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GELATINIZATION OF WAXY, NORMAL AND HIGH AMYLOSE CORN STARCHES

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Abstract
The influence of amylose content on water uptake and solubility behaviors and percent degree of gelatinization of waxy (Amioca), normal and high amylose (Hylon VII) corn starches were investigated. Gelatinization was also followed by differential scanning calorimetry and the transition temperatures and enthalpies were determined. The results were discussed in relation to underlying mass transfer processes during gelatinization. It was determined that water uptake and solubility of starches decreased as the amylose content increased. Amioca exhibited the highest water uptake, attributed to its low amylose content. The water uptake and solubility of Hylon VII were low at all temperatures. The highly associated structure of Hylon VII and also the presence of lipid complexes were considered as the factors influencing its gelatinization behavior. Therefore it was concluded that the mass transfer processes for gelatinization was mainly influenced by free amylose for network formation rather than the proportion of amylose in starch.

Keywords: Amioca; Normal corn starch; Hylon VII; Gelatinization; Mass transfer.

MUMSU, NORMAL VE YÜKSEK AMİLOZ İÇERİKLİ MISIR NİŞASTALARININ JELATİNİZASYONU

Özet
Bu çalışmada mumsu (Amioca), normal ve yüksek amiloz içerikli (Hylon VII) mısır nişastalarının su kazancı ve çözünürlük davranışları ile jelatinizasyon derecelerine amiloz içeriğinin etkisi araştırılmıştır. Jelatinizasyon diferansiyel taramalı kalorimetri yöntemi ile de incelenmiş, geçiş sıcaklıkları ve entalpileri belirlenmiştir. Jelatinizasyon süresince meydana gelen değişiklikler kütle aktarımı yaklaşımıyla değerlendirilmiştir. Nişastadaki amiloz miktarı azaldıkça su alma ve çözünürlük miktarının azaldığı belirlenmiştir. En yüksek su alma Amioca da izlenmiş ve bu durum düşük amiloz içeriği ile ilişkilendirilmiştir. Hylon VII de gözlenen molekül içi assosiyasyon ve lipit varlığının jelatinizasyon davranışında belirleyici rol oynadığı izlenmiştir. Elde edilen sonuçlar ışığında, jelatinizasyon sırasındaki kütle aktarım olaylarının nişastadaki amiloz oranından çok ağ yapıda yer alabilen serbest amiloz miktarından etkilendiği sonucuna varılmıştır.

Anahtar kelimeler: Amioca; Normal mısır nişastası; Hylon VII; Jelatinizasyon; Kütle aktarımı.

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INTRODUCTION

Corn starch is a valuable ingredient to the food industry, being widely used as a thickener, gelling agent, bulking agent and water retention agent (1). On the basis of amylose and amylopectin ratio, corn starch can be separated into normal, waxy and high amylose corn starch. Normal corn starch consists of about 25% amylose and 75% amylopectin fraction (2).

During processing of starch many changes occur in the physicochemical properties. The most important one can be considered as gelatinization. Starch granules are insoluble in cold water, but they absorb water in aqueous medium reversibly (3, 5). When starch granules are heated in excess water, at a certain temperature, the swelling becomes irreversible and the structure of the granule is altered significantly (4, 6). This process is called gelatinization and several theories have been proposed for its mechanism, but yet a universally accepted definition of the process is not available (7).

Semi-crystalline starch granules are primarily composed of two biopolymers, i.e. the essentially linear amylose and the highly branched amylopectin. It has been shown that structural features such as amylose content and crystallinity are related to events associated with gelatinization:starch granule swelling, amylose and/or amylopectin leaching, loss of radial (birefringence), supra-molecular (crystallinity) and molecular order (2, 8, 9). In literature, contradictory results were found with regard to the influence of amylose on gelatinization properties. Gelatinization enthalpies (expressed on starch or amylopectin basis) are either not related to (10-12) or decreased (8, 13, 14) with amylose content. The semi-crystalline nature of starch granules, as detected by X-ray diffraction studies, consists mainly of short amylopectin chains which form double helices associated into clusters forming crystalline lamellae. Recent reports showed that the crystallinity decreased with increasing amylose content in corn starches (15-16). However the precise role played by the amylose in the structure of starch granule is still unclear (8). Apart from the influence of amylose content on gelatinization, gelatinization temperatures and/or enthalpy increase with the crystallinity. This holds for waxy starches of different botanical origin (17) as well as for low and high amylose corn starches (8).

