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TECHNOLOGÓW ŻYWNOŚCI

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Suplement

Nr 4(33) Kraków 2002 Rok 9

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X INTERNATIONAL STARCH CONVENTION

Kraków 11-14 czerwca 2002 r.

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tel./fax (012) 280-71-51/266-92-69
e-mail: msroda@uci.agh.edu.pl

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OD REDAKCJI

Szanowni Państwo,

przekazujemy Państwu Suplement nr 4(33) kwartalnika „Żywność", w którym opublikowano artykuły będące pokłosiem X International Starch Convention (X Międzynarodowej Konferencji Skrobiowej).

Teksty artykułów są wydrukowane w języku angielskim, ze streszczeniem w języku polskim, ponieważ Konferencja miała charakter międzynarodowy.

Wyrażamy nadzieję, że zamieszczone w tym Suplemencie artykuły, przybliżą Państwu aktualny stan badań nad skrobią i zostaną przyjęte z zainteresowaniem.

Kraków, grudzień 2002 r.

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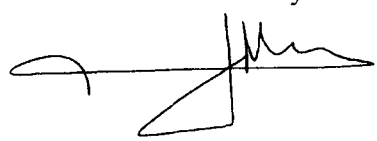

Tadeusz Sikora

This collection of the papers is a regular issue of the "Żywność" ("Food") journal, and at the same time. This volume plays a role of the Proceedings of the IX International Starch Convention held in Cracow on 11–14 June 2002.

The Proceedings can be regarded as a document that provides ample information on a number of topics with respect to structural properties of starch granules and starch gels.

This publication is made possible by the authors, the participants of the X ISC, and the efforts of the reviewers. On behalf of X ISC Organizing Committee I acknowledge their contribution with appreciation.

Prof. Dr. Piotr Tomasik, D.Sc.
(Head of X ISC Organizing Committee)

X INTERNATIONAL STARCH CONVENTION

Cracow, June 11–14, 2002

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THE STABILITY OF LIPID-AMYLOSE COMPLEXES UPON ENZYMATIC HYDROLYSIS OF WHEAT STARCHES OF DIFFERENT CHEMICAL COMPOSITION

Summary

Effect of chemical composition of wheat starches applied for glucose production on the susceptibility of lipid-amylose complexes to enzymatic digestion was studied. Influence of various types of enzymatic preparations (Termamyl S α-amylase, AMG E glucoamylase, G-zyme lysophospholipase) and temperature of hydrolysis were tested. Enzymatic starch degradation was carried out in the differential MICRO DSC III scanning calorimeter (Setaram, France) within the temperature range of 20–120°C and at the scanning rate of 1°C per minute. The scanning profile was as follows: 20–105°C, 105–105°C (5 min), 105–95°C, 95–95°C (60 min), 95–60°C, 60-60°C (48 hrs), 60–40°C, 40–120°C (I heating), 120–40°C (I cooling).

The enthalpy of decomposition of lipid-amylose complexes and the filtration rate of resulting glucose syrups were taken as the measure of efficiency of wheat starches liquefaction with the α-amylase and their further hydrolysis to glucose by means of glucoamylase and lysophospholipase.

Chemical composition of enzymatically digested wheat starches had an impact on the degree of the lipid-amylose complexes decomposition, and physicochemical properties of starch hydrolysates. Though one of the wheat starch preparations contained more lipids, the filtration rate of its hydrolysates exceeded that of hydrolysates produced from the starch displaying lower lipid content. It probably resulted from the higher susceptibility of the lipid-amylose complexes present in this starch, to the enzymatic degradation. DSC data suggested the possibility of dependence of pathways of the complexes decomposition on polymorphs of those complexes.

Introduction

Physicochemical features of starch hydrolysates are influenced by botanical origin of starch and its chemical composition [1, 10, 11, 17]. In processing of wheat starch these properties are particularly affected by the lipid content. Wheat starch con-

Technical University of Lodz, Institute of Chemical Technology of Food, Stefanowskiego 4/10, 90-924 Lodz, phone: (42) 631 34 58, e-mail: justynar@snack.p.lodz.pl
** Department of Physical Chemistry, University of Lodz, Pomorska 165, 90-236 Łódź*

tains approximately 1% of lipids, mainly lysophospholipids (85–95% of the total content) that form complexes with amylose [2, 5, 6]. Virtually, total phosphorus in starch granules belongs to phospholipids. Surface lipids are localized in the outer surface of starch granules. Because of oxidation they are responsible for an unpleasant (so called "corn") odor of wheat starch [12, 13, 14].

Amylose-lipid complexes reduce starch swelling and solubilization, and decrease water-binding and, therefore, they obstruct an access of amylolytic enzymes to starch molecules. Thus, they have a negative effect on production of glucose syrups. In consequence, a lower degree of starch saccharification as well as turbidity and haze in the syrups appear resulting in reduced filtration rate and glucose syrups yield [1, 10, 11].

Amylose complexed with lipids is much less susceptible to enzymatic hydrolysis than free amylose. Complete decomposition of these complexes requires an excess of amylolytic enzymes and/or much longer time of the process [9]. An accessibility of amylose-lipid complexes to enzymatic digestion remains unclear, although the problem of their hydrolysis in starch gels, has been an intensively studied. This prompted us to examine the stability of amylose-lipid complexes upon enzymatic digestion of wheat starch of different chemical composition, and compare properties of the hydrolysates obtained. Starch preparations used in the studies were treated with TERMAMYL S α-amylase, AMG E glucoamylase and G-zyme lysophospholipase, at variable time and temperature.

Materials and methods

The composition of wheat starch preparations, subjected to enzymatic hydrolysis is presented in Table 1.

Table 1

Composition of examined wheat starch preparations

Component	Wheat starch No. 1 [% of s.s.]	Wheat starch No. 2 [% of s.s.]
Amylose	25.5	21.0
Lipids	0.64	0.79
Protein	0.81	0.82
Total pentosans	0.29	0.32
Soluble pentosans	0.013	0.021
Phosphorus	0.034	0.029

Enzymatic preparations applied for starch digestion were as follows:

- TERMAMYL S α -amylase (120 KNU per g) – 0.6 g per 1000 g s.s.;
- AMG E glucoamylase (300 AGU per 1 ml) – 0.015 ml per 90 ml;
- G-zyme G 999 lysophospholipase (1000 U per g) – 0.015 ml per 900 ml.

Hydrolysis of 33% wheat starch gels with above enzymes was conducted in the differential scanning calorimeter MICRO DSC III (Setaram (Caluire, France), at temperatures ranging from 20 to 120°C, and at the scanning rate of 1°C/min. The process was run according to the following scanning profile: 20–105°C, 105–105°C (5 min), 105–95°C, 95–95°C (60 min), 95–60°C, 60–60°C (48 hrs), 60–40°C, 40–120°C (I heating), 120–40°C (I cooling).

The process of starch depolymerization was also carried out in a pressure converter. Parameters of the experiments: initial temp. 105°C – 5 min, pressure – 0,098 Mpa. Subsequenty, temperature was decreased to 95°C and at this temperature liquefaction was conducted by 1 h.

The degree of decomposition of the amylose-lipid complexes during enzymatic liquefaction and saccharification of starch was determined by means of:

- an estimation of the enthalpy of their degradation using the differential scanning calorimetry (DSC);
- filtration rate measurements (the filtrate's volume per units of time and filter surface area)

Filtration experiments were carried out at 60°C. 100 ml samples of starch hydrolysates were taken at determined time intervals and the concentration of each sample was brought to 33°Bx. The samples were filtered through a fluted filter paper of a diameter of 205 mm, and an area of 3.3011 mm^2, constant for all tests. The filtering surface area was 3.1420 mm^2. The filtrate volume measurements were done every minute within the period of 10 minutes.

