Article

New Approach To Study Starch Gelatinization Applying a Combination of Hot-Stage Light Microscopy and Differential Scanning Calorimetry

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ABSTRACT: To overcome the difficulty of the original polarizing microscope-based method in monitoring the gelatinization of starch, a new method for dynamically monitoring the gelatinization process, integral optical density (IOD), which was based on the digital image analysis technique, was proposed. Hot-stage light microscopy and differential scanning calorimetry (DSC) techniques were coupled to study the dynamic changes of three types of starches: type A (corn starch), type B (potato starch), and type C (pea starch), during the gelatinization process in an excess water system. A model of response difference change of crystallite could represent the responding intensity of crystallization changes in the process of starch gelatinization. Results demonstrated that three crystalline types of starch underwent a process of swelling, accompanied with gradual disappearing of the crystallite. This difference was mainly associated with the diversity and composition of the starch structure. The IOD method was of advantage compared to the previous traditional methods that are based on a polarization microscope, such as counting the particle number and calculating polarization area methods, because it was the product of two parameters: optical density and area, which would be a response of both light intensity and area of birefringence light. The single peak in DSC corresponded to the combination of crystalline helix—helix dissociation and the reduction of the molecule helix—coil transition, while the gelatinization degree measured by the IOD method mainly corresponded to the helix—helix dissociation. The gelatinization mechanism could be revealed clearer in this study.

KEYWORDS: Starch, gelatinization, dynamic monitoring, integral optical density, digital image analysis technique, hot-stage light microscopy

■ INTRODUCTION

Gelatinization is a term used to describe the irreversible changes that accompanied the disruption of the starch granular structure;[1] it is one of the most important features of starch and the most common use of natural phenomena in our daily life and production. Although the sequence of structural transformations that the starch granule undergoes during this order—disorder transition has been extensively researched from the beginning of the 19th century, none of the published starch gelatinization theories can fully and adequately explain the exact mechanism of sequential structural change that starch granules undergo during gelatinization.[2] Recent evidence suggests that starch gelatinization occurs over a wider temperature range rather than as a sudden order—disorder transition taking place within a narrow temperature range[3] and varied widely because of the difference of starch sources. After a variety of thermal processing and unit operations, starch granules undergo a process of swelling accompanied with gradual disappearing of the crystalline, affecting the functional and structure properties of the food. Therefore, monitoring and controlling the starch gelatinization degree is of important significance.

Many physical, chemical, and enzyme experimental tools and techniques, such as polarizing microscope (measuring the loss of birefringence), differential scanning calorimetry (DSC), thermomechanical analyzer (monitoring the change of thermal enthalpy), nuclear magnetic resonance (NMR), etc., can be used in charactering starch gelatinization and related properties. With different parameters, these methods can characterize starch gelatinization properties from different perspectives. The measured physical and chemical properties are of some differences, unique but interrelated. Many researchers have compared different gelatinization analysis methods for further elaboration of the mechanism of the starch gelatinization process. Liu et al.[4] investigated the order—disorder transition that occurs upon heating an aqueous suspension of starch granules using DSC, X-ray crystallinity, and birefringence methods (with counting the particle number method); the results showed that the decrease in crystallinity occurs both before the birefringence of granules starts to disappear and after all birefringence is lost. Cooke and Gidley[5] studied different starches after defined thermal pretreatments, quantified the starch residual molecular and crystalline order by spectroscopy and X-ray diffraction, and compared to DSC. The comparison suggested that the enthalpy of gelatinization primarily reflected the loss of molecular (double-helical) order. Banks et al.[6] developed a general procedure to measure the degree of gelatinization in samples over a broad concentration range. The birefringence, DSC, X-ray, and amylose—iodine complex formation were combined first to demonstrate that the gelatinization temperature measured by four methods was in accordance under high moisture of the water—starch system. As

a popular apparatus on studying gelatinization, the polarizing microscope takes advantage of the crystal properties of starch, showing birefringence under polarization light and observing the ordered structure of starch granules under 500 nm wavelength.[7] In tradition, losing polarization for 2 and 98% is defined as the onset and final gelatinization temperatures of the particles, respectively,[8] and the quantity method, counting the disappearing of starch granules, is applied. However, the birefringence does not disappear in a short time during the process of starch gelatinization, and the quantity is not even a geometrical parameter. As a result, the real situation of starch gelatinization cannot be characterized.

