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Transformation of Water Clusters in Wet Starch under Changing Environmental Conditions

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Abstract—Transformation of the water cluster distribution in wet potato starch (with a water content of 27 to 45%) at temperatures that ranged from –50 to +80°C was studied by differential scanning calorimetry. A significant difference was observed between the transformations in the temperature ranges below and above 0°C. Both cooling and heating at $T < 0$°C enabled a reorganization of the initial size distribution of water clusters characteristic for room temperature. These changes could lead to an increase of the average cluster size during both crystallization and melting. The transformation intensity depended on the water content and scanning rate and differed between the native and amorphous states of starch. In this case, the cluster-size distribution remained unimodal. However, heating of wet native starch to temperatures close to the point of transition into the amorphous state (75–80°C) induced a bimodal distribution due to the emergence of large water clusters; thus, the heterogeneity of the water distribution within the native granules increased.

Keywords: calorimetry, starch, water clusters, crystallization, melting, size distribution

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This work is a continuation of calorimetric studies of the thermal properties of freezable water (FW) dispersed in wet starches. Some of these studies were reported earlier [1, 2]. These reports also provided a description of the distinctive architectural features of native starch granules based on the concepts presented in [3, 4]. The present study was focused on the distinctive features of granule structure related to the heterogeneity of the water distribution in granules [5, 6]. An uneven distribution of water in the granule volume has been established. Alternating zones with different density termed “growth rings” are found in granules that vary in size from 1 to 100 μm, with the size depending on the origin of the starch. The width of these zones is less than one micrometer. The amorphous phase is the predominant component of rings with a lower density, whereas the crystalline phase predominates in the denser rings. The size of each individual crystallite is approximately 10 nm. The content of crystallohydrate water (that is, unfreezable or bound water) in the nanocrystallites is fixed rigidly for each crystal lattice type that occurs in natural starches (types A and B) [7]. The water content in the amorphous phase of the native granule can vary in a broad range, but remains lower than 50% for any starch type. This part of water is freezable if the starch moisture content exceeds 23–24%, that is, supercooling below the T_m leads to water crystallization [4]. Studies [8–15] showed that this water is highly dispersed and occurs in nanometer-size clusters with melting parameters that are dependent on cluster size.

The main objective of the present work was to perform a more detailed investigation of water cluster transformation in wet starch under the influence of changing environmental conditions. Changes of cluster size upon a change in component concentrations in a starch–water system were discussed earlier [1]. This problem is mainly related to the change in the boundary between freezable and unfreezable water in both starches and other biopolymers [1, 16, 17]. The transformation of the water cluster distribution in starch samples of varying humidity upon temperature changes both below and above 0°C relative to the distribution characteristic of starch at room temperature is considered in the present study. Moreover, the process of attainment of the equilibrium water concentration after various thermal impacts on both native and amorphous starch is discussed. Considerable attention is paid to the reversibility (or irreversibility) of all processes being discussed.

Research on water cluster transformation in a biopolymer matrix is of great significance for both fundamental and applied research. The results of such studies are of the greatest importance for understanding

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Abbreviations: FW, freezable water; DSC, differential scanning calorimetry.

the physical processes that accompany freezing or thawing of starch-containing substances. The results can be used to select specific conditions that ensure the preservation of various biopolymers and biological tissue viability after storage at low temperatures. The study of FW crystallization and melting can also contribute to the clarification of the cause of the deterioration of consumer qualities of starch-containing foods, cosmetics, and pharmaceuticals exposed to increasing temperatures upon storage at temperatures both lower and higher than 0°C.

METHODS

The experimental procedure was described in detail in the previous studies [1, 2] devoted to the manifestation of size effects during dispersed water melting and crystallization in native and amorphous starches with varying hydration degrees. The studies were performed by differential scanning calorimetry (DSC) in a DSC111 device (SETARAM Instrumentation, France). The measurements were carried out both in the heating mode at temperatures that ranged from –50 to 110°C (narrower temperature subranges within this range were used for specific tasks) and in the cooling mode at temperatures that ranged from 25 to –50°C. The rate of sample heating and cooling was usually 5 degrees/min. The water concentration in the investigated “potato starch–water” system ranged from 27 to 45%.