Results and discussion

Majority of preceding studies on the behavior of amylose-lipid complexes during enzymatic hydrolysis of cereal starch (mainly from barley) were carried out at temperatures below starch gelatinization temperature, and using preparations of α-amylase [8, 15]. Also model amylose-lipid complexes, obtained in the reaction of starch (mainly from potatoes) with fatty acids or lysophosphatidylcholine, were treated with preparations of α-amylase or glucoamylase [3, 4, 7, 16].

Our studies were focused on determination of an effect of chemical composition of starch on the stability of amylose-lipid complexes, whose presence hampers fitration of starch hydrolysates.

Despite the larger lipid content in the wheat starch preparation No. 2, in comparison to the preparation No. 1 (Table 1), filtration rates of wheat starch No. 2 hydrolysates were higher (Fig. 4). It mainly resulted from a better susceptibility of the amylose-lipid complexes, present in the suspension of the starch No. 2, to the attack of the α-amylase preparation, upon the step of liquefaction that was observed by means of differential scanning calorimetry.

Fig. 1 presents thermograms of melting of the native wheat starch preparations No. 1 and 2. In case of wheat starch No. 1, an endothermal shift at low temperature (T_m of 58.8°C, ΔH_m of 3.68 Jg^{-1} – I heating) perhaps corresponds to melting of the crystalline layer of wheat starch (gelatinization), and the peak at high temperature (T_m of 100.9°C, ΔH_m of 0.68 Jg^{-1} – I heating) seems to result from the dissociation of the amylose-lipid complexes. The enthalpy of gelatinization of the wheat starch No. 2 is the same as that of the starch No. 1 slurry, but the enthalpy of the amylose-lipid complex decomposition is higher (ΔH_m of 0.72 J g^{-1}, T_m of 101.3°C). Although higher value of the enthalpy of the complex dissociation indicates its higher concentration, the rate of filtration of the hydrolysate from the starch No. 2 was higher. As stated above, it most probably resulted from a higher susceptibility of the amylose-lipid complexes to enzymatic digestion with α-amylase during starch liquefaction. This property might be a consequence of a lower amylose content and structure of the complex, dependent on the position of the lipid molecule inside of the amylose helix.

Amylose-lipid complexes can recover their structure upon cooling of wheat starch slurries, and the peaks presented in Fig. 1 confirm this phenomenon (I cooling). Dependently on wheat starch composition, peaks of different shapes could be observed, indicating that various polymorphs of the regenerated complexes could appear on cooling.

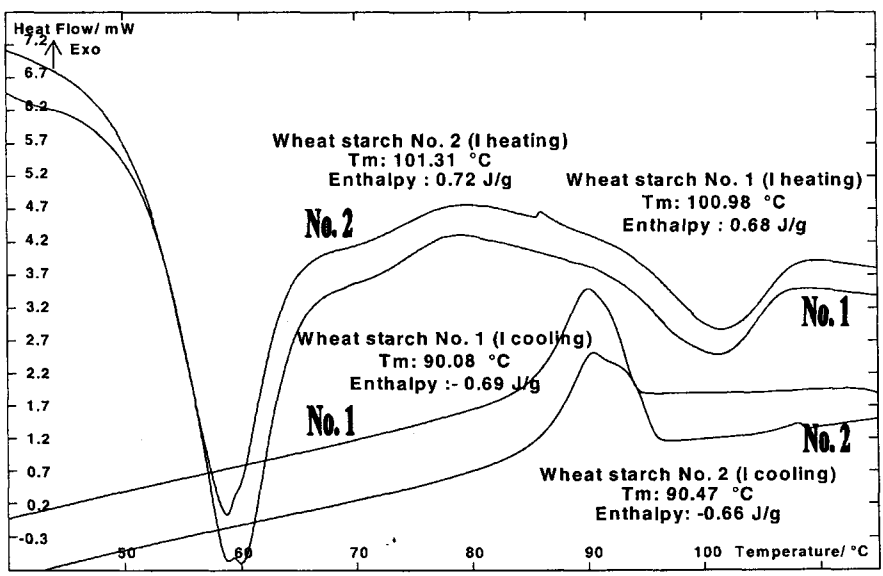

Fig. 1. DSC thermograms of native wheat starch preparation no 1 and no 2.

Fig. 2 depicts endotherms of decomposition of amylose-lipid complexes present in the wheat starches suspensions (No 1 and 2, respectively) digested with

TERMAMYL S α-amylase (0.6 g per 1000 g s.s.). In wheat starch No.1 slurry, subjected to treatment with the α-amylase, the peak corresponding to the complex decomposition has a larger area (higher value of the enthalpy: ΔH_m of 0.30 Jg^{-1}, T_m of 100.9°C). Thus, degree of decomposition of this complex is lower than for the starch No. 2 suspension (T_m of 98.9°C, ΔH_m of 0.06 Jg^{-1}).

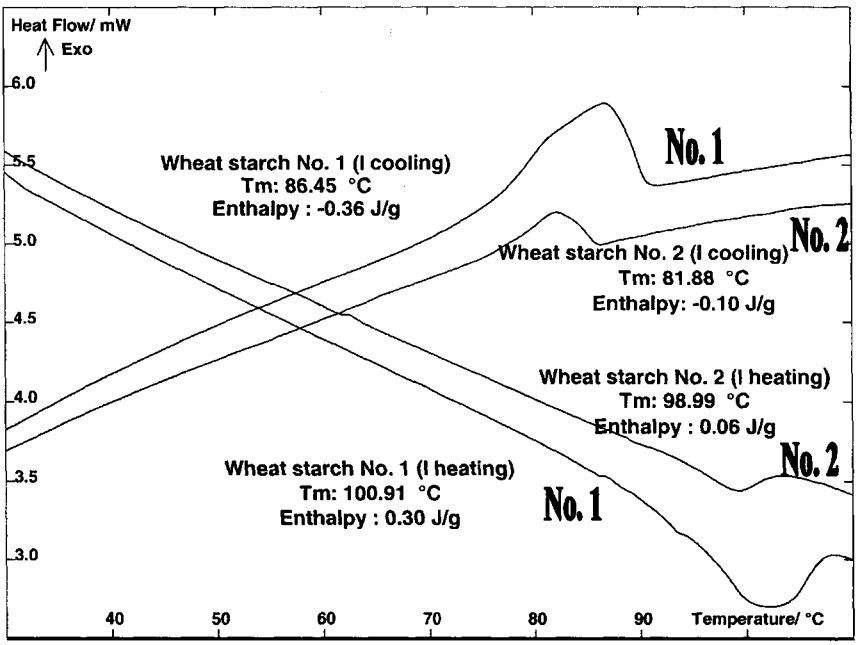

Fig. 2. DSC thermograms of wheat starches suspensions no 1 and no 2 digested with Termamyl S α–amylase.

Such degree of decomposition of the amylose-lipid complexes of the starch No. 2 during enzymatic liquefaction with the TERMAMYL S α-amylase coincides with higher filtration rate of the hydrolysate in comparison to the syrup derived from starch No. 1 (Fig. 4).

The stage of starch liquefaction is also followed by regeneration of the amylose-lipid complexes (upon cooling of the slurries), as can be deduced from peaks presented in Fig. 2.

