CHAPTER 5

Starch Gelatinization

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Contents

Abstract

Starch occurs as highly organized structures, known as starch granules. Starch has unique thermal properties and functionality that have permitted its wide use in food products and industrial applications. When heated in water, starch undergoes a transition process, during which the granules break down into a mixture of

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polymers-in-solution, known as gelatinization. The sequence of structural transformations that the starch granule undergoes during this order-to-disorder transition has been extensively researched. None of the published starch gelatinization theories can fully and adequately explain the exact mechanism of sequential structural changes that starch granules undergo during gelatinization. This chapter analyzes several published theories and summarizes our current understanding of the starch gelatinization process.

Key Words: Starch, gelatinization.

ABBREVIATIONS

DSC	Differential scanning calorimetry
NMR	Nuclear magnetic resonance
RS	Resistant starch
T_g	Glass transition temperature
T_o	DSC onset temperature
T_p	DSC peak temperature
T_c	DSC conclusion (or end) temperature
T_m	Melting temperature
TMA	Thermomechanical analysis
XRD	X-ray diffraction

I. INTRODUCTION

Starch is the major component of human diet and is also used for many food and nonfood/industrial applications (Gaillard, 1987; FAO, 2006a). A significant quantity of starch is further processed by subjecting it to various forms of chemical and physical modifications, resulting in starches with even greater functionality attributes (Zobel, 1992). Both unmodified (native) and modified starches are used as bulking agents, thickeners, stabilizers, viscosity builders, and gel formers. Starch is also used to produce various hydrolysis products, such as maltose, maltodextrins, and cyclodextrins, by acid and/or enzyme conversion methods.

Starch is obtained from a variety of plant sources. Corn, cassava, sweet potato, wheat, and potato are the major sources of food starch while sorghum, barley, rice, sago, arrowroot, etc. serve as minor sources of starch in different localized regions of the world (Gaillard, 1987; Ratnayake and Jackson, 2003). Raw starch granules do not disperse in cold water. This limits the use of raw native starches for food as well as industrial applications, and therefore starch is often cooked during product-manufacturing processes. Cooking (or heat treatment) causes structural and often molecular changes in granular and polymeric structures of starch.

Functional properties of starch are directly influenced by hydrothermal (heat and moisture) treatment or processing conditions. When raw starch granules are heated in water, the semicrystalline nature of their structure is reduced or eliminated and the granules break down, forming a viscous solution; solution viscosity depends on starch source and concentration. Heat-induced starch granule breakdown in water is known as gelatinization. The unique starch gelatinization processes, and the subsequent formation of viscous solutions when starch granules are heated in water, have fascinated researchers since the early 1800s. It is known that granules undergo a sequence of structural changes during gelatinization. These structural changes, however, are poorly understood and are the subject of frequent research studies.

Gelatinization is described as a transition of starch granules from an ordered state to a disordered state. This chapter critically evaluates and summarizes the ''evolution'' of currently accepted theoretical descriptions of starch gelatinization process.

II. STARCH: IMPORTANCE AND SOURCES

Starch is one of the most abundant plant polysaccharides and is a major source of carbohydrates and energy in the human diet (Zobel and Stephen, 1995). Starch is the most widely used hydrocolloid in the food industry (Wanous, 2004), and is also a widely used industrial substrate polymer. Total annual world production of starch is approximately 60 million MT and it is predicted to increase by additional approximately 10 million MT by 2010 (FAO, 2006b; LMC International, 2002; S. K. Patil and Associates, 2007). Corn/maize (*Zea mays* L.), cassava (also known as tapioca—*Manihot esculenta* Crantn.), sweet potato (*Ipomoea batatas* L.), wheat (*Triticum aestivum* L.), and potato (*Solanum tuberosum* L.) are the major sources of starch, while rice (*Oryza sativa* L.), barley (*Hordeum vulgare* L.), sago (*Cycas* spp.), arrowroot (*Tacca leontopetaloides* (L.) Kuntze), buckwheat (*Fagopyrum esculentum* Moench), etc. contribute in lesser amounts to total global production.

Starch is isolated from plant sources by various methods (Ratnayake and Jackson, 2003; Zobel, 1992). The method of starch isolation depends on the nature and composition of the raw material source. Most food starches are isolated and purified on a commercial scale and then used as ingredients by food manufacturers. Commercial food starches are generally classified based on both botanical origin and functionality. With the increasing availability of modified starches prepared for specific food applications, starch manufacturers tend to emphasize and market starch with a secondary focus on botanical source. In fact, for food manufacturers, ingredient functionality is the most important consideration when choosing starch for food products. Starch functional properties depend on many factors that include both granular and molecular (polymeric) characteristics. Starch physicochemical properties depend on both its botanical source and any form of physical or chemical modification applied to starch during its preparation into an ingredient. Extensive research on starch has focused on its botanical sources, functionality, chemistry, and nutritional/health effects.

III. STARCH STRUCTURE AND CRYSTALLINITY

Native or raw starch occurs as small semispherical (microscopic) structures called granules. The size, shape, and molecular arrangement inside the granules depend on the species, cultivar, variety of the source plant, and the genetic–environment interactions (Ratnayake and Jackson, 2003; Trubell, 1944; Zobel, 1988a). Regardless of the plant source, all starches are made up of d-glucopyranose-based polymeric structures. In starch polymers, individual glucose units are linked together by either α(1-4) or α(1-6) glycosidic bonds. The starch biosynthetic pathway generally results in two different kinds of polymers being formed, namely, amylose and amylopectin. Amylose is essentially a linear polymer with α(1-4) linkages [and 2–5% α(1-6) bonds] and has a molecular weight less than 1×10^5. Amylopectin is a branched polymer, which has both α(1-4) and α(1-6) bonds with a molecular weight ranging from 50 to 500 million (Banks and Greenwood, 1975; Thomas and Atwell, 1999). The molecular weights and degrees of branching in a given starch polymer depend on its source (Blanshard, 1987; Zobel, 1992; Zobel and Stephen, 1995). The linear branch chains of the amylopectin molecule contain an average of 20–25 α(1-4)-linked anhydroglucose residues. These linear branches are linked by α(1-6) glycosidic bonds to form a highly branched structure (Hizukuri, 1986; Manners, 1989; Fig. 5.1). For detailed information on how starch polymers are synthesized in plants, readers may refer to reviews and articles by Denyer *et al.* (1997), Smith *et al.*, (1997), Erlander (1998), Kossmann and Lloyd (2000), Ball and Morell (2003), James *et al.*, (2003), and Preiss (2004). The polymer composition of starch is genetically controlled; mutations are found naturally or plant breeding techniques can be used to obtain *high-amylose* or high-amylopectin (*waxy*) starches (Blennow, 2004; Bogracheva *et al.*, 1999; Tetlow, 2006). The basic structure of the starch granule has been recognized and documented in the literature since the late 1800s (Czaja, 1978; Donald, 2004; French, 1944; Frey-Wyssling, 1969; Jane, 2006; Kraemer, 1902; Meyer and Gibbons, 1951; Oates, 1997; Trubell, 1944; Vermeylen *et al.*, 2004; Wang *et al.*, 1998). Early reports were mainly based on optical microscopy results, which

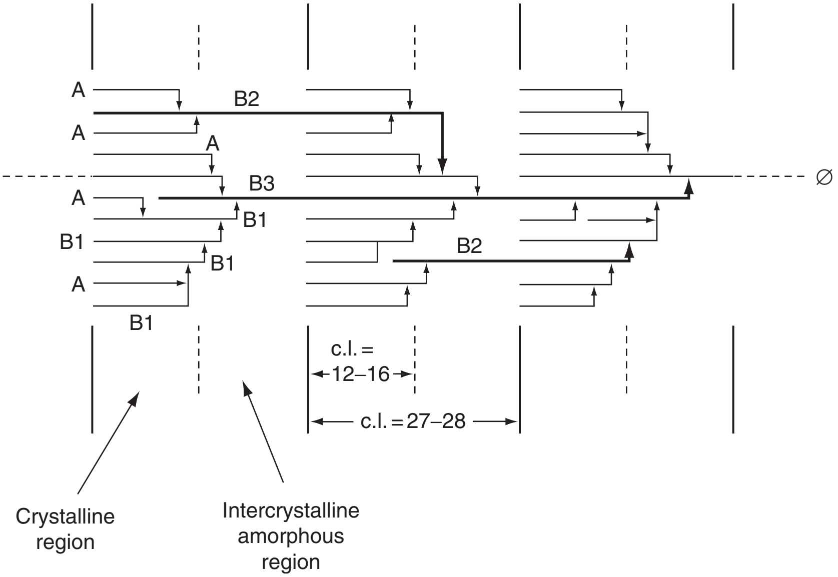

FIGURE 5.1 Cluster model of amylopectin. A and B denote nomenclature of branch chains, Φ=reducing end, c.l. = chain length in degree of polymerization. Reprinted from Carbohydrate Research, Vol. 147, Hizukuri (1986), Polymodal distribution of the chain lengths of amylopectin, and its significance, Pages 342–347, with permission from Elsevier.

revealed granule shapes, sizes, and the presence of growth rings (Fig. 5.2) inside granules from different sources (Leach, 1914). Although the existence of growth rings is obvious in starch granules, the biochemical origins and physiological importance of these rings are poorly understood. Concentric growth rings or striations inside the starch granule were initially thought to be a result of "periods of relative inactivity of deposition of starchy material" during granule synthesis (Trubell, 1944). Later, it was proposed that these growth rings were formed as a result of a "photosynthetically controlled supply of starch precursor" in wheat starch, but not in potato starch (Buttrose, 1962). Subsequent research during the last few decades has not completely explained the origin of growth rings, although the enzyme activity (starch synthases) involved in the process are well documented (Fig. 5.3). More recent reports (Pilling and Smith, 2003) indicate that a variety of factors, including circadian rhythms, physical mechanisms, and diurnal rhythms, control growth ring formation in starch granules.

A. Birefringence and starch crystallinity

Starch granules, when observed under polarized light, exhibit an optical birefringence pattern known as a "Maltese cross" (Fig. 5.4), which implies a high degree of molecular order within the granule (Greenwood, 1979).

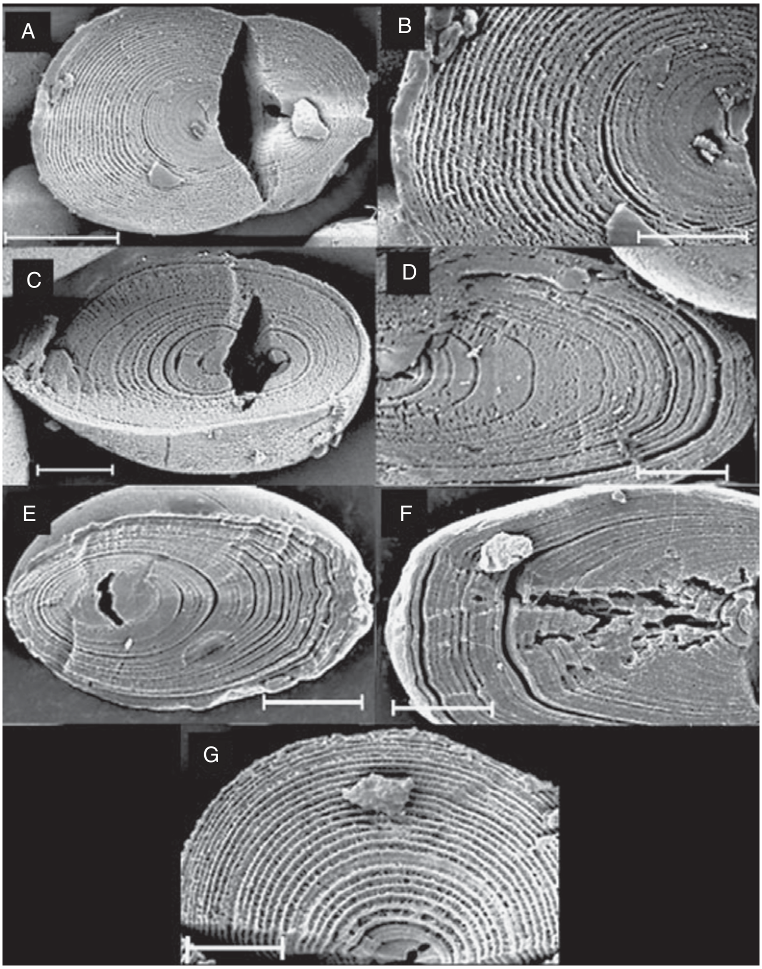

FIGURE 5.2 Growth rings in potato starch granules developed under different environmental conditions. Scale bars on A and G represent 10 μm; all other scale bars represent 5 μm. (A and B) Plant grown in 16 h of light at 18 °C and 8 h of dark at 15 °C. (C and D) Plant grown in constant light and constant temperature (18 °C). (E and F) Microtuber grown in continuous darkness at 25 °C for 12–16 weeks. (G) Plant grown in 20 h of light at 18 °C and 20 h of dark at 15 °C (Pilling and Smith, 2003; reproduced with permission from American Society of Plant Biologists).