From the point of view of mass transfer, two simultaneous processes, namely the diffusion of water into granule and the leaching of soluble polysaccharides from the matrix, take place during gelatinization. Both processes are known to be affected by the structural properties, especially amylose/amylopectin content of the starch. However, few researches have been carried out, relating gelatinization process to the mass transfer occurring, and the data concerning the effect of starch structure on the mass transfer were very rare.

Briefly, the objectives of this study were [1] to determine X-ray diffraction patterns, water uptake (WU), %solubility (%S) and percent degree of gelatinization (%DG) of starches with different amylose contents (waxy, normal and high amylose corn starches), [2] to study their gelatinization properties by differential scanning calorimetry (DSC), and [3] by using the data obtained, to discuss the results from the point of view of mass transfer occurring during gelatinization.

MATERIALS AND METHODS

Materials

Three kinds of corn starches with different amylose contents were used in this study. Normal corn starch was purchased from Sigma Chemical CO. Amioca (waxy corn starch) and Hylon VII (high amylose corn starch) were donated by National Starch & Chemical Limited. Amylose contents of starches were approximately 1-2 % for Amioca, 21-24 % for normal corn starch and 63% for Hylon VII, as declared by their producers. Moisture contents of starches were determined using standard AOAC (18) method and found as 11.4±0.1, 11.2±0.1 and 11.9±0.1% (w/w) for Amioca, normal corn and Hylon VII, respectively.

Water Uptake and Solubility

WU, %S and swelling power (SP) were determined in duplicate by the modified method of Leach *et al.* (19). According to this method, starch suspension (1.0%, w/v) was heated at the temperature interval of 50-95 °C with a 5 °C increase in this temperature range. Heating lasted for 30 minutes with low level of stirring (200 rpm). Then the sample was cooled to room temperature in an iced water bath and centrifuged (5000 rpm, 15 minutes). Subsequently, the supernatant and sediment were separated. The supernatant was evaporated and dried to the constant weight (130 °C, 1 hour) and the dry matter obtained was used to calculate %S, expressed as gram soluble per gram dry starch. The sediment, on the other hand, was weighed to determine SP (g sediment /g dry sediment) with correction for solubles. The %S and SP were calculated as follows:

$$\% S = \frac{\text{Weight of solubles}}{\text{Weight of dry starch}} \times 100 \qquad \text{Equation (1)}$$

$$SP = \frac{\text{Weight of sediment}}{\text{Weight of dry starch} \times \left[1 - \frac{\%S}{100}\right]} \qquad \text{Equation (2)}$$

The SP and WU are related parameters. Therefore it is possible to arrange SP equation to obtain an expression for water uptake (g water / g dry sediment)

$$WU = SP - 1 \qquad \text{Equation (3)}$$

Percent Degree of Gelatinization

In order to determine the degree of gelatinization, the procedure based on the amylose-iodine blue method was used (20). This method takes into account the amylose released during gelatinization. This amylose reacts with iodine to form a blue colour complex measured at 600 nm. For this purpose, raw starch is completely dispersed in 0.5 N KOH while partially gelatinized one in 0.2 N KOH. The ratio of the two absorbances obtained from partially gelatinized and raw starch samples were used to calculate the % DG.

Differential Scanning Calorimetry

DSC experiments were carried out using TA Q20 model DSC apparatus (TA Instruments, USA). The calorimeter was calibrated with indium (melting point = 156.6 °C, ΔH = 28.5 J/g). The DSC runs were operated under nitrogen gas atmosphere (30 mL/min) and an empty pan was used as the reference.

The hydrated starch samples were prepared as follows: approximately 3 mg starch was weighed in an aluminum pan and distilled water was added subsequently. Then the weight of contents was controlled continuously until the desired moisture content was attained. Afterwards, the pan was hermetically sealed and allowed to stabilize at room temperature overnight before heating in the calorimeter.