Fig. 3 shows thermograms of suspensions of both the starches, treated for 48 hrs with AMG E glucoamylase and G-zyme lysophospholipase. None amylose-lipid complex was detected in the product of digestion of the starch No. 2. It coincides with its high filtration rate. On the contrary, the starch No. 1 hydrolysate contained very poorly degraded amylose-lipid complex (T_m of 99.6°C, ΔH_m of 0.20 Jg^{-1}). Saccharification of this starch caused only a slight decrease in the enthalpy of decomposition of the complex

(ΔH_m of 0.30 Jg^{-1} after liquefaction). The presence of the stable amylose-lipid complexes, despite the treatment with enzymes, hampered filtration of the final product.

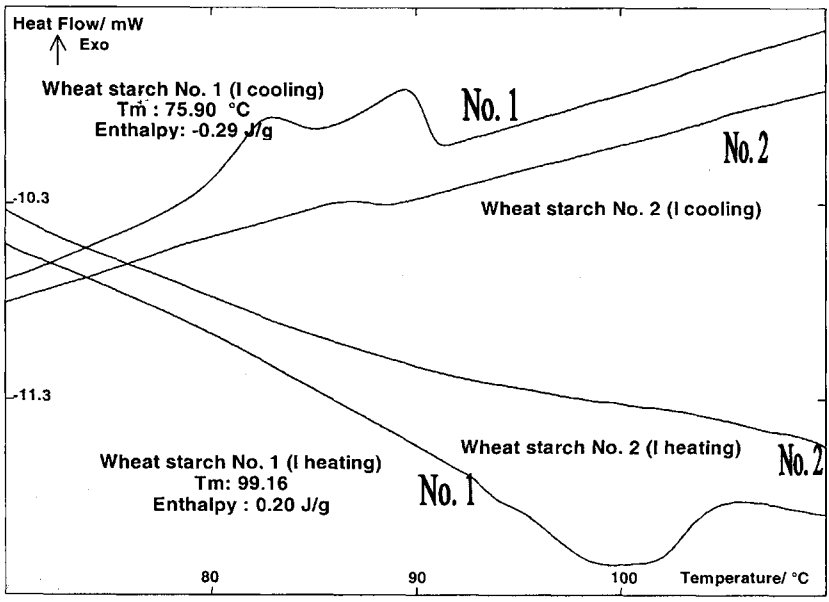

Fig. 3. DSC thermograms of wheat starches suspensions no 1 and no 2 treated for 48 hrs with AMG E glucoamylase and G – zyme lysophospholipase.

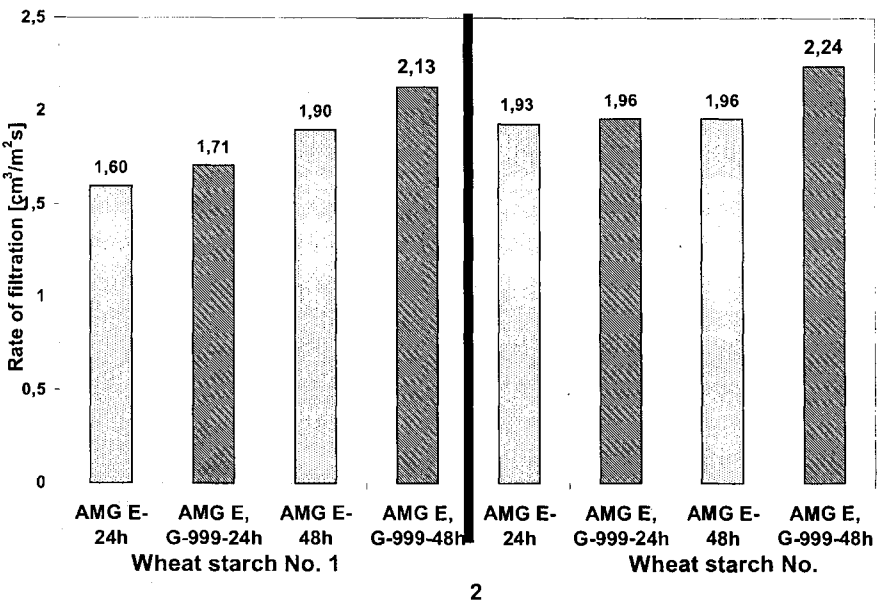

Fig. 4. Rate of filtration of glucose hydrolysate obtained from wheat starches no 1 and 2 treated for 48 hrs with AMG E glucoamylase or AMG E glucoamylase and G – zyme lysophospholipase.

Conclusions

Results of our studies indicate that:

1. Chemical composition of wheat starches subjected to enzymatic hydrolysis affects the degree of decomposition of the amylose-lipid complexes present in starch granules, and the rate of filtration of glucose syrups.
2. Liquefaction of two different preparations of wheat starch with TERMAMYL S α-amylase increased degree of decomposition of the complex in the preparation No. 2 (ΔH_m of 0.06 Jg^{-1}), though it contained more lipids, in comparison to the starch No. 1 (ΔH_m of 0.30 Jg^{-1}). Therefore, filtration of hydrolysate of the latter starch was difficult.
3. Chemical composition of wheat starch affects the susceptibility of the amylose-lipid complexes to enzymatic degradation. Saccharification of the liquefied starch No. 2 resulted in a total decomposition of these complexes, whereas in the liquefied starch No. 1 only a minor dissociation was observed after treatment under the same conditions.

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TRWAŁOŚĆ KOMPLEKSÓW AMYLOZOWO – LIPIDOWYCH W PROCESIE ENZYMATYCZNEJ HYDROLIZY SKROBI PSZENNEJ O RÓŻNYM SKŁADZIE CHEMICZNYM

Streszczenie

Celem badań było sprawdzenie wpływu składu chemicznego surowca skrobiowego, użytego w procesie enzymatycznej hydrolizy skrobi pszennej do glukozy, na podatność kompleksu amylozowo-lipidowego na rozkład, w zależności od zastosowanego preparatu enzymtycznego (α-amylaza TERMAMYL S, glukoamylaza AMG E, lizofosfolipaza G-zyme) oraz temperatury procesu.

Proces hydrolizy z wykorzystaniem wyżej wymienionych preparatów enzymatycznych prowadzony był w różnicowym kalorymetrze skaningowym typu MICRO DSC III firmy Setaram w zakresie temperatur od 20°C do 120°C i szybkości skanowania 1°C/min. zgodnie ze skanami: 20°C – 105°C, 105°C – 105°C (5 min), 105°C – 95°C, 95°C – 95°C (60 min), 95°C – 60°C, 60°C – 60°C (48 godz.), 60°C – 40°C, 40°C – 120°C (I grzanie), 120° – 40°C (I chłodzenie).

Miarą efektywności wpływu działania preparatu α-amylazy w etapie upłynniania i glukoamylazy z lizofosfolipazą w etapie scukrzania, zastosowanych w procesie hydrolizy skrobi pszennych o różnym składzie chemicznym, na stopień rozkładu kompleksu amylozowo-lipidowego, był pomiar entalpii ich rozpadu metodą różnicowej kalorymetrii skaningowej (DSC) oraz pomiar szybkości filtracji uzyskanych hydrolizatów glukozowych.

Badania wykazały wpływ zróżnicowanego składu chemicznego skrobi pszennej poddanej procesowi enzymatycznej hydrolizy na stopień rozkładu kompleksu amylozowo-lipidowego oraz na właściwości fizykochemiczne uzyskanych hydrolizatów glukozowych. Pomimo wyższej ogólnej zawartości lipidów w jednej ze skrobi, szybkość filtracji hydrolizatów z niej uzyskanych była wyższa niż hydrolizatów otrzymanych ze skrobi o mniejszej zawartości lipidów. Wiąże się to z większą podatnością kompleksu tej skrobi na rozkład pod wpływem zastosowanych preparatów enzymatycznych. Dane DSC sugerują, że mogą istnieć różne drogi hydrolizy kompleksów amylozowo-lipidowych w zależności od ich postaci polimorficznej.