RESULTS AND DISCUSSION

Thermograms of the processes of freezable water melting and crystallization in potato starch for various water concentrations are shown in Fig. 1. First and foremost, these data clearly demonstrate the existence of hysteresis between FW melting and crystallization in both native and amorphous starch. The values of the temperature and heat of these processes derived from the results of thermogram processing were published earlier [1, 2], and the complex pattern of the dependence of these parameters on water content in starch was reported. Size effects were characteristic of FW melting and crystallization heats in both native and amorphous starch, whereas a size effect on the temperatures of these processes was observed only in the case of native starch. The dependence of water cluster melting and crystallization temperatures in amorphous starch followed a different pattern.

At the same time, experimental demonstration of the differences between melting and crystallization parameters of water clusters in both states of wet starch supported cluster rearrangement upon heating and cooling. In other words, there was a change in the distributions of water clusters that are characteristic of the native and amorphous states of starch at room temperature. (Importantly, all samples were incubated at room temperature for 24 h after preparation, so that moisture distribution in the bulk starch and within the granules could reach an equilibrium).

Analysis of the shape of FW melting and crystallization curves recorded in starches with different moisture content received considerable attention in the present work. Importantly, we believe that the shape of FW melting curves derived from DSC of starch samples is reflective of the size distribution of water clusters, although scanning conditions affect curve shape [1, 2]. Similar concepts underlie the analysis of melting curves of other crystalline nanostructures, such as nanocrystallites of partially crystalline synthetic polymers [18–20], crystalline structures of starch proper [21, 22], and ordered secondary structures of biopolymers [23, 24]. However, it is worth mentioning that our attempts to transform the shape of the FW melting curve in native starch by incubating the sample at varying temperatures near the transition range or within this range yielded negative results, whereas experiments of this kind are known to induce significant changes in the melting curves of polymer and biopolymer crystalline structures [18–24].

Therefore, it is completely natural to address the phenomenon reflected by the curve of FW crystallization obtained by DSC of a biopolymer. The direct connection between crystallization temperature and water cluster size that is usually observed in the case of melting processes [1, 2, 25, 26] cannot be traced in this case. The intensity of supercooled liquid crystallization is reportedly determined by two kinetic factors: the nucleation rate and the crystal growth rate, or, more precisely, by the ratio of the rates of these processes and the degree of overlap of the processes on the temperature axis at a given cooling rate [27]. However, we believe that it is entirely possible to trace the relative changes in cluster size using the data on the changes of the FW crystallization temperature and heat of crystallization as dependent on the FW content in the crystal and the thermal impact.

The data we obtained indicate the possibility of the transformation of the initial water cluster distribution in starch with a fixed moisture content upon both heating and cooling. The intensity of a possible transformation depends on a number of factors, such as the water concentration in the starch, heating and cooling rates, and the temperature of starch incubation in a temperature range that corresponds to the native state of the biopolymer.

Water Cluster Transformation in Native and Amorphous Starches at Temperatures Below 0°C

Comparison of FW melting and crystallization temperatures in starches with varying moisture content. As emphasized above, comparison of FW melting and crystallization curves in potato starch with a fixed moisture level revealed dissimilar arrangements of the curves in the heating and cooling thermograms in the

