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Dynamics of molecular clusters in biomatrix and starch of wheat grain during the ripening process

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ABSTRACT

Gravitation mass spectroscopy was used to investigate how the long-range order (LRO) in biomatrix and starch of wheat grain (kind B, North Germany) change during ripening. A strong change of the molecular cluster (domain) ensembles in the biomatrix was concluded to take place at the time of fast grain growth. The changing dynamics of the water cluster ensembles containing from 12 to 1889 molecules in a young biomatrix was given. The micellar structure in the biomatrix, in which the polysaccharide synthesis proceeds, shall be modelled. The molecular mass cluster distributions in different starch types of a different grain ripe degree were analyzed. LRO of molecular cluster ensembles up to 3.2 million Dalton and up to 4 billion Dalton was concluded to be differently for different starch types. The dynamics of the reverse cluster destruction in the temperature interval from 298 to 523 K was shown. The melting enthalpies for the simplest starch clusters, which build micellar and super-micellar structures in LRO were calculated. Starch types were found to be highly sensitive against heating (selective influence on LRO) and mechanical (up to 516 Pa) influences. Depending on the wheat ripening stage the starch types are different in the polymerization degree in the amylopectin branches furthermore, they are different at the LRO level in granula.

KEYWORDS

Long-range order;
Clusters;
Biomatrix;
Grain;
Water;
Structure;
Starch;
Pressure.

INTRODUCTION

If we understand how water LRO in biomatrices and starch nanostructures in grain shall be formed then it should be possible to get an idea about the mechanism of the wheat ripening process and then to develop a plan how the process can be steered and further to look for a possible starch synthesis. At this time there is no information concerning the dynamics of the structure change in the grain biomatrix and starch during the wheat ripening process.

Structure differences in starch of similar wheat types were previously investigated by calorimetry[1] where the thermic characteristics of melting of single starch molecular fragments were determined and their structures given. These structures are little different from the classic suggestion of Banks and Mair[2].

Amylopectin granula are assumed to be in the crystal

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state however, XRD patterns aren't distinctive and show a high amorphous halo, that is an indication for a low structured polymer[3,4]. On the other side from the physical chemistry of polymers it is known that branched polymers don't crystallize or only badly particularly then if it is a polymer with very hard chains which in addition has branches to which belong polysaccharides. Crystal structures of these polymers are characterized by many defects where the branches are thrown out from the crystal lattice[5].

It is well known that the fold conformation of macromolecule fragments in branches is thermodynamically more favorable than the unfold one. The macromolecules' folding and the folding of their branches leads to a cluster structure formation[6], which is determined by gravitation fields, molecular clusters (domains) shall be built at the expense of energy clusters in physical vacuum[7]. The last one is caused by the heterogeneity of the physical vacuum at stationary gravitation wave level[8]. Additionally, as it was shown earlier by the authors, molecular clusters in biomatrices and starch interact actively with gravitation radiation from celestial bodies[9] therefore, this fact shouldn't be ignored.

The aim of the present work was to understand the formation process of LRO in biomatrices and starch of wheat grain during the ripening.

MATERIALS AND METHODS

The wheat type B growing in North Germany (Mecklenburg/Vorpommern) was used as investigation object where grains were collected from the lower ear part (Figure 1, 20 pieces). The sensor of the Zubow gravitation mass spectrometer (GMSZ, earlier called as flicker noise spectroscopy[15] was placed directly inside the grain and LRO scanning in biomatrix was carried out according to the method described in[8,9]. Remember, that the dividing of clusters in collapsed and expanded forms, was interpreted using the energy state of cluster interaction with surroundings. Starch was obtained from 20 wheat ears. First the grains were ground in distilled water, then the suspension was squeezed out, and the liquid part was taken for sedimentation where the precipitate of the second decantation was washed with distilled water. The obtained white powder was dried at 298 K on air and finally over silicagel. The grain samples were collected from 20 random ears every second day (afternoon) on the very same agricultural area. LRO analysis was recorded not later than 15 minutes after sampling. The air temperature (T, K) in the agricultural area at sampling time from July 11th to August 22nd, 2010 was described by the approximation $T = 10^{-5}t^4 - 1.5454t^3 + 93584t^2 - 3\cdot10^9t + 3\cdot10^{13}$, where t – calendar data from July 11th to August 22nd, 2010. The summer in 2010 was characterized by high temperatures up to 310 K (July 11th) and dryness. The thermal properties of starch were analyzed using sample heating under air-free atmosphere (6 K/min) and the pressure influence was recorded at 298 K.

RESULTS AND DISCUSSION

The base characteristics for the development of the wheat grain biomatrix are shown in Figure 1. In the area of grain growth, there are two events in LRO, which leads to a decreased cluster kinds' number (marked with horizontal bracket). Let's discuss these events in detail (Figure 2).