A digital image analysis technique has been hired for a number of areas, such as medicine, geological, and food quality.[9−12] Through the collection of related digital photos and the use of professional image analysis software on the target area of concern, such as quantity, size, color, and light intensity, the relevant data are analyzed. Optical density (OD) is widely used in the biomedical area, especially molecular biology; it can indirectly reflect the relative amount of immunoreactive substance in the sample and is often used to measure the positive values in the cells by staining.[13] The integral optical density (IOD) values (defined as the sum of OD values of all pixels within that area) within a certain part of the sample are proportional to the amount of positive material contained within the site.[14] Starch birefringence optical experienced diminution to whole disappearance in the gelatinization process. The crystal structure of starch granules is defined as "positive material", whose strength is proportional to its birefringence brightness under polarized light. Professional image processing software Image-Pro Plus 5.0 is used in the analysis of digital pictures filmed under birefringence light. The IOD value is calculated to reflect the strength of the starch crystalline structure and dynamically and quantitatively characterize the changes of the crystallization degree in the gelatinization process.

Starch can be mainly divided into types A and B according to the geometrical characteristics of the unit cell, the double helix bulk density, and the number of different bound water contents in the crystal structure.[15,16] Two types of crystallite, or polymorph structures, A and B, have been identified in starch granules, which can be distinguished by the packing density of double helices. A polymorphs are denser than B.[17] Starches from different species have either A (e.g., maize), B (e.g., potato), or both A and B (e.g., pea) types of polymorphs.[18] In this respect, maize starch is termed A type, potato starch is termed B type, and pea starch is termed C type. In A-type polymorphs, the double helices are closely packed together with a small amount of structural water, and in B-type polymorphs, the double helices are more loosely packed together with proportionately more water.[19] If C-type starches consist of A- and B-type granules, then it is likely that their properties will be intermediate between those of A- and B-type starches. If, on the other hand, both polymorphs exist within all granules,[20] then C-type starches will have unique properties dependent upon the arrangement of these two polymorphs in the granule.

To date, no studies have carried out the use of IOD digital image analysis technology on starch gelatinization process analysis. The present study, taking advantage of this new method to dynamically and quantitatively monitor the crystal disappearance, demonstrates that the starch gelatinization process, in this case, in the combination of hot-stage light microscopy and the DSC technique, can be realistically reflected and clearly revealed.

■ MATERIALS AND METHODS

Materials. Native starches were of food grade and used in this study. Corn starch (type A) was purchased from Guangdong Pengjin Industrial, Ltd. (Guangdong, China). Potato starch (type B) was obtained from Haotian Group, Ltd. (Jiangsu, China). Pea starch (type C) was purchased from Yantai Oriental Protein, Ltd. (Shandong, China). Chemicals and solvents used in this work were of analytical grade.

Hot-Stage Light Microscopy. Starch dispersions (starch/distilled water = 1:4, dry basis) equilibrated for 2 h were sealed between two glass coverslips using Dow Corning 732 sealant before being replaced in the hot stage (model THMS600, Linkam, Surrey, U.K.).

Each specimen in the hot stage was observed under a polarization microscope (model BHS-2, Olympus Vanox, Japan) equipped with a digital camera, which can display live video of birefringence granules in real time. A temperature programmer was connected with the hot stage to control the heating progress from 40 to 90 °C at a rate of 2 °C/min. Live pictures were captured every 5 °C when below 60 °C, while live pictures were captured every 2 °C above 60 °C. Each image (2048 × 1536 pixels) was saved as 12-bit TIFF image file, without compression.

