids content. The low water content also makes enzymatic treatments, such as debranching, difficult. For these reasons, many studies on the formation of resistant starch use a starch to water ratio of 1:10 (w/v).

Other substances in the food system

Food systems often contain a multitude of compounds that can have impacts on amylose retrogradation. Lipids and sugars have been reported to inhibit RS3 formation (Germani 1983; Czuchajowska and others 1991; Philpot and others 2006). The addition of sugar in a gelatinized starch solution makes the gelatinized starch molecules more stable through hydrogen bonding and therefore less likely to retrograde (Spies and Hoseney 1982; Slade and Levine 1987). In a system containing lipids, there is competition between amylose-lipid complex formation and amylose retrogradation (Czuchajowska and others 1991). Slade and Levine (1987) stated that amylose-lipid complex formation is favored over amylose aggregation.

In vitro Starch Digestion

Starch has been classified into three distinct nutritional categories, rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) based on their resistance to enzymatic digestion (Englyst and others 1992). Rapidly digestible starch (RDS) is hydrolyzed into glucose within the first 20 min of incubation. Slowly digestible starch (SDS) is digested

between 20 min and 120 min. Resistant starch (RS) is then categorized as the starch that resists enzyme degradation for 120 min.

The method proposed by Englyst and others (1992) is an indirect method that measures the glucose released from enzymatic digestion and calculates the resistant starch as the difference between the enzymatically digested starch at 120 min and the total starch content. The Englyst method attempts to mimic in vivo digestion of starch by using a variety of starch digesting enzymes. The three nutritional categories of starch in the Englyst method are defined by the time they can resist enzymatic hydrolysis.

Starch Films and Cereal Coating

The need for biodegradable and natural polymers for film applications has spurred an interest in starch films. Most applications of starch films have centered on food packaging and preservation. Historically, starches have been used to create biofilms because of their ability of form films with superior mechanical and barrier properties. Low moisture permeability is desirable in other food applications, most notably coatings for ready-to-eat cereals.

Ready-to-eat (RTE) cereals include a wide variety of groups such as puffed, flaked, presweetened, or regular (Bartolomei and Thesing 1998). Ready-to-eat breakfast cereals are usually coated with a sugar-based solution to prevent moisture absorption, which can lead to staling and the loss of crispness. One of the most important factors for consumer appeal of RTE cereals is the texture after being submerged in milk (Calandro and Murray 1992). The time a cereal can retain its desired crispness after being added to milk is known as the “bowl life” (Bartolomei and Thesing 1998). Ready-to-eat cereals can also be classified into presweetened and unsweetened varieties. Presweetened cereals are sweetened by coatings to avoid having the

sweetener incorporated into the cereal itself, which can cause adverse attributes to the cereal (Bartolomei and Thesing 1998). Cereals that are not sweetened are still usually coated to decrease moisture absorption, increase bowl life, and add a desired sheen. The process of making cereals is dictated by the type of cereals, but most processing techniques involve similar basic techniques. In the production of flaked cereal the ingredients are mixed, cooked, cooled/dried, flaked, toasted, coated, and dried (Fast and Caldwell 2000). The end product is RTE cereal with low moisture content of less than 5% (Bartolomei and Thesing 1998).

The coatings used in the cereal industry range from fruit juices to metallic salt mixtures (Bone and others 1986; Carpenter and others 1989). Sugar solutions are the most common coating used in the cereal industry because of their sweetness, hygroscopicity, and ease of crystallization. A mixture of glucose and maltodextrins has replaced sucrose solutions as the preferred coating in most cereal applications (Sheng and Widicus 1986). These sugar solutions, nevertheless, present problems such as a high tackiness, carmelization, and the plugging of the spray nozzles used to spray the solution to cereal (Caldwell and others 2000). Consumer acceptance of sugary cereals is also diminishing because consumers are associating sugar cereal products with dental caries and hyperactivity. The major hurdle of creating a low sugar replacement for coating ready-to-eat cereals is the loss of the desirable texture, flavor, and color properties that sugar coated RTE cereals posses (Green and Nowakowski 2005). Alternative coating technologies have been developed using other substances, such as phytoglycogen (Anderson and others 2003) or maltodextrin (Long and Chatel 2006). These results have proved the viability of larger molecules as cereal coatings.

CHAPTER 3

EFFECTS OF β-AMYLOLYSIS ON THE RESISTANT STARCH FORMATION OF DEBRANCHED CORN STARCHES

ABSTRACT

Retrograded amylose is resistant to digestion by amylolytic enzymes, which is known as resistant starch type III (RS3). This study investigated the effect of β-amylase hydrolysis on the formation and physicochemical properties of RS3 from debranched corn starches. Three types of corn starch (Hylon VII, Hylon V, and common corn) were first gelatinized and then hydrolyzed using β-amylase to varying degrees. The resultant hydrolyzed starch was debranched with isoamylase and then exposed to 3 times of temperature cycling at 135/133/133°C for 30 min and 95°C for 24 hr to promote RS formation. The proportions of amylose and amylopectin long and short chains were affected by the β-amylase treatment and varied with starch type. The crystallinity of retrograded starches decreased after 2.5 and 4 hr but then increased after 14 hr of β-amylolysis. A broad endotherm from approximately 45 to 120°C and a small endotherm above 150°C were noted for all retrograded starches. The β-amylase treatment increased the end temperature of the broad endotherm. All three corn starches had increased RS contents after moderate β-amylolysis with Hylon V having the highest RS content at 70.7% after 4 hr of β-amylolysis. The RS content was positively correlated with amylose and amylopectin long chains, but negatively correlated with amylopectin short chains. The results suggest that RS3 formation is affected by starch composition as well as starch structure and can be increased by moderate β-amylolysis.

INTRODUCTION

Resistant starch is defined as the fraction of starch that escapes digestion in the small intestine of healthy people (Englyst and others 1992). This portion of starch is nutritionally relevant because it has been shown to contribute to the colonic heath in animal models and humans (Asp 1994; Eerlingen and Delcour 1995; Phillips and others 1995; Cummings and others 1996). Resistant starch is classified into four major categories by the mechanism in which they are resistant to digestion. Resistant starch type I (RS1) is physically inaccessible starch, type II (RS2) is native granular starch, type III (RS3) is retrograded starch, and type IV (RS4) is chemically modified starch.

RS3 is primarily composed of retrograded amylose because of its strong tendency to reassociate (Berry 1986; Siljestrom and others 1989; Sievert and Pomeranz 1989; Eerlingen and others 1993). Therefore, amylose content is a main factor governing the formation of RS3 (Berry 1986; Sievert and Pomeranz 1989). There are several ways to further increase RS3 formation in starch. Debranching of starch results in all linear glucans, which more readily to reassociate (Berry 1986; Henley and Chiu 1994). In addition, autoclaving (Berry 1986; Sievert and Pomeranz 1989, Henley and Chiu 1994) and temperature cycling (Sievert and Pomeranz 1989; Ozturk and others 2009) have been shown to increase RS3 production. Temperature cycling refers to the process of autoclaving a starch slurry to a temperature >120°C and then storing it at a specified temperature to promote retrogradation. A storage temperature of 95-100°C produced higher amounts of RS than lower temperatures (Eerlingen and others 1993; Ozturk and others 2009). The high autoclaving temperatures melt less stable structures, and the subsequent storage cycles would promote the formation of more stable RS3 structures (Ozturk and others 2009). Higher autoclaving temperatures produced products slightly higher in resistant starch than

products autoclaved at lower temperatures (Berry 1986; Sievert and Pomeranz 1989). The number of autoclaving cycles also correlated with RS3 content (Sievert and Pomeranz 1989; Ozturk 2009). However, a decrease in RS3 was found when autoclaving temperature was above 134°C (Sievert and Pomeranz 1989).

Although amylose content is the predominant factor, amylose degree of polymerization (DP) has also been shown to affect RS3 formation. Eerlingen and others (1993) reported that resistant starch increased with increasing amylose DP up to 100 glucose units and then leveled off (Haralampu 2000). Gidley and others (1995) corroborated this by showing the minimum DP for double helix formation was 10 glucose units. However, these results were obtained using purified amylose instead of debranched starch that contains linear glucans with a broader DP range. Modifying the chain length of native starch to promote RS3 formation has shown mixed results. Hasjim and Jane (2009) found that a mild acid hydrolysis increased the RS content of extruded common corn starch. They hypothesized that the shorter, acid-modified starch molecules had more mobility and therefore reassociated more easily. Koksel and others (2011) reported that the RS content of acid-treated high-amylose starch did not increase under various storage conditions to promote retrogradation, however, when immediately dried a slight increase in RS content was observed.

Acid-hydrolysis creates random cleavage of starch in contrast to more controlled cleavage by β-amylase, which is an exo-enzyme that cleaves the α-(1-4) linkages from the non-reducing ends. In this study, corn starches of varying amylose contents were modified by the controlled hydrolysis of β-amylase to various degrees, in combination with debranching by isoamylase. The effects of β-amylase hydrolysis on the formation of RS and the physiochemical properties of the resultant starches were investigated.

MATERIALS AND METHODS

Materials

Corn starches with different amylose contents were used in this study. Hylon VII (70% amylose) and Hylon V (50% amylose) were provided by National Starch LLC. (Bridgewater, N.J., U.S.A.). Common corn starch (~25% amylose) was obtained from Cargill Inc. (Hammond, Ind., U.S.A.). Beta-amylase from *Bacillus cereus* (activity 2484 U/mg) and D-glucose assay kit were purchased from Megazyme International (Wickow, Ireland). Isoamylase from *Pseudomonas amylodermosa* (activity $>1.25\times10^6$ U/g) was purchased from Hayashibara Biochemical Laboratories Inc. (Okyama, Japan). Porcine pancreatin (pancrease activity 200 U) and amyloglucosidase from *Aspergillus niger* (≥ 300 U/mL) were purchased from Sigma-Aldrich Chemical (St. Louis, Mo., U.S.A.).

Enzymatic Treatments

Twenty grams of starch was suspended in 400 mL of 100 mM acetate buffer (pH 6.5) in a boiling water bath for 30 min, and then autoclaved at 135°C for 30 min to become fully gelatinized. The starch solution was equilibrated in a 40°C water bath, added with 200 μL β-amylase, and then incubated for 0, 2.5, 4, or 14 hr to achieve varying degrees of hydrolysis. After the incubation, the starch was precipitated with 2 L of ethanol and then centrifuged at 7000 ×g for 20 min. The supernatant was collected for the determination of the hydrolysis degree by measuring the total carbohydrate content using the phenol-sulfuric method (Dubrois others 1956). The sediment was rinsed with a small amount of water and kept at 50°C for 30 min to evaporate any residual ethanol. The samples were then freeze-dried to allow for easier dispersion in buffer for the subsequent debranching treatment.

For the debranching treatment, the β-amylase-treated starch was dispersed in 200 mL and autoclaved at 135°C for 30 min to ensure complete dissolution. The amount of β-amylase-treated starch varied between 11 and 18 g because of viscosity variation from varying degrees of hydrolysis. The dispersion was then added with 200 mL of 200 mM acetate buffer (pH 3.5) and isoamylase (300 U/g starch) and incubated at 45°C with agitation for 48 hr to achieve complete debranching.

Temperature Cycling

The enzyme-treated starch solution was autoclaved at 135°C for 30 min, equilibrated in an oven at 95°C for 24 hr, and then autoclaved again at 133°C for 30 min and equilibrated at 95°C for 24 hr. The second autoclaving temperature was 133°C to avoid melting any already formed RS (Ozturk and others 2009). The autoclaving-oven cycle was repeated one more time to result in a total of 3 temperature cycles. After the third cycle the starch was transferred to watch glasses and dried in a forced-air oven at 50°C for 48 hr. The dried sample was ground in a cyclone mill (UDY Corporation, Ft. Collins, Colo., U.S.A.) to fit through a 0.25-mm mesh.