H. M. BARANOWSKA, R. REZLER

TEMPERATURE CHARACTERISATION OF STARCH AND STARCH-PROTEIN DISPERSIONS

Summary

The aim of the study was to analyse changes in the parameters describing molecular dynamics of water in starch and starch-protein dispersions taking place in the process of gelation. The study was performed by the nuclear magnetic resonance (NMR) method for starch dispersion samples of the concentration of 0.10g/cm^3 (*Triticum durum* wheat starch) and starch-protein dispersion samples (gluten obtained from wheat) of the concentration c = 0.1g/cm^3. NMR measurements were performed in the range of 20°–70°C. The parameters describing molecular dynamics of water in retrograded gels obtained at 70°C and 100°C were also determined.

The process of starch gelation was found to occur already at temperatures lower than 70°C. The spin-lattice relaxation times were observed to decrease despite of increase in temperature. It suggested a decreased mobility of water molecules in the system studied. It would result from the formation of spatial lattice formation already in the process of gelation. In the starch-protein samples, the relaxation time, T_2, slightly increased with increase in temperature over the whole range studied. In this system water molecules had unlimited mobility, which suggested a lack of gelation. These results were confirmed by analysis of the relaxation times for both systems of retrograded gels obtained at different temperatures. For the starch gel, the relaxation times were identical at both temperatures of gelation. For starch-protein gel the relaxation times are longer for the gel obtained at 70°C than at 100°C. This observation confirmed temperature of 70°C was insufficient for lattice formation.

Introduction

Gelatinization is a transformation occurring on heating of starch-water dispersions. Above certain temperature, starch granules swell and alter their structure [1, 2, 6]. A gradual loss of birefringence occurs, and the low molecular weight components leach into water. During this process, the secondary bonds that maintain the granule structure are broken and the micelar network is pulled apart.

Department of Physics, Agricultural University of Poznań, 60-637 Poznań, Wojska Polskiego 38/42, Poland, rezler@owl.au.poznan.pl

Several methods can be used to follow the gelatinization process: loss of birefringence, increase in viscosity, and susceptibility to enzymatic degradation, decrease in enthalpy, and loss of X-ray diffraction pattern. Among them only susceptibility to enzymes and variation of enthalpy are used in quantification of the extent of gelatinization. These methods, however, are either time-consuming (enzyme susceptibility) or show poor reproducibility (calorimetry).

Lelievre and Mitchell [7] showed that heating of starch-water dispersions above 60°C, resulted in an increase in the relaxation time measured by pulsed-proton nuclear magnetic resonance (NMR). This behaviour was attributed to an increase in the mobility and hydration of starch polymers, suggesting an increase in the number of protons linked to the liquid phase. Although the relaxation time due to the protons in the solid phase was not measured, it would be lower than those in the liquid phase. Thus, in a starch-protein sample with different degrees of gelatinization and different content of proteins, there would be different relations between the protons in the liquid phase and those in the solid phase. Thus, a study of gelatinization of starch water-protein systems with involvement of the ratio of the protons in the liquid and those in the solid phase as determined by pulsed-proton NMR.

In this study possibility of using pulsed-proton NMR to measure the degree of gelatinization of starch-protein systems was recognised. Simultaneously, gelatinization kinetics of wheat starch-gluten-water systems was investigated.

Material and methods

Materials: Wheat starch (*Triticum durum), (*Sigma, MC = 9%) and starch-protein (gluten from wheat containing 80% protein and 7% fat), (Sigma, MC = 8%) dispersions.

Sample preparation: The total concentration of the polymers was 0.10g/cm3. The starch-proteins mixtures were prepared following the starch to protein ratio of 9:1, 8:2, 7:3, 6:4, 5:5. Directly after preparation the samples were placed in NMR tubes and closed.

The NMR experiment was performed on a pulse NMR spectrometer ELLAB Poznań operating at 15 MHz. The spin-lattice relaxation times T_1 were measured applying the inversion-recovery pulse sequence (π–τ–$\pi/2$).The pulse distance τ was changing from 10 to 5300 ms. The repetition time TR was 10 s. The spin-spin relaxation times T_2 were measured by the CPMG pulse train. The distance between τ pulses was established for 3 ms, the number of spin-echos was 50. The CracSpin calculated program [11] was used to obtain the vales T_1, while T_2 values were calculated by the fit to the formula:

$$M_{x,y} = M_0 \left\{ \exp \frac{-\tau}{T_2} \right\}$$

where: M_0 is the equilibrium value of magnetisation, T_2 the spin-spin relaxation time. Temperature was controlled with precision of ±0.5°C.

Results and discussion

Recognition of molecular mechanisms of changes in the starch structure which took place at different levels of its organisation under hydrothermal treatment is of great importance. Gelation is the most important processes occurring in starch systems at moderate temperatures and in the presence of water, temperature of gelation is one of the most important parameters characterising the starch system.

The temperature dependencies of the spin-lattice relaxation times are shown in Fig. 1.

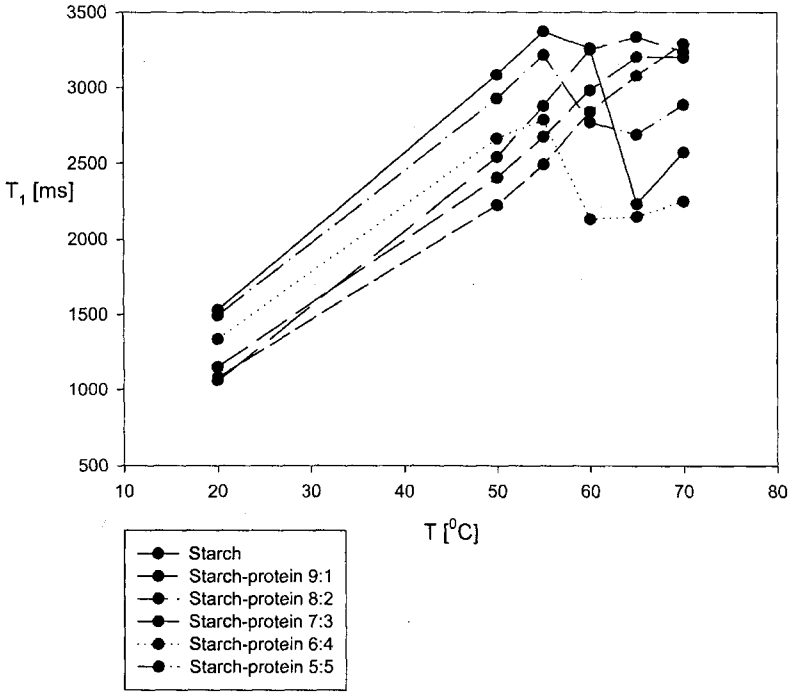

Fig. 1. The temperature dependencies of the spin-lattice relaxation times.

For starch dispersions, above +60°C the T_1 relaxation time decreases in spite of increase in temperature.