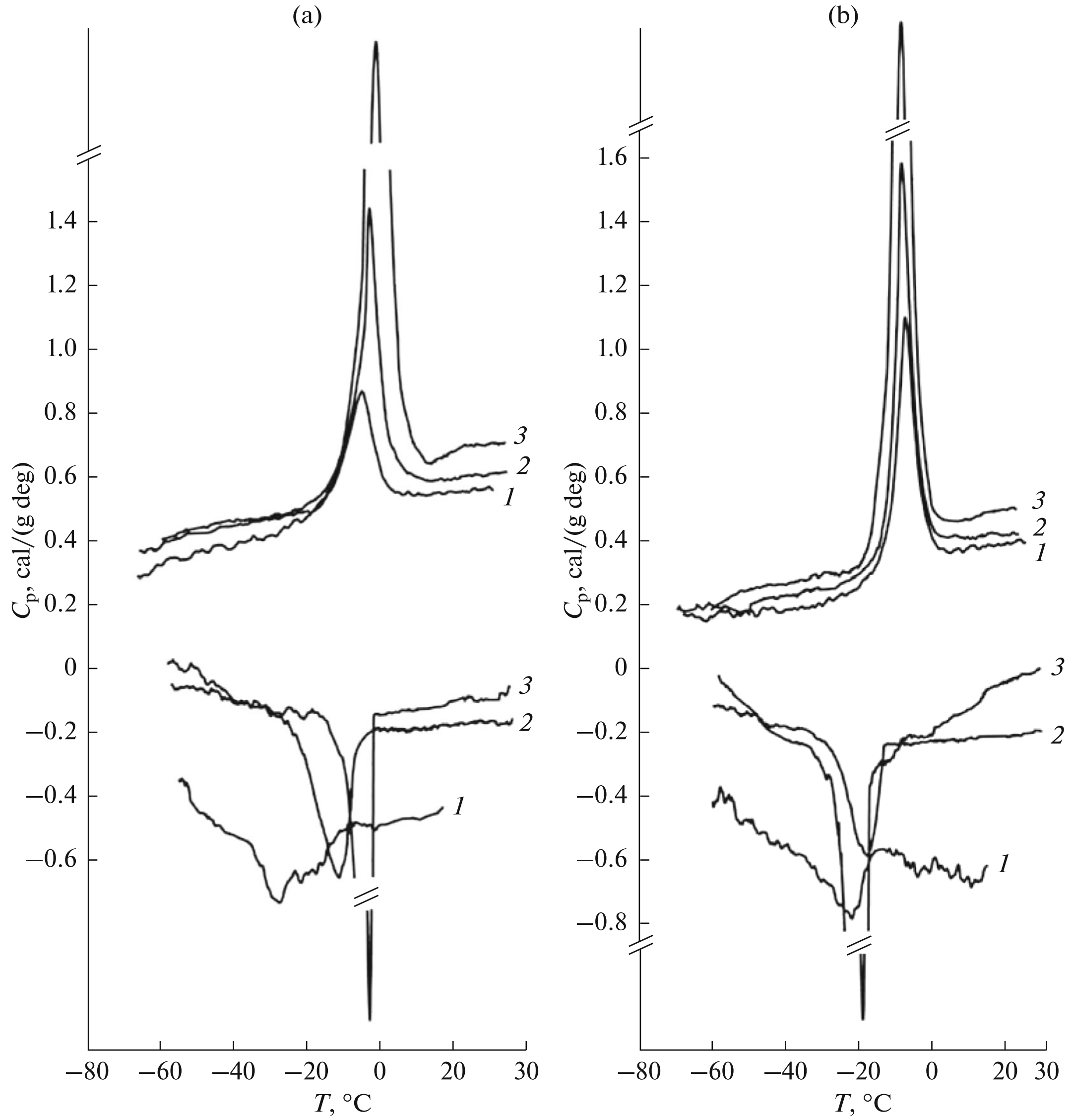

Fig. 1. Heating/cooling thermograms in the FW melting/crystallization range for native (a) and amorphous (b) potato starch samples at different water concentrations (%): (*1*) 30; (*2*) 35; (*3*) 45; $V_{\text{heat}} = V_{\text{cool}} = 5$ degrees/min.

entire range of examined water concentrations (Fig. 1).

Let us first discuss *the native state*. The temperature at which the FW melting process started (T_0) remained practically unchanged (−20°C) as the degree of starch hydration increased from 27 to 45%. Assessment of the size of clusters with this melting point based on the well-known Gibbs–Thomson equation yielded a value on the order of 1 nm, whereas a size of approximately 40 nm was predicted for FW clusters that had a T_m of −1°C. Let us also mention that the estimate is affected by the value of the heat of melting, and larger values are obtained for water cluster sizes if the actual heat of FW melting in starch (obtained in experiments) is taken into account.

As shown in Fig. 1a, FW melting curves for starch samples with a lower moisture content are located inside the melting curves of the samples with a higher moisture content. The half-width of the melting curves ($\Delta T_m/2$) does not change very much with increasing water concentration, decreasing from 9 to 6°C.

The minimal moisture level that allowed the relatively reliable detection of the exothermic crystallization maximum at the scanning rate we used was 29%, in contrast to the results of FW melting in potato starch [2]. The final temperature of FW crystallization during cooling shifted from −40 to −20°C as the hydration degree of the starch increased from 30 to 45%, and the temperature T_{cr} of the maximum at the FW crystallization curve changed from −30 to −2°C.