Birefringence (or double refraction) is the decomposition of a light ray into two rays when it passes through certain types of crystalline material. This occurs only when the material is anisotropic, that is, the material has different characteristics in different directions. Amylose and amylopectin polymers are organized into a radially anisotropic, semicrystalline unit in the starch granule. This radial anisotropy is responsible for the distinctive

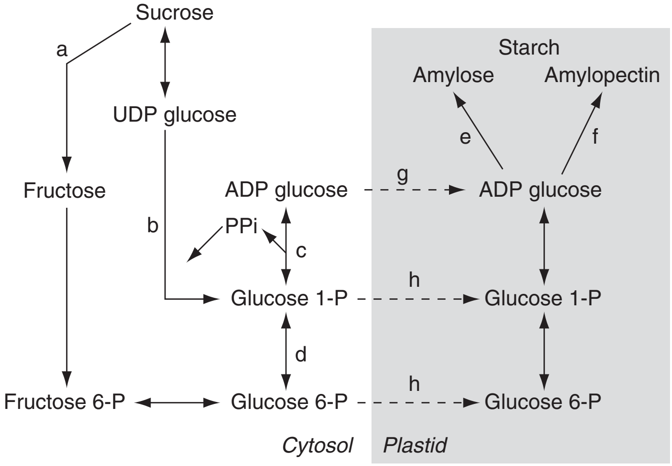

FIGURE 5.3 Biochemical pathway of starch biosynthesis from sucrose. The major metabolites and enzymes involved in the conversion of sucrose to starch in storage organs. Carbon enters the plastid either as a hexose phosphate or as ADPglucose. Enzymes are: (a) sucrose synthase; (b) UDPglucose pyrophosphorylase; (c) ADPglucose pyrophosphorylase; (d) phosphoglucomutase; (e) starch synthase (GBSSI); (f) starch synthase and starch-branching enzyme; (g) ADPglucose transporter; (h) hexose phosphate transporter. PPi: inorganic pyrophosphate (Smith *et al.*, 1997; Reprinted with permission from the Annual Review of Plant Physiology and Plant Molecular Biology, Volume 48, ©1997 by Annual Reviews. www.annualreviews.org.)

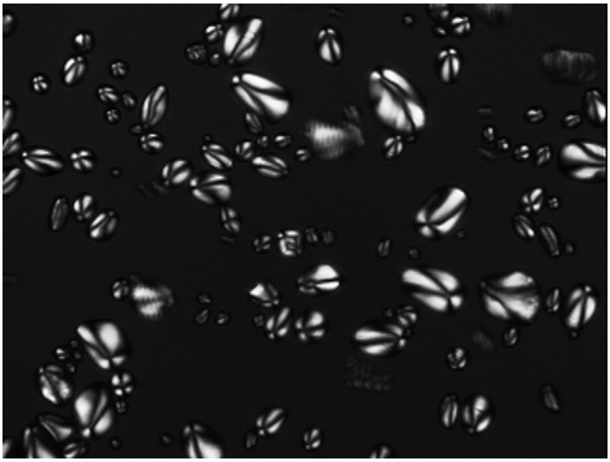

FIGURE 5.4 Potato starch granules viewed under polarized light (magnification, ×400). Reprinted from Encyclopedia of Food Sciences and Nutrition (Second Edition), Jackson (2003b), Starch – Structure, properties and determination, Pages 5561–5567, with permission from Elsevier.

Maltese cross (Blanshard, 1979). The crystallinity of starch is caused essentially by amylopectin polymer interactions (Banks and Greenwood, 1975; Biliaderis, 1998; Donald, 2004; Hizukuri, 1996). An illustration of currently accepted starch granule structure is given in Fig. 5.5. It is believed that the outer branches of amylopectin molecules interact to arrange themselves into ''crystallites'' forming crystalline lamellae within the granule (Fig. 5.5; Tester *et al.*, 2004). A small number of amylose polymers may also interact with amylopectin crystallites. This hypothetical structure has been derived based on the cluster model of amylopectin (Hizukuri, 1986; Robin *et al.*, 1974; Fig. 5.1).

The crystallinity exhibited by starch granules can be measured, both qualitatively and quantitatively, by X-ray powder diffraction (Eliasson *et al.*, 1987; Hizukuri, 1961; Jauncey and Pennell, 1933; Katz, 1928; Mizuno *et al.*, 1998; Nara *et al.*, 1978; Ziegler *et al.*, 2005; Zobel, 1988b). Degree of crystallinity and the nature of crystalline and noncrystalline structures and their relationship within granules are major factors that determine starch properties. Starch crystallinity, determined by X-ray diffraction (XRD), is influenced by starch moisture content; positions and intensities of radiation maxima are affected by the degree of hydration (Cleven *et al.*, 1978; Waigh *et al.*, 1997). Trubell (1944) suggested that changes in X-ray patterns were due to water entering into the crystalline structure and anisotropy, and therefore was due to ''crystal-like'' structures within granules. Qualitative measurement is usually the determination of XRD patterns (Gernat *et al.*, 1990; Zobel, 1988b; Zobel *et al.*, 1988; Fig. 5.6). Quantitative measurements include polymorphic composition and degree of relative crystallinity compared to a given reference crystalline material (Bogracheva *et al.*, 1997; Cairns *et al.*, 1997; Nara *et al.*, 1978; see Box 1 for more details on starch XRD).

B. Granular structure

Although the origin of granular assembly is being further refined (Pilling and Smith, 2003; Smith, 2001; Smith *et al.*, 1997), it is generally accepted that granules contain amorphous and crystalline domains arranged in alternating concentric rings that create a semicrystalline environment within the granule. It is also understood that the crystalline domains are mainly composed of amylopectin while bulk amorphous domains are made up of amylose traversed by noncrystalline regions of amylopectin. The crystalline patterns of starch are classified into three distinct groups, **A, B,** and **C** based on their polymorphism. During late 1970s, Wu and Sarko published a series of articles (Sarko and Wu, 1978; Wu and Sarko, 1978a,b) elucidating the nature of these **A, B,** and **C** polymorphs in starch crystalline structure. The **A** polymorph crystallizes in an orthogonal unit cell with slightly distorted hexagonal packing and 8 water molecules per unit cell, whereas the **B** polymorph crystallizes in a hexagonal

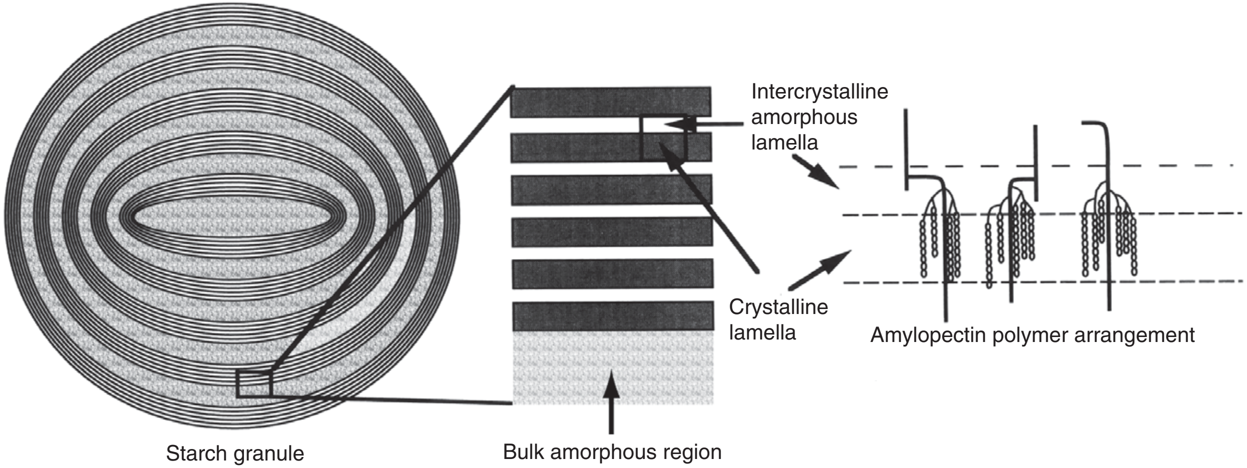

FIGURE 5.5 Schematic diagram of starch granule structure. Adapted from Donald et al. (1997), Starch – Structure and functionality, ISBN 0854047425, with permission from Woodhead Publishing Ltd., Cambridge, UK.

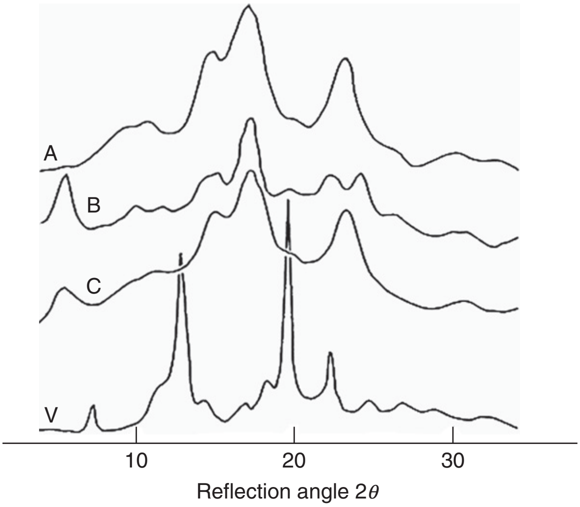

FIGURE 5.6 Classes of X-ray diffraction patterns exhibited by different starches. In general, the A pattern is exhibited by cereal starches. The B pattern is exhibited by tuber (potato) starches, and the C pattern is exhibited by root (cassava) and legume (pea) starches. The *Verkleisterungspektrum* or V pattern is exhibited by amylose–lipid complexes. Zobel (1988b), Starch crystal transformations and their industrial importance. Starch/Stärke, 40, 1–7. Copyright Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission.

unit cell with a more open hexagonal packing and 36 water molecules per unit cell (Fig. 5.8). The **C** polymorphic structure is a mixture of **A** and **B** unit cells and, therefore, is considered to be intermediate between **A** and **B** forms in packing density and structure. These different polymorphisms display distinctly different XRD patterns (Fig. 5.6).

IV. STARCH GELATINIZATION THEORIES AND MODELS

The properties exhibited by starch during gelatinization are governed by several factors, including granule size and shape. When raw starch is heated in excess water, granules swell and lose their birefringence. After the granules are swollen to a maximum volume, they burst dispersing ''starch substance'' forming a colloidal dispersion in water (Alsberg, 1928).