In aqueous dispersions of starch heated above the gelation temperature the irreversible process of swelling takes place, accompanied by disappearance of the crystalline ordering and release of amylose to the solution [3, 4, 8, 10]. The degree of the crystal structure of the system is determined by the contribution of bihelical forms among the high-molecular starch components. The temperature dependence of this

contribution reveals a transition from the ordered helical fragments of the polymer chains to the state of spatially disordered coil. Some of the water molecules penetrating into the swelling starch grains become immobile and bound to the starch hydroxyl groups unshielded in the process of gelation. Simultaneously, the crystal phase melts and the concentration of the active segments of the amorphous lattice decreases with increase in temperature, causing a deshielding of more hydrophilic group. They bound more water molecules limiting their dynamics. This influences the temperature dependence of T_1 relaxation time (Fig. 1).

The spatial lattice of macromolecular gel starts forming under conditions when stable bonds can form among fragments of different macromolecules. This process occurs on cooling of hot starch gels of sufficient both concentration and degree of homogeneity. The intermolecular bonds form as a result of coiling of neighbouring fragments of the macromolecule chains taking place on decreasing temperature, and their association to bihelical forms, characteristic of native and retrograded starch. In order to determine the effect of the temperature of gelation on the final structure of the macromolecular gel, the relaxation time was measured for starch systems gelled at +70°C and +100°C. The measurements were performed at +20°C at 24 hours after the completion of gelation. The T_1 relaxation time values obtained were within the error limit (±5%) for both systems (Table 1). This result leads to a conclusion that in spite of different temperatures of gelation, the spatial lattices formed as a result of the retrogradation processes are similar and temperature of +70°C is sufficient for starch gelation within the concentration range studied.

For the starch-protein systems two different courses of temperature dependencies of T_1 relaxation time were observed. For the systems of starch-to-protein concentrations ratio of 9:1, 8:2 and 7:3, the value of T_1 relaxation time increased with increase in temperature. This dependence was interpreted as follows. The water molecules initially bound with the protein are released as a result of the protein denaturation process above +50°C [5, 9]. At the same time, because of the uncoiling of the starch molecules, the water molecules take part in the formation of the lattice. In general, these two processes evoked no macroscopic change in the water molecules dynamics. The situation is different for the systems containing starch and protein at the concentration ratio of 6:4 and 5:5, for which the temperature dependencies of T_1 relaxation time revealed maxima and minima in the temperature range studied. At the beginning, the T_1 value increases with temperature increasing up to +55°C, which suggested the release of water molecules as a result of protein denaturation and an increase in their mobility. A significant decrease in T_1 observed at +60°C for the system of the ratio of 6:4 and at +65°C for the system of the ratio of 5:5, was probably related to the uncoiling of starch chains as well as participation of water molecules in the lattice formation and bonding of some of the water molecules at the hydrophilous sites of the peptide chains de-

shielded as a result of protein denaturation. Further slow increase in T_1 is explained as a consequence of an increase in the mobility of those water molecules which are not involved in the lattice formation.

Table 1

The spin-lattice and spin-spin relaxation times for starch and starch-protein dispersions before and after gelation.

	T_1 [ms]			T_2 [ms]		
	Before gelation	After gelation at 70°C	After gelation at 100°C	Before gelation	After gelation at 70°C	After gelation at 100°C
Starch	1527	669	680	718	191	176
Starch-protein 9:1	1060	1040	796	894	792	484
Starch-protein 8:2	1154	1142	886	689	621	447
Starch-protein 7:3	1083	1059	960	788	978	334
Starch-protein 6:4	1337	1020	928	927	531	462
Starch-protein 5:5	1492	1161	1108	894	1213	519

The temperature dependencies of spin-lattice relaxation times are shown in Fig. 2.

The T_2 relaxation time slightly increases with increasing temperature for all the systems studied. The most significant change in T_2 was noted in the range 20–50°C. It is interpreted as being related to increase in mobility of the water molecules not involved in the network formation.

Conclusions

1. The transition from the ordered spiral forms of the fragments of the polymer chains to the state of spatially disordered coil, taking place in the starch systems above 60°C facilitates the process of bonding of the water molecules to the hydroxyl groups of starch (unavailable at lower temperatures). It leads to a decrease in the water molecule dynamics.
2. In spite of different temperatures of gelation, the spatial lattices formed as a result of the processes of retrogradation are similar and the temperature of +70°C is sufficient for gelation of starch in the systems without proteins in the concentration range studied.
3. For starch-protein mixtures of the same total concentration, temperature of +70°C is insufficient for gelation of the starch in the system.

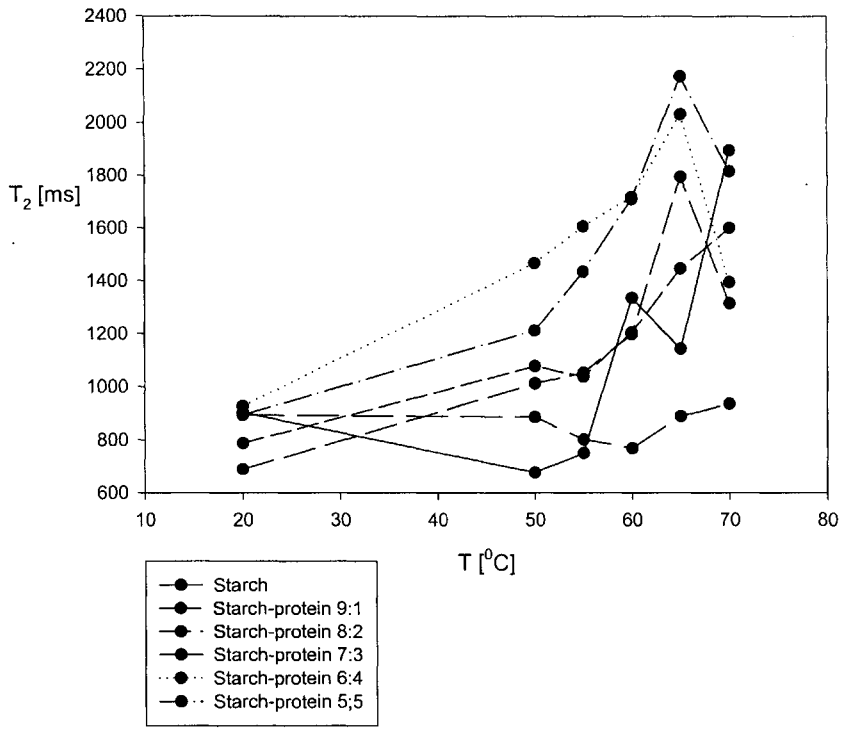

Fig. 2. The temperature dependencies of the spin-spin relaxation times.

4. There are two different ranges of starch-protein concentrations under which the dynamics of the water molecules depends on the concentration of protein in the system.
5. The relaxation times determined for starch-protein gels at 70°C are longer than these at 100°C. It confirms that for such systems the temperature of 70°C is insufficient for the lattice formation.