The amount of water added to the starch was adjusted accordingly to obtain different water:starch ratios of 1:1, 2:1 and 3:1 (w/w). The original moisture content of the starch sample was also taken into account in calculating the above-mentioned starch-water ratios. The onset (T_o), peak (T_p) and completion temperatures (T_c) of the gelatinization endotherm and also the enthalpy associated with gelatinization were obtained from endotherm. The adequate amount of water required for complete gelatinization was determined as water:starch = 3:1.

X-Ray Diffractometry

The X-ray diffraction patterns of starch samples were obtained on a Phillips X-ray diffractometer (PW 1140). Patterns were recorded from a diffraction angle (2θ) of 5-35 °C at a scan speed of 2 °C/min. In order to measure the relative crystallinity of starch in the X-ray diffractogram, the method proposed by Köksel *et al.* (21) was used.

Statistical Analysis

Experimental data were subjected to one-way analysis of variance (ANOVA), using SPSS version 11.5 (SPSS Inc., USA). Treatment means were tested separately for least significant difference (LSD) test.

RESULTS AND DISCUSSION

X-Ray Diffraction Patterns

The A-type X-ray diffraction patterns were obtained for Amioca and normal corn starch (Figure 1). The B plus V type of X-ray diffraction pattern was observed for Hylon VII. The distinct peaks in the spectra demonstrate the crystalline nature of granules. In addition, Hylon VII showed a peak at 2θ of 19-21°, suggesting a crystalline structure of amylose-lipid complex in the granules. The percent crystallinities for Amioca and normal corn starch have been calculated as 38 and 35, and for Hylon VII, it was determined as 8%. These results indicated that no correlation was observed between % crystallinities and amylose contents of starches. Of the three corn starches studied, Hylon VII had the lowest X-ray crystallinity. However, low X-ray crystallinity is not necessarily related to poorly ordered starch molecules, but may be the result of small-size crystallites in the granules.

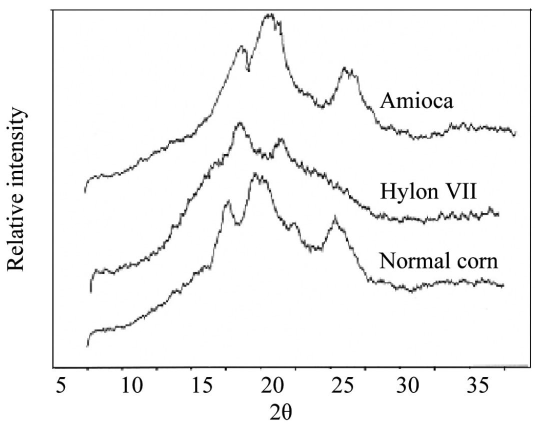

Figure 1. X-Ray diffraction patterns of corn starches.

Water uptake (WU) and Solubility (%S)

The WU and % solubility curves at different temperatures are presented in Figure 2a and b. The results revealed that both the WU (diffusion of water into granules) and %S (leaching soluble polysaccharides, amylose leaching) depended on the temperature and the type of starch studied. The observable difference between WU and %S of starches was detected after the temperature of 70 °C and 65 °C respectively. Amioca always had the highest WU. The water uptake of normal corn starch was always lower than that of Amioca and higher than that of Hylon VII. The inverse correlation between WU and amylose content led to the conclusion that water uptake was mainly decreased as the amylose content increased. This result is in accordance with what has been reported by the other authors (22-24). Colonna & Mercier (22) stated that there exists a relationship between water uptake and solubilization processes. This is evident from WU and %S curves in Figure 2a and b. Similarly, an inverse correlation between %S and amylose content was observed at temperatures less than 90 °C. In other words, %S also decreased as the amylose content of starch increased.