A. Early studies

In the 1940s, researchers identified that when starch is heated in water there were three distinct stages in the granule disruption process. During the first phase, water is slowly and reversibly absorbed by granules,

BOX 1

X-RAY DIFFRACTION

In early 1900s, it was discovered that starch gave an X-ray spectrum of a crystalline, not amorphous, substance (Katz, 1928). Researchers suspected that starch exhibited both amorphous and crystalline characteristics in XRD experiments, elucidated mainly from a theoretical point-of-view (Sponsler, 1923). An article published by Katz and van Itallie (1930) was paid great interest by researchers and caused XRD to be used as a ''popular'' analytical tool in the mid- to late twentieth century. In this article, Katz and van Itallie (1930) explained how starches can be classified into three different groups: A, B, and C based on the XRD patterns. XRD can estimate, qualitatively and quantitatively, both crystalline and amorphous phases of starch granules. Therefore, theoretically speaking, the loss of granular structural order and changes in both amorphous and crystalline domains during gelatinization can be monitored and estimated by XRD (Fig. 5.7). Developments in knowledge, techniques, and instrumentation, since Katz and van Itallie's findings, have permitted better, reliable, and highly reproducible procedures for X-ray analyses of starch. Both small-angle and wide-angle XRD have been extensively used during last two decades to characterize starch properties. Comprehensive reviews (Chandrasekaran, 1998; Zobel, 1988b) and numerous theories (Cairns *et al*., 1997; Eliasson *et al*., 1987; Garnat *et al*., 1990; Hizukuri, 1961; Jenkins and Donald, 1997; Nara *et al*., 1978; Mizuno *et al*., 1998; Schreiner and Jenkins, 1983) on how to determine starch relative crystallinity and polymorphic composition using XRD have been published. When starch granules are completely gelatinized, they exhibit an amorphous XRD pattern (with no peaks) as opposed to native or partially gelatinized starch that displays XRD profiles with peaks (Fig. 5.7). The peaks in XRD profiles are known to represent the crystallinity, and the large ''background'' area of the profile represents the amorphous phase of starch. During gelatinization, starch crystallinity is lost at a rate and to an extent depending on the intensity of hydrothermal treatment applied. Although XRD has traditionally been used as a tool to characterize starch (at static temperatures), it can also be used to determine changes during gelatinization of starch. However, there are some drawbacks to using XRD in starch gelatinization experiments.

Gelatinization, as we understand now, is not only associated with crystalline order, but is also influenced by structural changes in the amorphous region. XRD does not detect or account for the structural changes that occur in the amorphous regions of the starch granules.

continued

BOX 1 (*continued*)

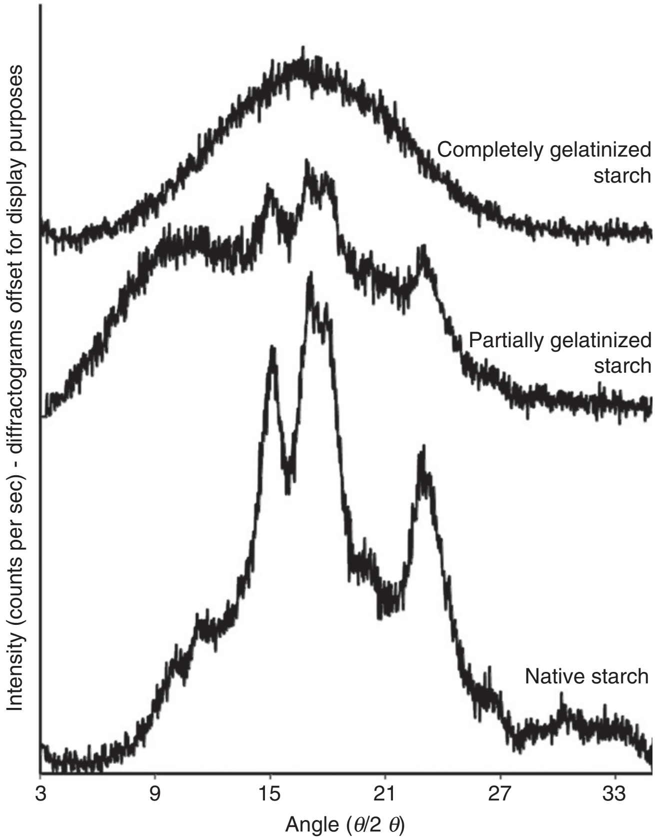

FIGURE 5.7 X-ray diffraction profiles of native (ungelatinized), partially gelatinized, and completely gelatinized (amorphous) tapioca starch. Reprinted from Carbohydrate Polymers, Vol. 67, Ratnayake and Jackson (2007), A new insight into the gelatinization process of native starches, Pages 511–529, 2007, with permission from Elsevier.

(A) X-Ray powder diffraction requires relatively dry samples. Therefore, to measure the degree of gelatinization by XRD, some post-processing of gelatinized samples is required. Consequently, the results of X-ray analysis may depend on the nature and extent of sample changes that occur during the postgelatinization treatments such as moisture removal and preservation.

(B) X-Ray intensities (or peak intensities) depend on sample moisture content. Therefore, to obtain reproducible data, the moisture contents of all the samples analyzed in the experiment should be constant (usually between 10–18% w/w) depending on the analytical requirements.

(C) A standard, universally acceptable method is not used to estimate the % relative crystallinity of starch by researchers. The differing procedures of estimating % relative crystallinity have produced conflicting data interpretations (Cheetham and Tao, 1998; Mizuno *et al.*, 1998; Nara *et al.*, 1978).

(D) We have found that the method of Wakelin *et al.* (1959), as used by Nara *et al.* (1978) to calculate the % relative crystallinity, is a reliable and reproducible procedure to determine the degree of gelatinization of starch by XRD powder diffractometry. This procedure, however, is not straightforward and needs suitable reference samples for 100% crystalline and 100% amorphous material, and appropriate computer software to analyze data.

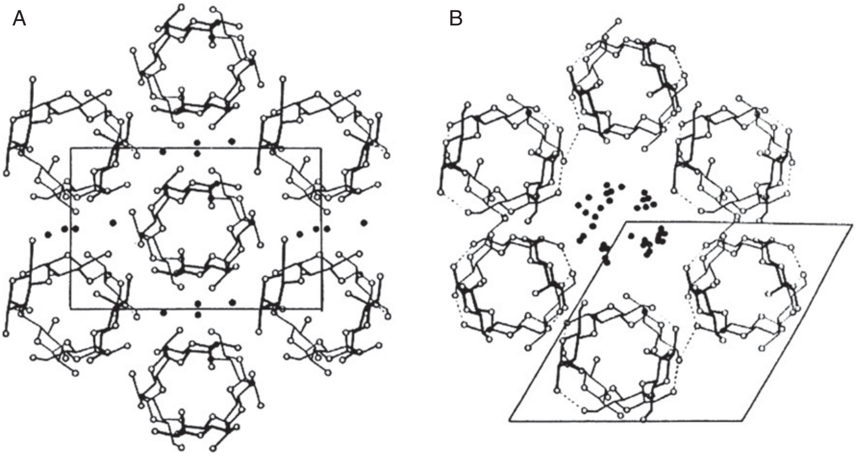

FIGURE 5.8 Unit cells (outlined in each diagram) and helix packing in A and B polymorphs of starch. Reprinted from Carbohydrate Research, Vol. 61, Wu and Sarko (1978b), The double helical molecular structure of crystalline A-amylose, Pages 27–40, with permission from Elsevier.

swelling them to a limited extent. The viscosity of such a suspension does not increase noticeably during this stage. These granules, if subsequently dehydrated, retain their birefringence and return to their original appearance (French, 1944, 1950). The second phase of swelling starts within a small temperature range, characteristic to the particular starch, in which granules increase their volume to a larger extent by absorbing considerable amounts of water while rapidly losing birefringence. During this second phase, which is marked by a sudden rise in viscosity of the starch–water suspension, a small amount of starch becomes solubilized. After traversing the critical temperature at which the second phase changes occur, granules do not return to their original shape when cooled and/or dehydrated. During the third phase of swelling, at further increasing temperatures, the granules become formless sacs and the more soluble portion of starch leaches out into the suspension. Ultimately, this suspension is filled with swollen granule sacs, and contains very few intact granules. On cooling, this mixture has the capacity to form gels (French, 1944, 1950). Early studies failed to reveal the exact mechanism of starch gelatinization. Before the structure of the starch granule was characterized in detail, some researchers discovered that granules, which had been solubilized by the action of acids, were no longer capable of swelling. However, these granules could be disintegrated and dispersed in hot water (French, 1944, 1950; Trubell, 1944).

With an increasing understanding of starch granular structure, crystallinity, and polymorphic forms (Zaslow, 1965), it was established that gelatinization begins in the more water-accessible amorphous regions of the granule where intermolecular bonding is weak. The use of a Kofler hot stage microscope with polarized light became the method of choice to measure gelatinization, as the loss of birefringence could be easily detected (Leach, 1965). By the 1960s, it was discovered that the initial change in granular structure during gelatinization was swelling without physical disintegration. Researchers suggested that the degree and kind of association within the granules controlled the swelling behavior of a particular starch (Leach, 1965). It also was observed that some cereal starches, such as regular cornstarch, sorghum, and rice, exhibited a two-stage swelling pattern. This phenomenon was linked to the possible presence of two distinct groups of internal bonding forces inside granules, that is, amorphous and crystalline regions (Leach, 1965). With the wide use of polarized light hot stage microscopy to determine starch gelatinization, the gelatinization temperature was defined as the temperature at which 98% of the granules lose birefringence when viewed under a polarized light microscope (Collison, 1968; Watson, 1964). Goering *et al.* (1974) investigated uncertainties associated with using birefringence loss to define starch gelatinization temperature by comparing the technique to a glucoamylase enzyme treatment procedure (Shetty *et al.*, 1974; Table 5.1). The discrepancies in measurements were found to be caused by the limitations of measuring birefringence and the nonhomogenous structure of different starch granules within a given sample. See Box 2 for more information on estimation of starch gelatinization by enzymatic methods (Table 5.2).

B. Semi-cooperative theory

Marchant and Blanshard (1978) studied starch gelatinization using a light scattering system to measure birefringence. This small-angle light scattering device (Marchant *et al.*, 1977) was used to measure the intensity of

TABLE 5.1 Comparison of birefringence loss and degree of gelatinization in different starches[a]

Starch	Temperature (°C)	Loss of birefringence (%)	Degree of gelatinization (%) by glucoamylase method
Corn	65	2	13
	70	89.6	51
	75	100	66
Wheat	55	46.8	9
	60	77.3	27
	65	100	70
	70	–	81
Rice	75	88.7	61
	80	99.3	72
	85	100	77
Potato	60	0	3
	65	0	36
	70	45	44
	75	100	55
	80	–	64

[a] Adapted from Goering *et al.* (1974).

total scattered light which is the summed average of the polarization crosses from birefringent granules. They suggested that three processes (1) water diffusion into the starch granule, (2) loss of birefringence or ''hydration facilitated melting,'' and (3) granular swelling predominantly after loss of birefringence occur during gelatinization. They proposed that the loss of birefringence was a result of ''solvation-assisted helix to coil transition.'' Marchant and Blanshard (1978) also proposed that helices, which cause birefringence, are aggregated together by hydrogen bonding and are radially oriented within the starch granule. They disputed theories that described starch gelatinization as a highly cooperative or ''all-or-none'' process. According to Marchant and Blanshard (1978), starch granules are not coherent crystal structures. Whole granules do not act as the primary thermodynamic unit, but the crystallites within the granule are basic to their thermal behavior, while amorphous regions also play a distinctive role. They further indicated that previously published theories did not account for the granular swelling that occurs before phase transition. Based on previously published reports, Marchant and Blanshard (1978) concluded that the starch granule was a highly concentrated and condensed gel system in which junction zones between polymers make crystallites. The stability of those crystallites and the polymer chain conformations within the amorphous regions were described as mutually

BOX 2

GELATINIZATION MEASUREMENT BY ENZYMATIC METHODS

An estimation of starch gelatinization using glucoamylase was reported by Toyama *et al.* in 1966. The degree of starch gelatinization was estimated by selective digestion of gelatinized starch using glucoamylase [E.C. 3.2.1.3] enzyme followed by determination of D-glucose concentration (Shetty *et al.* 1974). The principle behind this method is that glucoamylase does not attack intact/raw starch granules. Shetty *et al.* (1974) argued that the enzymatic gelatinization measurement method they proposed was more accurate and sensitive compared to other methods because: (1) enzymatic methods are more substrate (glucose) specific and, therefore, prevent unnecessary carbohydrate degradations during analyses; and (2) glucoamylase converts gelatinized starch into glucose as opposed to other enzymes, such as α-amylase or β-amylase which convert starch into larger molecules (maltose or dextrins). According to the method of Shetty *et al.* (1974), the degree of starch gelatinization can be estimated as

$$\%\ \text{Starch}\ gelatinization = [(X - B) \times 100]/T$$

where X=percentage of starch removed by glucoamylase digestion, B=correction factor (from digestion of intact starch by glucoamylase), and T=total starch percentage (estimated by dispersion in 90% DMSO followed by glucoamylase digestion).