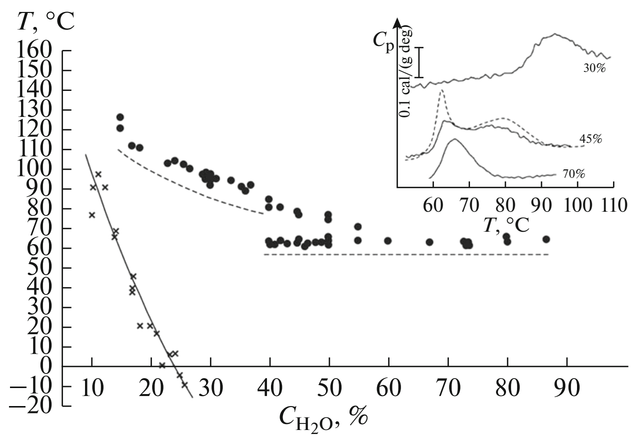

Fig. 2. The dependence of amorphization and vitrification temperatures (circles [24] and crosses [31], respectively) on the content of moisture in potato starch. The dashed line corresponds to the temperature at the beginning of the amorphization process (T_0). The inset shows the thermograms of heating of native potato starch samples with different moisture contents. The solid line corresponds to V_{heat} = 5 deg/min, and the dashed line, to V_{heat} = 1 deg/min.

As a result, there was almost no overlap between FW crystallization curves for starch samples with a moisture content of 30 and 45%, in contrast to the melting curves (Fig. 1a). Moreover, our data show that the half-width of FW crystallization curves ($\Delta T_{cr}/2$) decreased sharply in the above-mentioned range of starch humidity values. The decrease was five- to sixfold, from 20 to 3–4 °C. The results we obtained show that the crystallization process is faster in starch samples with a higher FW content. Moreover, these results suggest that significant changes in the initial size distribution of water clusters characteristic of the samples at room temperature occur upon cooling and crystallization in samples with a high moisture content (45%). The presence of the smallest clusters was clearly evident from the curves recorded at a moisture content of 30%, but not from the thermograms of samples with a moisture content of 45%. Joining of small clusters to larger clusters apparently occurred in this case, and water redistribution within starch granules ensued. Nanoclusters with a heat of crystallization of the order of 2.5 cal/g were fully assimilated, whereas those with a heat of crystallization of the order of 20 cal/g were assimilated only partially. This process appears to be natural, since the decrease of the surface energy of water clusters is thermodynamically favorable. These data are also indicative of the high mobility of the smallest supercooled water clusters at temperatures ranging from –40 to –20°C.

The amorphous state of starch. The difference between FW melting and crystallization curves dependent on the moisture content was only in the case of native starch. As follows from Fig. 1b, the melting curves of small water clusters in amorphous starch with a moisture content ranging from 27 to 45% were located within the melting curve of large clusters, similarly to the curves for native starch. However, FW crystallization in amorphous starch occurred in the same temperature range independent of moisture content (Fig. 1b) and thus differed significantly from crystallization in the native starch. As indicated in [2], the latter phenomenon is naturally related to the properties of the biopolymer matrix itself. Various hydrated biopolymers (collagen [28, 29], globular proteins and DNA [30, 31] and starches [31–33]) are known to assume a vitreous state after thermal disruption of the native structure. The vitrification temperature of amorphous starch depends greatly on the moisture content, similarly to that of denatured biopolymers, and lies below 0°C as far as the concentration range addressed in the present study is concerned [31] (Fig. 2). The dependence of the amorphization temperature (nanocrystallite T_m) on the degree of starch hydration [32] is shown in the same figure (and discussed below). Thus, the amorphous biopolymer matrix is in a highly elastic state in the temperature range we investigated and the molecular mobility of the polymer chains and the starch–water system as a whole is higher in the case of amorphous starch than in the case of native starch. This leads to changes in the conditions for FW crystallization in amorphous starch.

Thus, the conditions for the recording of FW crystallization used in the present study enabled the detection of the increase of water cluster size upon the cooling of native starch granules in the temperature range below 0°C.

