CALORIMETRIC STUDY OF THE NATIVE AND POSTDENATURED STRUCTURES IN STARCHES WITH DIFFERENT DEGREE OF HYDRATION

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DSC studies of melting process of annealed native structures and postdenatured ones in low-amylose starches with different degrees of hydration were carried out. The starch recrystallization at different thermal treatments of the samples was studied both after the complete and partial destroy of native structures. It has been shown that native structures as well as postdenatured ones possess the ability to perfection, which is most clearly seen at the annealing at temperatures inside their melting ranges. The results obtained demonstrate that at the same duration of annealing the process of crystal perfection for secondary starch structures proceed more intensively compared to the native ones. The presence of the remained native structures in partial melt in contrast to the remained gel ones restricts the ability of the recrystallized structures to perfection.

Keywords: ability to perfection, annealing, DSC, recrystallized and native structures, starches

Introduction

Starch is a unique biopolymer, close to synthetic polymers both on relative simplicity of chemical structure and on the character of supermolecular structures. In this connection the study of the melting and formation processes of starch native and postdenatured structures is mostly based on the fundamental concepts of melting and recrystallization in partly crystalline synthetic polymers. It is generally accepted that the melting temperature of polymer crystallites indicates the degree of the crystalline perfection. The decrease of the crystalline perfection is caused both by local and/or structural defects and by very small sizes of the crystallites [1, 2]. As the polymer crystallites are metastable systems [3] it is possible that with a time span or under the heating the crystallite perfection processes will take place. They can lead to the decrease of contain of defects or increase of crystallite sizes, both processes causing the increase of the melting temperature [1, 2].

Up to now it is known that the native starch structures, in contrast to other biopolymers, under their prolonged annealing at temperatures slightly below the melting range, as well as within the melting range, are able to the improvement of the quality of crystallites [4–9]. How this process is displayed in calorimetric experiments with the used standard scanning rates is the question which stays still under discussion.

In the present paper we report the results of the DSC studies both of melting process of annealed native granules and of recrystallization and thermal destruction of postdenatured structures for low-amylose starches with different degrees of hydration. The starch recrystallization at different thermal treatments of the samples was studied both after the complete and partial destroy of native structures. It was also interesting to study the ability of postdenatured starch structures to the improvement of their quality and to compare with native crystallites under similar thermal conditions.

These data on evolution of the starch melting curves, in turn, were compared with the corresponding results for other biological and synthetic polymers as well. As a whole, the results obtained have allowed us to get a deeper insight in the nature of the processes accompanying the melting process of native and non-native ordered starch structures. For the discussion in the present work we have used both our new studies in this field and some results published earlier.

Experimental

We have studied the potato starch from Aldrich (USA), and rice starch 'Lazurnyi', and also potato amylopectin starch received from the RAS Institute for Biochemical Physics. The content of amylose in potato and rice starches, according to Morrison, was equal to 27.6 and 13.3%, respectively. In potato amylopectin starch

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it was not more than 1%. Water content in starch–water systems was mainly changed from 40 to 75%.

All studies were performed using the Setaram DSC-111 differential scanning microcalorimeter with sensitivity of $3 \cdot 10^{-5}$ J s^{-1}. Temperature was controlled with a precision of ± 0.2°C. The error in values of melting heat for the wide melting curves was not more than 5%. The used scanning rates were varied from 0.2 to 5°C min^{-1} according to the particular task. The details of the experiments one can read in our other paper in [10].

Results and discussion

The results of our DSC studies of the melting of native potato [11] and rice starches, and potato amylopectin starch with different water content are in a good agreement with those of other researchers for the concentration ranges where the published data are available [12–15]. For all studied starches on passing from the samples with water excess to ones with intermediate humidity the shape of the melting curves transforms from singlet into the doublet. Inspite of the classical character of such transformation its nature is still under discussion. The main question is either the doublet character of the melting curves results from kinetic [14] or thermodynamic [16–18] factors.

As it is mentioned above it can be accepted that the melting curves of ordered starch structures as well as in the case of partly crystalline synthetic polymers reflect the distribution of crystallites on the melting temperatures T_m, that is on the quality of crystallites and/or their sizes.