DSC. Gelatinization parameters of starch were detected using a Perkin-Elmer DSC 8000 (Norwalk, CT) equipped with a refrigerated cooling system and Pyris operation software (Perkin-Elmer). Melting point and enthalpies of indium were used for temperature and heat capacity calibration.

The ratio of starch to distilled water and heating scan program was made the same as in the hot-stage experiment. Starch slurry (starch/distilled water = 1:4, dry basis) equilibrated for 2 h. The nitrogen access speed was 20 mL/s. The spectrum of starch gelatinization enthalpy was analyzed by the Pyris Software accompanied with the DSC instrument. The DSC measurement of each specimen has been performed in triplicate, and the results are presented as the mean.

Degree of Gelatinization (DG). Each parameter of gelatinization, such as the ratio of starch/water and heating program, was well-controlled. It was worth stressing that the heating rate was chosen at 2 °C/min to minimize any temperature lag because of the large mass of the steel pan.[21] Although the two techniques of studying starch gelatinization used in this study were based on different theories and performed in different experimental instruments, they were indeed detecting the same process.

(1) IOD method:

DG_I is the percentage of IOD values decreased from the initial state of starch at a specific temperature point. The IOD value of each digital picture was calculated by the Image-Pro Plus 5.0 software.

DG based on the IOD value was calculated as follows:

$$\text{background correction: } C = A - B \tag{1}$$

$$DG_I\ (\%) = (1 - C/C_0) \times 100\% \tag{2}$$

where A is the original IOD value (IOD value calculated from the original digital image), B is the background IOD value (IOD value calculated from the original digital image when all of the birefringence disappeared), C is the real IOD value (IOD value of birefringence light derived from the specific crystal structure of starch in the digital image), and C_0 is the initial real IOD value (IOD value of birefringence light derived from the specific crystal structure of starch in the initial digital image). In this study, the IOD value of 40 °C was set as the initial IOD value.

(2) DSC enthalpy change integral method (DSC method):

DG_D is the percentage of area integrated from the initial point of the thermal peak (t_0) to a specific temperature point to the total thermal peak area. DG based on the DSC thermogram was obtained as follows:

$$DG_D\,(\%) = \int_{t_0}^{t} f(x)\mathrm{d}x \qquad (3)$$

where t is the specific temperature point and t_0 is the initial point of thermal peak. In this study, t_0 was 40 °C.

Model of Response Difference of Crystallite Change (MRDCC). The MRDCC is a new characterization of the crystallization change degree in the starch gelatinization process. It characterizes that the starch gelatinization speed changes with the temperature (the crystallization change degree on a certain range of temperatures). A similar starch crystal structure corresponds to the same kind of peak type.

The peak of crystalline change is the diversified peak curves of MRDCC defined as the peak of crystalline change, and its multimodality corresponds to the diversity gelatinization behavior of starch granules.

The peak height is the variation of the crystallization degree in a certain temperature range, gelatinization speed, $\%/\Delta T$ for units (% is the gelatinization degree difference, and ΔT is the range of temperatures corresponding to the peak height).

For the drawing method, the MRDCC at a certain temperature is defined as the degree of gelatinization (DG_I) of this temperature measured by the IOD method minus that of the previous temperature. The temperature is chosen for the horizontal axis, while the response difference change of crystallite is chosen for the vertical axis, fitting the scatter with the tension spline function, and the response difference change of crystallite is obtained.

In this experiment, for example, below 60 °C (including 60 °C), the peak height of the difference change of crystallite at a certain temperature, such as 55 °C, is the result of the gelatinization degree at 55 °C minus the gelatinization degree of the previous temperature at 50 °C; above 60 °C, the peak height of the difference change of crystallite at a certain temperature, such as 68 °C, is the gelatinization degree at 68 °C, subtracting the gelatinization degree of the previous temperature at 66 °C.

At least triplicate runs were made of each sample; the result was calculated and analyzed by Excel software, and curves were obtained by drawing and fitting the Curve Expert-Pro.