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TEMPERATUROWA CHARAKTERYSTYKA DYSPERSJI SKROBIOWYCH I SKROBIOWO-BIAŁKOWYCH

Streszczenie

Celem podjętych badań była analiza zmian parametrów określających dynamikę molekularną wody w dyspersji skrobiowej i dyspersjach skrobiowo-białkowych w trakcie procesu kleikowania. Badania przeprowadzono na próbkach dyspersji skrobiowej o stężeniu 0,10 g/cm^3 (skrobia pszenicy *Triticum durum*) i skrobiowo-białkowych (gluten uzyskany z pszenicy) o stałym stężeniu mieszaniny c = 0,1 g/cm^3. Pomiary prowadzono w zakresie temperatur 20°–70°C. Dodatkowo porównano parametry określające dynamikę molekularną wody w zretrogradowanych żelach kleikowanych w temperaturze 70°C i 100°C. Do badań wykorzystano technikę magnetycznego rezonansu jądrowego. Stwierdzono, że skrobia kleikuje w temperaturze niższej niż 70°C. Zaobserwowano spadek wartości czasu relaksacji spin-sieć mimo wzrostu temperatury. Sugeruje to, że w układzie obniżona została mobilność molekuł wody. Jest to efektem formowania się struktur przestrzennych sieci już w trakcie kleikowania. W układzie żeli skrobiowo-białkowych w całym analizowanym zakresie temperatury wartości czasów relaksacji T_2 nieznacznie rosną ze wzrostem temperatury. Molekuły wody w tym układzie nie mają ograniczonej mobilności co sugeruje brak efektu kleikowania. Powyższe wyniki potwierdza analiza wartości czasów relaksacji obu układów zretrogradowanych żeli kleikowanych w różnej temperaturze. Wartości czasów relaksacji żelu skrobiowego są takie same w obu zakresach temperatury kleikowania. Czasy relaksacji żelu skrobiowo-białkowego są dłuższe w przypadku kleikowania w temperaturze 70°C niż w przypadku kleikowania w temperaturze 100°C. Potwierdza to, że temperatura 70°C jest niewystarczająca do uformowania sieci takiego układu.

G.B. PLENZLER, D.M. NAPIERAŁA, H.M. BARANOWSKA

HYDRATION OF STARCH AND PROTEIN SEEDS IN EARLY PHASE OF GERMINATION

Summary

Seed swelling in the first phase of imbibition involves mainly development of seed colloids. Chemical affinity of such colloids to water differs depending on the surface properties of the macromolecules. The biopolymer surface could perturb the dynamic and static state of water. For this reason the structure and composition of seeds, especially proteins, starch, and lipid content, can control the course of the swelling process.

The study presents the microscopic and macroscopic parameters describing the swelling pea seeds and triticale grains. Differences in corresponding parameters were observed. Measurements of water uptake rate in both species showed higher water uptake in triticale grains compared to that in pea seeds in the first step of the process but lower in the subsequent phase. The results of pulse ^{1}H-NMR measurements have revealed two groups of water protons, each in a different magnetic environment responsible for a different relaxation rate. These two groups correspond to water molecules differing in mobility, such as free and bound water, respectively.

The difference in results obtained for triticale and pea are related to size, different permeability of seed envelopes, different mobility of seed water and chemical content mainly determined by starch. Its structure and physicochemical properties are also very important.

Introduction

Starch is the major carbohydrate used extensively in food industry as a water binder, thickener, texturizer, emulsifier and gelling agent. It resides in plant tuber and seed endosperm where it occurs as granules [11], each typically containing several million amylopectin molecules accompanied by a higher number of smaller linear amylose molecules. The relative proportions of amylose to amylopectin depend on the natural source of starch [7]. Of the two components of starch, amylose is more useful as a hydrocolloid. Its expanded chain conformation [5] is responsible for the high vis-

Department of Physics, Agricultural University of Poznań, 60-637 Poznań, Wojska Polskiego 38/42, gplenzler@woodcock.au.poznan.pl

cosity of water soluble starch and hydrophobic inner surface, which is not able to hold water strongly and the water molecules can be easily replaced by more hydrophobic ones such as lipids and aromatic compounds.

Seed swelling process in the first phase of imbibition mainly involves hydration of seed colloids. Chemical affinity of colloid forming macromolecular compounds to water depends on their surface properties [5, 6, 8]. The biopolymer surface could perturb the dynamic and static state of water near the surface. For this reason the structure and composition of seeds, especially the proteins, starch, and lipid content, can control the course of the process. The starch-water interactions are very important for the behaviour of the system. The interactions involve the molecular mobility of water which can be determined by ^{1}H-NMR spectroscopy [1, 3].

In the present study we have compared the macroscopic (the rate of water uptake) and microscopic parameters (transverse relaxation time of two fraction of water) describing the process of germination in triticale and pea seeds, belonging to starch and protein rich starch seeds, respectively.

Material and methods

Pea seeds, cv. Sześciotygodniowy (six-weeks old) and triticale grains, cv. Presto from and SH Choryń and CNOS Poznań, respectively, were used for all the experiments. Five groups of 20 seeds each were placed on Petri dishes and moistened with distilled water and then incubated in a germination chamber at stable temperature of 294 K. Relative mass change and relative water content (g H_2O/g dry mass) were measured with E50 S Gibertini balance (Italy), in standard conditions.

The state of water in seeds was studied in relation to moisture content by the laboratory made pulse NMR spectrometer, operating at 30 MHz using the CPMG pulse sequence (90°–τ–180°). The results of the experiments were analyzed using a non-linear least-squares curve-fitting procedure. Each set of data was fitted to the one-, two- or three term exponential and the best fit was taken based on the chi-square and correlation coefficient value.

Results and discusion

Water sorption kinetics in pea seeds and triticale grains are presented in Figure 1. As can be seen, the water uptake rate in triticale is higher than in pea in the first phase of this process and then decreased. It may stem from a higher relative surface of individual seeds and different seed cover capacity to transport water in both types of seeds studied. Moreover, triticale belongs to starchy seeds of high starch content (68% carbohydrates and 11% proteins), whereas pea is characterised by high protein content (53% carbohydrates and 24% proteins) [4]. The effect observed in Fig. 1 is consistent

with general trend in which the oleic seeds absorb less water than seeds containing more starch and, first of all, protein seeds [10].

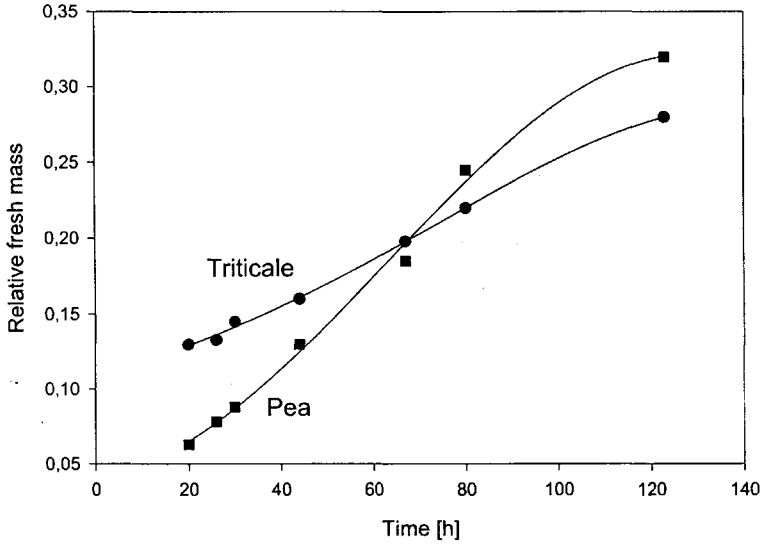

Fig. 1. Water uptake kinetic in pea and triticale seeds.

^{1}H-NMR studies of the seed hydration have been most often performed on NMR-spectrometers designed to observe the signal from liquids [2, 3]. This method is useful for water entrapment (caging) in tissues or for in-situ water circulation investigations [1]. Two-dimensional high-resolution NMR experiments and molecular dynamics calculations [2] have indicated that in biopolymer systems and biological tissues it is usually sufficient to consider three states of water, this is, structural or bound water, surface water and bulk water. Structural water is that hydrogen bonded inside of groves and cavities of globular proteins and polysaccharides, which plays an important role in determining the structure and dynamics of the biopolymer chains. The second fraction of water molecules sorbs at the biopolymer surface. In globular protein there are three types of water adsorbing sites, deriving from three types of amino acid residues: charged, polar and non-polar. The charged or dissociating groups chemisorb individual water molecules with high affinity [8]. The permanently available water molecules can form clusters around the polar groups, with decreasing affinity to water molecules. As more moisture was available, water aggregated over non-polar residues. This surface water extends for several molecular layers from the surface of macromolecules and was extremely mobile. The bulk of the cell water is normal liquid water and exists only when the water content in seeds exceed 0.8 g H_2O/g dry mass. Since the water content

in our samples is lower than that required for bulk water appearance. This water component is beyond of our current considerations.