Goering *et al.* (1974) used the enzymatic method of Shetty *et al.* (1974) to determine degree of starch gelatinization and discovered that the enzymatic method indicated significantly higher degrees of gelatinization compared to optical birefringence techniques. Most commonly available commercial glucoamylase preparations are contaminated with small amounts of α-amylase; this might cause ''raw starch digestion'' resulting in an overestimation of percent gelatinization.

independent, with the crystallites being essentially ''isolated'' units in terms of their energy relationship with each other. They also suggested that starch polymer chains in the amorphous regions were susceptible to rearrangement under appropriate conditions; these rearrangements would impact the granule's gelatinization characteristics. Considering the nonuniform nature of granular structure and distinctive roles of amylose and amylopectin, Marchant and Blanshard (1978) described the gelatinization as a ''semi-cooperative'' process. In its first phase, the semi-cooperative process describes a situation where a significant portion of granule crystallites are melted and the resultant changes induce the remaining crystallites, along with the entire granular structure, to disintegrate in a second phase. According to Marchant and Blanshard (1978),

TABLE 5.2 Starch gelatinization (in excess water) parameters reported by various researchers[a]

Starch type	Onset (°C)	Peak (°C)	End (°C)	Enthalpy (J/g)
Regular corn	64.0	69.0	75.5	13.0
High-amylose corn (70% amylose)	68.9	80.5	106.1	11.5
Waxy corn	66.0	70.7	78.4	15.5
Wheat	57.1	61.6	66.2	10.7
Rice (~15% amylose)	61.5	70.0	78.6	7.1
Waxy rice	76.1	81.1	87.0	19.2
Oat	60.0	63.5	70.5	13.5
Potato	61.6	65.9	79.4	17.0
Sweet potato	67.3	72.7	79.6	13.6
Yam (*Dioscorea alata*)	70.2	74.4	80.9	20.9
Cassava/Tapioca	63.9	70.5	82.7	8.5
Black bean	66.9	76.5	83.0	12.4
Chickpea	59.4	64.7	71.1	9.7
Lentil	60.7	66.1	76.1	12.6
Navy bean	66.0	75.1	85.0	13.2
Smooth pea	60.8	66.9	73.4	13.8
Pinto bean	72.0	75.0	81.0	15.4
Proso millet	69.0	73.9	81.8	14.9
Amaranth (*Amaranthus hypochondriacus*)	63.0	70.0	78.0	10.5

Adapted from Donovan (1979), Jackson (2003a), Jyothi *et al.* (2005), Cruz-Orea *et al.* (2002), Hoover *et al.* (2003), Hoover and Ratnayake (2002), Varavinit *et al.* (2003), Atichokudomchai *et al.* (2002), Liu et al. (2002), Valetudie *et al.* (1995), Shi and Seib (1992), Yanez *et al.* (1991), Tomita *et al.* (1981), Liu *et al.* (2005), Jane *et al.* (1999).

[a] The values given in this table represent randomly selected data from many studies or a particular cultivar/variety used in the study. Starch phase transition parameters, measured by DSC, may change depending on many factors, including variety, cultivar, hybrid, starch:water ratio in the sample, and scanning/experimental conditions.

energy characteristics of the crystallites in any given starch granule lie within a narrow range resulting in an approximately 1.5 °C gelatinization range for a single granule. However, in a population of starch granules, the gelatinization range is much wider due to differences between granules. The temperature range required to gelatinize a starch sample, therefore, reflects the differences in energy characteristics of its constituent granules. According to Marchant and Blanshard (1978), during synthesis, it is possible for an individual starch granule to have a more consistent physiological environment to acquire a relatively uniform set of internal crystallites, which would be different in character from other individual granules. Although this argument does not directly affect their semi-cooperative gelatinization theory, it also should be noted that at any given time not one but many starch granules are being synthesized in the same physiological environment. Therefore, it is possible to have granules containing crystallites with similar characteristics in a given starch sample. Such an argument is also consistent with starch gelatinization as expressed by a differential scanning calorimetry (DSC) endotherm peak, which often is the result of many individual granules melting simultaneously.

With the application of differential thermal analysis (DTA) and DSC to starch characterization and analysis (Stevens and Elton, 1977; Wootton and Bamunuarachchi, 1979), researchers had new tools to study the gelatinization process and associated granular structural changes. In the classical method to measure starch gelatinization using DSC, a small sample is heated with excess water in a hermetically sealed pan against a reference pan (see Box 3 for details). DSC allowed researchers to use a wide range of moisture levels to study gelatinization, which was impossible with birefringence studies. Moreover, with DSC, it was possible to express the heat energy required for granular structure phase transition during gelatinization.

C. Water availability theory

Donovan (1979) studied starch gelatinization at both low and high moisture levels using DSC. He observed that as moisture levels were decreased (water:starch volume fractions less than 0.45) DSC endotherms become biphasic. The first (typical) endotherm decreased in area, and the second (high temperature) endotherm shifted to even higher temperatures as water content was decreased (Fig. 5.9). Donovan (1979) suggested that these two endotherms represented two distinct mechanisms by which ordered regions of starch granule undergo hydration facilitated phase transition. This explanation assumes that the crystalline regions are the same throughout the starch granule and that the phase transition mechanism depends only on moisture availability. Donovan (1979) argued that the single endotherm at excess water content occurs due to an order–disorder phase transition of starch granules. He further explained that granule swelling occurred due to water imbibition into amorphous regions first, which facilitated the ''stripping'' of starch chains from crystallite surfaces.[1] ''Stripping starch chains form the surfaces of

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[1] Starch crystallinity is caused by parallely arranged starch polymers which act as crystals. No evidence has been presented in published literature to describe the actual physical nature of ''starch crystallites.'' It should not be considered that starch granules contain ''crystals'' or ''crystallite particles'' formed by starch polymers. Starch crystallinity represents the relative arrangement of starch polymers in granules, not the presence of physical ''crystals.''

BOX 3

DIFFERENTIAL THERMAL ANALYSIS AND DIFFERENTIAL SCANNING CALORIMETRY

DTA is a technique that involves heating or cooling a sample, compared with a reference, under identical conditions, and recording the differential temperature against time or temperature. Absorption or evolution of heat, caused by changes in the sample during heating/cooling can be observed relative to the reference, which is normally an inert material. Although the DTA technique is widely used to investigate thermal transitions of different compounds, there were strong discrepancies and confusion related to establishing reaction kinetics based on DTA results (Kissinger, 1957; Reed *et al.*, 1966; Thompson, 1966). DTA experiments on thermal behaviors of starch and other carbohydrates were first reported in 1950s (Morita, 1956a,b; Morita, 1957; Morita and Rice, 1955). With the development of analytical instruments, DSC operating based on heat flux (TA Instruments, New Castle, DE) and power compensation (Perkin-Elmer, Waltham, MA) mechanisms controlled by computers became more commonly available. DSC permitted precise control and measurement of energy changes during thermal transitions compared to older DTA instruments (Androsch *et al.*, 2000; Danley, 2003; Dong and Hunt, 2001). The differences between DTA and DSC techniques and instrumentation have been well explained (Brown, 2002; Riga and Collins, 2000).

Stevens and Elton (1971) published an early study using DSC to investigate the starch gelatinization process. They characterized a variety of starches and discovered that the DSC profiles reveal both qualitative (nature of water absorption into the granules, effects of damaged starch on thermal behavior, etc.) and quantitative (energy required to gelatinize) data. The DSC instruments that were available during the early 1970s used relatively large samples (500 mg) (Stevens and Elton, 1971), and manually controlled heating and cooling mechanisms required considerable time to complete several scans. Different types of thermal references, such as fine glass beads with silicone oil (Stevens and Elton, 1971); water or buffer (Donovan, 1979); empty sealed pan, that is atmospheric air (Eliasson, 1980; Wada *et al.*, 1979; Wootton and Bamunuarachchi, 1979); and liquid paraffin (Evans and Haisman, 1982), were used in early DSC starch gelatinization experiments. The rationale for selecting a particular thermal reference type was usually unreported, and there was not an agreement on when and why a particular thermal reference should be selected. In order to obtain reliable starch thermal property data, Yu and Christie (2001) and Randzio *et al.* (2002) indicated that careful consideration of sample

continued

BOX 3 (*continued*)

preparation (weight or volume ratios of water and starch), pan type, and scanning conditions are extremely important.

Numerous studies have been conducted on starch phase transitions using DSC as the primary or only experimental tool. Some theories and mechanisms suggested for starch phase transition have been proposed solely based on DSC experiments (Donovan, 1979; Evans and Haisman, 1982; Nakazawa *et al.*, 1984; Pravisani *et al.*, 1985; Randzio *et al.*, 2002; Zhong and Sun, 2005). Not having a general consensus on a standard practice for conducting DSC experiments has created confusion and discrepancies in data interpretation; there is still an ongoing debate on what happens to starch structures and the molecular associations during specific stages of DSC enthalpic transitions.

crystallites'' was merely a hypothesis for which there is no substantial direct experimental evidence.

Donovan (1979) argues that when sufficient water is present at low temperatures, water is nonuniformly distributed within the starch granules and that there are high concentrations of water present near some crystallites. As these localized high concentrations of water are heated, Donovan suggested that the crystallites in nearby areas undergo polymer stripping from their surfaces, causing a single DSC endotherm. It could be possible that at low moisture levels, some crystallites melt at low temperatures by water-facilitated plasticization that allows optimum hydrogen bonding transfer from starch polymer clusters to water. Some crystallites would also melt at high temperature without the plasticizing effect of water; this may be a realignment of crystallites by hydrogen bond transfer among starch polymers. This theory does not explain, however, why low moisture levels facilitate melting of some crystallites as a group (representing a distinctly separate high-temperature endotherm) rather than just resulting in a broadened endotherm.

In the presence of sufficient (i.e., excess) water, all the crystallites are pulled apart by swelling and therefore none remains to be melted at high temperatures, eliminating the high-temperature endotherms. Donovan used Flory's crystallite melting theory (Flory, 1953) to explain these observations. Flory's theory, discussed in Section IV.G, applies to perfect crystals at equilibrium. Starch, on the other hand, is semicrystalline, and the distribution of amorphous regions within the granule is not uniform. Donovan's theory of crystallite melting (1979) by two different mechanisms at low moisture levels is appealing, and it is reasonable to assume that Donovan's explanation refers more to removal of starch

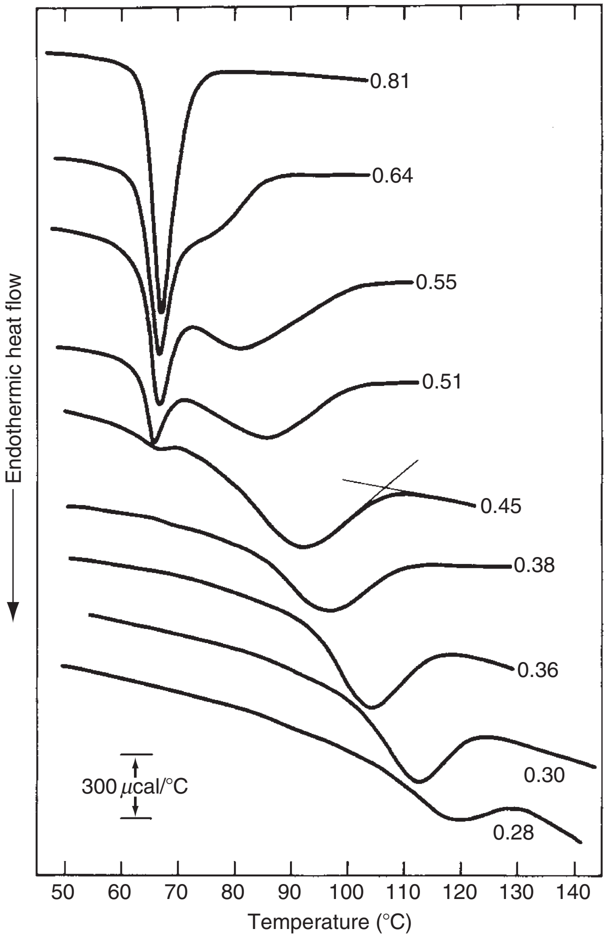

FIGURE 5.9 DSC profiles of potato starch at different water contents (volume fraction of water indicated next to each profile). Heating rate=10 °C/min. Donovan (1979), Phase transitions of starch-water system. Biopolymers, 18, 263–275. Copyright Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission.

polymers from crystallite arrangements due to increased temperature in the presence of water. Consequently, it cannot be argued that crystalline melting in starch granules during gelatinization is an absolute result of water availability (i.e., diluent), although water absorption by amorphous regions likely impacts subsequent structural changes during heating. Donovan's results (1979) confirm the importance of water availability as a fundamental property in the phase transition process of starch granules.