The IOD method characterizes the change of the degree of crystallization in the process of starch gelatinization; it is a unified response of the crystallinity change of starch granules aggregate within a certain time. It can be deduced that the crystal structure change in the process of gelatinization will tend to the normal distribution reflected from the multi-crystalline peak curves, because of the similar structure and/or component of starch granules corresponding to the same gelatinization course, and the gelatinization process may be in line with the normal distribution.

■ RESULTS AND DISCUSSION

Birefringent of Granules and the DG Measured by the IOD Method. As shown by the results of the gelatinization process in Table 1, with the rise of the temperature, three kinds of granules underwent a process of swelling accompanied with gradual disappearance of birefringence light. Not every starch granule gelatinized simultaneously; it led to a state diversity at every temperature. During the whole gelatinization process, the starch granule represented three kinds of states: totally gelatinized (birefringence light totally disappeared), partially gelatinized (decrease of light intensity and/or area of birefringence light), and ungelatinized (without any apparent/detectable change under the birefringence light). The majority of the starch was in the stage of partially gelatinized.

As seen from Figure 1, it was known that three curves reflecting three kinds of starches experienced different degrees of crystallization from gradually decreased to completely eliminated in the gelatinization process. There was no significance difference in pre-gelatinization (<60 °C) and late gelatinization (>68 °C) of A- and B-type DG_I. However, when

Table 1. Micrographs of A-, B-, and C-Type Starches at Different Temperatures (40, 55, 60, 64, 66, 68, 72, and 76 °C) during the Heating Process under Polarized Light and Corresponding DG_I (%)

T/°C	A-type	B-type	C-type
40 °C			
DG_I	0	0	0
55 °C			
DG_I	3.2%	2.9%	9.2%
60 °C			
DG_I	8.0%	6.7%	22.9%
64 °C			
DG_I	17.3%	37.1%	49.9%
66 °C			
DG_I	35.5%	56.8%	59.9%
68 °C			
DG_I	66.6%	76.2%	70.7%
72 °C			
DG_I	98.7%	96.6%	88.4%
76 °C			
DG_I	100%	99.9%	97.3%

they were in the partial gelatinization (60−68 °C), DG_I of type B was greater than that of type A. With regard to type C, the DG_I was greater than types A and B before 66 °C but reversed after about 68 °C. The rising rate of A- and B-type DG_I
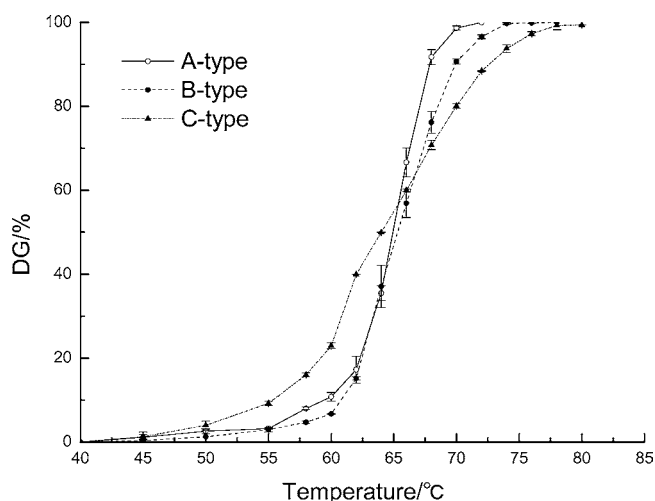
Figure 1. Relationship between the temperature and DG_I of A-, B-, and C-type starches during the heating process.

increased sharply at 64 and 62 °C, respectively, and reduced gradually after 68 °C together. Before 62 °C, the rising rate of C-type DG_I experienced an increased process, then reduced and stabilized at a certain value, and gradually reduced to zero after 72 °C.