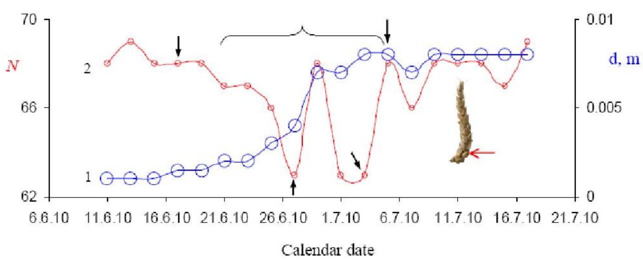

Figure 1. Change of the maximal wheat grain size (curve 1, d, meter) and number of cluster kinds in grain biomatrix (curve 2, *N*) during the ripening process. Diapason of cluster ensemble masses up to 3.2 million Dalton. Active events of grain growth are described by horizontal bracket. The water content in biomatrix after July 17th, 2010 was lower than 50 wt. %. The grain to be analyzed is marked with a horizontal bracket.

As shown in Figure 2 the GMSZ spectra differ of each other strongly. While the spectrum of June 17th, 2010 shows a distribution of collapsed water clusters, sub-micellar and micellar structures[6,9] (-*f*) that are typically for a stable biomatrix, the other spectra cannot be characterized in such a way. The grain size enlargement was therefore concluded to influence LRO in biomatrix, which at this time highly changed and whose clusters mainly consisted of expanded structures

(+ *f*). After forced ripening and grain size stabilization because of strong weather dryness at July 5th, 2010, LRO in bimatrix approached LRO of June 17th, 2010

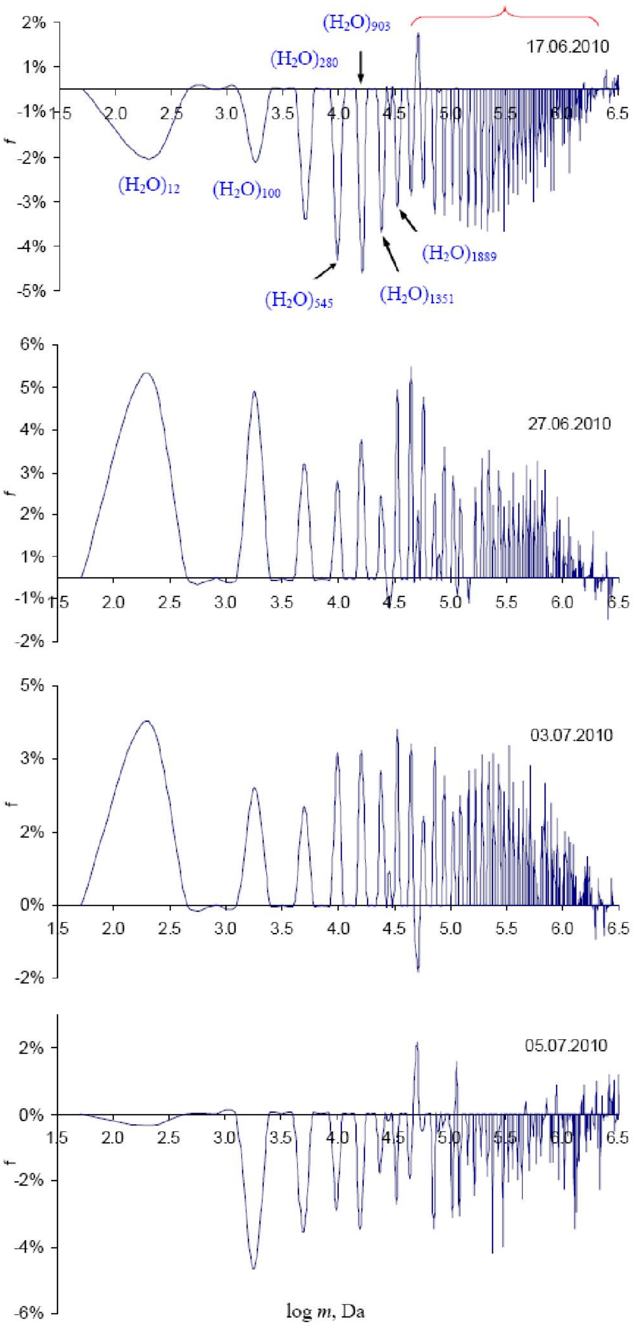

Figure 2. GMSZ spectra of wheat grain biomatrix at the time that is marked by arrows in Figure 1.
Diapason of cluster ensemble masses up to 3.2 million Dalton. Weak shock waves. The Zubow constant amounts to 6.4·10^{-15} N/m. The interval of clusters, sub-micellar and micellar structures built by polysaccharides and proteins are marked by a horizontal bracket.

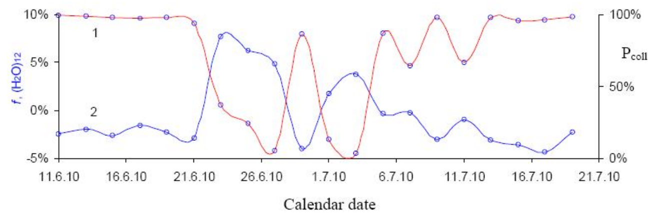

Figure 3. The rate of collapsed clusters (1, P_{coll}) and signal intensities (2, *f*) of the base water cluster in wheat grain biomatrix during ripening.

and matched it at July 13th, 2010 almost.

To understand the processes in the biomatrix during the ripening period the data shown in Figure 3 shall be discussed. At intensive grain growth (Figure 1) a strong decrease of the density of all clusters in an ensemble including the base water cluster was observed. $(Í_2Î)_{12}$[10] gets a positive value *f*, being an indication for its forced expansion.