Comparison of the heats of FW melting and crystallization in native and amorphous starch samples. An earlier comparison of the observed values of the heat of water cluster melting and crystallization per unit sample mass showed that Q_m was always higher than Q_{cr} for FW in native and amorphous potato starch [2]. The values of the heats of all the processes under consideration are listed in Table 1 for a discussion of the relative changes in the values. The values were inferred from the smoothed $Q(C_{H_2O})$ curves based on experimental data from [2]. These values (normalized to the total sample weight) will be used below and referred to as “the heat of FW melting” and “the heat of FW crystallization” in starch.

As evident from Table 1, the difference $Q_m^N - Q_{cr}^N$ is 1–2 cal/g for native starch and $Q_m^D - Q_{cr}^D$ = 3–5 cal/g for amorphous starch. It is important to understand that Q_m refers to the total amount of the melted FW independent of the heating conditions, and Q_{cr} refers to the amount of water that “managed” to crystallize under the specific experimental conditions. Table 1 also shows that Q_m^D, the heat of FW melting in amorphous starch, is always higher than that for native starch ($Q_{\mathrm{m}}^{\mathrm{N}}$) at the same moisture content. Importantly, the $Q_{\mathrm{m}}^{\mathrm{D}}$ values shown were obtained upon FW melting immediately after the transition of starch into the amorphous state. The $Q_{\mathrm{m}}^{\mathrm{D}}$ values decreased significantly after 10 to 20 h [1, 2].

Finally, the heat values of the transitions we investigated could be arranged in the following order for every humidity value under the experimental conditions used: $Q_{\mathrm{m}}^{\mathrm{D}} > Q_{\mathrm{m}}^{\mathrm{N}} > Q_{\mathrm{cr}}^{\mathrm{N}} > Q_{\mathrm{cr}}^{\mathrm{D}}$. In this case, the difference between the values decreases as the moisture content in starch increases and the absolute values of heat increase.

Thus, the data presented above demonstrate that the heat of melting increases as a result of heating of a water cluster set that crystallizes upon cooling. Different processes that may lead to this result can be considered. First, the crystallization of supercooled water clusters that did not have sufficient time to crystallize at the cooling rate used can be the cause of the increase of Q_{m}. Secondly, an increase in Q_{m} can occur due to a further increase in the size of crystallite nuclei formed during the cooling process. Finally, small cluster recrystallization during heating can lead to an increase in Q_{m}. Heating may evoke the melting of the smallest clusters that are crystallized first upon cooling: that is, these clusters are transformed into supercooled water clusters and subsequently fused to larger (frozen) clusters. It appears impossible to separate these components of the process that lead to additional crystallization of the clusters in the system under investigation. However, we were able to record this process in experiments. The comparison of heating thermograms for native and amorphous samples with a moisture content of 37% revealed an appreciable additional heat release in the amorphous sample (relatively to the native sample) prior to the beginning of FW melting, with this being indicative of an additional crystallization process (Fig. 3). Moreover, the heat of FW melting increased and the T_{m} of FW was slightly shifted towards higher temperatures; this was indicative of the increase of the number of larger clusters.

Let us recall that an increase in the size of the initial crystallites during the melting process was observed in many studies of synthetic polymers [19, 20], including a study in which one of the authors of the present work was involved [18]. The melting curves observed in the experiments were shown to have a doublet structure if the rates of heating and rearrangement in the crystallite system were comparable. The shape of the doublet depended on the heating rate. However, the observed value of the heat of fusion remained constant (independent of the heating rate). Let us note that Q_{m} dependence on crystallite size in synthetic polymers was not observed in calorimetric studies or reported by any researcher. The heat of melting of minimal-size water clusters in the starch–water system investigated was at least two times less than the Q_{m} of pure water, as shown in earlier studies [1, 2, 21]. This is the reason that the increase of the average cluster size and the subsequent increase of FW Q_{m} upon the heating of starch were so distinct.