Structure of Enzyme-Treated Starch

The enzyme-treated starch (12 mg) was dissolved in 3 mL of 100% DMSO under boiling and stirring for 3 hr and allowed to cool overnight with stirring. The sample was filtered through a 0.45-um filter, and 200 uL of the filtrate was injected into a HPLC system consisting of a Waters 515 HPLC pump, Waters 2410 refractive index detector, and 2 size-exclusion columns (Shodex OH-804 and OH-802 Showa Denko K.K. Kawasaki, Japan) used in tandem with 0.1 M ammonium acetate at 0.5 mL/min as the eluent. Dextran standards of 4400, 9900, 21400, 43500, 196,000, and 277,000 Da and glucose were used to establish the calibration curve.

Physiochemical Properties of Enzyme-Treated Starch

The X-ray powder diffraction was measured using a Phillips analytical diffractometer (Phillips, Almelo, Netherlands). The samples were scanned from 4° to 35° (2θ) at 45 kV and 40 mA with a step size of 0.02°. The background was subtracted from the diffractogram by drawing a straight baseline tangentially to the curve at 5°(2θ). A relative crystallinity can be determined by comparing the area under the crystalline peaks with the area of the amorphous under the peaks.

Thermal properties were assessed by a differential scanning calorimeter (DSC, Perkin-Elmer Co., Norwalk, Conn., U.S.A.). Approximately 10 mg (db) of starch was weighed into a stainless steel DSC pan, and 20 µL of deionized water was added by a microsyringe. The mixture was hermetically sealed and equilibrated at room temperature for at least 24 hr prior to heating from 25°C to 180°C at 10°C/min. An empty pan was used as the reference. The onset, peak and end gelatinization temperatures and enthalpy (ΔH) was calculated from the endotherms.

The proportion of rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) were determined by following the method of Englyst and others (1992), except that invertase was not used because of the absence of sucrose in starch.

Structure of Resistant Starch

The chain-length distribution of the RS residue from the method of Englyst and others (1992) was analyzed with HPLC. Three mL aliquots of the samples after 120 min hydrolysis was added to 30 mL of ethanol, and then centrifuged at 4000 ×g for 10 min. The supernatant was discarded, and the precipitate was freeze-dried. Ten mg of the freeze-dried hydrolysate was added to 3 mL of water, heated in a boiling water bath for 1 hr, and injected into a HPLC system equipped with a guard column, a Carbopac PA1 analytical column, a pulsed amperometic

detector, and an AS40 autosampler (Dionex-ICS-3000, Dionex Corp., Sunnyvale, Calif., U.S.A.). The eluents and eluent gradient were setup according to the method of Kasemsuwan and others (1995).

Statistical Analysis

The data were analyzed by using JMP software (SAS Institute Inc., Cary, N.C., U.S.A.). Comparison of means was executed using Tukey's test and bivariate analysis was performed using the Pearson-product moment approach.

Results and Discussion

β-amylase Hydrolysis

Fig. 3.1 displays the hydrolysis profiles of the three corn starches during the β-amylase treatment over 14 hr. Common corn starch had the lowest hydrolysis degree among the starches at 2.5 hr, which was not expected because of its higher proportion of amylopectin than Hylon V and VII, which consists of more non-reducing ends for β-amylase to hydrolyze. The slow initial rate of hydrolysis for common corn starch was attributed to its higher viscosity after gelatinization compared with those of Hylon V and Hylon VII, which hindered the enzyme diffusion. All three starches exhibited a similar degree of hydrolysis after 4 hr. At the end of the 14 hr, the hydrolysis degrees of common corn, Hylon V, and Hylon VII were 40.5, 35.5, and 30.1 %, respectively, which correlated with their amylopectin contents.

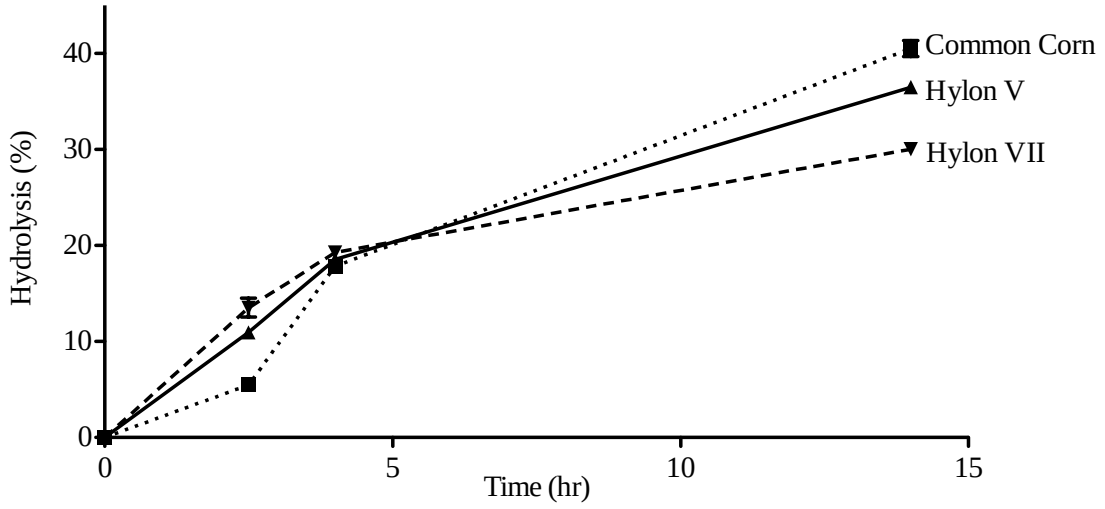

Fig. 3.1. Hydrolysis of corn starches with varying amylose contents by β-amylase over time.

Structural Characteristics of Enzyme-Treated Starches

The normalized molecular-size distributions of the enzyme-treated starches are displayed in Fig. 3.2, and the proportion and corresponding peak DP are listed in Table 3.1. Amylose was eluted first before 27 min (Fraction, Fr. I), followed by amylopectin long chains (Fr. II) and then amylopectin short chains (Fr. III and Fr. IV).

There were notable changes in proportions of each fraction for all starches during β-amylolysis. The Fr. I of all starches displayed a similar trend of an initial increase after 2.5 hr, but a decrease after 14 hr of β-amylolysis. The extent of changes, however, varied with starch type. Hylon V had the most increase in Fr. I after 2.5 hr (18%), whereas Hylon VII had the least (4.6%). Overall, the Fr. I was least affected by β-amylase hydrolysis in Hylon VII and most affected in Hylon V.

The Fr. II, amylopectin long chains with DP of approximately 20-210, increased with increasing β-amylolysis in both common corn starch and Hylon VII, but decreased in Hylon V from 45.6 to 37.6 % from 4 hr to 14 hr of β-amylolysis. For Fr. III, amylopectin short chains with DP of approximately 9-2, Hylon VII displayed a continual decrease as β-amylolysis increased. Common corn starch and Hylon V exhibited an initial decrease followed by an increase at higher β-amylase hydrolysis levels. The Fr. IV, amylopectin short chains with DP of approximately 5-9, decreased with increasing hydrolysis time for common corn starch and Hylon VII, but decreased initially before then increased at the 4 and 14 hr hydrolysis times for Hylon V.

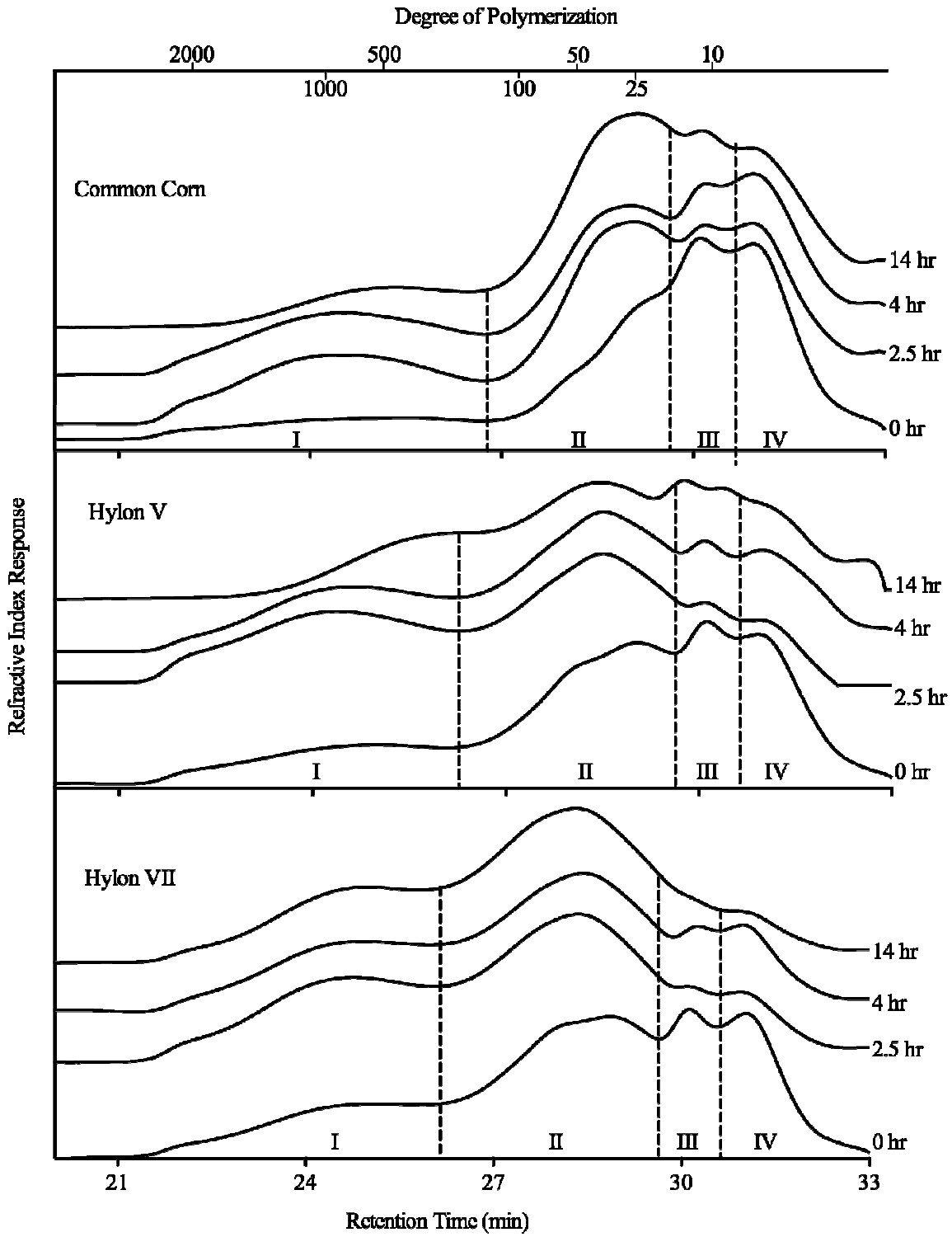

Fig. 3.2. Molecular-size distributions of common corn starch, Hylon V, and Hylon VII after β-amylase hydrolysis for varying times and then debranched by isoamylase.

Table 3.1

Distribution of Chain Lengths of Enzyme-Treated Starch

Sample	β-amylase hydrolysis (hr)	Properties	Fraction I	Fraction II	Fraction III	Fraction IV
Common Corn Starch	0	Peak Area (%)	11.6	28.2	24.6	35.6
		DP_{peak}	856	37	11	6
	2.5	Peak Area (%)	22.3	32.9	15.8	29.1
		DP_{peak}	824	27	11	6
	4	Peak Area (%)	22.9	40.0	9.9	27.3
		DP_{peak}	818	26	11	7
	14	Peak Area (%)	11.1	41.2	17.3	25.5
		DP_{peak}	475	25	12	7
Hylon V	0	Peak Area (%)	16.4	39.6	20.1	23.9
		DP_{peak}	895	26	12	6
	2.5	Peak Area (%)	34.4	45.8	9.9	9.8
		DP_{peak}	878	39	12	19
	4	Peak Area (%)	25.8	45.6	10.0	19.2
		DP_{peak}	669	39	12	6
	14	Peak Area (%)	22.9	37.6	12.6	26.8
		DP_{peak}	275	40	15	10
Hylon VII	0	Peak Area (%)	22.1	43.2	16.4	20.4
		DP_{peak}	832	30	12	6
	2.5	Peak Area (%)	26.7	51.5	6.8	14.5
		DP_{peak}	654	42	11	6
	4	Peak Area (%)	31.0	52.1	7.0	9.9
		DP_{peak}	646	43	12	6
	14	Peak Area (%)	27.2	58.8	6.4	7.5
		DP_{peak}	520	44	11	6

The Fr. I peak DP decreased from 856 to 475 for common corn starch, from 895 to 275 for Hylon V, and from 832 to 520 for Hylon VII after β-amylolysis for 14 hr. Common corn

starch showed a slight decrease in peak DP of amylopectin long chains (Fr. II) with increasing β-amylase treatment. Both Hylon V and VII showed an initial sharp increase in peak DP of Fr. II and then no change with further β-amylolysis. For all three starches the peak DP of Fr. III and IV remained relatively unchanged with varying β-amylase hydrolysis durations.