As visible from Figure 3 there are two events at June 23rd and June 27th, 2010 which are seen as one large overlapped signal. These events were identified by some water clusters too. They were assumed to concern not only the base water cluster but the complete water cluster ensemble in biomatrix. These two earlier events are the beginning of LRO changing in biomatrix as well as the start of the ripening.

To get a first idea on the mechanism of processes proceeding in biomatrices the results from Figure 4 shall be discussed. As shown the average molecular cluster mass $(Ì_{GMS}=\Sigma(|f|\cdot m))$ achieved its highest value at June 23rd, 2010 (filled point) which permits to distinguish earlier events by using the mechanism. Because the cluster masses remained unchanged at this time the rise of M_{GMS} has to be ascribed to an increased absolute sum $\Sigma(|f|)$. This effect could be explained by a reinforced interaction of the clusters with their surroundings only[8]. The average cluster mass remains constant even then when the part of collapsed clusters (Figure 3) and number of cluster kinds (Figure 1) change strongly as it was the case on June 27th, 2010 and July 3rd, 2010. The events of this time should be connected less with the surroundings' interaction but rather have been caused by some decreasing of cluster kinds' number (Figure 1).

Thus, the hierarchy of the events taking place in LRO of biomatrix could be represented as follows: strengthening of clusters' interaction with surroundings, destruction of some cluster kinds, rearrangement of clusters in ensemble and recovery of some earlier destroyed clusters. The processes in LRO of the growing grain biomatrix were considered from the position of micelle structure in which the role of surfactants (SAS) was played by simple proteins and lipids (Figure 5).

In the first event (Figure 4, I) in biomatrix, the cluster kinds' numbers and the part of collapsed clusters decreased while the interaction of clusters with their surroundings rose. Here the water clusters were

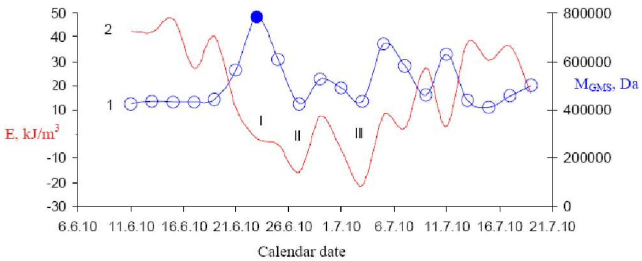

Figure 4. Average cluster molecular mass (1, M_{GMS}) and energy (2, E) of the cluster ensemble (up to 3.2 million Dalton) in biomatrix. The device calibration was carried out according to the evaporation energy of water (44.1 kJ/mol). (Ì$_{GMS}$=Σ(|*f*|·*m*)), where Σ is the mathematical symbol for sum of all clusters (from N = 1 up to N in this investigated ensemble), *m* – cluster mass in Dalton.

assumed to be destroyed under the influence of SAS which led to the formation of the simplest sub-micellar structures[6] in the grain seed that then coagulated to a micellar structure at June 27th, 2010 (event II, Figure 4). At this time the *N* value declined a little (Figure 1, marked with arrow)[11] whereas the cluster interaction with surroundings remained unchanged.

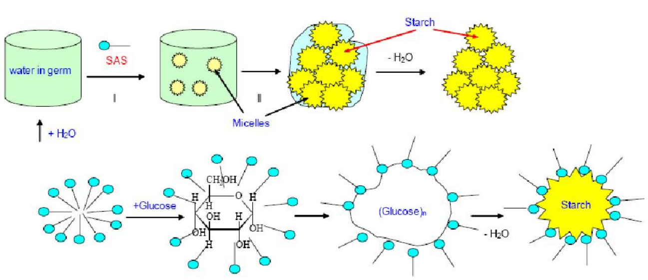

Figure 5. Models for the formation mechanism of micellar structures in wheat grain biomatrix during ripening and for starch synthesis.

in sub-micellar structures which were then, depending on their filling degree with glucose, transformed to micellar and later to super micellar structures. Condensation of glucose into ordered micelles led to starch formation (granula) where proteins, lipids, SAS and salts were concentrated on the micelles' surface; from what later a shell between granula forms.

To understand structural changes in starch during wheat ripening, LRO of polysaccharides was investigated under heating[9] and pressure[11]. Both by heating and pressure the movement of individual clusters as well as the thermodynamic stability of all cluster ensembles as an unit can be influenced. First it shall be discussed how the integral characteristics of the cluster distribution in starch for different ripening stages

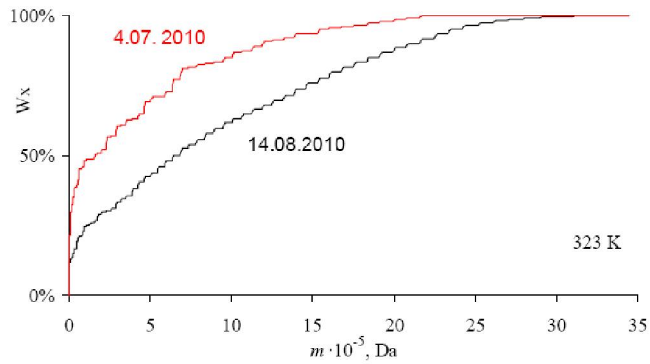

Figure 6. Integral curves for cluster fraction distributions (W_x) in starch, extracted from wheat grain at July 4th and August 14th, 2010. Heating up to 323 K. where $W_x = |f \cdot m|_i + |f \cdot m|_{i+1} + ... + |f \cdot m|_N$, *m* - cluster mass in Dalton, *i* – cluster number from 1 to *N*.