D. Crystallite stability theory

Evans and Haisman (1982) investigated the gelatinization behavior of potato starch by studying starch granule equilibrium states with glycerol and sodium hydroxide solutions, using DSC and colorimetric (blue dextran exclusion) methods. They argued that starch gelatinization was related essentially to crystallite melting within starch granules and explained the biphasic endotherm (Section IV.C) as a result of melting least stable crystallites first, thereby removing some of the constraints that restrict the granule from absorbing additional external water.

According to Evans and Haisman (1982), there are two phases of water in starch granules: (1) the ''tightly'' bound phase, approximately 20% of the amount, absorbed by granules; and (2) a ''loosely'' bound phase that is available for gelatinization. They found that the DSC measured onset temperature (T_o) increased rapidly below 0.6 g water per 1 g starch (~29% water volume fraction) and stayed essentially constant between 0.6 and 2.0 g water per 1 g starch (29–57% water volume fraction) (Fig. 5.10). Changes observed in T_o and T_p, and the peak separation pattern

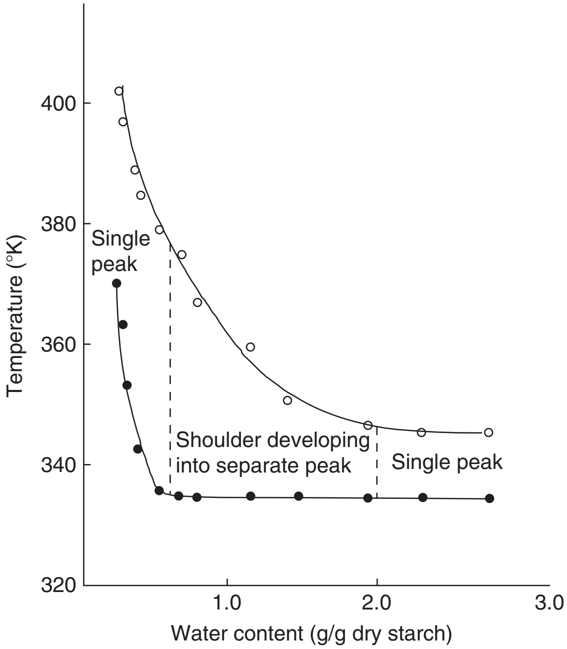

FIGURE 5.10 Change of gelatinization temperatures (initial=●, final=o) of potato starch based on water content. Evans and Haisman (1982), The effect of solutes on the gelatinization temperature range of potato starch. Starch/Stärke, 34, 224–231. Copyright Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission.

that depended upon water content, were depicted as evidence for existence of more-stable (perfect) and less-stable (less-perfect) crystallites in starch granules. Evans and Haisman (1982) used Flory's theory (1953) of polymer melting in the presence of a diluent to explain starch gelatinization. They assumed that Flory's theory can be applied to the melting behavior of less-perfect crystals (starch). They also assumed that volume fractions in Flory's equation should be based on granules rather than the entire starch–water system because once the starch granules are swollen to their maximum capacities, changes in the external medium (water) would not affect granule composition. Flory's theory, however, applies to hypothetical perfect crystals at equilibrium (see Section IV.G for details).

According to Evans and Haisman (1982), the gelatinization process starts with melting of the least stable crystallites (corresponding to the T_o in DSC endotherms) with subsequent progressive melting into more-stable crystallite domains. They also assumed that the melting of less-stable crystallites at lower temperatures releases constraints on more-stable crystallites, and makes those crystallites and remaining crystallites melt faster at lower temperatures than they might ordinarily melt. This argument is essentially a combination of Marchant and Blanshard's semi-cooperative theory (1978) and Donovan's water availability theory (1979). Evans and Haisman (1982) also argued that the gelatinization temperature range (the difference between the onset and end temperatures) is determined by the distribution of least stable crystallites in the granules. They explained that the biphasic endotherm at low moisture levels was a result of cooperative melting of some crystallites in the presence of sufficient water followed by ''true'' melting of remaining crystallites in the absence of free water. They claimed that Donovan's theory (1979) was invalid because the ''stress'' created by swelling of amorphous regions could not occur within a very narrow temperature range, and such a stress could not rapidly rise as crystals melt.

According to more recent research (Ratnayake and Jackson, 2007; Sahai and Jackson, 1999; Vermeylen *et al*., 2006), it is quite obvious that an assumption that DSC endotherms represent crystallite melting during starch gelatinization process is not valid. If DSC endotherms of starch–water systems represent only crystallite melting, water absorption into starch granules should occur without any energy utilization. In reality, however, this is not the case. Despite the limited and reversible water absorption that occurs initially, much of the water absorption-driven starch granule swelling occurs only when starch–water mixtures are heated. This means that starch granules need energy to absorb water and swell, that is, it is an endothermic process. Evans and Haisman (1982), however, assumed not only that Flory's theory can be applied to starch, which is semicrystalline and not in equilibrium during gelatinization, but also that the DSC endotherm is an absolute result of crystallite melting. There is sufficient recent research evidence (Ratnayake and Jackson, 2007; Zobel *et al.*, 1988) to suggest that starch "crystallinity" measured by XRD and DSC do not measure the same or similar phenomena, but are assessing two different phenomena that take place during the gelatinization process.

By the 1980s, it had been established that starch gelatinization involved starch granule hydration and swelling, birefringence loss, crystallite melting or loss of crystallinity, polymer leaching, and irreversible loss of granular structure. It had been found that structural and thermodynamical changes in the starch granules during gelatinization could be monitored by various analytical techniques such as the birefringence end point method, viscosity measurements, amylose-iodine binding methods, enzymatic digestibility, nuclear magnetic resonance, light scattering/extinction, solubility and sedimentation methods, colorimetric methods (Congo red and blue dextran), XRD, and DSC (Ghiasi *et al.*, 1982; Lund, 1984; Shiotsubo, 1984; Shiotsubo and Takahashi, 1984; Zobel, 1984).

E. Sequential phase transitions (first amorphous and then crystalline) theory

Nakazawa *et al.* (1984) conducted low-temperature treatment-in-water[2] studies on regular rice starch using DSC and detected that at temperatures below T_o, the amorphous phase became mobile but the crystalline region did not. Some amorphous portions were restructured into more-stable forms. T_p shifted to higher temperatures as holding times increased when the sample was treated a below its T_o (Fig. 5.11). They also observed that the transitions associated with granular restructuring were detected as a sharp peak at high T_p (Fig. 5.11) but they were not detectable by XRD (Fig. 5.12) and concluded that the structural features detected by DSC and XRD were independent of each other. In regular rice starch, they observed a biphasic endotherm in DSC when starch–water mixtures are held for certain periods at constant temperatures. These endotherms merged into a single endotherm with a higher T_p, when held for longer times (Fig. 5.11).

Nakazawa *et al.* (1984) suggested that starch granules are not at equilibrium with water during gelatinization, especially when DSC is used to determine gelatinization properties. To obtain an equilibrium condition they annealed rice starch granules in water for different time and temperature combinations. DSC gelatinization temperatures (T_o and T_p) were observed shifting to higher temperatures with an increasing degree of annealing (Fig. 5.13). According to Nakazawa *et al.* (1984) the energy

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[2] Some of these low-temperature treatments meet the hydro-thermal conditions of classic starch annealing procedures. Annealing is normally conducted below gelatinization temperatures in the presence of excess water (Jacobs and Delcour, 1998; Tester and Debon, 2000; Ozcan and Jackson, 2003).

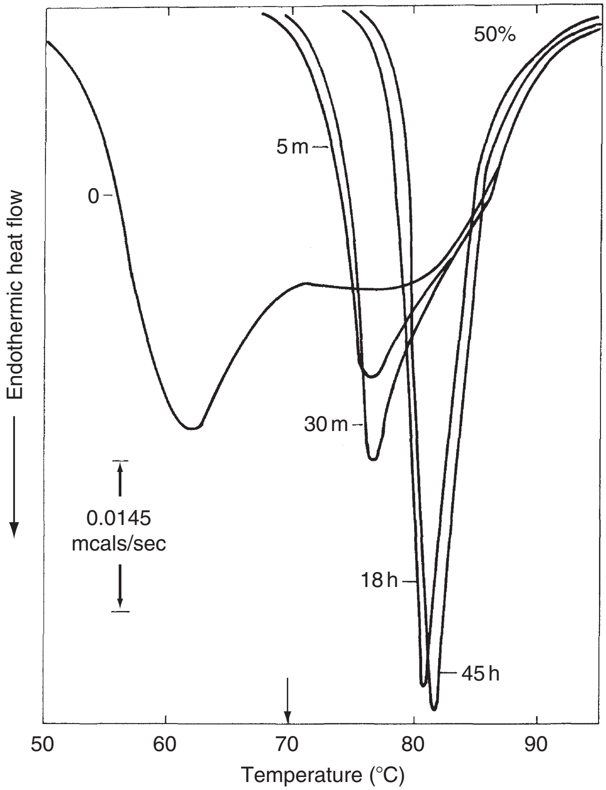

FIGURE 5.11 DSC endotherms of rice starch annealed at 70 °C (times are indicated next to each profile) [adapted from Nakazawa *et al.* (1984); reproduced with permission from Japan Science and Technology Agency].

provided during lower temperature treatments rearranged the starch polymers, and at high temperatures (75 °C) the crystallite structures became completely amorphous (Fig. 5.12). Their results also showed that approximately 80% amylopectin-containing (~20% amylose) native starch required more energy to become amorphous during high-temperature treatments compared to 100% amylopectin-containing starch. They also concluded that increased water facilitates the mobility of starch molecules so that the crystalline to amorphous transition takes place at lower temperatures.

Nakazawa *et al.* (1984) argued that when starch–water mixtures (30–50% starch) are held at a certain temperature (55–80 °C), for a certain period (0–45 h), and depending on the time–temperature combination, starch granules increase their amorphous portion and decrease their crystalline portion. These amorphous and crystalline phases melted sequentially during DSC phase transition experiments. Their experiments

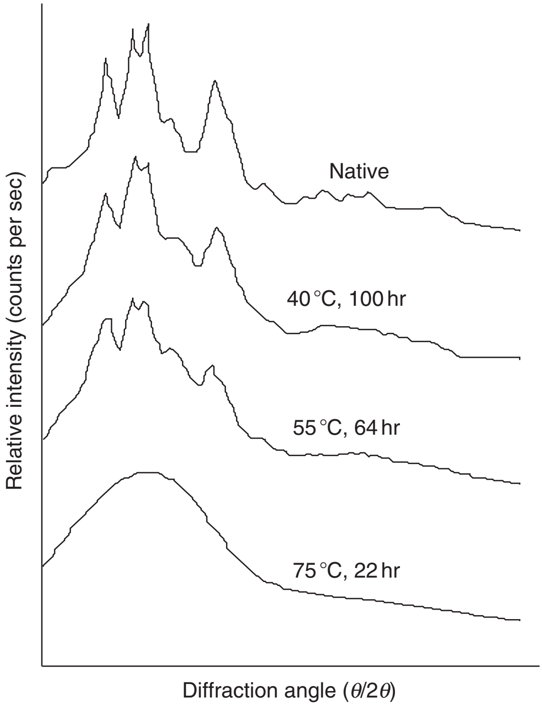

FIGURE 5.12 X-ray diffraction patterns of annealed rice starch (treatment conditions are indicated above each profile) [adapted from Nakazawa *et al.* (1984); with permission from Japan Science and Technology Agency].

showed that when starch–water mixtures are held above gelatinization temperatures (75 °C), the second endotherm disappeared (Fig. 5.13). Nakazawa *et al.* 's argument (1984), however, does not confirm their own sequential phase transition theory. If longer times and high (above gelatinization) temperatures decreased the crystalline:amorphous ratio within starch granules, the resulting granules should show single DSC endotherms at correspondingly lower transition temperatures (because supposedly amorphous regions undergo ''gelatinization'' or phase transition at lower temperatures). Their DSC results (Fig. 5.14), however, indicated that the peak temperature (T_{P}) increased and the enthalpy changed without a particular pattern with increasing subgelatinization temperatures. This observation could be better explained by the crystallite stability theory (Evans and Haisman, 1982).