DSC study of the ability of the native starch structures to the perfection

For this purpose primarily the data on evolution of the melting curves of the native starch with 50% water content, which are doublet, were obtained at the variation of sample heating rate [11]. The temperature positions of the both maxima of the doublet native starch melting curve practically do not depend on the used heating rates which is in contrast to the cases of both biological [20] and synthetic polymers [1–3]. These results are in good agreement with the results of other investigations [14, 20]. For biopolymers a decrease of the melting temperature at a decrease of the heating rate has, in our opinion, the entropy origin and can be attributed to the disordering of the polymer chains in the melt forming after the denaturation. Such conclusion has been confirmed by the study of the thermostability of postdenatured structures forming in proteins. It has been shown that the slower is the heating, the stronger a structural memory is obliterated in the

melt [21]. While for synthetic polymers the increase of the melting temperature with the lowering of the heating rate is caused by processes of structural improvement of the initial crystallites. Thus, the comparison with biological and synthetic polymers allows to suppose that independence of the melting curves in the case of starches on the used heating rates is a result of superposition of the processes of the crystalline structure improving and gradual loss of a structural memory in the melt at a decrease of heating rate which, to our delight, are nearly compensated.

Annealing of native starch structures inside their melting temperature range

The dependence of Q_m and T_m of the remnants of the native starch structures for samples annealed at fixed temperature inside the melting range on the annealing duration was examined. It was found that in this case for all starches studied with the increase of the annealing time, for example from 3 min to 5.5 h, the melting curves of remnants were shifted to higher temperatures and became narrower (Fig. 1A). At the same time, the melting heat Q_m, at least, did not increase. Thus, the annealing improved the crystalline ordering of non-molten native structures but did not increase their amount. However, it should be noted that up to now the available in literature data on behavior of Q_m at the starch annealing are contradictory. The character of the T_m changes of the native remnants, found in our study, agrees with results of other studies [22]. Annealing prolonged well over 10 min resulted in shifting melting curves of annealed native
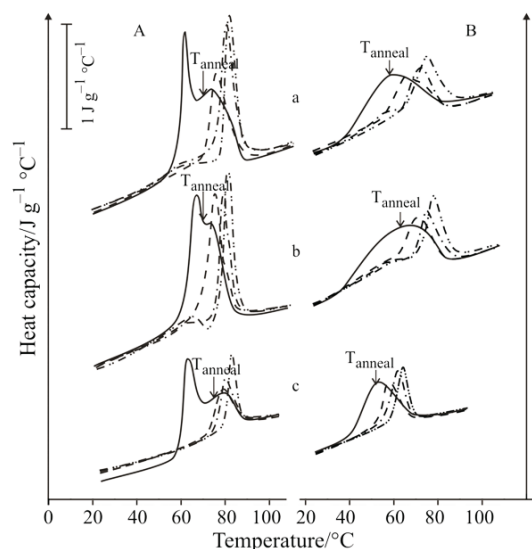

Fig. 1 The annealing of the A – native and B – non-native ordered starch structures during the different time: —— 0, --- 3, —·—·— 80 min, —··—··— 5.5 h. a – potato starch, b – potato amylopectin starch, c – rice starch. C_{H_2O}=50%. V_h=2°C min^{-1}

structures of starch beyond the range of melting temperature of initial native structures. Thus, the thermodynamic behavior of annealed native structures of low-amylose starches was similar to that of crystallites in partly-crystalline synthetic polymers [1, 2] but it essentially differed from the results for some other biopolymers. As it is known in small globular proteins and in fibrillar protein collagen the annealing at temperature within the denaturation range only decreased amount of the ordered structures [20, 23]. In other words, in proteins in contrast to starches no improvement of the crystalline quality was observed.

Formation and melting processes of ordered non-native starch structures

As it is well known after complete thermal destruction of native starch structures (gelatinization) the recrystallization process (retrogradation) takes place. The thermal properties of ordered secondary starch structures formed from the melt are strongly dependent on conditions of their heat treatment and storage.

The recrystallization in amorphous phase after the complete destruction of native structures in three chosen low-amylose starches was studied. The secondary ordered structures were formed in water–starch systems with water content, which was varied from 75 to 40%. Figure 2 represents the results on the change of the melting curves of the non-native ordered structures as storage time of samples at T_{room} was extended for all studied starches with humidity ~50%, as an example.

It has been shown, that ordered secondary starch structures were formed by crystallites of lower quality compared to native structures and, as a rule, they had a broader distribution of T_m at all water concentrations under study. It should be noted that all ordered non-native structures in starch were completely restored when conditions for their formation were reproduced. It resembled behaviour of the melting structures of semi-crystalline synthetic polymers [2, 3] and distinguished them from the irreversible melting of native starch structures.