changed. The integral curves for the cluster fraction distribution (W_x) in starch extracted from wheat grain at July 4th and August 14th, 2010 appear to be identical hence supporting the cluster formation mechanism in starch according to the grape bunch principle[12]. However, there are some differences in the cluster mass ranges of 500,000 and 1,500,000 Dalton becoming clearer at starch heating (Figure 6).

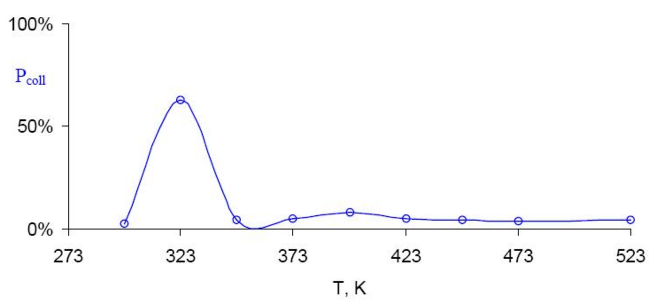

Figure 7. Part of collapsed clusters (P_{coll}, the part of signals with negative *f* value) in starch extracted from wheat grain at July 23rd, 2010.

As visible from the Figure 6 the starch cluster fractions of various ripening grades are characterized by different thermal stability. In the fully ripe grain, starch clusters were more expanded though at heating up to 323 K they were transformed to collapsed ones. The part of collapsed clusters in starch at the ripening stage at July 23rd, 2010 shall be discussed (Figure 7).

At 325 K the part of collapsed clusters in starch rises strongly and then falls fast again, however (Figure 7). After cooling down the sample from 523 to 298 K, P_{coll} agrees with P_{coll} at the beginning of the heating process. This behavior could indicate on a reversibly destroyed cluster interaction with the surroundings proceeding at melting of specific fractions of the hard polymer chains. The lower the cluster interaction with their surroundings the more dense they are, and conversely that means the interaction with surroundings causes more expanded structures. However, the GMSZ spectra aren't identical completely before and after heating, some differences are detectable with integral characteristics, only.

On the other side, unknown melting processes in starch were described at 325 K[1]. The nature of these processes isn't clear up completely yet, endothermic reconstruction processes in tertial structures of starch at LRO level could proceed at these low temperatures, however. The melting enthalpy of starch chains' fragments (2.6 kJ/mol) from Kozlov[1] used for the calibration of the GMSZ spectrometer, related to the highest point in the curve (Figure 7). Several heating effects' curves for starch at different ripening stages are shown in Figure 8. During ripening, a LRO reconstruction was observed to proceed in starch of the grain biomatrix which leads to the domination of some melting/crystallizing structures at different temperatures.

„Young" starch (curves 1 and 2) is characterized by an exothermic crystallization process (clusters' densification and ordering) at 373 K (I, curve 1), that has been shifted to 348 K (II) on July 13th, 2010. Besides, there is an endothermic effect at 425 K (III) that has to be seen only as melting of polymer chains' fragments, because up to 523 K polymer destructions weren't observed by TG/DTA[13,14]. At July 23rd, 2010 extracted

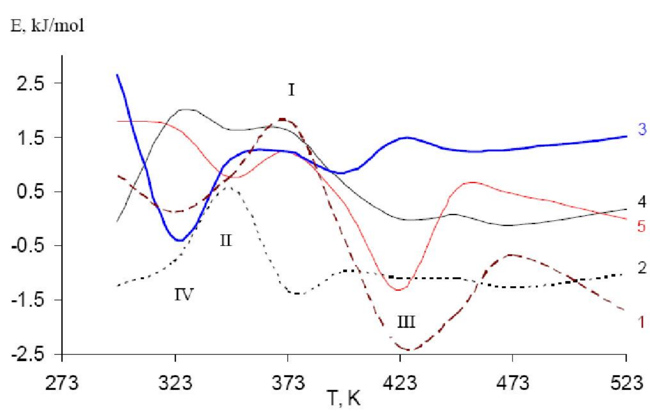

Figure 8. Thermal effects of different starch types extracted from wheat grain at different ripening stages at heating. 1 – July 4th, 2010, 2 – July 13th, 2010, 3 – July 23rd, 2010, 4 – August 4th, 2010, 5 – August 14th, 2010.