X-ray Diffraction

The X-ray diffraction patterns and relative cyrstallinities of retrograded enzyme-treated starches are shown in Fig. 3.3. Most starches with the exception of debranched-only common corn starch showed a B-type cyrstalline polymorph. Debranched-only common corn starch showed a A-type characteristic with major peaks at 2θ = 15.3, 17.2, 18.2, and 23.2° and minor peaks at 10, 11.5, 14.2, 19.5, 22.3, and 26.3°, but changed to a B-type pattern with major peaks at 17.2, 22.2, and 24.3°, and minor peaks at 14.2, 15.3, and 19.5° when the β-amylase treatement was applied. The β-amylase treatment had little impact on the crystalline structure of high-amylose corn starches. The intenisty of the peaks at 14.2 and 19.5° remained relatively unchanged for all starches, but the peak at 15.3° seemed to be strongly affected by the β-amylase treatment. In high-amylose staches the peak at 15.3° was reduced at 2.5 and 4 hr hydrolysis, but increased with 14 hr hydrolysis. For common corn starch this peak significantly decrased after 2.5 hr hydrolysis, reduced further after 4 hr hydrolysis, but re-appeared after 14 hr β-amylase treatment. All three starches shared a similar X-ray pattern and relative crystallinity after 14 hr of β-amylolysis.

The formation of the A-type crystalline polymoph has been shown to be favored with shorter chains amylose and higher temperatures (Gidley and Bulpin 1987; Pfannemuller 1987). The A-type pattern displayed by debranched-only common corn starch was attributed to the high

proportion of amylopectin short chains. The β-amylase treatment decreased the proportion of short chains, and therefore the transition to a B-type polymorph appeared to be associated with an increase in long chains after the degradation of short chains. In addition, the increase in intesity at 15.3° in all starches, when β-amylase hydrolysis time increased to 14 hr from 4 hr, indicates the increase of A-type polymorph with an increase in amylopectin short chains (Fr. III and IV) as reported in Table 3.1.

A trend was noted among all starches that the crystallinity initially decreased with 2.5 and 4 hr hydrolysis but then increased after 14 hr of hydrolysis. Common corn starch had the highest crystallinity after debranching alone, most likely because of its large proportion of short chains (Fr. III and IV) (Table 3.1.), which promoted the formation of the A-type polymorph. After 2.5 - 4 hr of β-amylase hydrolysis, a significant proportion of short chains in common corn starch was degraded, thus resulting in decreased crystallinity. However, after 14 hr of hydrolysis, long chains were degraded to short chains that again contributed to the increase in crystallinity.

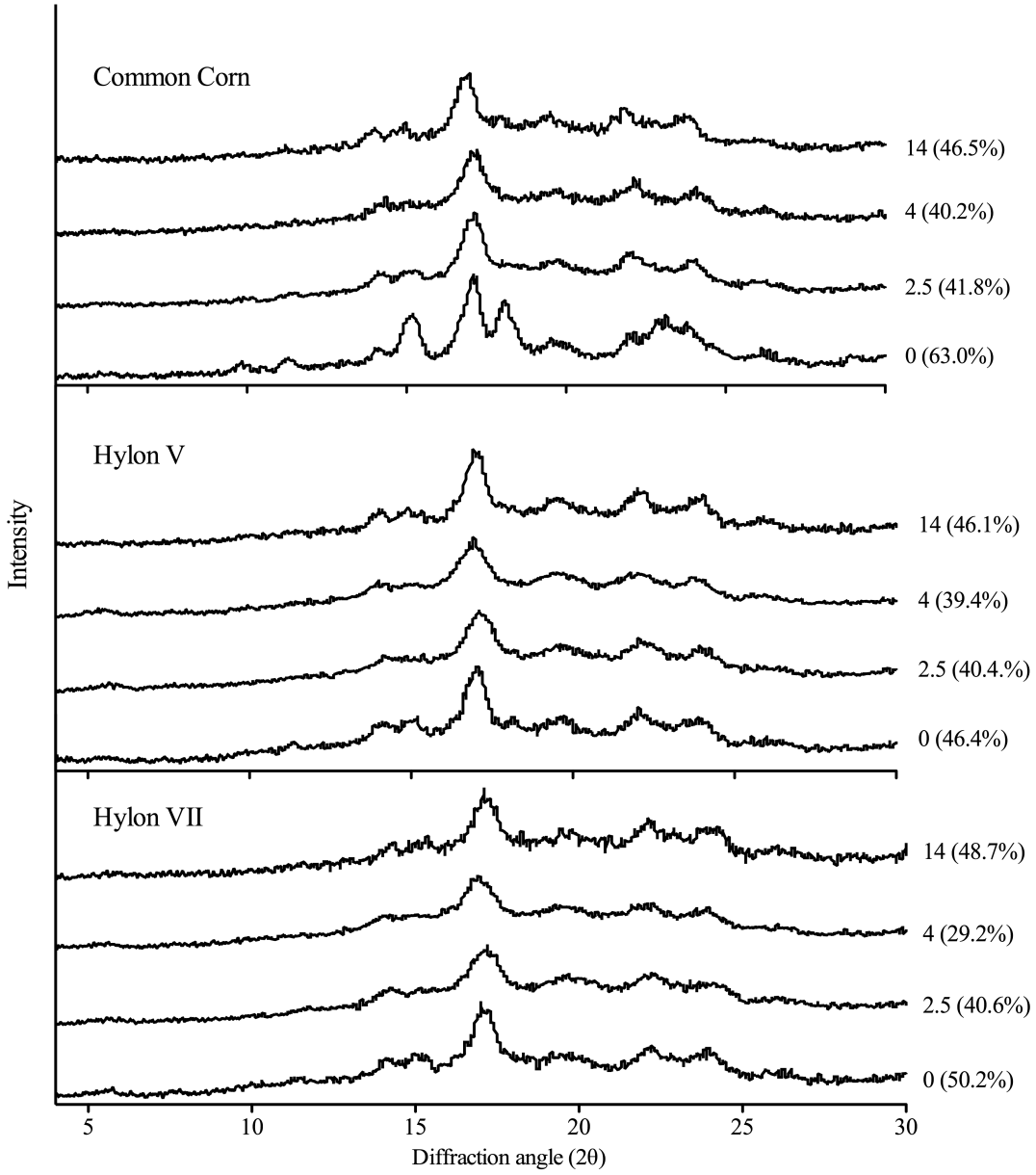

Fig. 3.3. X-ray diffraction patterns of retrograded debranched corn starches with varying times (hr) of β-amylolysis. The relative crystallinity values are shown inside the parentheses.

Thermal Properties

All three retrograded debranched-only starches showed a broad endotherm from approximately 45 to 120°C and a small endotherm above 150°C. The β-amylase treatment shifted the end temperature of the broad endotherm to a higher temperature and sometimes resulted in multiple smaller peaks. Moates and others (1997) reported that the melting temperature of crystals from short chains, such as the ones from retrograded debranched amylopectin, increased from 57 to 119°C as chain length increased from 12 to 55 glucose units. It is assumed that the multiple small peaks in the temperature range between 50 and 120°C corresponded to the three distinct populations of amylopectin chains (Fr. II, III, and IV in Fig. 3.2). The small differences in the temperature range and size of these small peaks could be due to the differences in their chain length distributions of the amylopectin chains (Fig. 3.2).

The endotherm above 150°C, which is associated with the melting of amylose double helices (Sievert and Pomeranz 1989), was the most pronounced for debranched-only Hylon VII, presumably because of its high amylose content. When β-amylase was applied, the high temperature endotherm became larger. Two possible factors could contribute to the increase of this endotherm with β-amylolysis. One is that the degradation of amylopectin short chains (Fr. III and IV) might allow improved amylose reassociation because the presence of large amounts of amylopectin short chains could interfere with amylose complexation. The other possibility is the long amylose chains might not be optimal but became more suited for reassociation after the β-amylase treatment.

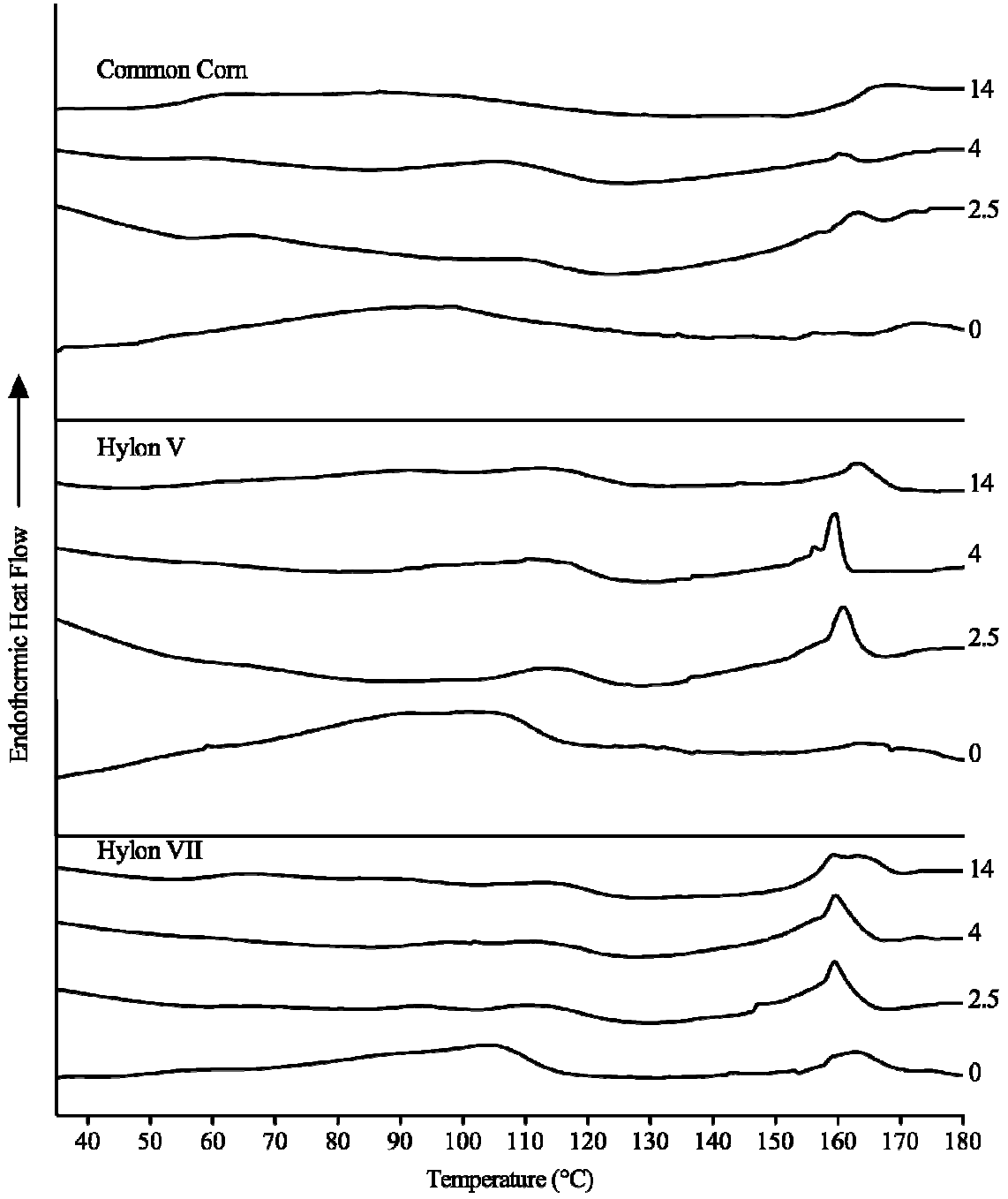

Fig. 3.4. DSC thermograms of different enzymatically-treated corn starches. Number corresponds to time (hr) of the β-amylase treatment.