It was established that the character of changes of the melting curves depending on storage duration of gelatinized samples for potato amylopectin and rice starches as well as for potato starch studied before [24] was practically the same at different water content. For all starches the data obtained have shown that the growth rate of non-native ordered structures at small storage times (~1 h) in water–starch systems with intermediate humidities is faster than in systems with water excess. The immediate reheating after the gelatinization of starch samples with water content ~50% and lower always leads to appearance a small additional endotherm in the corresponding temperature range on the DSC curves in contrast to systems with water excess. It means that after the melting of native starches at intermediate humidities the structural memory on the mutual orientation of A-chains of amylopectin persists [24].

Figure 2 shows that during retrogradation of the studied gelatinized starches with water content ~50% at T_{room} the essential change of distribution of crystallites over their quality took place. After storage for a few days the recrystallized starch structures were always formed by crystallites of lower quality. The similar character of the T_m dependence on the storage time of starch was also observed in the other studies of retrogradation [25]. Further after prolonged storage of starches at T_{room}, over 1 month since their gelation, the melting curves shifted to the higher temperatures and/or became narrower. This proves the ability of ordered non-native starch structures to the perfection for all studied starches similarly as it takes place in partly-crystalline synthetic polymers at long time of crystallization [1, 2].
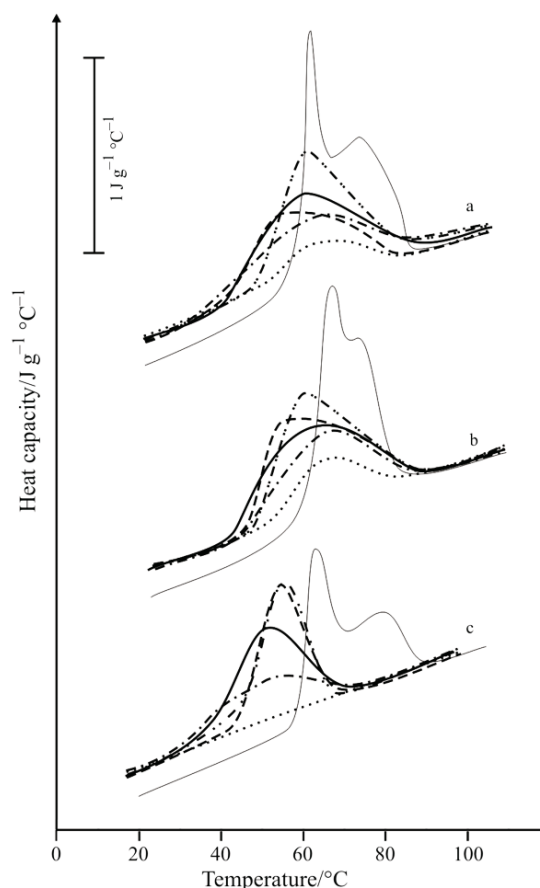

Fig. 2 Retrogradation of different starches at the storage (T_{room}=15°C) during the different period: the heating of native starch (— with double peaks), rescan ⋯ – just after gelatinization, –·–·– – 1 day, — — – 7 days, --- – 1 month, –··–··– – 3 months. a – potato starch, b – potato amylopectin starch and c – rice starch. C_{H_2O}=50%. V_h=2°C min^{-1}

Our experiment has shown that the starch gels with different water content (75–40%) formed on prolonged storage time had the melting temperatures close to each other. Moreover, the same results were obtained in a substantially wider interval of water concentration (75–26%) at which gels were prepared. The given concentration was afforded either by water addition or drying the samples. The data obtained led to a conclusion that water concentration did not influence the quality of forming gel. However, it is not correct.

Here it is important to emphasize that in the experiment discussed above we, as well as in the available literature dedicated to starch retrogradation, compared the gel structures grown and molten under the same concentration conditions. This led to that the systems at intermediate humidities differed simultaneously by formation and melting conditions. Figure 3 represents, besides the results of such 'traditional' calorimetric study of starch gels (curve 1), the results on T_m of gels grown at the same (50%) but molten at different water concentrations (curve 2) and shows the considerable difference between them. Note, that the latter dependence is similar to that for the initial native starch crystallites (curve 3) [12, 26].