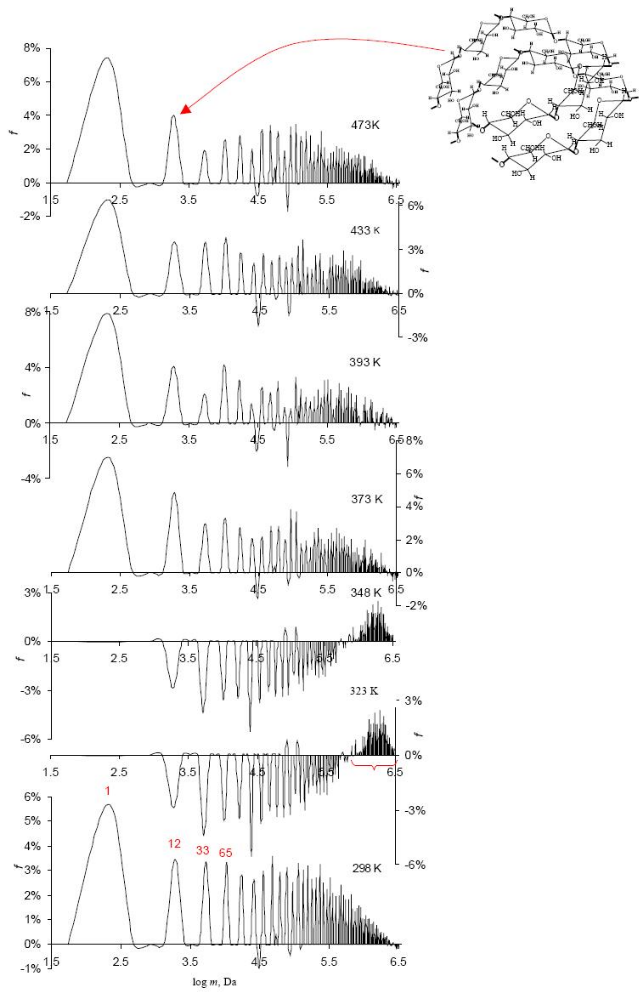

Figure 9. Change of cluster signals in starch extracted from wheat grain at July 23rd, 2010. On the right side the model of the base starch cluster (12 units' spiral of α-D-glycopyranose, 2 meanders) is shown[11,15]. Cluster ensemble up to 3.2 million Dalton, 6 K/min. Zubow constant is equal to $6.8\cdot10^{-15}$ N/m. Weak shock wave (δ < 1 N/m^2). The number of α-D-glycopyranose units in a cluster is given over the signal. Low pressured powder in air-free atmosphere.

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starch is described by one endo effect at 325 K (IV, curve 3), only. In starch from old wheat grain (August 4[th], 2010), there are only low exothermic effects of crystallization at 325 and 373 K, that were observed in starch of much older grains (curve 5) too. Though the latter shows a strong endothermic peak at 423 K ascribed to melting.

The GMSZ spectra of starch extracted from wheat grain at July 23[rd], 2010 are given in Figure 9. Already a temperature increase by 25 K leads to a radical rearrangement of LRO in the polysaccharide. The main part of the simplest clusters and some sub-micellar clusters (up to log *m* = 5.5) became free of their interaction with surroundings and got collapsed while the micellar structures (log *m* > 5.8) remained unchanged. The integral cluster parts' distribution (Figure 10) is charac-

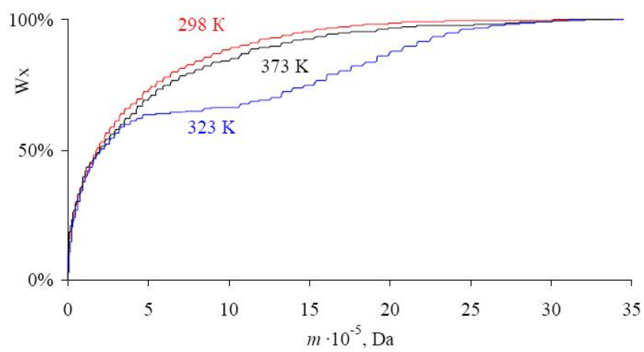

Figure 10. Integral cluster parts' distribution in starch extracted from wheat grain at July 23[rd], 2010 at 298, 323 and 373 K.

terized by domination of super micellar structures in the mass interval marked by a horizontal bracket in Figure 9, which is caused by melting of sub-micelles and micelles. The state remained unchanged up to 373 K. After that and because of an increased cluster interaction with surroundings the collapsed clusters were destroyed to expanded forms.

Sub-micellar and micellar structures were found to partially disappear at heating the polysaccharide up to 323 K (mass interval from 0.5 million to 2.5 million Dalton) while a further heating up to 373 K recovered the distribution state at 298 K. The melting of clusters seems to be accompanied with their partial destruction where some clusters should be rebuilt which is the base for the formation of new LRO at higher temperatures. The super micellar structures that are marked with a horizontal bracket in Figure 9 are characterized to be unstable too and they were destroyed to new sub-micellar and micellar structures at temperatures higher 373 K. Here the signals of α-D-glycopyranose members showed an interesting behavior (1, Figure 9) they are represented in the whole temperature range as expanded structures besides at 323 and 348 K. At these temperatures, the signals seem to be a super position of signals reflecting the units' state both in small and super molecular clusters.