The ^{1}H-NMR spin-spin relaxation time study (T_2) in triticale grains and pea seeds (Figs. 2 and 3) distinguished between two states of water. The relaxation time T_2 for two fractions of water took higher values for pea seeds than for triticale grains. For the two groups of seeds the humidity dependence of transverse relaxation time was not linear.

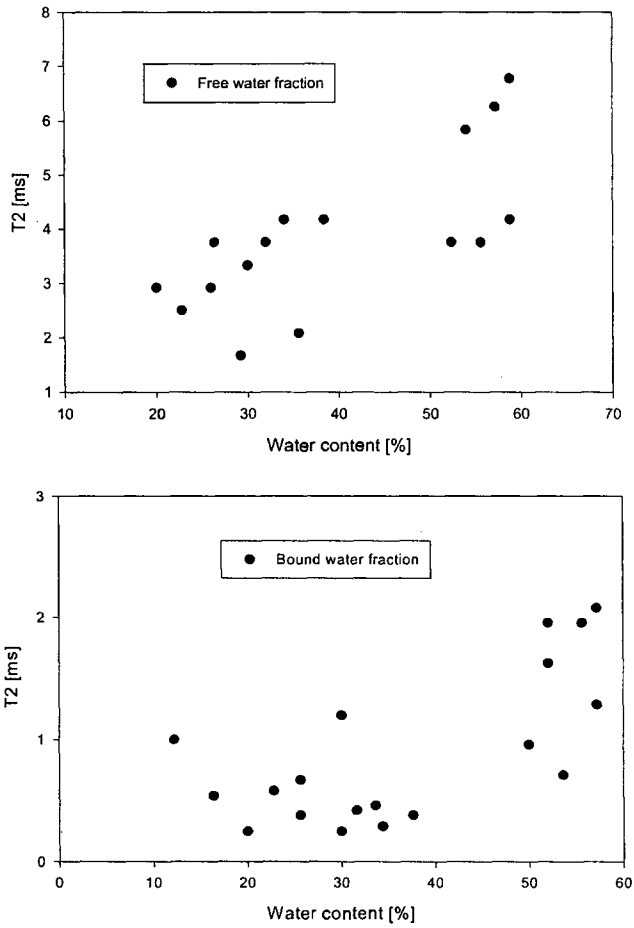

Fig. 2. Transverse relaxation times T_2 for bound and free water fraction in triticale grain.

The compact molecules possessed a reduced mobility as compared to less compact molecules. The reduced mobility provided a longer relaxation time, i.e. a time in which the molecules must return to equilibrium. Observed differences in transverse

relaxation time T_2 in pea and triticale could be explained as a result of diverse in starch structure in both investigated species.

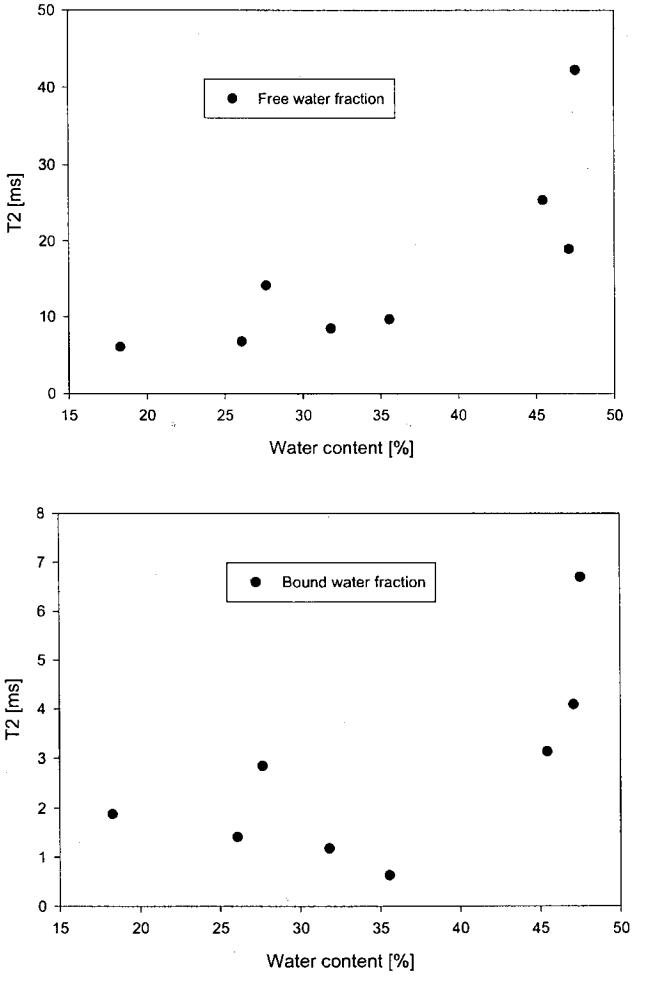

Fig. 3. Transverse relaxation times T_2 for bound and free water fraction in pea seeds.

Starch granules are complex structures consisting of crystalline and amorphous areas. The short chains in the amylopectin molecule are organised into double helices some of which are crystallized into lamellae. These lamellae alternate with the amorphous layers [11]. Amylopectin double-helical chains can either form the more open hydrated hexagonal crystallites (type B), the denser crystallites (type A) or, such as in pea, contain both polymorph forms: A and B [11]. Such structures determine the mobility of water in studied systems.

Conclusions

Presented investigations have shown that well documented differences in chemical content and structure of starch granules in pea and triticale correspond to differences in microscopic parameter – transverse relaxation time T_2 – in those seeds. The used NMR method is nondestructive and can be adapted to other noninvasive investigations of in vivo starch systems.

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HYDRATACJA NASION SKROBIOWYCH I BIAŁKOWYCH WE WCZESNEJ FAZIE KIEŁKOWANIA

Streszczenie

Pęcznienie nasion w pierwszej fazie polega głównie na hydratacji koloidów nasiennych. Powinowactwo chemiczne tych związków do wody różni się zależnie od właściwości powierzchniowych ich makromolekuł. Powierzchnie biopolimerów zakłócają dynamiczny i statyczny stan wody. Z tego względu struktura i skład nasion, w szczególności zawartość protein, skrobi oraz lipidów może warunkować przebieg procesu pęcznienia.

Proces pęcznienia nasion grochu i ziarniaków pszenżyta opisano w przedstawionych badaniach za pomocą parametrów makroskopowych (względny przyrost masy) oraz mikroskopowych (czasy relaksacji spin-spin).

Grawimetrycznie określony pobór wody przez nasiona pszenżyta, początkowo większy niż w grochu, w późniejszej fazie obniżał się. Metodą spektroskopii ^{1}H-NMR ujawniono obecność dwóch frakcji wody, które znajdując się w różnym otoczeniu różniły się ruchliwością, opisaną przez czasy relaksacji spinowo-spinowej. W przypadku grochu ten parametr mikroskopowy był zdecydowanie większy niż dla pszenżyta. Obserwowane różnice są dyskutowane przy uwzględnieniu różnic w wymiarach nasion i ziarniaków, różnej przepuszczalności okryw nasiennych oraz przede wszystkim różnic w składzie chemicznym, w którym dominujący udział ma skrobia.