The differences on the structure of three kinds of starches led to its gelatinization process diversity. The current state of understanding the gelatinization demonstrates that, in excess water, the first step is for the amorphous growth rings to suck in water and rapidly expand in size. Consequently, because the system is widely cross-linked through the amorphous backbone, the coupled semi-crystalline lamellae is then disrupted, reducing the crystallinity of the granule and causing it to lose its birefringence.[7] A partial loss of crystallinity at the lower temperatures shown in Table 1 was due to the slight expansion under hydrothermal conditions and the double helices stripping off the sides of the crystallites. At a high temperature, starch granules expanded sharply and possessed enough energy to encourage the double helices to strip off the crystallites. The B-type DG_I increased fiercely at a lower temperature than A-type DG_I; therefore, more gelatinization endotherm occurred at a lower temperature for B-type starch compared to A-type starch. It was postulated that potato starch (B type) contained phosphorus in the form of phosphate monoesters that occurred on the starch molecule as negatively changed groups. The ionic repulsion generated by these groups weakened the association between the molecules and increased the granule water-binding capacity and swelling power.[22] The increasing rate of A-type DG_I was faster than that of B-type DG_I in the middle term of gelatinization; with regard to this behavior, our knowledge about this part was highly limited. One of the reasons, however, may be the existence of the amorphous and crystalline regions of the B-type granule. The different parts of the amorphous region had different glass transition temperatures during heating, and as a consequence, the mobility of this region increased over a wide range of temperatures.[23] Another possible reason for the DG_I increasing rate difference of the two types of starch was that the amorphous growth rings of the B-type starch were denser and contained less water than those of the A-type starch.[24]

According to the study by Bogracheva et al.,[23] the gelatinization behavior of C-type granules began from the hilum area and then spread quickly through the central part of the granules, where the B polymorph was seated. The outer parts of the granule contain the denser A polymorphs and, therefore, had a higher temperature of disruption. The center part of the crystalline structure of the C-type starch was lost quickly in the gelatinization initially, but in comparison to the A and B types, the outer layer of the C crystal structure disappeared more slowly, so that the C-type starch had lower DG_I and DG_I rising rate after a high temperature ($\geq$68 °C). The C-type starch could better resist expansion early in the gelatinization than A and B types;[15] however, it came out with higher DG_I. This might be due to the B polymorphs, which expand first during gelatinization, reducing the gelatinization temperature of the nearby crystalline areas and accelerating the disintegration process.[19] Therefore, it was considered that the polycrystalline distribution of the C-type starch as well as a dynamic balance between the acceleration effect and expansion suppression characteristics determined the diversity gelatinization stage of the C-type starch.

Comparison of the Four Kinds of Methods on DG Measurement. It is well-known that applying a hot-stage microscope to measure the loss of birefringence was based on counting of the granule amount (DG_C), which lost their Maltese cross. However, before total gelatinization, the Maltese cross of the partially gelatinized starch granule is deforming with the swelling of starch and loss of crystalline order. Thus, there was not clear boundary to define if the Maltese cross really disappeared, while actually many partially gelatinized starch granules already had not identifiable "cross" at all. Although a traditional granule counting method might be still workable and relatively accurate to measure the gelatinization temperature, we could not deny that a parameter based on the amount of particle was a limitation to reveal the real state of starch under gelatinization.

The polygon area of the birefringent zone of granules was selected by Parada and Aguilera[11] as the microstructural parameter of reference when considering its good correlation with DG. It was a progress compared to the previous counting method; however, the truth was that not only the relative unmelted starch granule bulk represented as area that decreased during gelatinization but also the intensity of the remaining area.

IOD was brought in because it was the product of two parameters: optical density and area, which would be a response to both light intensity and area of birefringence light. Therefore, DG of the starch granule especially together with the partially gelatinized zone could be detected and quantitatively analyzed. With regard to the curve of DG_I, the starch granules occurred with slight expansion at a lower temperature (50 °C), especially those with broken particles, expanding earlier and more violently, accompanied with significant weakening of the birefringent. At the same time, as could be seen from Table 1, there were more than 1000 starch granules that could be observed in a picture, so that the method achieved a higher density of observation under the microscope while with reduced experimental work and enhanced statistical significance.