In the next the GMSZ spectra of "young" starch in a cluster ensemble up to 4 billion Dalton shall be discussed (Figure 11). As visible huge clusters of defined molecular masses were cut from the mass ensemble after heating. In the base polysaccharide, at 298 K in the area of super micellar structures > lîg *m* = 6.5, the

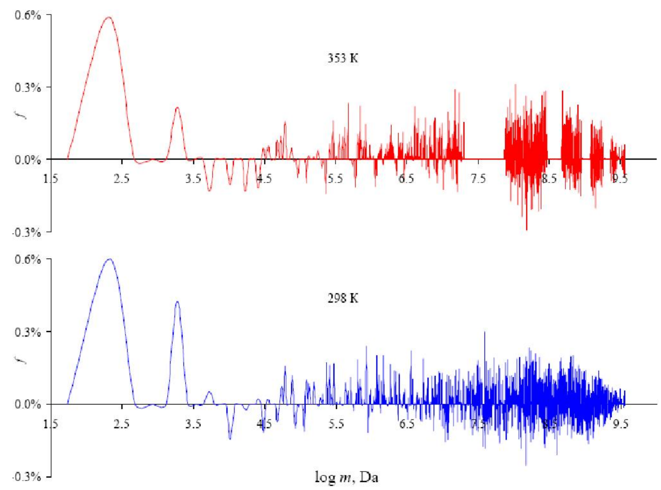

Figure 11. Change of cluster signals in „young" starch extracted from wheat grain at July 4[th], 2010.
Cluster ensemble up to 4 billion Dalton, 6 K/min. Zubow constant is equal to $6.8 \cdot 10^{-15}$ N/m. Low pressured powder in air-free atmosphere. Weak shock wave (ð < 1 N/m^2).

signals are randomly, at the first glance, indicating on poorly organized clusters expanded as well as collapsed. However, they were destroyed at heating and recovered at cooling. Here a melting process of nanostructures proceeds that has to be understood as breakup of clusters independently on their form or interaction with surroundings. How these regularities shall be for starch from wheat grain of different ripening?

Beside the spectra of July 4[th], 2010 (Figure 11) the analogue spectra at July 23[rd], 2010 are given in Figure 12. Melting processes in the polymer proceed in the area of super micellar structures though; the sample heating didn't lead to cluster destruction in the range of large masses as found for "young" starch (Figure 11). On the other side, the cluster distribution in the area of super micellar structures was found to be more uni-

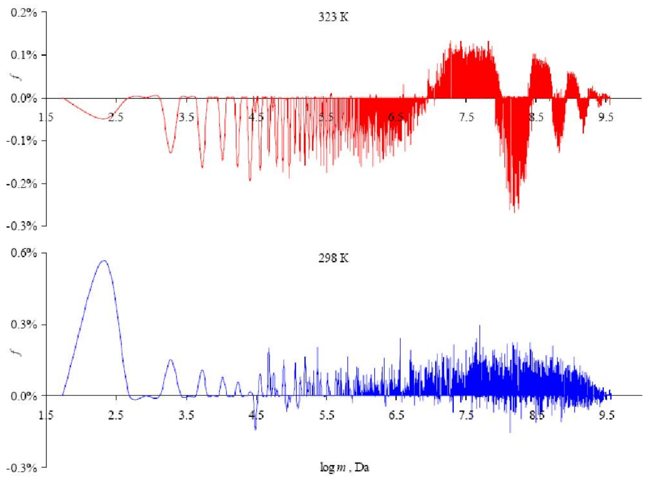

Figure 12. Change of cluster signals in „young" starch extracted from wheat grain at July 23rd, 2010. Cluster ensemble up to 4 billion Dalton, 6 K/min. Zubow constant is equal to 6.8·10^{-15} N/m. Low pressured powder in air-free atmosphere. Weak shock wave (ð < 1 N/m^2).

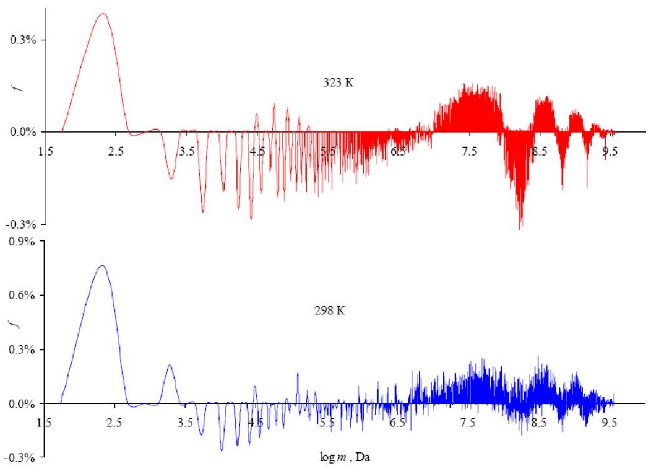

Figure 13. Change of cluster signals in „young" starch extracted from wheat grain at August 14th, 2010. Cluster ensemble up to 4 billion Dalton, 6 K/min. Zubow constant is equal to 6.8·10^{-15} N/m. Low pressured powder in air-free atmosphere. Weak shock wave (ð < 1 N/m^2).

formly than in "young" starch.

The final starch analysis was made on August 14th, 2010 (Figure 13). As shown the heating process is accompanied with strong LRO reconstructions especially, in the super micellar region though, the processes in "old" starch strongly differ from those in "young" one. This difference can be applied for a qualitative polysaccharide analysis furthermore, for understanding the LRO formation mechanism.