Thus, our study ultimately showed that both cooling and heating in the temperature range below 0°C could lead to an increase in the size of water clusters presented in the starch sample at room temperature; this indicated the sufficiently high motility of nano-

Table 1. The values of heat of all the processes. Smoothed $Q(C_{\mathrm{H_2O}})$ curves based on the experimental data from [2] were used to calculate the values

$C_{\mathrm{H_2O}}$, %	$Q_{\mathrm{m}}^{\mathrm{N}}$, cal/g	$Q_{\mathrm{cr}}^{\mathrm{N}}$, cal/g	$Q_{\mathrm{m}}^{\mathrm{D}}$, cal/g	$Q_{\mathrm{cr}}^{\mathrm{D}}$, cal/g
30	3.6	2.5	5.5	2.5
32	4.0	2.7	5.8	2.5
34	5.2	3.7	6.8	2.9
36	7.1	5.3	8.7	4.0
38	9.5	7.7	11.0	6.0
40	12.4	10.8	13.5	8.8
42	15.4	14.3	16.0	12.2
44	18.8	17.8	18.8	15.6

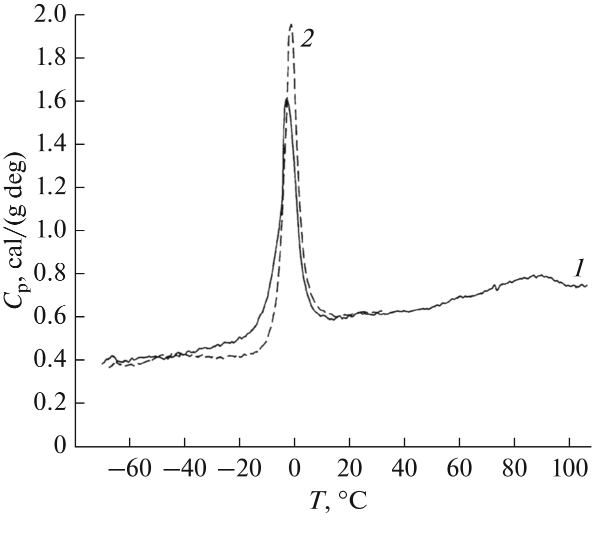

Fig. 3. Heating thermograms for native (*1*) and amorphous (*2*) potato starch samples with $C_{\mathrm{H_2O}} = 37\%$. $V_{\mathrm{heat}} = 5$ deg/min.

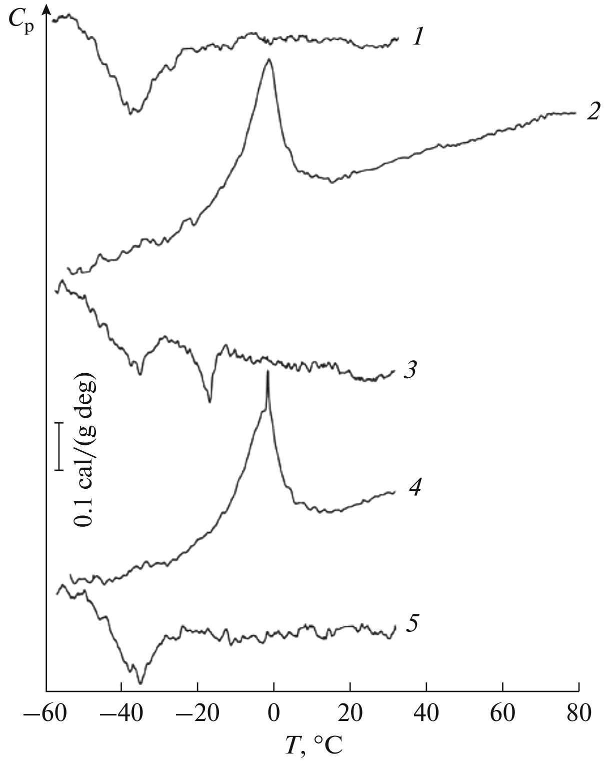

Fig. 4. Direct and reverse scanning thermograms in the melting/crystallization temperature range for a native potato starch sample subjected to different types of thermal pretreatment: (*1*) cooling of the original sample; (*2*) heating of the original sample to $T = 85°C$; (*3*) cooling immediately after heating to a high temperature; (*4*) heating of a preheated sample; (*5*) cooling of the sample after 1 day of storage at room temperature (20°C); $C_{H_2O} = 30\%$, $V_{heat} = V_{cool} = 5$ deg/min.

sized water clusters in the system under investigation at $T < 0°C$. One can assume that the cluster-enlargement process can be prevented if the cooling rate is much higher (for instance, 100 degrees/min) than the cluster consolidation rate ([34] and references therein). The process of water cluster enlargement can reportedly lead to damage in biological tissues. However, it is very difficult to thaw tissue at a rate this high; therefore, cluster consolidation and biological tissue rupture are almost inevitable. The search for approaches to minimize such consequences is an important task for medicine and certain branches of industry.