The comparison of the curves 1 and 2 in the Fig. 3 allowed to evaluate the influence of varying water content on the thermal properties of starch ordered secondary structures. One can see that the gel formed at 50% water content and then dried up to 30% of water had T_m above that of gel initially formed and molten at the same (30%) concentration. While the T_m of the 50% gel molten in the presence of 73% of water had T_m lower than T_m of gel formed directly under conditions of excess of water. These results unequivocally pointed to better organization of the secondary structures formed after gelatinization in excessive water than that formed at intermediate and low humidities. Thus, at the comparison of thermal properties of starch gels it is very important to know and differ the concentration conditions at which they were formed and then those molten.
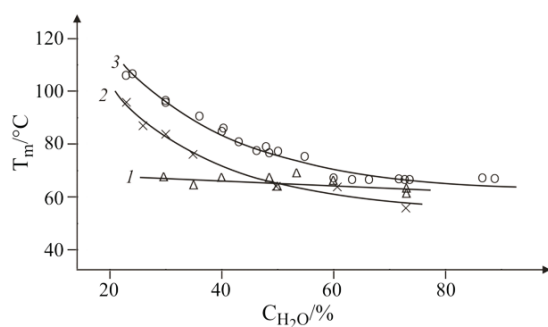
The study of the recrystallized structures formed in amorphous phase after the partial destroy of native and non-native starch structures

First, we shall discuss the results on the formation and melting of secondary structures in the presence of the remaining annealed native crystallites. In this case after the storage at T_{room} of the annealed native samples for all studied starches the melting curves attain the bimodal character. The narrow high-temperature maximum in these curves corresponds to the melting of the remnants of annealed native structures, whereas the broad low-temperature maximum corresponds to the melting of secondary structures recrystallized in the partial melt during the storage. It has been found that at variation of the heating rate, the duration and temperature of the storage the formed gel structures and the remained native structures behave as two independent systems which do not transform one into the other (Fig. 4). At the change of the heating rate from 0.5 up to 5°C min^{-1} the temperature shift ΔT between the corresponding maxima remains to constants (Fig. 4a). It is seen from Fig. 4b that at the initial stages of storage at T_{room} the amount of secondary structures (the value of Q_m) increases on the expense of the low quality crystallites which is evidenced by the lowering of T_m whereas Q_m and T_m of the native structures do not change. It has been also found that the annealing of the secondary structures, formed from the partial melt, at temperatures close to the beginning of the melting range (35°C) brings up the shift of the low temperature maximum to higher tem-
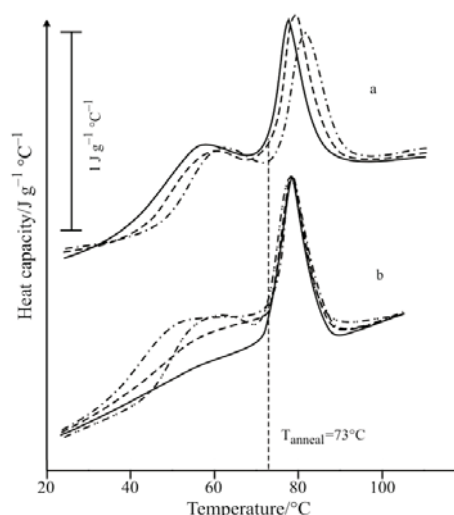

Fig. 4 a – The influence of the heating rate on the bimodal curves for potato starch. — – 0.5, --- 2 and –·–·– – 5°C min^{-1}. b – The influence of storage duration at T_{room}=15°C on the thermal properties of the annealed potato starch sample: — – 0 min, --- 1 day, 7 days, –·–·– – 1 day storage with additional annealing at T_{anneal}=35°C during 4 h. T_{anneal}= 73°C. C_{H_2O}=50%. V_h=2°C min^{-1}

Fig. 3 Concentration dependence of the T_m for native potato starch and its gels aged during 7 days at T_{room}. 1 – gels formed and molten at the same humidity, 2 – gel formed at 50% of water, 3 – initial native starch

peratures and narrows this maximum which evidences the improvement of quality of ordered gel structures. At the same time such treatment has no effect on the melting of remnants of native starch structures (Fig. 4b). It should be emphasized that in these experiments the maximum achievable T_m of secondary structures is limited by the presence of remnants of native structures. Besides, in this case even at the substantial increase of storage time the melting curve preserves the doublet structure which also indicates that the native structures which are present after the annealing in the melt restrict the ability of the forming gel structures to the improvement of their quality.