At starch samples' heating up to 523 K and to adapt to the new thermodynamic conditions, some reorganization processes in LRO take place. However because of an unstable LRO the processes cannot always be interpreted definitely. For example, the GMSZ spectra sequence of starch on July 13th, 2010 (period of intensive grain growth, Figure 1) shows for the heating temperatures from 298 to 523 K strong changes in the cluster ensemble but without selective cluster reaction on temperature like for "young" and "old" starches. It could be a special case of a structure not organized completely here.

The polymerization degree (PD) of starch branches (amylopectin) can be approximately estimated by using the average cluster molecular mass and number of cluster kinds the clusters decomposes into at heating. The polymerization degree for starch extracted from wheat grain of different ripening is given in Figure 14. A correlation between PD and temperature of wheat grain sam-

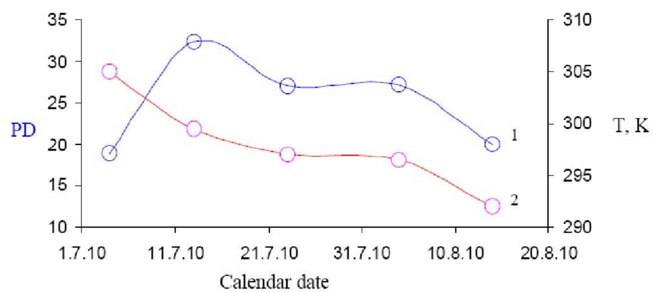

Figure 14. Changes of the polymerization degree in branches of starch (1) extracted from wheat grain of different ripening stages and change of the air temperature (2) at grain sampling from the agricultural field.

pling was observed after July 13th, 2010, only. Before this time, the PD is smaller, what is explained with a beginning growth of amylopectin branches. Here in young grain, the branches' growth in amylopectin seems to dominate over falling temperature. The PD declining after July 13th, 2010 was explained to be connected with a temperature debranching proceeding at temperatures higher 298 K[9] and leading to rapid polymer ageing.

At heating starch extracted from grains of different ripening stages it was observed that both some old clusters disappeared and new ones arose. The dynamics of these processes at starch heating from 298 to 323 K is shown in Figure 15.

New clusters' arising and old ones' disappearance has to be understood as the result of a destroyed clusters' interaction with their surroundings comparable with coagulation/melting. As visible from Figure 15 arising of new clusters and disappearance of old ones don't proceed synchronously especially explicitly for "young" starch. However, for ripe wheat grain (August 14th, 2010), the number of disappearing clusters is nearly equal to that one of arising clusters in starch. This case occurs too, when the curves 1 and 2 cross each other on July 26th, 2010. The highest number of new cluster kinds on August 4th, 2010 was assumed as LRO reconstruction. After heating up to 523 K the differences between the curves 1 and 2 became marginal for all starch types.

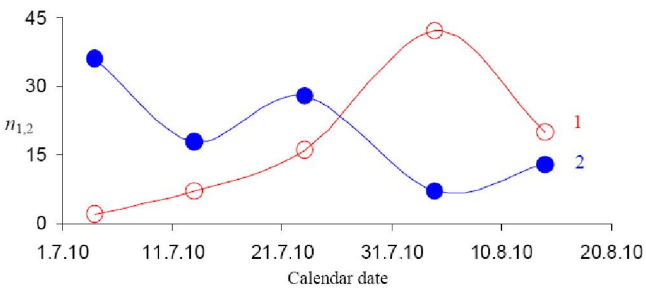

Figure 15. Cluster kinds' dynamics in starch extracted from wheat grain of different ripening. Heating from 298 to 323 K. 1 - arising of new cluster kinds (n_1) and 2 - disappearance of old ones (n_2).

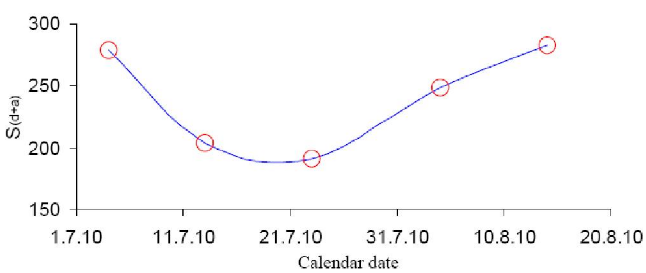

Figure 16. Whole number of mobile cluster kinds ($S_{(d+a)}$) in starch of differently ripened wheat grain. Polymer heating from 298 to 523 K. Cluster ensemble up to 3.2 million Dalton.

It would be of interest to see how change the dynamics of the whole number ($S_{(d+a)}$) of mobile cluster kinds (sum of appearing ($S_à$) and disappearing (S_d) cluster kinds in an narrow temperature interval, 25 K) in starch from differently ripened wheat grain (Figure 16).

The whole number of mobile cluster kinds in "young" and "old" starch seems to be nearly equally though, for the remaining starch types, it reduced by 40 %. These events were assumed as dynamics of two processes: intensely growing polymer chains in "young" starch and chains' debranching in "old" starch. Here the debranching in solid granula is less caused by enzymes but it is rather the result of mechanical disintegration because of rising interne tensions in the branched polymer. The mobility of enzymes in a solid and dry granule is highly limited. Many unlinked clusters or at least clusters that are weakly linked with surroundings seem to be in "young" starch however, they are in "old" starch too, as the result of mechanical decomposition of amylopectin branches (curves 1 and 5 in Figure 8). This conclusion proofs the model for the starch nanostructure as grapes' bunch.