Figure 2 showed the DG of potato starch calculated by different methods. DG varied from the measuring method. At every temperature, DG_I was the biggest, followed by DG_A, DG_C, and DG_D. The reason why DG_I was greater than DG_A was mainly attributed to the partially gelatinized starch granule, which lost its specific crystalline order at a certain degree but
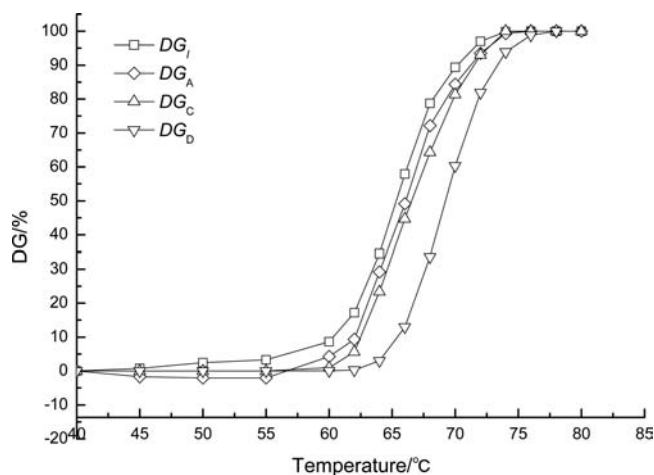
Figure 2. DG of potato starch detected by four methods.

did not yet lead to the disappearance of birefringence light. As shown in Figure 1, most granules were on the state of partial gelatinization during the middle stage of gelatinization. With the area method (DG_A), those remaining weaken zones were identified as the same as those ungelatinized zones. In other words, DG was underestimated by the area method.

Therefore, in the three kinds of methods based on optical microscopy, the IOD method could dynamically measure starch crystallization, truly and accurately reflecting the state of the starch gelatinization process under a specific temperature with higher accuracy and sensitivity.

DSC followed the gelatinization process in the aspect of net enthalpy change (ΔH), which came from a combination of endothermic melted, ordered structures and exothermic granular swelling. On the whole, those specific ordered structures, which could be observed as birefringence, were only one component of the overall ordered structures melted endothermicly. DG evaluation based on overall net enthalpy, DG_D, would be the lowest compared to the other three methods.

MRDCC. Figures 3 and 4 were obtained by the DSC technique and MRDCC, respectively. As shown in Figure 3, the endothermic heat flow peak of A-, B-, and C-type starches was typically a single peak, with the peak gelatinization temperature
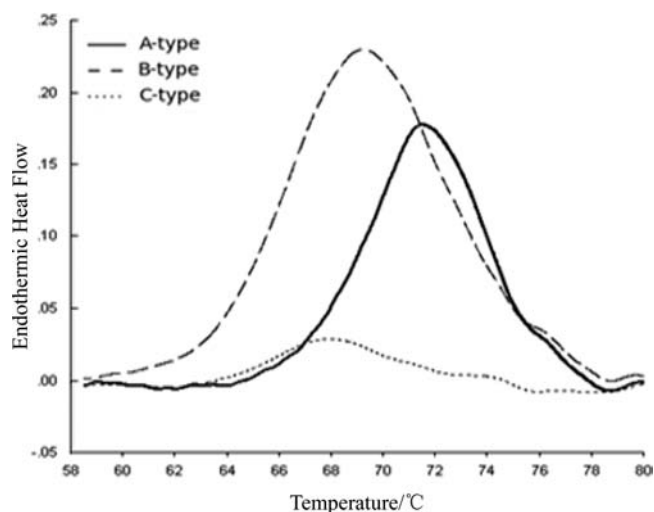

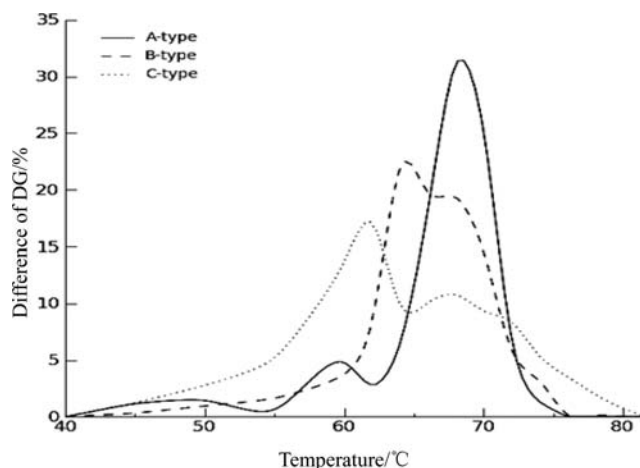**Figure 3.** DSC scanning thermogram of A-, B-, and C-type starches.