Second, we shall discuss the results on the annealing of postdenatured structures of starch at temperatures inside their melting interval. It has been found that, as in the case of annealed native structures, their ability to the structural improvement for all studied starches at such annealing manifests most clearly. The increase of t_{anneal} at the fixed temperature does not change the melting heat Q_m, as it is a case for the native structures annealed in similar conditions, for all studied starches. The melting curves become narrower and shift to the higher temperatures (Fig. 1B). Note, in these experiments we have tried to approach the conditions of annealing for secondary structures (its duration) to those for native structures discussed above. It has been found that the similar duration of annealing inside the melting range leads to considerably higher increase of the T_m for the secondary structures than that for the annealed native ones despite the fact that in all cases the T_{anneal} for the former were lower than those for the later. In order to minimize the possible errors in the determination of T_m for annealed samples at the scanning stop we compared the values of T_m, which were obtained after their annealing at different duration, with the T_m at the minimum time of annealing (3 min) guaranteeing the achievement of thermoequilibrium in the sample. Figure 5 demonstrates that for all studied low-amylose starches at such annealing the rate of the improving of crystalline quality for the secondary ordered structures is much higher than that for the native ones.

It should be noted that there exists one more difference between the native and secondary structures annealed inside their melting ranges. As the experiment showed the presence of the annealed secondary starch structures in the partial melt, in contrast to the annealed native ones, does not hinder the formation in the future of the gel identical to the parent one, which proves once more the reversibility of the melting process of starch gels.

The comparison of the ability to the perfection of recrystallized and native structures subjected to similar thermal treatment and storage conditions allowed
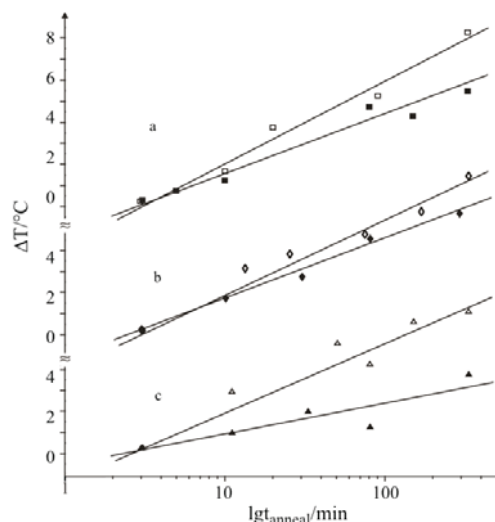

Fig. 5 The shift of the melting temperature $\Delta T = T_m - T_{m/3\ min}$ for the 1 – annealed native and 2 – annealed gelled samples dependent on the time of annealing. a – the potato starch, b – the potato amylopectin starch and c – the rice starch

us to assume that the structural transformations, leading to the improvement of quality of the ordered structures, resulted not only from the decrease of the number of defects on the interface between the crystalline and amorphous phases, as it is accepted in the majority of available studies [27, 28]. It is natural to assume, that the structural imperfections can be located on all crystallite surfaces (both face and side) as well as inside the crystallite. In later case both the structure of double helixes and its packing into the crystal lattice can be distorted. The role of every kind of such defects in native and secondary structures will be different. It can be supposed that in native starch the spatial arrangement of the axis of amylopectin molecules which form a part of crystallites, being the basic elements of crystalline lamellar structure of starch [29, 30], is correlated. As a result, the decrease of the surface energy of native starch crystallites due to the fusion of even relatively small regions of the side surfaces, without the change of the width of crystallite inside the crystalline lamella, will cause an increase of their T_m. At the same time such process is practically impossible in gels due to the non-correlated orientation of the axis of amylopectin molecules after starch gelatinization. However, the decrease of the amount of defects in the secondary starch crystallites which results in the increase of their T_m can be expected due to that an efficient convolution of double helixes and a packing of them into the crystallites at the annealing in gel structures occurs much better than in native ones.

Conclusions

As a whole, the results obtained have allowed us to understand deeper the nature of the processes accompanying the melting both of native and non-native ordered starch structures. For all low-amylose starches studied the native structures as well as postdenatured ones possess the ability to perfection, which is most clearly seen at the annealing at temperatures inside their melting ranges. It has been established that for all starches the rate of the improvement of crystal quality for such annealed secondary structures is much higher than that for the native ones at similar thermal treatment and storage conditions. There exists the essential difference between the native and secondary structures annealed at temperatures inside the melting ranges in their influence on the recrystallization process in the partial melt. The presence of the annealed gel structures in the melt does not hinder the formation in the future of the gel identical to the parent one. In contrary, the presence of the remnants of native starch structures in the melt restricts the ability of the recrystallized structures to the perfection.

The comparison of the behavior of recrystallized and native starch structures subjected to similar thermal treatment and storage conditions has allowed to assume that the structural transformations, leading to the improvement of the crystal quality, which is expressed in the increase of their T_m, are connected not only with the change of the crystallite surface but also with the change inside the crystallite, in particular, with the efficient convolution of double helixes and a much better packing of them into the crystallites.

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