Digestibility

Table 3.2 summarizes the fractions of RDS, SDS, and RS of enzyme-treated starches assuming their total amount of starch is 100. The procedure for producing RS3 was modified from Ozturk and others (2009), in which RS contents ranged from 41 to 58 % as measured by Approved Method 32-40 (AACC International 2000). In the present study, increased RS3 production was anticipated because of the incorporation of enzymatic treatments and temperature cycling. For the debranching-only treatment, Hylon V had a higher amount (58.7%) of RS than Hylon VII (50.7%), which was also reported by Ozturk and others (2009). All starches showed significantly higher RS contents after the β-amylase treatment for 2.5 and 4 hr, but their RS contents decreased after 14 hr of β-amylase treatment. Debranched and β-amylase-treated (4 hr) common corn starch produced more RS (55.5%) than debranched-only Hylon VII (50.7%). Although amylose content is one of the most important factors for starch to form RS3, these results indicate that other aspects of starch structure appear to be as important in RS3 formation. Hylon V had a higher RS content than common corn starch and Hylon VII at 2.5 hr hydrolysis, and had the highest RS content along with Hylon VII after 4 hr of hydrolysis. Within each starch type, the highest RS level was found at 4 hr hydrolysis. The present results support the findings of Hasjim and Jane (2009) and Koksel and others (2011), and their different conclusions were attributed to their uses of different extents of hydrolysis and starch types.

The changes in RS seem to negatively correlate with the changes in RDS for common corn starch and Hylon VII, but negatively correlate with the changes in SDS for Hylon V. Debranched-only common corn starch had the highest RDS (62.1%) and also had the only with a strong A-type polymorph (Fig. 3.3) when compared with lower RDS and the B-type polymorph of the other starches. The RDS and SDS of common corn starch decreased with 2.5 and 4 hr β-

amylolysis, but then increased with further treatment of 14 hr. During β-amylolysis, the RDS levels in Hylon V only slightly changed, while the SDS in Hylon VII stayed relatively constant. Amylopectin short chains after debranching has been associated with SDS formation (Robin and others 2008; Cai and Shi 2009, 2010). Robin and others (2008) reported that SDS formation was highest for chains with a peak DP of 32. However, recrystallization is also greatly affected by the retrogradation conditions, particularly temperature. In the present study, most Hylon V had a higher SDS than common corn starch for the same hydrolysis condition, suggesting that amylopectin structure may have a greater impact than amylopectin quantity in SDS formation. For common corn starch and Hylon V, their SDS significantly decreased with the 2.5 and 4 hr β-amylolysis. However, with further hydrolysis the SDS of Hylon V increased to the level of the debranched-only one. The increase in SDS was attributed to the degradation of amylopectin long chains to short chains. For Hylon VII the SDS did not significantly change with the β-amylase treatment, probably because its high amylose content compensated for the degradation of amylopectin.

Hylon VII is commonly used in the commercial production of RS3 due to its high amylose content, but it has a high gelatinization temperature and is not as readily available as common corn starch. The β-amylase treatment of common corn starch might provide an alternative way of producing RS3.

Table 3.2

Nutritional Properties of Retrograded Enzyme-treated Corn Starches*

Sample	β-amylolysis (hr)	RDS (%)	SDS (%)	RS (%)
Common Corn Starch	0	62.1a	9.4b	28.6h
	2.5	49.9b	4.5bc	45.6g
	4	41.8c	2.7c	55.5ef
	14	51.0b	4.6bc	44.5g
Hylon V	0	25.4ef	20.6a	58.7de
	2.5	27.4def	7.8bc	64.7bc
	4	28.1def	2.5c	70.7a
	14	24.6f	17.0a	58.4de
Hylon VII	0	40.0c	8.7bc	50.7f
	2.5	29.3de	8.6bc	62.1cd
	4	25.0ef	6.6bc	67.9ab
	14	30.2d	8.3bc	61.5cd

Means of 3 replications. Values with same latter in same column are not significantly different ($p < 0.05$). RDS = rapidly digestible starch, SDS = slowly digestible starch, RS = resistant starch, RS = 100 – (RDS + SDS).

In an effort to better understand how starch chain lengths correlated with its digestibility, a bivariate analysis was performed on starch structures (Fr. I to IV in Table 3.1) and digestibility (Table 3.2), and the results are displayed in Table 3.3. The RS content was positively correlated with Fr. I and II and negatively correlated with Fr. IV, and the opposite trend was noted for the RDS with negative correlation with Fr. I and II and positive correlation with Fr. IV. The SDS did not show any significant correlation with any starch fraction. Others have reported that amylopectin branch chains are integral for SDS formation, which was not observed under the conditions of this experiment, which could be attributed to the high storage temperatures used.

The conditions to promote SDS formation often involve storage at lower temperatures (Guraya and others 2001a, 2001b; Miao and others 2009), which have been shown to increase retrogradation of debranched amylopectin.

Table 3.3

Bivariate Analysis of Starch Structure and Digestibility

Fraction	RDS	SDS	RS
Fraction I	-0.6916*	-0.3485	0.8409*
Fraction II	-0.5809*	-0.3049	0.6428*
Fraction III	0.0486	0.2739	-0.0873
Fraction IV	0.7017*	0.2780	-0.7848*

* Statistically significant ($p < 0.05$)

The minimum and optimum chain lengths on amylose reassociation and RS3 formation have been well studied (Pfannemuller and Bauer-Carnap 1977; Gidley 1987; Eerlingen and others 1993). Pfannemuller and Bauer-Carnap (1977) reported that the maximum rate of aggregation took place in amylose molecules of DP 80. Gidley (1987) demonstrated that the minimum DP for amylose double helix formation was 10 glucose units and later reported that amylose molecules with DP values above 100 were likely to form networks that were more sensitive to enzyme digestion. Eerlingen and others (1993) showed that amylose chain length had no significant effect on RS formation once it reached DP 100. Gidley and others (1995) reported that RS3 was comprised of amylose chains of DP 10-100.

When trying to analyze which chain lengths are most important for the formation of RS, Fraction I and IV showed the largest correlation. Fraction I continually decreased in DP with

increasing β-amylase treatment, but RS did not increase with continual β-amylase treatment. With this information it is logical to infer that the chain length of Fr. I is not the primary factor for RS formation. Increases in RS seem to be most affected by the amount of starch in Fraction I and IV. In the present study, some starches showed an overall decrease in the proportion of short chains because the long chains were not degraded to short chains at the same rate as short chains were hydrolyzed in to saccharides. Conversely, in some cases an increase in short chains was observed as longer amylopectin chains are degraded to shorter lengths and short chains. Therefore, because of the polymodal nature of debranched amylopectin chains, an increase in β-amylase hydrolysis does not always correspond to a higher proportion of short chains. Understanding the degradation of amylopectin using β-amylase helps explain how a very strong negative correlation was shown between Fraction IV but most samples saw a decrease in RS as β-amylase treatment increased from 4 to 14 hr. During this same time period most samples displayed an increase in Fr. IV, as molecules from Fractions II and III were degraded further, which suggests that the amount of short chains (Fr. IV) is an important factor in RS formation.

RS Residue Structure

Table 3.4 presents the average chain length and chain-length distribution of the recovered RS from the digestibility study. The average DP of recovered RS residue ranges 21.3 to 26.4 with the DP 13-24 as the predominant fraction. In general, the 14 hr samples had smaller proportions of DP 36+ fraction. Small changes were noted in the average DP between the RS residue from common corn starch and those from the two high amylose corn starches. It has been reported that RS chain length is independent of the starting chain length prior to starch retrogradation (Eerlingen and others 1993). The present results of slight differences in average RS chain length with respect to starch source and β-amylase treatment might be due to

differences in the method of recovering the RS. Eerlingen and others (1993) recovered the RS residue after subjecting RS to a modified version of the dietary fiber method (AOAC 1985), whereas this study recovered the RS residue after the Englyst method (1992). However, the small DP range of RS residue between DP 21 - 27 supports the lamellar model (Jolley 1970) for polymer crystallization and the micelle model for starch reassociation by Jane and Robyt (1984). In these proposed models the molecules were proposed to align over short crystalline regions (~24 glucose units) interdispersed with amorphous regions. Once these RS molecules were exposed to digestive enzymes, the amorphous regions were digested, and the recovered resistant starch had a narrow range of DP corresponding to the length of the helical portion of the molecule involved in RS formation (Eerlingen and others 1993).

Table 3.4

Chain-length Distribution of Recovered Resistant Starch*

Sample	β-amylolysis (hr)	Average DP	DP 6-12	DP 13-24	DP 25-36	DP 36+
Common Corn	0	22.3 ± 0.3	17.6 ± 1.0	46.7 ± 0.2	25.1 ± 0.5	10.6 ± 0.3
	2.5	22.9 ± 0.6	17.8 ± 1.8	43.4 ± 0.7	26.7 ± 1.1	12.1 ± 1.4
	4	24.4 ± 0.9	14.6 ± 1.2	40.3 ± 1.4	30.4 ± 1.1	14.7 ± 1.5
	14	22.1 ± 0.9	17.2 ± 1.3	46.6 ± 1.6	27.4 ± 0.7	8.8 ± 0.2
Hylon V	0	21.3 ± 0.8	23.9 ± 1.3	42.2 ± 3.6	24.2 ± 0.7	9.7 ± 4.2
	2.5	26.5 ± 0.1	11.1 ± 3.1	37.4 ± 3.3	30.9 ± 1.3	20.6 ± 1.4
	4	26.4 ± 0.0	10.0 ± 0.3	39.1 ± 0.5	31.2 ± 0.2	19.7 ± 0.4
	14	23.5 ± 0.2	15.8 ± 0.9	42.8 ± 0.1	28.7 ± 0.4	12.6 ± 0.2
Hylon VII	0	26.1 ± 0.1	10.7 ± 0.4	39.8 ± 0.6	30.4 ± 0.3	19.2 ± 0.5
	2.5	24.0 ± 0.1	15.6 ± 2.8	41.6 ± 2.8	28.6 ± 1.2	14.2 ± 1.1
	4	25.0 ± 1.7	13.3 ± 2.1	40.6 ± 4.2	29.3 ± 1.5	16.8 ± 4.9
	14	24.0 ± 0.4	17.6 ± 1.1	38.8 ± 0.3	28.4 ± 0.6	15.1 ± 0.5

* Means of duplicate measurements with standard deviations.

CONCLUSIONS

This study demonstrates that both chemical composition and structure of starch affected RS3 formation in debranched starch. Debranched amylose-containing corn starches had increased RS contents with the incorporation of a mild β-amylase treatment. The mild β-amylase treatment hydrolyzed amylopectin short chains, which interfere with reassociation of linear chains, and thus increased the proportions of amylose and amylopectin long chains, which contributed to RS formation. However extensive β-amylolysis might create new amylopectin short chains and/or shorten amylose and amylopectin long chains to become not as optimal to form RS under the temperature cycling conditions in this study.