Figure 4. Relationship of the IOD response difference value of A-, B-, and C-type starches with temperature.

being 71.8, 69.1, and 69.9 °C, respectively. As seen from Figure 4, three curves showed obvious multiple peaks. Type A had two distinct peaks; the peak temperatures were 59.7 and 68.4 °C (our parallel error was less than 5%; the peaks that were less than 5% had been ignored). The second peak temperature was lower than the DSC scan diagram for 3.4 °C. B had a significant peak at 64.4 °C, lower than the DSC for 4.7 °C, while a shoulder peak was located on the right. The C-type starch had two clear peaks; the peak temperatures were 61.8 and 67.7 °C, respectively. It was described that the C-type starch possessed the property of polycrystalline and the gelatinization behavior of multi-stage. We could suggest that the first peak corresponded to the B polymorphs within the C-type starch and the second peak corresponded to the A polymorphs outer moiety of the C-type crystal. In comparison to the pure A- and B-type starches from Figure 4, these two peak temperatures were relatively low for 2.6 and 0.7 °C. To summarize, the MRDCC figure reflected that not only the C-type starch had crystallized diversity because of its special composition but also A- and B-type starches, traditionally viewed as a single crystal type of starch, showed crystal diversity. It could be seen from the left-side peak of the main peak of the A-type starch, as well as the right shoulder peak of the main peak of the B-type starch. It was worth noting that the right shoulder peak of the B-type starch might relate to the big and small particles presented in polarization images. The peak gelatinization temperature of the A polymorphs in the C-type starch was not far from the pure A-type starch (0.7 °C), while the peak gelatinization temperature of the corresponding B polymorphs was lower by 2.6 °C; this might relate to the more porous structure portion of the B polymorphs in the C-type starch center and the water swelling earlier in the gelatinization. The use of the proposed model provided an improved understanding of diversity crystallite and multi-stage in the gelatinization of starch.

Relationship between DG_I and DG_D. With the establishment of a rectangular sit coordinate system, which corresponded to DG_I and DG_D at the same temperature point, DG_I acted as the abscissa and DG_D acted as the ordinate. It reflected the relationship between the crystalline decrease (DG_I) and the enthalpy change (DG_D) gelatinization process. The correlation R^2 values of three DG curves were 0.964, 0.990, and 0.988. Figure 5 displayed that the DG characterized by the IOD method was faster than the DSC method; namely, DG_D
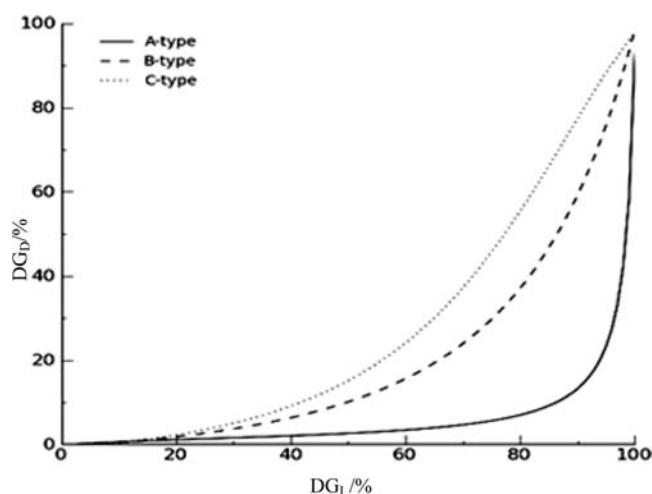
Figure 5. Relationship between DG_I and DG_D of A-, B-, and C-type starches at the same temperature.

increased slowly in the former stage of DG_I and rapidly increased in the later stage. This trend was most obvious in A-type starch; before the DG_I was up to 90%, DG_D completed only 20% and increased dramatically in the later stage of DG_I (>90%). With regard to B-type starch, DG_D and DG_I showed smaller differences; DG_I was 70% when DG_D slowly increased to about 20%, and then the increasing rate rose fast subsequently. The DG trend of C-type starch was similar to B, but DG_D is higher than B under the same conditions.