Colonna and Mercier (1985) suggested a theory, similar to sequential phase transition theory of Nakazawa *et al.* (1984), after studying a variety of starches using DSC and amylography (viscosity measurements during heating/cooling). They argued that the low-temperature endotherms of

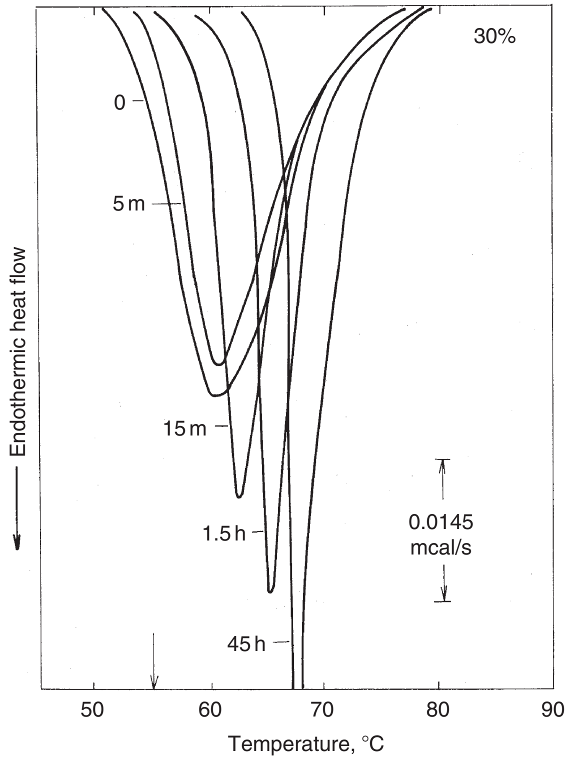

FIGURE 5.13 Effect of annealing (degree of annealing increases along X axis) on DSC transition temperatures of rice starch held at 55 °C for specific times (indicated next to each profile) (Nakazawa *et al.*, 1984; reproduced with permission from Japan Science and Technology Agency).

multipeak DSC profiles represented a "normal gelatinization transition" (although it was not fully outlined in the published report, it could be assumed that this transition occurred in the presence of sufficient water to act as the diluent for some crystallites), and endotherms at high temperatures correspond to "true melting" of starch crystallites. Colonna and Mercier (1985) attributed the first transition to disorientations of the amorphous region without crystallite melting (Fig. 5.15). However, in their illustration (Fig. 5.15), the bidirectional arrows associated with the second transition seem surprising since crystallite melting during gelatinization is irreversible unless it undergoes retrogradation (a recrystallization phenomena that is not thought to include the same crystallization sites as those found in native granules). Colonna and Mercier (1985) indicated that at sufficient water contents, starch polymers in amorphous regions increased their mobility, causing irreversible swelling in areas

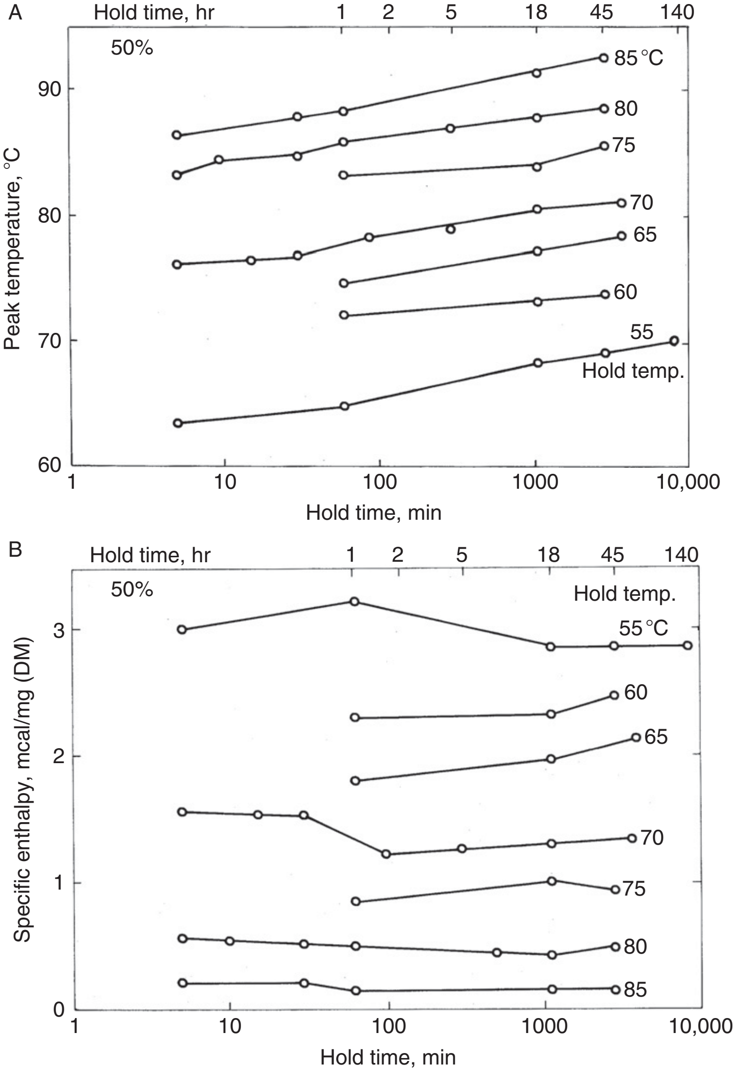

FIGURE 5.14 (A) Change of T_p based on annealing temperature and time. (B) Change of enthalpy based on annealing temperature and holding time (Nakazawa *et al.*, 1984; reproduced with permission from Japan Science and Technology Agency).

partially restricted by crystallites. Upon a further increase in temperature, crystallite melting, considered independent of swelling, allowed more polymer chain movement to destabilize the entire granular structure. A similar theory was suggested by Pravisani *et al.* (1985), who investigated potato starch gelatinization using DSC and microscopy. Nakazawa *et al.* (1984) did not consider granular structural changes that take place

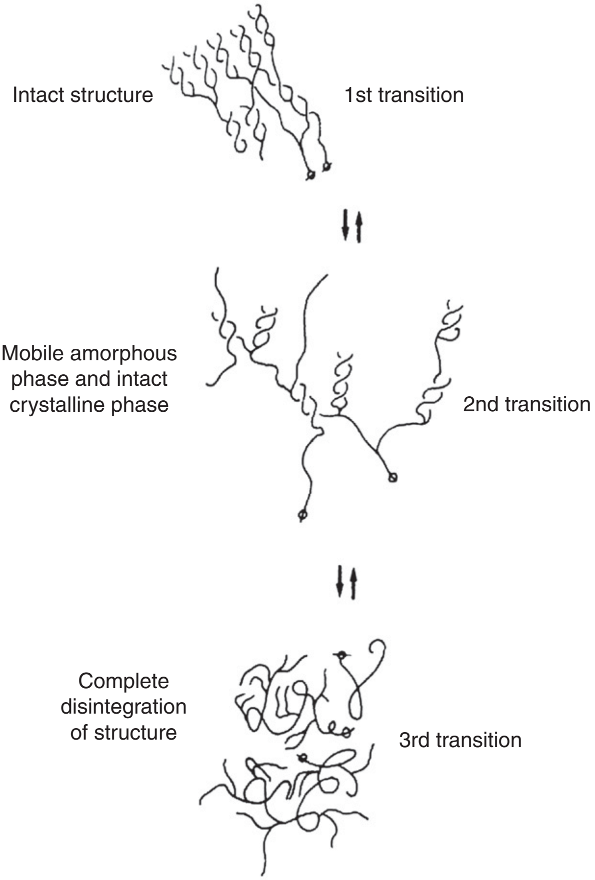

FIGURE 5.15 Phase transitions of starch structures during gelatinization. Reprinted from Phytochemistry, Vol.24, Colonna and Mercier (1985), Gelatinization and melting of maize and pea starches with normal and high-amylose genotypes, Pages 1667–1674, with permission from Elsevier.

during annealing (holding starch–water mixtures for long durations at specific time–temperature combinations)[3] to explain their observations. There is sufficient evidence in the literature to support the observed starch structural changes, especially in the intermolecular bonds, during annealing (Kohyama and Sasaki, 2006; Qi *et al.*, 2004; Tester *et al.*, 2001). Although Nakazawa *et al.*'s results (1984) do not adequately explain the nature of sequential phase transition of amorphous and crystalline portions in starch granules during gelatinization, their observations highlight

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[3] The term ''annealing'' is not completely applicable to all the treatments they used because some of the holding temperatures are above the gelatinization temperature of rice starch.

the limitations of using DSC alone to determine gelatinization properties of starch. DSC explains the ‘‘net result’’ of what takes place during gelatinization in terms of energy dynamics, but does not provide a complete picture of the process.

F. Three-stage (partial melting, recrystallization, and total melting) phase transition theory

Biliaderis *et al.* (1986) argued that the previously mentioned gelatinization theories on solvent (water) facilitated starch crystallite melting were imperfect because they did not consider the semicrystalline nature of starch. According to Biliaderis *et al.* (1986), starch, being a semicrystalline material, undergoes reorganization during heating in DSC experiments. During slow DSC scans (between 1 and 5 °C/min) and after the onset (T_o) of the first peak, mobile polymers rearrange in the crystalline region. They also postulated that such a process could take place during annealing. They found that the biphasic endotherm observed by others could be forced to merge into a single endotherm at high heating rates (~20–30 °C/min). Biliaderis *et al.* (1986) argued that the reorganization of starch polymers during gelatinization was similar to annealing, and it was the reason for biphasic endotherms at low moisture levels. Therefore, according to these workers, the starch gelatinization process is composed of three phases: partial melting, recrystallization, and final melting. They argued that this three-phase theory better corresponded to nonequilibrium crystallite melting during gelatinization. They also reported that both annealing and recrystallization processes depended on moisture content and these processes became less prominent at high moisture levels. Biliaderis *et al.* (1986) also reported that DSC transitions did not truly represent initial semicrystalline structure phase transitions of starch, but represented a composite result of melting and reorganization that occurred during thermal analysis. Polymer mobility in the bulk amorphous region was restricted by its close association with some parts of crystalline domains. In addition, they suggested that the intercrystalline amorphous region (Fig. 5.5) does not carry ‘‘normal’’ amorphous characteristics due to strains imposed by crystalline domains. They argued that these structural differences associated with starch’s semicrystalline nature, compared to a completely crystalline material, explained the multiple melting profiles observed at low moisture levels. If this is the sequence of changes that takes place during starch gelatinization, applicability of the ‘‘fringed micelle’’ model (Billmeyer, 1984; Flory, 1953; Slade and Levine, 1987, 1995; Wunderlich, 1973) becomes invalid because that model assumes, for gelatinization purposes, that starch structures are two-phase systems in which crystallites are dispersed in a homogenous amorphous matrix and that the two phases behave independently.

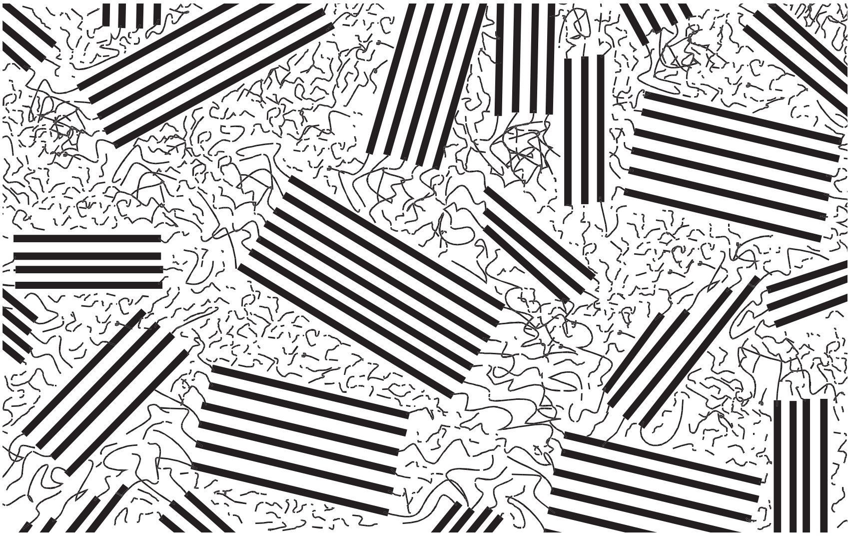

FIGURE 5.16 Spontaneous ordering of linear polymers in a semicrystalline material. Bundles of dark lines represent crystalline regions. Reprinted from Flory (1953): Paul J. Flory, Principles of Polymer Chemistry, Copyright © 1953 Cornell University Press and Copyright © 1981 Paul J. Flory. Used by permission of the publisher, Cornell University Press.