CHAPTER 4

APPLICATION OF ENZYME-TREATED CORN STARCHES IN BREAKFAST CEREAL COATING

Abstract

Presently ready-to-eat cereals are coated with high levels of sugar coating to extend the bowl life. Because of health concerns of added sugar, there is a need to identify alternative coating materials. This study was designed to test the efficacy of debranched corn starches with varying amylose contents as a cereal coating. Hylon VII (70% amylose), common, and waxy corn starches were gelatinized and debranched, and then sprayed onto ready-to-eat breakfast cereal flakes. The surface morphology, milk absorption, texture, and digestibility of coated cereals were determined. A starch film of 50-130 μm was observed with scanning electron microscopy on the surface of the cereals coated with Hylon VII. All starch-coated cereals had a lower milk absorption value than the uncoated and glucose-coated controls. Among starch coatings, common corn starch and Hylon VII resulted in lower milk absorption than did waxy corn starch. After soaking in milk for 3 min, the peak force and work to peak of the cereals coated with corn starches were higher than those of the controls. The cereals coated with Hylon VII were found to have an increase in dietary fiber content. The results suggest that debranched amylose-containing corn starches could extend the bowl-life of ready-to-eat cereals.

Introduction

The process of making ready-to-eat (RTE) cereals is dictated by the type of cereals, but most processes involve similar techniques of mixing, cooking, cooling, shaping, toasting, coating, and drying (Fast and Caldwell 2000). The end product has a low moisture content of less than 5% (Bartolomei and Thesing 1998). One of the most important factors that RTE cereals appeal to consumers is the texture after being submerged in milk (Calandro and Murray 1992). Consumers prefer RTE cereals with the ability to retain their texture in milk. The time a cereal can retain its desired crispness after being added to milk is known as the "bowl life" (Bartolomei and Thesing 1998). Therefore RTE cereals are usually coated with a sugar-based solution to prevent moisture absorption, which leads to staling and loss of crispness. Ready-to-eat cereals can be classified into pre-sweetened and unsweetened types. Pre-sweetened cereals often contain a sugar coating to avoid the incorporation of sweetener into the cereals, which can cause adverse attributes to the cereals (Bartolomei and Thesing 1998). Cereals that are not sweetened are still usually coated to decrease moisture absorption, increase bowl life, and add a desired sheen.

Although sugar solutions are widely used in the cereal industry because of their sweetness, low hygroscopicity, and ease of crystallization, they present problems such as high tackiness, carmelization, and plugging of the spray nozzles (Caldwell and others 2000). In addition, dietary sugar has been linked to obesity and tooth decay (Lewis and others 1992; Gibson 1993; Ruxton and others 1999; Johnson and Frayry 2001). The major hurdle of creating a low-sugar replacement for RTE cereal coating is the loss of the desirable texture, color, and flavor that sugar-coated RTE cereals posses (Green and Nowakowski 2005). Alternative coating systems have been developed using substances, such as phytoglycogen (Anderson and others 2003) or maltodextrin (Long and Chatel 2006). Phytoglycogen-coated cereals had slightly

increased bowl-life and decreased milk absorption, but only when compared to a water-coated control. Due to the importance of bowl-life to consumer acceptance and increasing demand for a low-sugar product, a variety of alternatives have been developed, such as fruit juice coatings and adding metallic salts of fatty acids to the dough, to extend the bowl-life of cereals (Bone and others 1986; Carpenter and others 1989).

Starch films form when gelatinized starch dispersions are cooled and starch molecules aggregate and reorganize (Liu and Han 2005). Starch films have been widely used as an edible film and coating because of their high performance and low cost (Wolff and others 1951; Jokay and Mitan 1969; Liu and Han 2002). However, starch films become brittle and lose flexibility over time from retrogradation (Yoshida and Hijiya 1973).

Amylose content is considered as the most important intrinsic factor affecting the properties of starch films (Tharanathan 2003). The amount of amylose in starch is strongly correlated with the mechanical and physical properties of starch films. Starches with higher amylose contents produce strong yet flexible films, but those with high amylopectin contents produce brittle films with poor mechanical properties such as low tensile strength and low elongation (Wolff and others 1951; Moore and Robinson 1968; Palviainen and others 2001). Furthermore, gelatinized starch dispersions tend to be viscous, thus forming thick films and trapping air bubbles, both of which are not desirable (Yoshida and Hijiya 1973).

It was hypothesized that starch films could function as a cereal coating to replace sugar because of the crystalline structure from starch retrogradation. Debranching of starch would decrease the viscosity of starch dispersion for ease of film formation and at the same time enhance reassociation and formation of crystalline structures. Therefore, the objective of this study was to investigate the film-forming properties of debranched corn starches with varying

amylose content as a cereal coating with respect to bowl-life extension and physicochemical properties.

Materials and Methods

Three types of corn starch with different amylose contents were used in this study. Hylon VII (70% amylose) and waxy corn starch (0% amylose) were provided by National Starch LLC. (Bridgewater, N.J., U.S.A.). Common corn starch (~25% amylose) was obtained from Cargill Inc. (Hammond, Ind., U.S.A.). Isoamylase from *Pseudomonas amylodermosa* (activity >1.25 $\times 10^6$ U/g) was purchased from Hayashibara Biochemical Laboratories Inc. (Okyama, Japan). For the determination of total dietary fiber content, porcine pancreatin and amyloglucosidase from *Aspergillus niger* (≥ 300 U/mL) were purchased from Sigma Chemical (St. Louis, Mo., U.S.A.). The D-glucose assay kit was purchased from Megazyme International (Wickow, Ireland). Wheaties® (General Mills, Minneapolis, Minn., U.S.A.) and 2% reduced fat milk (Hiland Dairy, Springfield, Mo., U.S.A.) were purchased from a local grocery store.

Preparation of coatings

A 10% (w/v) slurry was prepared by adding 6 g of starch to 60 mL of 100 mM sodium acetate buffer (pH 3.5). The resultant slurry was cooked in a boiling water bath for 30 min and then autoclaved at 135°C for 30 min to fully gelatinize the starch. The starch solution was incubated with isoamylase (300 U/g starch) at 45°C for 48 hr to ensure complete debranching. Thereafter, the starch solution was autoclaved at 135°C for 30 min to disperse any aggregates formed during the enzyme treatment.

The starch solution was poured into a 32 oz. plastic spray bottle with adjustable spray nozzle (Sprayco, Detroit, Mich., U.S.A.) directly after autoclaving. The spray nozzle was

adjusted to a mist, in which each spray dispensed approximately 1 mL of starch solution. Thirty grams of Wheaties® was placed on a 600-cm^2 stainless steel mesh tray and sprayed with 10 mL of the starch solution at 80°C on each side. The starch coating was applied in three separate applications with a drying step of 50°C for 2 hr between each coating. The tray was then placed in a forced air oven at 50°C for 24 hr to allow the coating to dry. The weight of the finished coated cereals was determined to calculate the amount of starch coated onto cereals. For a control, 6 g of glucose was added to 60 mL of water to create a 10% (w/v) glucose solution, which was applied in identical fashion to the starch coatings. An uncoated cereal was also included as a reference.

Surface Morphology

The surface appearance of cereals was photographed using a Nikon D300s camera with DX 18-2000 mm zoom lens (Nikon Corporation, Tokyo, Japan) on a black cloth background. The morphology of surface and cross-section of flakes were observed using a FEI XL 30 scanning electron microscope (SEM) (FEI Corporation, Hillsboro, Ore., U.S.A.). Cereals were sputter-coated in gold and mounted on a stub either vertically for cross-section viewing or horizontally for surface viewing using carbon tape.

Milk Absorption

Milk absorption was measured by following the method of Anderson and others (2002) with modification. Four grams of cereals was placed in 30 mL of milk at 8°C for 3 min, and then the cereals was removed from the milk and drained on a 2.8-mm stainless steel mesh screen. The percent milk absorption was calculated by dividing the absorbed milk weight, which was the weight difference between the original cereals and the drained cereals, with the original cereals weight.

Texture

The texture of the coated cereals was measured using a modified method of Anderson and others (2002). Four grams of each cereal sample was placed in 30 mL of 2% reduced fat milk at 8°C for 3 min. After the soaking period, the cereals was removed from the milk using a strainer, placed on a paper towel for 10 sec, and then placed into a 10-blade Kramer shear cell assembly for a MTS Alliance RT/1 Texture Analyzer (MTS Systems Corp. Eden Prairie, Minn., U.S.A.). The peak force to fracture and the work required to peak was measured by using the following conditions: a pretest speed of 5 mm/s, a test-speed of 10 mm/s, a post-test speed of 5 mm/s, and a distance of 120 mm/s.

Nutritional Properties

The cereals were crushed using a mortar and pestle, and the fraction passed through a 2.8-mm screen but retained on a 1.4-mm screen was collected and used for analysis. This size range was chosen as an approximation of masticated particle size based on the work of Hoebler and others (2000). The dietary fiber content was determined using AACC method 32-05 (AACC International 1991).

Statistical Analysis

One-way ANOVA was performed, and means were compared with Tukey's test using the JMP program (SAS Institute, Cary, N.C., U.S.A.).

Results and Discussion

Surface Morphology

Although the total amount of starch coating applied was 60 mL (6 g of starch or glucose) for 30 g of cereals, the final coated cereals only increased approximately 10% over the precoated

weight, suggesting that only half of the applied starch solution was coated onto the flakes, and the other half was lost during application.

Flakes coated with Hylon VII showed a slight frosty surface with visible signs of an opaque coating (Fig 4.1A). Flakes coated with common corn starch showed a similar appearance, but with a less pronounced frosting effect (Fig. 4.1B) yet an increase in gloss or sheen. Flakes coated with waxy corn starch (Fig. 4.1C) displayed a glossy appearance that was much less opaque than Hylon VII and with more sheen than common corn starch. Samples coated with glucose (Fig. 4.1D) were very similar to cereals coated with waxy corn starch, but had a slightly darker brown color.

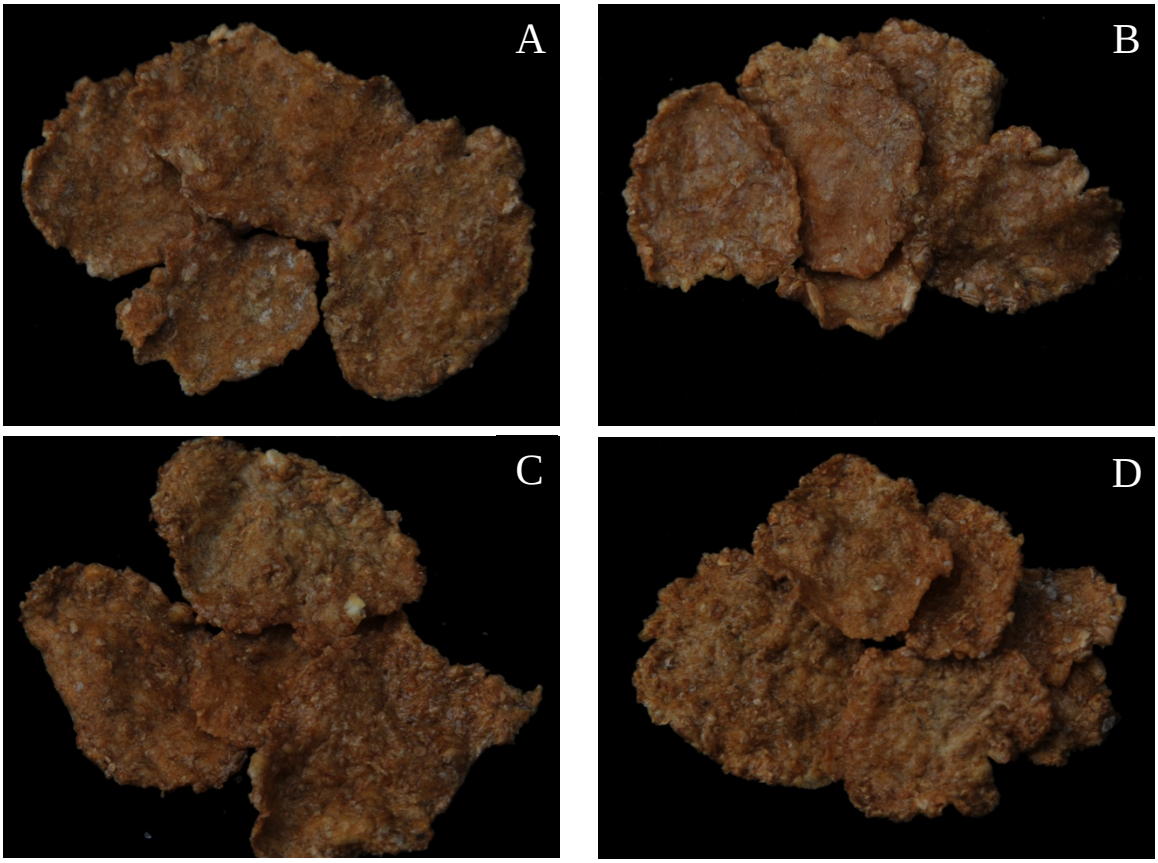

Fig. 4.1. Photographs of cereal flakes with various coatings: Hylon VII (A), common corn starch (B), waxy corn Starch (C), and glucose (D).