Deterioration of the consumer qualities of foodstuffs (including starch-containing ones) due to freezing has long been known [33] and the substantial role of the increase of water cluster size (related to thawing and freezing) in the process of food deterioration has been demonstrated reliably. Optical spectroscopy studies of cyclic thawing and freezing processes allowed a convincing demonstration of the increase in water cluster size in moist starch-containing foodstuffs at the micrometer level [35]. It is very difficult to separate these processes that exert strong effects on wet biopolymer properties. However, we believe that the present study provided a sufficiently clear demonstration of the role of water cluster redistribution in each of these processes at the nanoscale level.

Water cluster transformation in native and amorphous starch samples at temperatures above 0°C. The transformation of water clusters discussed above occurred upon heating and cooling at temperatures lower than 0°C. Let us now use the data on the changes of the shape of FW melting and crystallization curves in starch and on the possible effect of this process on the shape of the melting curves of the nanocrystallites of starch proper to discuss water cluster transformation upon heating of the samples at temperatures above 0°C.

The effect of heat treatment on the curves of FW melting and crystallization in starch. The experiments demonstrated the preservation of the unimodal character of the curves in native starch samples with different moisture content, regardless of all the differences between FW melting and crystallization curves. However, unimodality was perturbed if the native samples were heated to temperatures close to the point at which the melting of starch nanocrystallites (transition to the amorphous state) began. The dependence of the temperature of amorphization onset on the moisture content in the starch is shown in Fig. 2. The changes in the shape of FW melting and crystallization curves in a native sample with a moisture content of 30% heated to 85°C are shown in Fig. 4. A high-temperature component apparently emerges on the FW melting curve (curve *4*), whereas the Q_m value increases slightly. The bimodal character of the FW melting curve manifested in this case is clearly evident from a recording of FW crystallization (curve *3*). Therefore, one can conclude that heating of native starch samples within the temperature range of existence of native starch induces an increase of the heterogeneity of FW distribution in the granule over the native level. (Let us recall that the change of the average water cluster size upon the transition of starch into the amorphous state depends on the freezable water content in the sample. The average cluster size is larger than in native starch at a lower water content and smaller at a higher water content [1, 2]; this is determined by the respective increase of the transition temperature).

The bimodal character of the crystallization curves discussed above is most clearly manifested in the case of starch samples with a low content of freezable water (see Fig. 4, $C_{H_2O} = 30\%$). Earlier studies demonstrated a rather abrupt shift of the crystallization curves toward high temperatures upon an increase of FW content in the starch and a concomitant decrease of the distance between the two peaks of FW crystallization that corresponded to different cluster sizes. However, the bimodal character of the curves that emerged after heating of native starch with a higher moisture content (35%) to a temperature of the beginning of the transition into the amorphous state was still preserved, as evident from Fig. 5.

One should emphasize that the new heterogeneity that arises after the heating of native starch is reversible. Recovery of the initial size distribution of the water clusters was observed (compare curves *1* and *5* in Fig. 4), albeit after a relatively long period of time (approximately 10 to 20 h). As follows from these data, the attainment of water concentration equilibrium in a system of native starch and water takes much longer if large clusters with a T_m close to 0°C occur in the system (relative to the attainment of equilibrium in a system with small clusters).

The effect of water cluster transformation in starch with different moisture levels on the amorphization curve shape. The data on the change of the water cluster distribution in the native granules heated above room temperature are, in our opinion, of considerable interest for the understanding of the process of gelatinization (transition into the amorphous state) of starch proper. In spite of the existence of hundreds of studies that have addressed this process, the information on the role of water in the process is insufficient. Let us focus on one of the questions that arise upon the calorimetric studies of starch–water systems. Additional heat absorption is known to occur as these systems are heated above 60°C; X-ray diffraction analysis showed that this phenomenon leads to the disruption of native nanocrystalline structures in starch. Therefore, the endothermal DSC curve that corresponds to this process is often termed the melting curve. Let us note that there are considerable differences between the processes that follow the disruption of the native structure in starch samples with low and high moisture levels [3, 4].