The gelatinization enthalpy was a combination of the endothermic disruption process of short- and long- ordered structures and the exothermic process of granular swelling.[19] According to Waigh's side-chain liquid−crystalline theory,[7] in excess water (>40%, w/w), it could be considered that both the helix−helix dissociation and the helix−coil transition occurred simultaneously. The former corresponded to G endotherm (reduction of the starch crystal ordered structure), and the later corresponded to M1 endotherm (reduction of the molecule sequence structure); the single peak in the DSC diagram (Figure 3) was a combination of both of them. Polymer chains in the amorphous regions underwent the glass transition process, resulting in an entropic driven and endothermic swelling process, which physically destabilized the crystallites. From the study by Biliaderis,[25] we knew that molecular (double-helical) order was significantly greater than crystalline order in contributing to the DSC enthalpy; the research by Cooke and Gidley[5] also drew the same conclusion. At the same time, they also pointed out that the loss of the starch crystalline structure was mainly concerned with the early period of expansion of the starch granule and the loss of the double helix in the later expansion. Therefore, the gelatinization process reflected by DG_I mainly corresponded to the G peak, while DSC corresponded to the G and M1 peaks.

Starch had already occurred in various degrees of expansion before the DSC curve showed the start of the gelatinization temperature; this kind of thermal effect of expansion was often not obvious, but had a significant impact on the total crystallization. It could be suggested that DG_I started first and then DG_D. With a further increase of the temperature, there was enough energy to destroy the remote ordered structure of all that was left and lead to the spiral coil transition (loss of order molecular structure), and then DG_D increased constantly. Our results are in good agreement with the model

of starch gelatinization proposed by Chiotelli et al.,[22] who claimed that a liquid−crystalline transition (from smectic to isotropic) appears to start around 50 °C for wheat starch, i.e., before any endothermic event is visible with DSC. This transition corresponds to the onset of double-helical dissociation from the crystallites (decrease in long-range order).

Corn starch showed the largest DG_D hysteresis effect in Figure 5. Two possible explanations could be suggested for this behavior: Initially, in comparison to B-type starch, the amylopectin in the A-type starch was shorter in side-chain length and, therefore, showed a wider M1 peak; the author speculated that the originally merged G and M1 peaks in the temperature axis were further apart as a result. Second, the loss of crystallinity of corn starch was lower than the other two in contributing to the total enthalpy of the starch gelatinization. The curve of pea starch was not between the A and B types while in the upper position. This result might relate to the less crystalline amylopectin,[15] the existence of a double-helix structure, branched-lipid complexes, etc., in the pea starch; that is to say, crystallinity loss contributed more to the total enthalpy of the C-type starch gelatinization. We can deduce from this diagram that A-, B-, and C-type starches show a large difference in the gelatinization process. In short, different starches exhibited different DG characteristic curves because of their structure and composition differences.

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Notes
The authors declare no competing financial interest.

■ ABBREVIATIONS USED

DSC, differential scanning calorimetry; NMR, nuclear magnetic resonance; IOD, integral optical density; OD, optical density; DG, degree of gelatinization; DG_I, degree of gelatinization measured by the IOD method; DG_C, degree of gelatinization measured by counting the particle number method; DG_A, degree of gelatinization measured by calculating polarization area method; DG_D, degree of gelatinization measured by the DSC technique; G, reduction of the starch crystal ordered structure; M1, reduction of the molecule sequence structure; MRDCC, model of response difference of crystalline change

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