The representative SEM micrographs of cereals coated with Hylon VII and glucose (control) are shown in Fig. 4.2. The surface morphology of coated cereals was different for different coatings. Fissures were evident in the Hylon VII coating (Fig. 4.2A), which, however, was not observed in other starch and glucose coatings (Fig. 4.2C). A starch layer of approximately 50-130 μm was visible in the cross-section of Hylon VII-coated cereals with a dense structure in contrast to the loose, open structure of the cereal flakes (Fig. 4.2B). Only Hylon VII coating displayed a visible dense layer on the surface of the cereals. No film or coating was observed on the surface of flakes coated with debranched common or waxy corn starch and glucose (Fig. 4.2D). Because all starches were debranched, the most notable difference among the starches was the chain length distribution. Previously, Yoshida and Hijiya (1973) reported that starch with a higher proportion of long chains produced a better film than starch with high amounts of short chains. It is possible that linear amylose molecules with long degrees of polymerization (DP), when present in a large amount as in Hylon VII, reassociated with themselves rapidly and formed a distinct layer from the cereal surface. In contrast, linear amylopectin chains did not reassociate as easily as amylose, and short amylopectin chains might interfere amylose reassociation. Therefore, smaller starch molecules and glucose became part of the cereal surface instead of forming a dense layer with fissures.

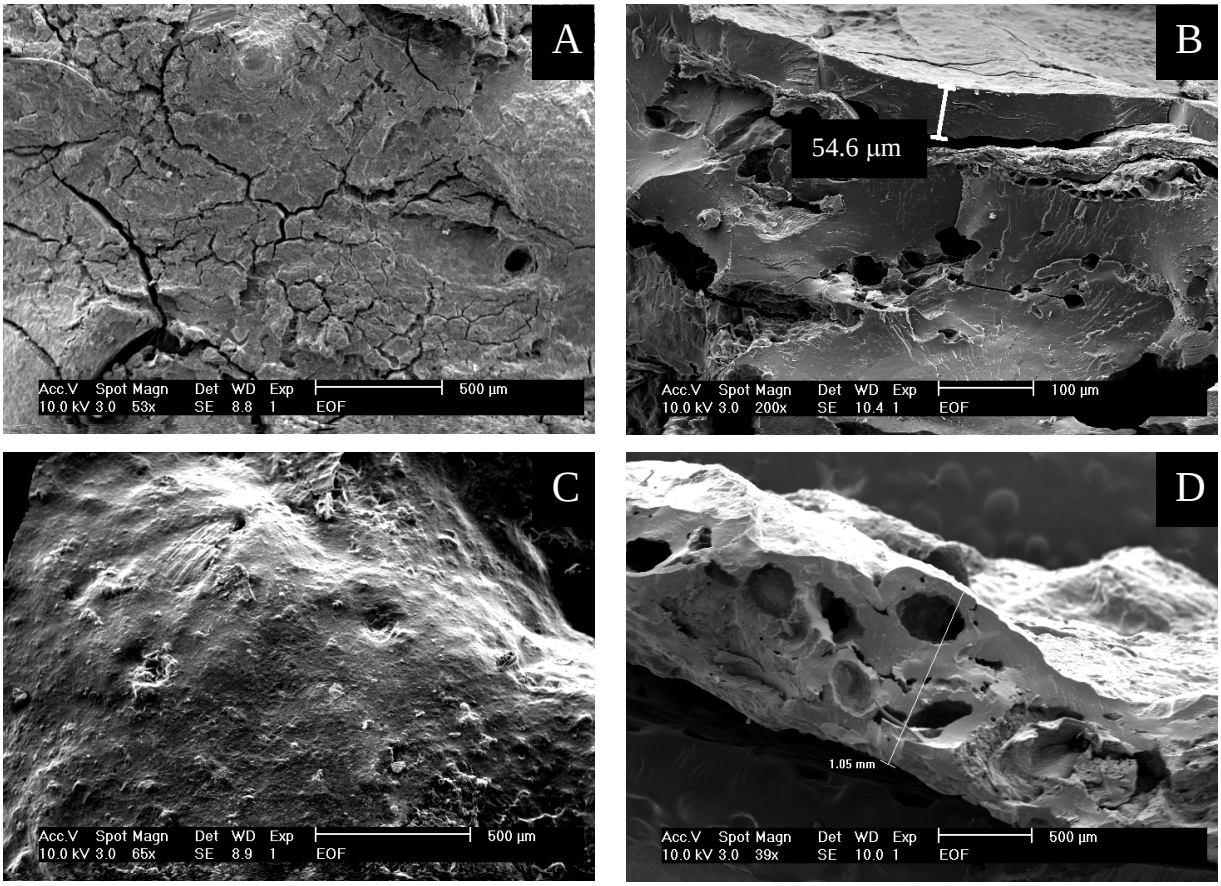

Fig. 4.2. SEM micrographs of cereal flakes with various coatings: Hylon VII (A & B) and glucose (C & D).

Milk Absorption

All three starch-coated cereals showed significantly reduced milk absorption compared with the two controls of glucose-coated and uncoated cereals (Fig. 4.3). Within the starches, cereals coated with waxy corn starch showed a slightly higher milk absorption value than those coated with common corn and Hylon VII. Yoshida and Hijiya (1973) reported that a high percentage of short chains (DP ≤ 100) in debranched starches of varying amylose contents had an adverse effect on starch film formation. Being 100% amylopectin, waxy corn starch consists of a majority of linear glucan chains with DP < 100, which would negatively impact its film forming properties. Because milk absorption is directly related to barrier properties of the

coatings, these results suggest that these three starches coatings had better moisture barrier properties than glucose.

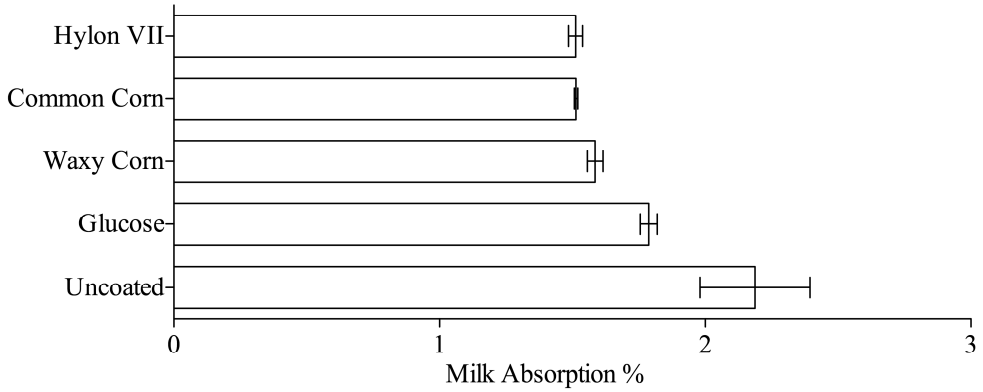

Fig. 4.3. Milk absorption of cereals with different coating materials after soaking in milk at 8°C for 3 min.

Textural Analysis

The glucose coating produced the hardest dry cereal, but also lost the most of its texture with milk soaking (Fig. 4.4). The peak force of the coated-cereals in the dry state increased with increasing amylose content of the starch coating, which was attributed to the better film-forming properties of amylose. Cereals coated with common corn starch and Hylon VII exhibited similar peak forces before and after soaking in milk, while cereals coated with waxy corn starch showed an increase in peak force with milk soaking. This increase was ascribed to the force required to extrude the wet cereal through the slots in the Kramer shear cell, in contrast of the dry cereals, which was not an indication of hardness. Both the glucose-coated and uncoated cereals displayed significant losses in peak force with milk soaking. The decrease in peak force was coupled with a loss of a crunching sound during the analysis. The peak force was negatively correlated with milk absorption, which was expected due to the effect of absorbed milk on the texture of cereals.

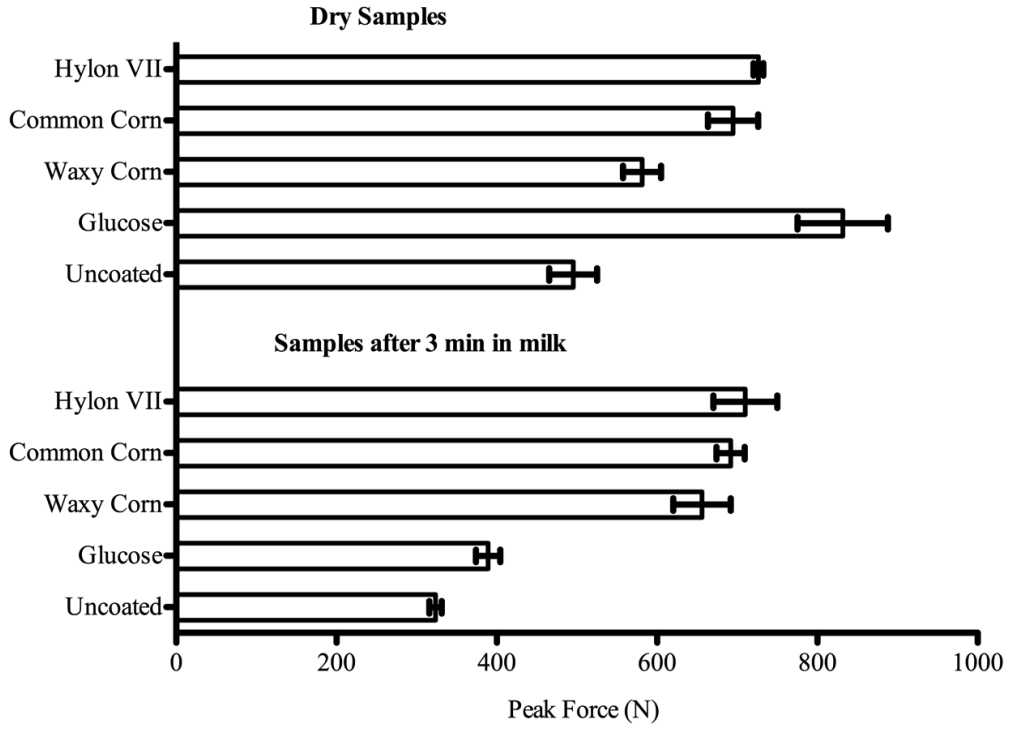

Fig. 4.4. Peak force of cereals with different coating materials before and after soaking in milk at 8°C for 3 min.

The work to peak (area under the force-displacement curve) followed the general trend of the peak force measurements (Fig. 4.5). Similar to the peak force results, waxy corn starch displayed an increase in the work to peak with milk soaking. However, the increase was more substantial in the work to peak measurement, which also indicates that the extrusion of wet cereal flakes was a significant factor in the textural analysis of the cereals soaked in milk. The extrusion of cereals through the slots of the shear cell broadened the force-displacement curve, thus increasing the work to peak at higher rates than the peak force.