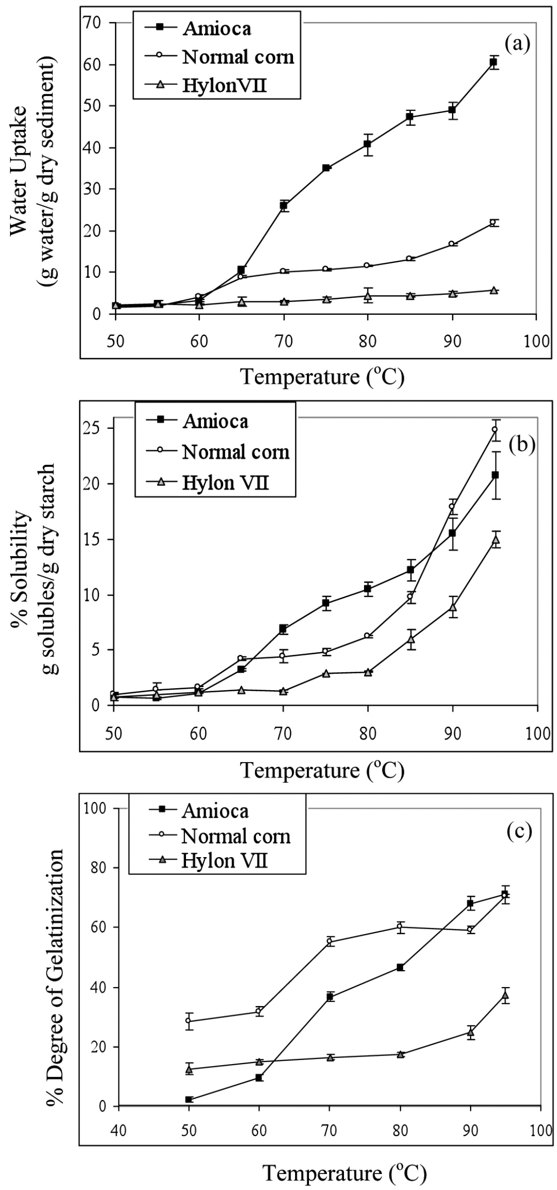

Figure 2. Effect of amylose content on (a) water uptake, (b) % solubility and (c) % degree of gelatinization of corn starches. (Mean values ± standard deviations are represented by bars)

% Degree of Gelatinization (%DG)

The % degree of gelatinization in the temperature range of 50-95 °C was followed by the spectrophotometric method based on the formation of amylose-iodine complex. In fact, %DG based on measurement of absorption peak at 600 nm can be taken as the ratio of solubilized amylose sites to the total amylose sites available in raw starch.

Taking this point into account, a comparison was made between the curves in Fig 1c. The % degree of solubilized amylose sites to the total in the raw starch was higher for corn starch, followed by Amioca. Although this ratio for Hylon VII was low at lower temperatures, it increased after 80 °C. This corresponded to the point where the increase in %S of Hylon VII started on the solubility curve (Figure 2b).

If the amylose was the only soluble portion in starch, similar behaviors should be observed in %S and %DG curves. However, when the curves in Figure 2b and c were compared, high %S of Amioca (nearly 20% of Amioca at 95 °C) indicated that mainly the amylopectin forms the soluble portion, since it only contains 1-2% amylose. Moreover no blue color but red coloration with iodine must be considered as an indication of amylopectin in soluble portion. Using %S and %DG data at 95 °C and taking amylose contents of starches into account, the percentage of amylose in the soluble portion has been calculated. The results obtained showed that the soluble portion of Amioca contained 6.8% amylose by weight. The same value for normal corn starch was 68.3% and the soluble portion of Hylon VII nearly contained only amylose. This was also evident from the gels obtained. For example, Amioca containing mainly amylopectin produced highly gelatinous dispersions when cooked and formed soft gels. In contrast, high amylose gels (normal corn starch and Hylon VII) were firm because amylose contributes gel strength and firmness and results in a tighter network.