The transformation of these melting curves upon the change of water concentration in the water–starch system is well documented. Many DSC studies, including those that we performed, have reported this effect [4, 15, 32, 33, 36–41]. Singlet endotherms were observed in systems that contained excess water (more than 60%), a doublet was observed at intermediate humidity levels (60–35%), and this doublet was converted into a singlet again as the moisture content decreased further. At the same time, the melting curves of native starch were shifted toward high temperatures as the moisture content decreased (see the inset in Fig. 2).

It is worthwhile to mention the two hypotheses (models) that are discussed most frequently in the numerous studies devoted to finding an explanation of these changes in melting curve shapes for starch nanocrystallites. Researchers naturally propose that the doublet structure of starch melting curves is related to the existence of crystallites with dissimilar properties. The first model emphasizes the differences in the hydrate environment of the crystallites [5, 36–38], whereas the second model focuses on the heterogeneity of the nanocrystallites proper [39–41].

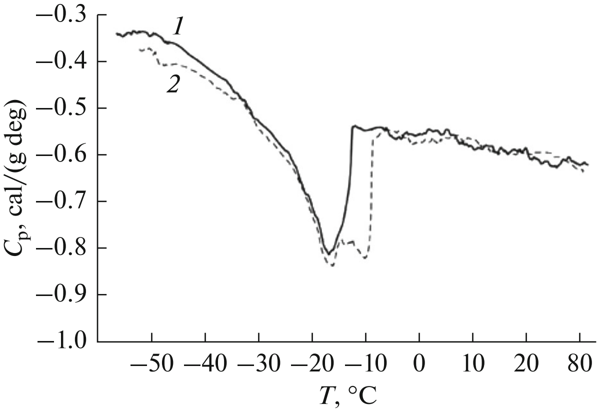

Fig. 5. The effect of heating of the native sample to T = 80°C on the shape of FW crystallization curve in potato starch: (*1*) cooling of the original sample; (*2*) cooling of the sample immediately after heating to T = 80°C; C_{H_2O} = 35%, V_{cool} = 5 deg/min.

The first model states that the localization of the aqueous environment of the crystallites is different. This does not imply a difference in the content of water of crystallization in the crystallites proper. This value is fixed in the investigated humidity range, although it varies between the different types of starch crystal lattices [7]. The water content in the amorphous phase that immediately adjoins the crystallites is a key factor in this model. As mentioned above, the amorphous phase of native starch granules is heterogeneous both at the nanoscale and on the microscale. The first model implies that the low-temperature peak of the doublet melting curve reflects melting of crystallites surrounded by an amorphous phase with a higher degree of hydration, whereas the high-temperature peak reflects the melting of crystallites with a lower degree of moisture [5, 36–38].

The second group of models is based on the concept of structural heterogeneity of starch crystallites proper associated with variation in the abundance of crystal lattice defects. It is natural to make such an assumption as the melting curves of starch recorded at different heating rates are compared [32] (see, for example, the inset for 45% in Fig. 2). The least-stable crystals (with the highest abundance of lattice defects) are believed to melt first and the amorphous phase formed from the disordered polysaccharide chains is believed to adsorb additional water. Consequently, a certain deficit of water emerges in the range of water concentrations under consideration here (27–45%), in contrast to the water excess discussed above. A temperature increase is required for the melting of the remaining (more thermostable) crystallites, since the total amount of water is fixed. The authors who support this model state that this requirement accounts for the emergence of the second peak in the endothermic doublet structure [39–41].

Notably, an unambiguous experiment that would demonstrate the superiority of one of the models has not been performed as yet.

The results presented in this study show that the heterogeneity in the size distribution of water clusters increases over the initial level as the starch samples are heated. The new distribution of water clusters could be observed if the scanning was stopped at temperatures close to the beginning of starch amorphization, the samples were cooled, and crystallization was monitored (Fig. 4). Additional larger clusters emerged as the starch granules were heated within the temperature range that corresponded to the existence of native starch. These clusters were not numerous, but the total surface area of the water clusters decreased as larger clusters emerged. Therefore, one can assume that the area of contact between the crystallites and the hydrated amorphous phase decreased as well, and that this contributed to the increase in the deficit of water required for crystallite melting.

In general, the data presented above demonstrate the essential role of the hydrate environment, which becomes more heterogeneous as the nanocrystallites are heated to T_m. However, the putative contribution of the crystallite system proper to the transformation of these melting curves should not be disregarded.

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