Thus, the ageing of starch in ripe wheat grain was assumed as a potential energy minimizing of polysaccharide chains and as formation of compact ordered nanostructures in LRO where the polymer chains partially shall be released from the crystallizing starch structures[5]. Logical, from July 11th to July 31st, 2010 the most compact LRO structures must be built in starch granula.

Let's return to the results of Figure 11. The inversion of expanded clusters (+ *f*) into collapsed ones (- *f*) in the whole mass interval of the cluster ensemble indicates on the clusters' dependency of each other in this ensemble additionally, on the formation of an unique energy field in which every cluster must subordinate itself to the other ones as a consistent energy pendulum[9]. If an ensemble is forced to change into another energy state e. g. at heating or under pressure then a corresponding reaction in form of LRO reconstruction has to be expected. As visible in Figure 11 after heating up to 323 K the cluster ensemble changed precisely, small clusters and large ones partially became more individually with less surroundings' interactions whereas another group of very large clusters (super micelles in expanded form) kept hold of their interaction with surroundings. The "melting" in starch as ascribed by Kozlov[1] is less the result of the double spiral decomposition but rather that one of an endothermic cluster reconstruction in the ensemble. Processes of forced LRO reconstruction in starch at heating from 298 to 523 K were observed to be accompanied by at least two endothermic effects of "pseudo melting" at 323...348 K and 448 ± 10 K, which strongly depends on the ripening grade of wheat grain. These properties of starch types can be applied for their identification, probably; however, it needs further investigations.

Starch was found to be sensitively to low pressure e. g. compression[11]. Shear forces were observed to destroy the polymer clusters' interaction with their surroundings which influences the whole oscillator ensemble. For understanding this phenomenon the GMSZ spectra of starch at different pressures are given in Figure 17. As visible the starch cluster ensemble remains stable to 18.4 Pa the following pressure increase changes highly the LRO structure, though. Here the changes are more strongly than those found for heating. Strikingly, at 23 Pa the α-D-glycopyranose units (1) and the spiral of two meanders (12 polymer units) started to interact actively with surroundings. This event was interpreted as expanded state of molecular clusters. On the other side, the cluster of 33 units remained in the collapsed state and larger clusters either disappeared or changed to a new thermodynamic balance

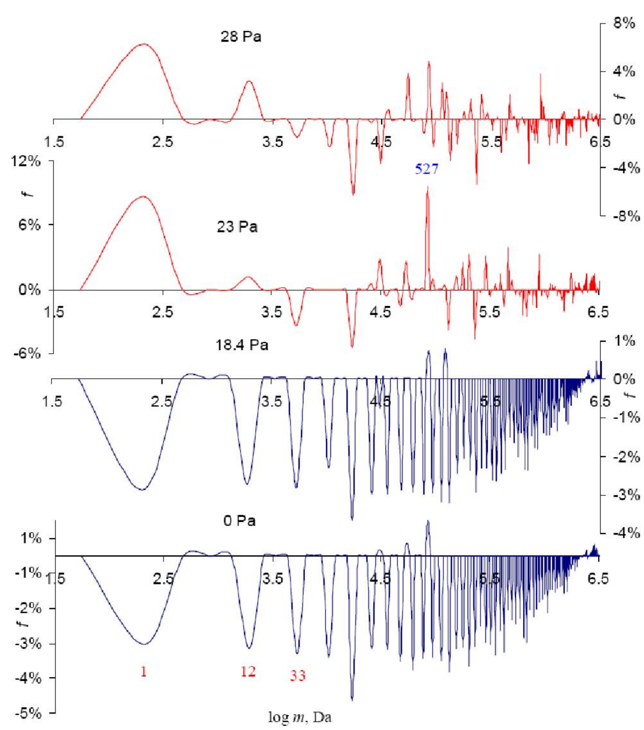

Figure 17. Influence of pressure on LRO in starch extracted from wheat grain on August 14th, 2010. Zubow constant is equal to 6.8·10^{-15} N/m. Weak shock wave (ð < 1 N/m^2), 298 K.

(expanded- collapsed). The expanded cluster with mass equally to 527 α-D- glycopyranose units was characterized by only one high signal at 23 Pa that was interpreted as relative stability to this pressure. It seems to belong to skeletal clusters of this starch cluster ensemble[11,1].

CONCLUSIONS

The results of this study showed that:

LRO in biomatrix of growing wheat grain is represented as molecular clusters that are in a dynamic balance with gravitation fields of surroundings, growing/ageing processes as well as weather conditions

The polysaccharide synthesis in the wheat grain biomatrix was found to proceed in micell structures that were transformed into large starch-containing super micell structures during ripening and ageing

LRO in starch was characterized to change during wheat ripening.

Starch samples extracted from wheat grain of different ripening grades are differently concerning their physicochemical properties

It should be possible in principle to synthesize starch during one to two weeks under laboratory conditions.

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