G. Application of Flory's theory to explain starch gelatinization

Numerous attempts (Cruz-Orea *et al.*, 2002; Donovan, 1979; Evans and Haisman, 1982; Lelievre, 1973) have been made to explain starch gelatinization and phase transition using Flory's theory of the melting point depression (Flory, 1953) in semicrystalline systems. According to Flory (1953), polymers having regular chain structures undergo spontaneous ordering referred to as crystallization (Fig. 5.16). This takes place when chain axes are aligned parallel to one another forming bundle-like structures (Fig. 5.16). The individual polymer chains may occur in a fully extended or a less-extended (spiral) configuration. Ordering of polymers to form crystals, sometimes, may not be accepted as genuine crystallization. In semicrystalline material, submicroscopic crystallites occur (imbedded) in a residual amorphous matrix. The proportion (%) of crystalline material depends on crystallization conditions (Flory, 1953).

The "degree of perfection" of crystallites is variable and depends on annealing conditions. According to Flory (1953), annealing is keeping the polymer at a temperature slightly below its melting temperature. When a polymer is annealed, depending on the time, temperature, and pressure combination, it is possible to obtain a range of polymer interactions from amorphous to crystalline and intermediate-level interactions between the two "extremes." These intermediate states are called "mesomorphic states," which are characteristic to semicrystalline material and are metastable (i.e., not at an equilibrium state) in nature. Annealing is known to both quantitatively and qualitatively improve crystalline order (Flory, 1953).

According to Flory (1949, 1953), the crystallite perfection is a measure of the amount of intermediate material located at the crystalline to amorphous transition boundary. Boundary regions or "intermediate-order" material presumably melts at lower temperatures than relatively more highly ordered internal regions (Flory, 1953). More perfect crystals have negligible amounts of intermediate-order material at the crystalline–amorphous transition region, resulting in a sharp melting point. Apart from the intermediate material at transition regions, crystallite size also plays an important role in melting point values. Small crystallites result in depressed melting points due to surface free energy (Flory, 1953). According to Flory (1953), melting point sharpness and reproducibility are indicators of the perfect crystalline state. The melting temperature (T_{m}) was described as the equilibrium melting temperature for a hypothetical perfect macroscopic crystal.

When Flory's theory (1953) of melting point depression is applied to starch gelatinization (or phase transition) in the presence of water, the situation can be described as follows. At equilibrium state, the chemical potentials between amorphous (μ_{u}) and crystalline repeating units (μ_{u}°) of two phases are equal:

$$\mu_u = \mu_u^o \tag{5.1}$$

The temperature at which this condition is satisfied is known as melting temperature or melting point (T_{m}). Melting temperature depends on the composition of the amorphous phase. In a starch–water system, water acts as a diluent and then T_{m} is regarded as the temperature at which the specific composition is similar to that of a standard solution. If the amorphous phase of starch is pure, the chemical potential (μ_{u}) is the same as its chemical potential at the standard state ($\mu_{\mathrm{u}}{}^{\circ}$) at the same temperature and pressure:

$$\mu_u \equiv \mu_u^o \tag{5.2}$$

The chemical potential of the polymer is affected by "impurities" such as solvents or copolymerized units. For an equilibrium condition in the presence of water as the diluent, the melting temperature of starch (T_{m}) would be lower because μ_{u} in the presence of diluent is less than μ_{u}°. For the starch–water system at equilibrium, the difference between the chemical potentials of the crystalline phase and the phase in the standard state (pure polymer at the same temperature and pressure) must be equal to the decrease in chemical potential of the polymer unit in solution relative to the same standard state (Flory, 1953). By considering the free energy of fusion per repeating unit and volume fraction of water (diluent), the melting point depression due to the presence of diluent can be expressed as follows (Flory, 1953):

$$\frac{1}{T_m} - \frac{1}{T_m^o} = \frac{R}{\Delta H_u} \frac{V_u}{V_1} (V_1 - \chi V_1^2) \tag{5.3}$$

where T_{m} = actual polymer melting point, T_{m}° = equilibrium melting temperature, R = gas constant, ΔH_{u} = enthalpy of fusion per repeating unit, V_{u}=molar volume of polymer repeating unit, V_1 = molar volume of diluent, and ξ = Flory-Huggins interaction parameter.

It is believed by most researchers, for the purpose of theoretical explanations, that the amorphous and crystalline domains of starch granules present in relatively mutually exclusive domains and the amorphous domains are not in an equilibrium state. Moreover, it cannot be assumed that water enters amorphous regions of the granules ''effortlessly'' (if it is the case, starch granules should, at least partially, disperse in cold water). Flory's theory is valid for systems under constant pressure. When DSC is used to evaluate starch–water systems, increasing heat also increases the pressure due to the increase in water temperature within the hermetically sealed pans. When starch is gelatinized in excess water, the water first acts as a diluent in the amorphous phase. When excess water is available, the fluid (or amorphous) phase never becomes a saturated phase; saturation is also a requirement of Flory's theory of melting point depression in semicrystalline material in the presence of a diluent. Polymers within the crystalline and amorphous regions are not homogenous (in terms of both polymer type and polymer chain length). The presence of homogenous polymer composition is a requirement to apply the theory to explain melting point depression. Flory's theory is applicable to a pure crystal of infinite size. Starch ''crystals'' are made up of much smaller size amylopectin branch interactions, which may be described as ''crystallite blocks'' (Takahashi and Yamada, 1998). The size of these ''crystals'' may depend on the amylopectin branch chain lengths (Jane *et al.*, 1999). These concerns raise questions on the applicability of Flory's theory to explain starch gelatinization.

V. STARCH ANNEALING AND ITS RELATIONSHIP TO GELATINIZATION

Starch annealing involves heating starches with sufficient hydration below their T_{o} to facilitate molecular mobility (Tester *et al.*, 2001). Annealing is defined as ''a physical treatment that involves incubation of starch granules in excess (>60% w/w) or at intermediate (40–55% w/w) water content during a certain period of time at a temperature above the glass transition temperature, but below the gelatinization temperature (Gough and Pybus, 1971; Jacobs and Delcour, 1998; Zeleznak and Hoseney, 1987). During annealing, granule composition remains essentially unchanged, but T_p increases significantly (Gomes *et al.*, 2004; Kohyama and Sasaki, 2006; Tester and Debon, 2000; Tester *et al.*, 2001; Fig. 5.13). Qi *et al.* (2004) studied the effect of annealing on gelatinization properties in an A-type cereal starch (waxy barley) using α-amylase and 2 M hydrochloric acid hydrolyses to remove amorphous domains, thus isolating crystalline regions. Annealing (48 °C for 7 days in excess water) increased the DSC melting enthalpy and narrowed the phase transition temperature range. Annealing starch in the presence of α-amylase did not significantly change the DSC melting enthalpy, but narrowed the transition temperature range. Onset, peak and end temperatures also increased in enzyme-treated samples compared to their native counterparts. Qi *et al.* (2004) suggested that annealing caused ordered crystalline domains and they entered into ''more optimally registered'' structures within the granules. This optimization of crystalline order, they concluded, controls the gelatinization properties of annealed starch by restricting hydration. However, it is not clear what structural changes or modifications take place within granules during so-called optimization or ''optimal registration'' processes. Other recent reports (Kohyama and Sasaki, 2006) also indicate that changes occur within crystalline regions during annealing. Marchant and Blanshard (1978) reported that starch polymer chains in the amorphous regions were susceptible to rearrangement under appropriate conditions, and that such rearrangements significantly affected the thermal behavior of starch granules. The nature of structural changes taking place inside starch granules during annealing is still under debate.

The results from DSC studies on starch annealing could be better explained by assuming an existence of nonmutually exclusive domains of crystalline and amorphous phases in starch. Most of the current theories on starch structure, gelatinization, and annealing assume discrete crystalline and amorphous domains in starch granules. Jenkins and Donald (1995) suggested that amylose, which is presumed to be mainly in the amorphous regions of the starch granules, could cocrystallize with amylopectin in the crystalline domains. If it is assumed that the amorphous and crystalline phases ''blend'' into each other gradually within an ''intermediate'' phase that has a semicrystalline structure (i.e., a crystalline structure ''disturbed'' by amorphous phase), the starch annealing phenomena could be explained differently. In the intermediate phase between the crystalline and amorphous domains, portions of amylopectin molecules are kept from making crystallites by interfering amylose molecules (or amorphous phase). When starch in excess water is heated below gelatinization temperatures (annealing treatment conditions), water first enters the amorphous regions creating increased polymer (amylose)

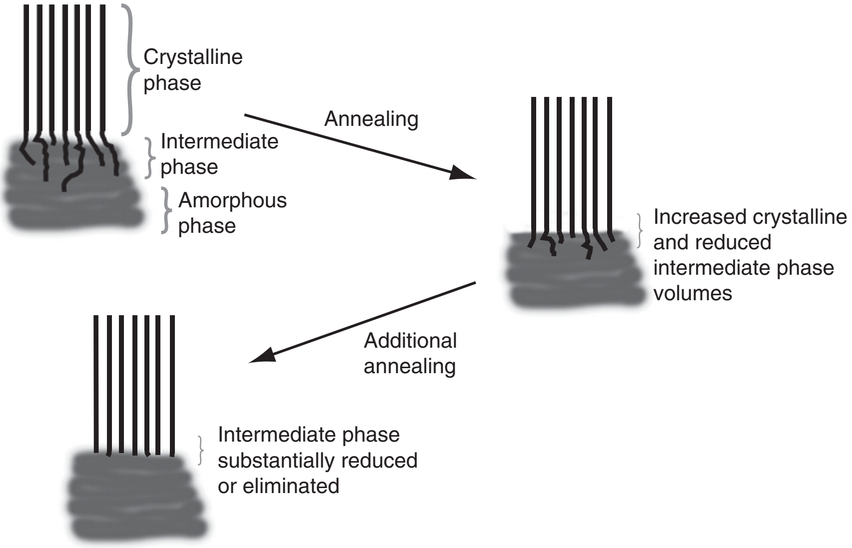

FIGURE 5.17 Proposed theory for the existence of an intermediate phase between amorphous and crystalline domains to explain starch annealing.

mobility. With increased annealing (i.e., treatment intensity), the polymers in the intermediate phase become more mobile. This increased polymer mobility reduces the interruptions to the crystalline phase and, with a potential decrease in the intermediate phase, the crystalline phase increases in size/volume yielding ''more perfect crystallites'' (the term used by many researchers to describe this phenomenon) (Fig. 5.17). This increased ''perfection'' and increased volume of the crystalline phase results in the observed larger, and sharper DSC enthalpies at higher peak temperatures in annealed starches (Fig. 5.13), compared to their native counterparts. In other words, ''imperfect'' molecular organizations at this hypothesized intermediate phase result in the wider and smaller DSC enthalpies seen at low peak temperatures (T_p) in native (nonannealed starches).