Gelatinization Studied by DSC

Gelatinization of starch has been recognized as a first-order irreversible reaction by the following scheme (25).

$$\text{Ungelatinized starch} + \text{water} \xrightarrow{heat} \text{gelatinized starch.}$$

Basic requirements for gelatinization are the ratio of the reactants and also the heat of reaction. Using different amounts of water per unit weight of starch, gelatinization experiments were carried out to determine the adequate amount of water so that water was not the limiting reactant in the reaction. The amount of water was determined to be 75% for Amioca and normal corn starch (Figure 3). At this composition, the peaks became narrower and did not show any baseline shift with symmetrical endotherms, especially in the case of Amioca. Therefore the heat uptake below the baseline represents only the enthalpy of the first-order crystalline melting transition (Figure 3). Table 1 shows that this value is 15.7 J/g dry starch for Amioca and 12.5 J/g dry starch for normal corn starch. Nearly the same values were found as 15.4 J/g dry starch for waxy corn starch and 12.3 J/g dry starch for normal corn starch by Jane *et al.* (26). Also in the same table are included the gelatinization temperatures (onset, T_0; peak, T_p and completion, T_c). The WU, %S and %DG curves indicated that the temperature of 65 °C appeared to be the critical temperature for all the thermal events leading to gelatinization. Moreover this point corresponded to the onset gelatinization temperatures of Amioca and normal corn starch (Table 1).

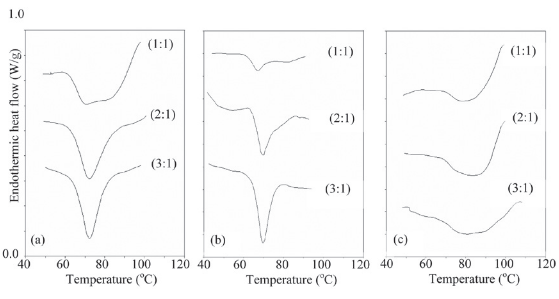

Figure 3. DSC thermograms of (a) Amioca, (b) normal corn and (c) Hylon VII starches at various moisture contents. Water: starch ratios are shown in brackets.

Table 1. Gelatinization properties of corn starches

Sample	T_0 (°C)	T_p (°C)	T_c (°C)	ΔH_g (J/g dry starch)
Amioca	64.6±1.1[a]	72.1±0.4[b]	80.5±0.7[b]	15.7±0.8[b]
Normal corn	64.6±0.2[a]	69.4±0.2[c]	74.5±0.3[c]	12.5±0.2[c]
Hylon VII	67.2±0.5[a]	88.4±0.1[a]	104.8±0.1[a]	18.1±0.1[a]

Water:starch= 3:1; All values shown are means ± standard deviations. Data with the same letter within a column are not statistically different at a (*P*<0.05) level.

A completely different behavior was observed in the case of Hylon VII. At all water ratios Hylon VII gave a single endotherm observed at a very broad temperature range (Figure 3c). Also among the starches, Hylon VII had significantly higher T_o, T_p and T_c. This behavior may be attributed to B-type crystalline form of amylopectin in high amylose starches, resulting in higher gelatinization temperatures. In addition the hydrogen bonding between the chains in high amylose structures results in highly internal associations (27). Due to this chemical hindrance, water uptake of Hylon VII was limited (Figure 2a and 3c). This means plasticization of Hylon VII by water is not adequate, as a result of which higher temperatures than the measured were required for complete gelatinization.

Mass Transfer Processes During Gelatinization

Initially, the hydration of starch granules involves the adsorption of water into amorphous regions of starch. During gelatinization, the starch granules swell several times its initial size, ruptures and simultaneously amylose leaches out from inside the granule. X-ray studies show that a three dimensional network is mainly formed by the leached out amylose. It is stated that likely the polymer existing in water compartments are mainly amylopectin (2;28). Therefore from the point of view of mass transfer, both the diffusion of water (WU) into granule and leaching of soluble polysaccharides (%S) from the matrix outcome as important processes leading and controlling gelatinization.

Although all the experimental conditions were the same, each starch exhibited different swelling and solubility behaviors. At this point, their structural differences should be considered because the physical structure of the starchy material plays an important role in the transport processes taking place within the sample. The first step in swelling is the penetration of water into the granule, which might be accompanied by swelling. It has been suggested that amylose plays a role in restricting initial swelling because this form of swelling proceeds more rapidly after amylose has first been exuded. Therefore swelling may be considered to occur as a result of two simultaneous processes, namely the diffusion of water into granule and leaching of amylose from it (1).