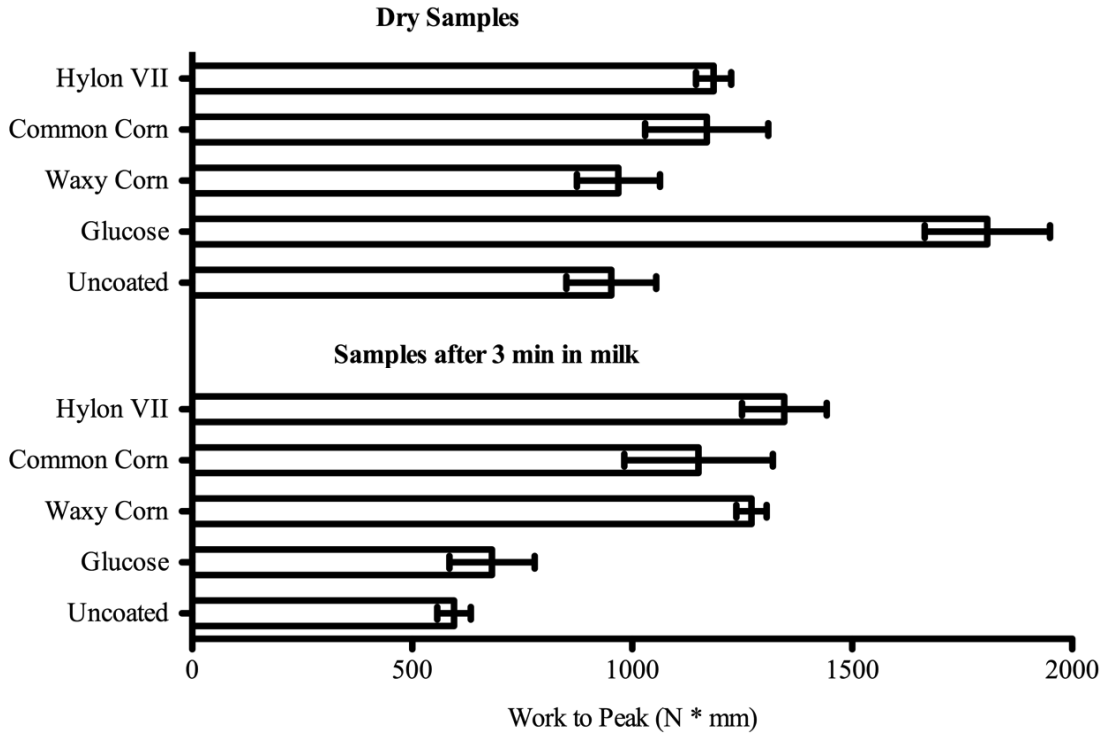

Fig. 4.5. Work to peak of cereals with different coating materials before and after soaking in milk at 8°C for 3 min.

Nutritional Properties

The dietary fiber contents of all cereals samples are presented in Table 4.1. In order to mimic the mastication experience, the particle size used for dietary fiber analysis was 1.4 - 2.8 mm instead of ground cereal flours. Although there was variation in the variation of the dietary fiber content, which was largely attributed to the particle size, the Hylon VII-coated flakes showed a significantly higher dietary fiber content than the others when analyzed using Tukey's test. The other coated cereals had similar dietary fiber content as the controls. These results suggest that the high amylose content in Hylon VII, when debranched, was capable of rapidly re-associate during the spraying and drying step of coating application to the extent that it formed a crystalline structure more resistant to enzymatic hydrolysis. These results also support the SEM

observations that only the Hylon VII-coated cereals showed a distinct starch layer, which was probably crystalline in nature and resistant to enzyme hydrolysis.

Table 4.1

Dietary Fiber Contents of Cereals with Different Coatings*

Coating	Uncoated	Glucose	Waxy Corn	Common Corn	Hylon VII
Dietary fiber content (%)	11.8 ± 0.4	12.9 ± 1.2	13.2 ± 1.0	13.6 ± 1.6	15.6 ± 1.3

*Means of triplicate measurements with standard deviations.

Conclusions

Debranched starches displayed better barrier properties than glucose when used as a flaked cereal coating. The increased ability of the debranched starches to reduce milk absorption was correlated with their increase in peak force and work to peak values. The formation of a layer of coating was only noted in cereals coated with debranched Hylon VII. The high amylose content in Hylon VII was ascribed to the rapid formation of a layer of coating and also resulted in a higher dietary fiber value. The use of debranched corn starches appears to be a viable alternative to sugar for extending the bowl-life of flaked cereal and at the same time increasing the dietary fiber content.

Acknowledgements

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CHAPTER 5

OVERALL CONCLUSION

This study demonstrates that chain length distribution of starch is important for RS and film formation. Debranched corn starches of varying amylose contents had increased RS contents with the incorporation of a mild β-amylase treatment. The mild β-amylase treatment hydrolyzed amylopectin short chains, which interfered with reassociation of linear chains, and increased the proportions of amylose and amylopectin long chains, which contributed to RS formation. However extensive β-amylolysis might create new amylopectin short chains and/or shorten amylose and amylopectin long chains to become not as optimal to form RS under the temperature cycling conditions in this study. Furthermore, debranched starches displayed better barrier properties than glucose when used as a flaked cereal coating. The amylose content of the starch coating did not have significant effects on milk absorption or texture, but did affect the surface morphology and dietary fiber content of the coated cereals. In conclusion, debranched corn starches showed promise as an alternative to sugar for extending the bowl-life of flaked cereal and as a source to produce RS3 when combined with a mild β-amylase hydrolysis.

CHAPTER 6

REFERENCES

American Association of official analytical chemists. 1985. Changes in methods: Total dietary fiber in foods. Enzymatic-gravimetric method. First action 43. A-14-43. A-20. Journal of the Association of Official Analytical Chemists. 68:399.

Anderson BA, Singh RP, Rovedo C. 2003. Use of phytoglycogen extracted from corn to increase the bowl-life of breakfast cereal. Journal of Food Process Engineering 26(3):315-322.

Asp NG. 1992. Resistant starch – Proceedings from the 2nd plenary meeting of EURESTA-European flair concerted action 11 on physiological implications of the consumption of resistant starch in man. European Journal of Clinical Nutrition 46:S1-S1.

Atwell WA, Hood LF, Lineback DR, Varrianomarston E, Zobel HF. 1988. The terminology and methodology associated with basic starch phenomena. Cereal Foods World 33(3):306

Bartolomei C, Thesing R. inventors; General Mills Inc. assignee. 1998 Jan 20. Method for preparing a sugar coated R-T-E cereal. US Patent 5,709,902

Berry CS. 1986. Resistant starch – formation and measurement of starch that survives exhaustive digestion with amylolytic enzymes during the determination of dietary fiber. Journal of Cereal Science 4(4):301-314.

Bone DP, Brophy K, Champion R, Meschewski S, McKinney CW. inventors; Quaker Oats Company, assignee. 1996 May 13. Ready-to-eat cereals. US Patent 4,588,596

Cai L, Shi Y-C, Rong L, Hsiao BS. 2010. Debranching and crystallization of waxy maize starch in relation to enzyme digestibility. Carbohydrate Polymers 81(2):385-393.

Cai L, Shi Y-C. 2010. Structure and digestibility of crystalline short-chain amylose from debranched waxy wheat, waxy maize, and waxy potato starches. Carbohydrate Polymers 79(4):1117-1123.

Calandro T, & Murray J, inventor. Nabisco brands, assignee. 1992 March 3. Process of making ready-to-eat cereals. US Patent 5,093,146.

Caldwell EF, Fast RB, Ievolella J, Lauhoff C, Levine H, Miller RC, Slade L, Strahm BS, Whalen PJ. 2000. Cooking of ready-to-eat breakfast cereals. Cereal Foods World 45(6):244+.

Carpenter T, Fisher W, Smith T, inventor. General Foods Corp., assignee. 1989 Nov 14. Coating cereal with fruit juice. US Patent 4,880,645.

Chung-Wai C, Henley M, Altieri P. inventors; National Starch and Chemical Investment Holding Corporation, assignee. 1994. Process for making amylase resistant starch from high amylose starch. US Patent 5,281,276

Czuchajowska Z, Sievert D, Pomeranz Y. 1991. Enzyme-resistant starch 4. effects of complexing lipids. Cereal Chemistry 68(5):537-542.

Dedeckere EAM, Kloots WJ, Vanamelsvoort JMM. 1993. Resistant starch decreases serum cholesterol and triacylglycerol concentration in rats. Journal of Nutrition 123(12):2142-2151.

Eberstein K, Hopcke R, Koniecznyjanda G, Stute R. 1980. DSC investigations of starches 1. feasibility of thermoanalytical methods to characterize starches. Starke 32(12):397-404.

Eerlingen RC, Crombez M, Delcour JA. 1993a. Enzyme-resistant starch 1. quantitative and qualitative influence of incubation time and temperature of autoclaved starch on resistant starch formation. Cereal Chemistry 70(3):339-344.

Eerlingen RC, Deceuninck M, Delcour JA. 1993b. Enzyme-resistant starch 2. influence of amylose chain-length on resistant starch formation. Cereal Chemistry 70(3):345-350.

Eliasson AC, Krog N. 1985. Physical properties of amylose monglyceride complexes. Journal of Cereal Science 3(3):239-248.

Englyst HN, Kingman SM, Cummings JH. 1992. Classification and measurement of nutritionally important starch fractions. European Journal of Clinical Nutrition 46:S33-S50.

Englyst HN, Trowell H, Southgate DAT, Cummings JH. 1987. Dietary fiber and resistant starch. American Journal of Clinical Nutrition 46(6):873-874.

Escarpa A, Gonzalez MC, Manas E, Garcia-Diaz L, Saura-Calixto F. 1996. Resistant starch formation: Standardization of a high-pressure autoclave process. Journal of Agricultural and Food Chemistry 44(3):924-928.

Evans A, Thompson DB. 2004. Resistance to alpha-amylase digestion in four native high-amylose maize starches. Cereal Chemistry 81(1):31-37.

Fannon JE, Bemiller JN. 1992. Strucutre of corn starch paste and granule remnants revealed by low-temperature scanning electron-microscopy after cryopreparation. Cereal Chemistry 69(4):456-460.

Faraj A, Vasanthan T, Hoover R. 2004. The effect of extrusion cooking on resistant starch formation in waxy and regular barley flours. Food Research International 37(5):517-525.

Gallant DJ, Bouchet B, Baldwin PM. 1997. Microscopy of starch: Evidence of a new level of granule organization. Carbohydrate Polymers 32(3-4):177-191.

Gallant DJ, Bouchet B, Buleon A, Perez S. 1992. Physical characteristics of starch granules and susceptibility to enzymatic degradation. European Journal of Clinical Nutrition 46:S3-S16.

Garcia-Alonso A, Jimenez-Escrig A, Martin-Carron N, Bravo L, Saura-Calixto F. 1999. Assessment of some parameters involved in the gelatinization and retrogradation of starch. Food Chemistry 66(2):181-187.

Germani R, Ciacco CF, Rodriguezamaya DB. 1983. Effect of sugars, lipids and type f starch on the mode and kinetics of retrogradation of concentrated corn starch gels. Starke 35(11):377-381.

Gibson SA. 1993. Consumption and sources of sugars in the diets of British schoolchildren – are high-sugar diets nutritionally inferior. Journal of Human Nutrition and Dietetics 6(4):355-371.

Gidley MJ. 1987. Factors affecting the crystalline type (Ac) of native starches and model compounds – a rationalization of observed effects in terms of polymorphic structures. Carbohydrate Research 161(2):301-304.

Gidley MJ. 1989. Molecular mechanisms underlying amylose aggregation and gelation. Macromolecules 22(1):351-358.

Gidley MJ, Bociek SM. 1985. Molecular organization in starches – a C-13 CP MAS NMR - study. Journal of the American Chemical Society 107(24):7040-7044.

Gidley MJ, Bulpin PV. 1987. Crystallization of maltooligosaccharides as models of the crystalline forms of starch – minimum chain-length requirement for the formation of double helices. Carbohydrate Research 161(2):291-300.

Gidley MJ, Cooke D, Darke AH, Hoffmann RA, Russell AL, Greenwell P. 1995. Molecular order and structure in enzyme-resistant retrograded starch. Carbohydrate Polymers 28(1):23-31.