VI. GLASS TRANSITION AND GELATINIZATION

When an amorphous material exists in a glassy state, it is hard and brittle. In a rubbery state, the material is soft and pliable. An amorphous material, at solid state (also referred to as glass), does not flow, but the molecules are randomly distributed as if they were in liquid state. When this ''glass'' is heated, it softens and eventually becomes a fluid. However, this is not a first-order transition and therefore occurs over a range of temperatures called the glass transition temperature (T_g). The state

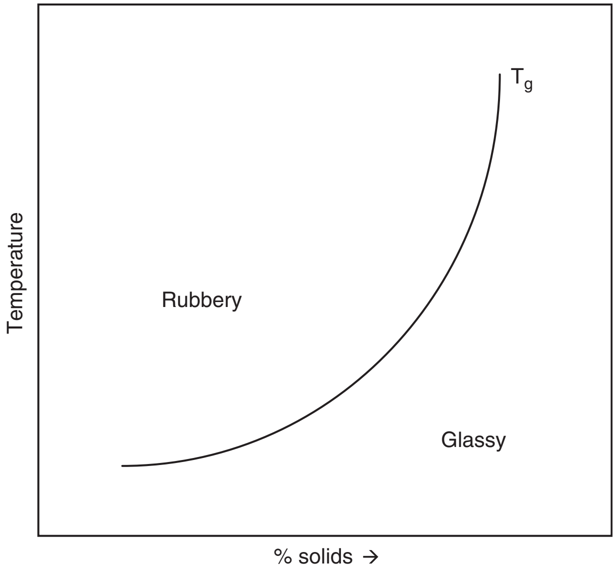

FIGURE 5.18 Glass transition diagram for an amorphous material.

(glassy or rubbery) of an amorphous material depends on its moisture content. When the corresponding plasticizing moisture content (W_g) increases (or the proportion of solids decreases), the glass transition temperature (T_g) decreases. Fig. 5.18 is commonly used to illustrate glass transition of an amorphous material. Slade and Levine (1991) proposed ''an idealized state diagram'' for an aqueous solution of a hypothetical, glass forming, small carbohydrate (glass forming, small carbohydrates are amorphous) to illustrate the critical locations of solute-specific subzero T_g (T_g') and the maximum practical amount of plasticizing moisture (W_g', which is calculated as [mass of solute−unfrozen water]/[weight % of water]) (Fig. 5.19). According to this theory, the kinetics of all constrained relaxation processes (such as translational and rotational diffusion) governed by the mobility of a water-plasticized polymer matrix in a glass forming system vary between distinct temperature/structure domains. These domains are divided by the glass transition. The material (water, solute mixture) below T_g is in the glassy solid state of very low mobility and very slow diffusion. At above T_g, but below T_m, the material is at a viscoelastic, rubbery liquid state of increased mobility and diffusion.

It has been suggested that glass transition is an important physicochemical event that controls the phase transition process of starch (Biliaderis, 1998). According to Biliaderis (1998), the ''fringe-micelle'' model (Fig. 5.16) does not permit assignment of a definite T_g for most starches. This is because the change in heat capacity during phase

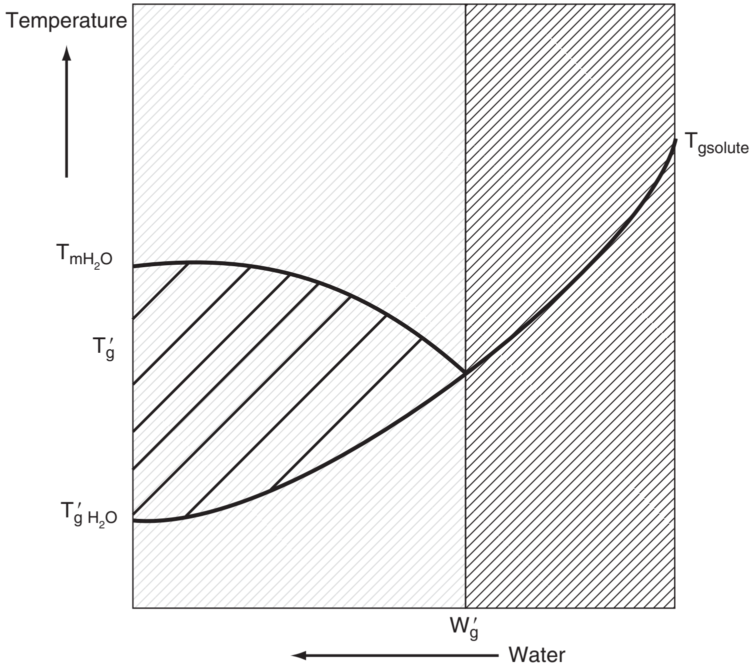

FIGURE 5.19 Schematic state diagram of temperature vs % weight of water for an aqueous solution of a hypothetical, glass-forming, small carbohydrate. Reprinted from Critical Reviews in Food Science and Nutrition, Vol.30, Slade and Levine (1991), Beyond water activity: Recent advances based on an alternative approach to the assessment of food quality and safety, Pages 115–359, with permission from Taylor and Francis (http://www.informaworld.com).

transition sometimes does not follow the change in crystallinity, although there are reports indicating that T_g increases with increasing crystallinity (Jin *et al.*, 1984; Kalichevsky *et al.*, 1992; Lim *et al.*, 2000; Zeleznak and Hoseney, 1987). T_g also depends on the moisture (water) content of the starch–water system (Mizuno *et al.*, 1998; Tananuwong and Reid, 2004; Zeleznak and Hoseney, 1987); increasing water contents decreases T_g (Fig. 5.19) in a high-moisture system. In a low-moisture system (8–30% moisture), T_g decreases with the increasing moisture content (Chung *et al.*, 2002). It is generally known that the glass transition is not always affected by changes in water content (starch:water ratio) because the plasticizing effect of water reaches a plateau after approximately 50% water (Huang *et al.*, 1994; Lim *et al.*, 2000; Tananuwong and Reid, 2004). Zeleznak and Hoseney (1987), contradicting the report of Maurice *et al.* (1985) and Biliaderis *et al.* (1986), suggested that the glass transition occurs at considerably lower temperatures than T_o. The argument was that the T_g

observed by previous authors was the sharp volume increase due to crystalline melting detected by TMA.

It is obvious that several factors, mainly the moisture content and degree of relative crystallinity, influence the T_g of a given starch. Matveev *et al.* (1998) studied glass transition in eight different starches and suggested that the observed positive changes in heat capacity during starch ''melting''are a sum of both the heat capacities of hydration and glass transition. They also hypothesized that the arrangement of amylose within the crystalline domains of starch granules, that is, the nature of amylose-amylopectin cocrystallization, is also responsible for the observed differences in T_g among different starches. It could be argued that the relative amounts of amylose and amylopectin in a given starch also influence the glass transition process. Relationships between the proportions of amylose/amylopectin and glass transition phenomenon have not been studied in detail. In food processing and other operations that use starch as a raw material, gelatinization takes place under conditions that make glass transition and related effects insignificant. Glass transition of high-moisture starch–water systems takes place below freezing (0 °C) temperatures and in low-moisture systems well below room temperature (<25 °C).

VII. CONTRADICTING THEORIES: WHAT IS GELATINIZATION?

The ordered state of the ''regular'' starch granule is adequately explained and well documented in the literature. Current theories, however, do not adequately explain changes in granular structure during gelatinization, especially in high amylose and waxy starches. Microscopic studies have revealed the presence of granular ''sacs'' or ''ghosts'' in starch–water dispersions after gelatinization (Debet and Gidley, 2007; Derek *et al.*, 1992; Gotilieb and Capelle, 2005). These ''ghosts'' or granule sacs undoubtedly influence the rheological and functional properties of gelatinized starch solutions. There is very limited information available on the transitions that take place from native granule to ''ghosts''; their structural and chemical composition is poorly characterized.

Sahai and Jackson (1999) studied the gelatinization behavior of regular cornstarch granules with differing sizes/densities using DSC. They found that DSC transition temperatures did not change significantly among different granule size/density fractions of cornstarch. However, melting enthalpies were different among fractions, indicating differences in granular structures. They also observed a decrease in the onset (T_o) and peak (T_p) with decreasing moisture levels (from 80% to 50%), and suggested that the ''least stable crystallites melt first'' theory provided in previous reports (Donovan and Mapes, 1980; Evans and Haisman, 1982) did not adequately explain starch gelatinization behaviors. In addition, studies on annealed starch fractions showed that the disappearance of secondary transitions in DSC were not clearly explained by the three-phase transition theory outlined by Nakazawa *et al.* (1984) and Biliaderis *et al.* (1986). Sahai and Jackson (1999) concluded that enthalpic transitions at various moisture levels did not represent a single phenomenon, but numerous changes occurring simultaneously. They further reported that one or more of these changes are predominantly responsible for DSC patterns at a given moisture level, and therefore any single prevailing theory is inadequate to explain starch gelatinization process.

There are numerous other reports published on starch phase transitions during gelatinization (Karlsson and Eliasson, 2003; Liu *et al.*, 2002; Randzio *et al.*, 2002; Tolstoguzov, 2003; Varavinit *et al.* 2003, Zhong and Sun, 2005). These studies reemphasize various aspects of previously published starch gelatinization theories. The explanations given to account for various thermal behaviors of starches, however, are inconsistent. This highlights the need for new approaches to explain starch gelatinization. Different starches undergo phase transitions differently. The nature, extent, and sequence of structural changes during phase transition depend on the original (native) structure, amylose to amylopectin ratio, and other polymer characteristics that can be attributed to the botanical source of a given starch and its growth environment. A universally acceptable model must be sufficiently comprehensive and flexible to explain the differing gelatinization processes of various starches.

The mechanism of starch phase transition partially depends on the amount of available water. It appears that the nature of structural changes that occur within starch granules at ‘‘low’’ (less than 65% v/v) water is different from what takes place at high or excess (above 65% v/v, generally around 80% v/v water is considered as ‘‘excess water’’ in DSC experiments) water levels. As a result, the same theory or model may not be suitable to describe what takes place at different water levels. Alternatively, it could be argued that the nature of the changes that take place in the starch granule structure during gelatinization are highly influenced by the amount of diluent (water) available. Depending on the amount of water present, the rates and extents of some mechanisms involved may change leading to contrasting observations at low- and high-water gelatinization experiments. Some reports, such as by Donovan (1979), suggest that there is a continuum of change in the gelatinization mechanism rather than a sudden change between low- and high-water systems (Fig. 5.9).

Starch gelatinization cannot be fully explained using DSC as the only experimental tool. As discussed in previous sections, DSC measures the net endothermic transitions that take place during starch gelatinization. It is known that the DSC parameters obtained for the same sample depend on heating rate (Fig. 5.20; Shiotsubo and Takahashi, 1984); transition

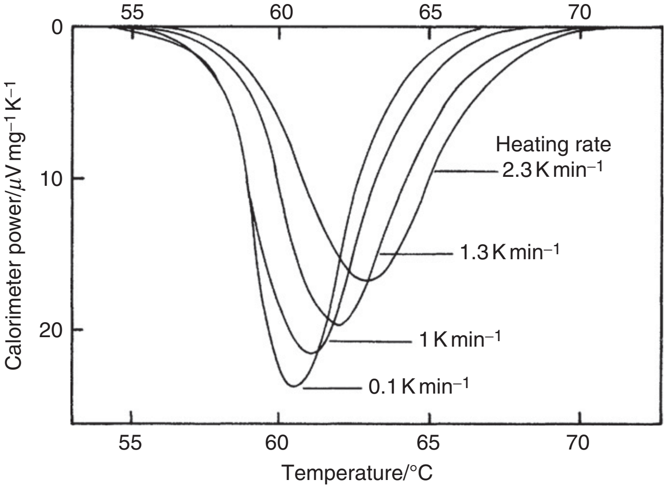

FIGURE 5.20 Change in DSC enthalpy depending on heating rate (Shiotsubo and Takahashi, 1984; reproduced with permission from Japan Science and Technology Agency).

temperatures (T_o, T_p, and T_c) increase with increasing heating rates. It is not possible to determine an appropriate or standard heating rate in DSC because very slow heating rates might impose an annealing-like effect on granules before gelatinization, and a very fast heating rate would increase the lag between the measured and the actual sample temperature. The use of different heating rates, starch:moisture ratios, and instrumentation limitations have added more confusion in interpreting starch gelatinization using DSC data.

Starch phase transitions occur in a wide temperature range. The phase transition process starts at temperatures as low as 35–40 °C, depending on the type of starch. In contrast to what was previously believed, it is now understood that amylose and/or amorphous phases also play significant roles in the phase transition process (Ratnayake and Jackson, 2007; Vermeylen *et al.*, 2006). Theories that describe gelatinization and phase transition in terms of crystallite melting, therefore, are unlikely to adequately explain the phenomena. In summary, it is evident that starch gelatinization is not an absolute result of crystallite melting. Hence, it should not be considered a simple order-to-disorder phase transition of starch structures.

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