According to the classical diffusion theory, diffusion rate of water into a solid depends mainly on its structural properties and also on temperature. Voids and pores in solids facilitate the diffusion of penetrate into the matrix. That the water uptake depends on the exudation of amylose from the polymer matrix can be explained by the formation of voids for the water to diffuse. Therefore the leaching of amylose from the polymer matrix becomes one of the controlling steps in the water uptake of the granules. For example, the highest water uptake exhibited by Amioca was a good example for this situation, which was attributed to its low amylose content leached out at the initial stages. As a result, water diffused relatively easily to its granule.

Hylon VII exhibited a completely different behaviour. Its water uptake remained nearly the same over the temperature range of 50-95 ^{o}C, but its percent solubility was affected by temperature increase that may be observed after 80 ^{o}C. One of the factors influencing the extent of swelling and leaching of solubles is known to be the presence of amylose-lipid complex (22, 24). Amylose forms an inclusion complex with lipids. V-type X-ray diffraction pattern in Figure 1 indicated that Hylon VII contained crystalline amylose-lipid complexes. Additionally, DSC studies also confirmed its presence by the melting endotherm of crystalline amylose-lipid complex in the temperature range of 104 ^{o}C and 125 ^{o}C (Data not shown). Thus the presence of endogenous lipids in starch may have an adverse effect on the swelling of individual granules by repelling the water. In addition, highly internal associations between chains via hydrogen bonding do not allow the penetration of water in Hylon VII. Therefore the polymer can not take up large quantities of water and do not swell (22).

Progressive leaching of amylose changes the microstructure of the polymer through the formation of pores via which the solubles are released, leading to an increase in soluble fraction. In fact, the leaching of soluble polysaccharides from the granule is far more complex than swelling because it depends on many processes such as swelling, the dissolution and diffusion of solubles. The amylose-lipid complexes are insoluble in water and require higher temperatures to dissociate. The dissociation of the complex was not found to be possible over the experimental temperature range studied, because the onset temperature of dissociation is 104 ^{o}C (Data not shown). Therefore it was concluded that the amylose involved in complex formation with lipids was prevented from leaching out. That the dissolution of amylose into water becomes the controlling step in the overall leaching process affected both the water uptake and solubility of Hylon VII in turn.

WU and %S can be used to assess the interactions between the starch chains, within the amorphous and crystalline domains of the starch granule (2). Hylon VII, with the least swelling, had comparable %S values at high temperatures with the other starches. This situation brings to mind that solubility of Hylon VII was limited on the surface of the granule, and a kind of surface gelatinization occurred by using the scarce amount of water that has penetrated to a limited extend into the granule. The broad endotherm observed and no effect of water on the peak in Figure 3c may be attributed to this phenomena. Despite the high and unrestricted swelling of Amioca, its %S values were not significantly different from the other starches tested. This indicates that diffusion rate of water into granules for Amioca was higher than solubilization rate (Figure 2a and b). It could be said that, on the contrary of Hylon VII, hydration occurred throughout the entire granule for Amioca.

For corn starch, a two-stage gelatinization curve was observed, similar to its water uptake and % solubility curves. This behavior indicates that neither swelling nor solubilization but both should be taken into account as effective factors on its gelatinization. Furthermore, higher %DG observed may be attributed to its high free amylose content.

CONCLUSIONS

The following conclusions can be derived from this study:

The highest water uptake was exhibited by Amioca and the least was exhibited by Hylon VII. High swelling of Amioca was attributed to its low amylose content and more open structure and as a result of which water diffuses relatively easily to its granule. Low swelling level of Hylon VII was explained by the presence of endogenous lipids in its granules and also by its high internally associated structure.

Since gelatinization is a diffusion controlled reaction, both the swelling and solubility outcome as important parameters among the factors affecting the extent of gelatinization. In gelatinization of normal corn starch, both processes seemed to be effective, while in the case of Hylon VII the solubilization of starch controlled the process. Another important point to be noted is that free amylose rather than the total in starch affected the gelatinization.

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