Green D, Nowakowski C. inventors; General Mills Inc. assignee. 2005 Dec 1. Low sugar presweetened coated cereals and method of prepartation. US Patent Application 2005/0266142 A1

Guraya HS, James C, Champagne ET. 2001a. Effect of cooling, and freezing on the digestibility of debranched rice starch and physical properties of the resulting material. Starch-Starke 53(2):64-74.

Guraya HS, James C, Champagne ET. 2001b. Effect of enzyme concentration and storage temperature on the formation of slowly digestible starch from cooked debranched rice starch. Starch-Starke 53(3-4):131-139.

Guraya HS, Kadan RS, Champagne ET. 1997. Effect of rice starch-lipid complexes on in vitro digestibility, complexing index, and viscosity. Cereal Chemistry 74(5):561-565.

Hamaker BR, Griffin VK. 1993. Effect of disulfide bond-containing protein on rice starch gelatinization and pasting. Cereal Chemistry 70(4):377-380.

Hanashiro I, Abe J, Hizukuri S. 1996. A periodic distribution of the chain length of amylopectin as revealed by high-performance anion-exchange chromatography. Carbohydrate Research 283:151-159.

Haralampu SG. 2000. Resistant starch - a review of the physical properties and biological impact of RS3. Carbohydrate Polymers 41(3):285-292.

Hasjim J, Jane JL. 2009. Production of resistant starch by extrusion cooking of acid-modified normal-maize starch. Journal of Food Science 74(7):C556-C562.

Hickman BE, Janaswamy S, Yao Y. 2009. Autoclave and beta-amylolysis lead to reduced in vitro digestibility of starch. Journal of Agricultural and Food Chemistry 57(15):7005-7012.

Hizukuri S. 1985. Relationship between the distribution of the chain-length of amylopectin and the crystalline – structure of starch granules. Carbohydrate Research 141(2):295-306.

Hizukuri S. 1986. Polymodal distribution of the chain lengths of amylopectins, and its significance. Carbohydrate Research 147(2):342-347.

Hizukuri S, Takeda Y, Yasuda M, Suzuki A. 1981. Multi-branched nature of amylose and the action of debranching enzymes. Carbohydrate Research 94(2):205-213.

Hoebler C, Devaux MF, Karinthi A, Belleville C, Barry JL. 2000. Particle size of solid food after human mastication and in vitro simulation of oral breakdown. International Journal of Food Sciences and Nutrition 51(5):353-366.

Holm J, Bjorck I, Ostrowska S, Eliasson AC, Asp NG, Larsson K, Lundquist I. 1983. Digestibility of amylose-lipid complexes invitro and invivo. Starke 35(9):294-297.

Hoseney RC, Atwell WA, Lineback DR. 1977. Scanning electron – microscopy of starch isolated from baked products. Cereal Foods World 22(2):56-60.

Jane JL, Robyt JF. 1984. Structure studies of amylose-V complexes and retrograded amylose by action of alpha-amylases, and new method for preparing amylodextrin. Carbohydrate Research 132(1):105-118.

Jane JL, Wong KS, McPherson AE. 1997. Branch-structure difference in starches of A- and B-type x-ray patterns revealed by their Naegeli dextrins. Carbohydrate Research 300(3):219-227.

Johnson RK, Frary C. 2001. Choose beverages and foods to moderate your intake of sugars: The 2000 Dietary Guidelines for Americans - What's all the fuss about? Journal of Nutrition 131(10):2766S-2771S.

Jolley JE. 1970. MICROSTRUCTURE OF PHOTOGRAPHIC GELATIN BINDERS. Photographic Science and Engineering 14(3):169-177.

Kasemsuwan T, Jane J, Schnable P, Stinard P, Robertson D. 1995. Characterization of the dominant mutant amylose-extender (AEL-5180) maize starch. Cereal Chemistry 72(5):457-464.

Kohyama K, Nishinari K. 1991. Effect of soluble sugars on gelatinization and retrogradation of sweet-potato starch. Journal of Agricultural and Food Chemistry 39(8):1406-1410.

Larsson K, Miezis Y. 1979. Possibility of dietary fiber formation by interaction between starch and lipids. Starke 31(9):301-302.

Lauritzen J, Hoffman JD. 1973. Extension of theory of growth of chain-folded polymer crystals to large undercoolings. Journal of Applied Physics 44(10):4340-4352.

Lewis CJ, Park YK, Dexter PB, Yetley EA. 1992. Nutrient intakes and body weights of persons consuming moderate levels of added sugars. Journal of the American Dietetic Association 92(6):708-713.

Liming C, Yong-Cheng S. 2010. Structure and digestibility of crystalline short-chain amylose from debranched waxy wheat, waxy maize, and waxy potato starches. Carbohydrate Polymers 79(4):1117-1123.

Liu Z, Han JH. 2005. Film-forming characteristics of starches. Journal of Food Science 70(1):E31-E36.

Long C, Chatel R, inventor. Pepsico, assignee. 2006 Dec 21. Reduced sugar RTE cereals with maltodextrin. US Patent Application 2006/0286223 A1.

Manners DJ. 1989. Recent developments in our understanding of amylopectin structure. Carbohydrate Polymers 11(2):87-112.

Mercier C, Charbonniere R, Grebaut J, Gueriviere J. 1980. Formation of amylose-lipid complexes by twin-screw extrusion of manioc starch. Cereal Chemistry 57(1):4-9.

Miao M, Zhang T, Qin X-T, Jiang B. 2009. Slow digestibility of cereal starch and postprandial glycemic response. Acta Nutrimenta Sinica 31(3):218-221.

Mikus FF, Hixon RM, Rundle RE. 1946. The complexes of fatty acids with amylose. Journal of the American Chemical Society 68(6):1115-1123.

Miller BS, Derby RI, Trimbo HB. 1973. Pictorial explanation for increase in viscosity of a heated wheat starch-water suspension. Cereal Chemistry 50(3):271-280.

Mitan FJ, Jokay L, inventors; American Maize Products-Corporation, assignee. 1969 Feb 18. Protective food coatings. US Patent 3,427,951.

Moates GK, Noel TR, Parker R, Ring SG. 1997. The effect of chain length and solvent interactions on the dissolution of the B-type crystalline polymorph of amylose in water. Carbohydrate Research 298(4):327-333.

Morrison WR, Gadan H. 1987. The amylose and lipid contents of starch granules in developing wheat endosperm. Journal of Cereal Science 5(3):263-275.

Ozturk S, Koksel H, Ng PKW. 2009. Characterization of resistant starch samples prepared from two high-amylose maize starches through debranching and heat treatments. Cereal Chemistry 86(5):503-510.

Ozturk S, Koksel H, Ng PKW. 2011. Production of resistant starch from acid-modified amylotype starches with enhanced functional properties. Journal of Food Engineering 103(2):156-164.

Palviainen P, Heinamaki J, Myllarinen P, Lahtinen R, Yliruusi J, Forssell P. 2001. Corn starches as film formers in aqueous-based film coating. Pharmaceutical Development and Technology 6(3):353-361.

Pfannemuller B, Mayerhof H, Schulz RC. 1971. Conformation of amylose in aqueous solution – optical rotatory dispersion and circular dichroism of amylose-iodine complexes and dependence on chain length of retrogradation amylose. Biopolymers 10(2):243-&.

Pfannemuller B. 1987. Influence of chain-length of short monodisperse amyloses on the formation of A-type and B-type X-ray-diffraction patterns. International Journal of Biological Macromolecules 9(2):105-108.

Pfannemuller B, Bauer-Carnap A. 1977. Electron-microscopic studies on fibrils formed from retrograded synthetic amyloses. Colloid and Polymer Science 255(9):844-848.

Philpot K, Martin M, Butardo V, Willoughby D, Fitzgerald M. 2006. Environmental factors that affect the ability of amylose to contribute to retrogradation in gels made from rice flour. Journal of Agricultural and Food Chemistry 54(14):5182-5190.

Ratnayake WS, Hoover R, Shahidi F, Perera C, Jane J. 2001. Composition, molecular structure, and physicochemical properties of starches from four field pea (Pisum sativum L.) cultivars. Food Chemistry 74(2):189-202.

Robin F, Merinat S, Simon A, Lehmann U. 2008. Influence of Chain Length on alpha-1,4-D-Glucan Recrystallization and Slowly Digestible Starch Formation. Starch-Starke 60(10):551-558.

Robin JP, Mercier C, Charbonn.R, Guilbot A. 1974. Linterized starches gel-filtration and enzymatic studies of insoluble residues from prolonged acid treatment of potato starch. Cereal Chemistry 51(3):389-406.

Ruxton CHS, Garceau FJS, Cottrell RC. 1999. Guidelines for sugar consumption in Europe: Is a quantitative approach justified? European Journal of Clinical Nutrition 53(7):503-513.

Sajilata MG, Singhal RS, Kulkarni PR. 2006. Resistant starch - A review. Comprehensive Reviews in Food Science and Food Safety 5(1):1-17.

Seib P, Woo K, inventors. Kansas State University, assignee. 2001 Oct. 11. Reversibly swellable starch products. US Patent 6,299,907.

Schmiedl D, Bauerlein M, Bengs H, Jacobasch G. 2000. Production of heat-stable, butyrogenic resistant starch. Carbohydrate Polymers 43(2):183-193.

Sheng YB, Widicus W, inventor. Quaker Oats Company. assignee. 1986 Sept. 30. Dipeptide sweetened ready-to-eat cereal and coating method. US Patent 4,614,657.

Sievert D, Pomeranz Y. 1989. Enzyme-resistant starch 1. characterization and evaluation by enzymatic, thermoanalytical, and microscopic methods. Cereal Chemistry 66(4):342-347.

Siljestrom M, Eliasson AC, Bjorck I. 1989. Characterization of resistant starch from autoclaved wheat-starch. Starch-Starke 41(4):147-151.

Slade L, Levine H. 1987. Starch and sugars as partially-crystalline, water-compatible polymer systems. Cereal Foods World 32(9):680-680.

Spies RD, Hoseney RC. 1982. Effect of sugars on starch gelatinization. Cereal Chemistry 59(2):128-131.

Takeda C, Takeda Y, Hizukuri S. 1989. Structure of amylomaize amylose. Cereal Chemistry 66(1):22-25.

Takeda C, Takeda Y, Hizukuri S. 1993. Structure of the amylopectin fraction of amylomaize. Carbohydrate Research 246:273-281.

Takeda Y, Hizukuri S, Takeda C, Suzuki A. 1987. Structures of branched molecules of amyloses of various origins, and molecular fractions of branched and unbranched molecules. Carbohydrate Research 165(1):139-145.

Tester RF, Karkalas J, Qi X. 2004. Starch - composition, fine structure and architecture. Journal of Cereal Science 39(2):151-165.

Tester RF, Morrison WR. 1990. Swelling and gelatinzation of cereal starches 1. effects of amylopectin, amylose, and lipids. Cereal Chemistry 67(6):551-557.

Tharanathan RN. 2003. Biodegradable films and composite coatings: past, present and future. Trends in Food Science & Technology 14(3):71-78.

Topping DL, Clifton PM. 2001. Short-chain fatty acids and human colonic function: Roles of resistant starch and nonstarch polysaccharides. Physiological Reviews 81(3):1031-1064.

Wolff IA, Davis HA, Cluskey JE, Gundrum LJ, Rist CE. 1951. Preparation of films from amylose. Industrial and Engineering Chemistry 43(4):915-919.

Yamaguchi M, Kainuma K, French D. 1979. Electron-microscopic observations of waxy maize starch. Journal of Ultrastructure Research 69(2):249-261.

Yoshida M, Hijiya H, inventors; Hyashibira Company, assignee. 1973 May 22. Process for the production of amylose films. US Patent 3,734,760.

Zabar S, Shimoni E, Bianco-Peled H. 2008. Development of nanostructure in resistant starch type III during thermal treatments and cycling. Macromolecular Bioscience 8(2):163-170.

Zobel HF. 1988. Molecules to granules – a comprehensive starch review. Starch-Starke 40(2):44-50.

Zobel HF, Young SN, Rocca LA. 1988. Starch gelatinization – an X-ray diffraction study. Cereal Chemistry 65(6):443-446.