LUCYNA SŁOMIŃSKA, ANNA GRZEŚKOWIAK-PRZYWECKA,
DANUTA WIŚNIEWSKA

LOW CONVERSION STARCH HYDROLYSATES

Summary

Starch liquefaction without significant increase in dextrose value was attempted. Potato, corn and pea high amylose starches were digested with thermostable alpha-amylase. Different reaction conditions (starch concentration, enzyme dosage, time reaction) were used in hydrolyses into DE < 10. Chemical and physical properties of obtained hydrolysates such as carbohydrate composition, iodine absorbance value, turbidity measurement, viscosity, gelling ability, swelling power were recognised. Comparison of properties of hydrolysates of similar DE indicated that the potato hydrolysates distinguish in the highest solubility and the lowest iodine absorbance. The high-amylose corn hydrolysates were characterised with the following properties: the highest swelling power and gelling ability. Pea hydrolysates had the highest viscosity.

Introduction

Starch hydrolysates are a mixture of reducing sugars. They are products a partial or total depolymerization of starch. Low converted starch hydrolysates (DE below 40) are usually produced by a two-stage process: the first stage of the hydrolysis – liquefaction – is carried out with acids or enzymes at elevated temperatures, the second stage of hydrolysis – saccharification – is carried with bacterial α-amylase to achieve a desired DE [2, 3, 4, 11, 12, 13, 20]. For many applications the functionality or suitability is enhanced when DE of the hydrolysates is relatively low (DE below 10). However, the production of these hydrolysates can be troublesome because of retrogradation of longer chain amylose fragments forming an insoluble haze.

The hydrolysates are commonly used in food industry as body agents, non-sweet fillers, carriers for synthetic sweeteners, flavour enhancers, additives for colouring

L. Słomińska, Starch and Potato Products Research Laboratory, ul. Armii Poznań 49, 62-030 Luboń; Institute of Biotechnology and Environment Protection, ul. Monte Cassino 21b, 60-561 Zielona Góra;
A. Grześkowiak-Przwecka, D. Wiśniewska, Starch and Potato Products Research Laboratory, Luboń

agents and so on [1, 6, 14, 16]. Functional properties of low DE starch hydrolysates depend on their production technology and kind of source of starch. High amylose corn starch hydrolysates made by enzymatic hydrolysis having DE between 5 to 15 distinguish in good gel strength – important attribute in a fat replacer [5]. They are also prepared by hydrolysing of a granular amylose starch in a strongly acid aqueous slurry at a temperature above 70°C [7]. Low DE potato hydrolysates as Paselli SA2 (Avebe) with a DE of 2 [17], C*Pur 01906 (Cerestar) with a DE of 3 [21] and Lycadex® (Roquette Freres) with a DE of 5 [19] are prepared by an enzymatic degradation of starch and have plastic, fat-like characteristic increased with applied hydrolysate concentration. Combination of low degree of polymerisation of oat hydrolysates and beta-glucan content gives unique functional properties of the products [8,9].

In this research properties of various low converted starch hydrolysates obtained with alpha amylase are compared.

Materials and methods

Enzyme

Commercial enzyme Termamyl 120 L, a mixture of outstanding heat-stable alpha-amylase produced by selected strains of *Bacillus licheniformis*, was used. Its activity was 120 KNU/g, where 1 KNU was the amount of enzyme that hydrolysed 5.26 g starch per hour using Novo's standard method for determination of α amylase (substrate – soluble starch, calcium content of solvent – 0.0043 M, reaction time – 7–20 min, temperature - 37°C, pH – 5.6 [15].

Source of substrate

Potato starch (Potato Enterprise in Łobez), corn starch (National Starch and Chemical), pea starch (DPS, Starch Protein and Service), were used as a substrate for enzyme action (Tab. 1).

Table 1

Composition of starches used as substrates.

Starch components	Potato starch	Corn starch	Pea starch
Moisture (%)	13.0	12.6	11.0
Lipids (% d.s.)	0.04	0.29	0.24
Proteins (% d.s.)	0.05	0.64	0,79
Amylose (% d.s)	21	70	66
Amylopectin (% d.s)	79	30	34

Experimental procedure

Aqueous starch slurries of the concentration of 15 and 20% were prepared, their pH was adjusted to 6.5, and enzyme (0.013 –0.055 KNU/g d.s.) was admixed. The suspension was maintained at 95°C for 10 – 45 min. The inactivation of enzyme was conducted with citric acid. The reaction mixtures were analysed after 10, 20, 30 and 45 min hydrolysis.

The following analyses were performed for each sample:

- the amount of reducing groups by modified Schoorl-Rogenbogen method [18],
- carbohydrate composition by High Performance Liquid Chromatography. (HPLC apparatus (Waters 600E, USA) was used with a Aminex HPX – 42A column (7.8 x 250 mm), Bio-Rad. Elution of carbohydrate was detected with a differential refractometer. Samples (20 μl) were injected into the column and eluted with water at temperature 85° at a velocity of 0,5 ml/min),
- viscosity (Brookfield Model DV-II – spindle # 4 and 5 at 20,50 and 100 min^{-1}) was measured at 20% concentration at 25°C,
- iodine absorbance value defined as the optical density at 500 nm using a 4 cm cell in Pye Unican SP 500 Series 2 spectrophotometer,
- solubility and swelling power by Leach method 10],
- gelling ability (Instron 1140 – spindle 50, penetration speed – 100 rev/min, penetration depth – 50 mm).

Results and discussion

Role of enzyme concentration and reaction time

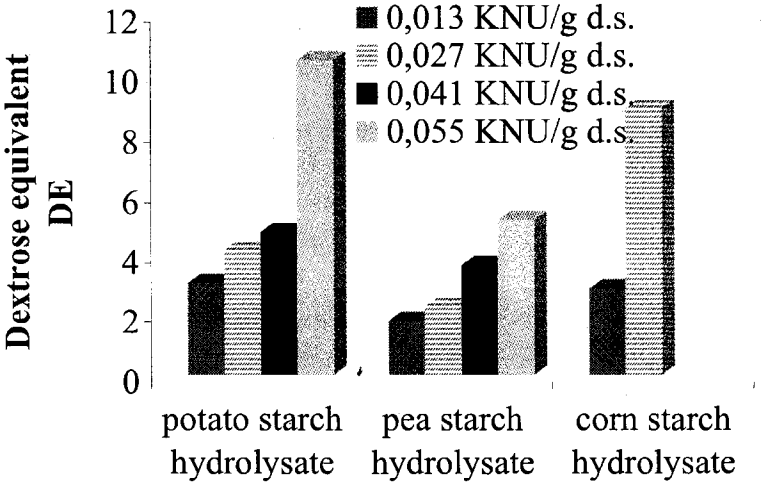
Starch hydrolysates were obtained by means of α-amylase treatment of potato starch, high amylose corn starch, and pea starch. The applied enzyme dose varied from 0.013 to 0.055 KNU/g d.s. As low enzyme dosage as 0.013 KNU/g d.s. produced hydrolysates of DE from 2 to 4 (Fig. 1A).

Production of these hydrolysates required different reaction time, this is, 10, 20, and 30 min for potato, corn, and pea starch, respectively (Fig. 1B).

Viscosity

The tested starches were treated by α-amylase in dosage 0.013 KNU/g d.s. within 30 min. Comparison of viscosity value for hydrolysates obtained from the starches characterised similar DE indicated that the highest viscosity shown the pea hydrolysate.

A)



B)

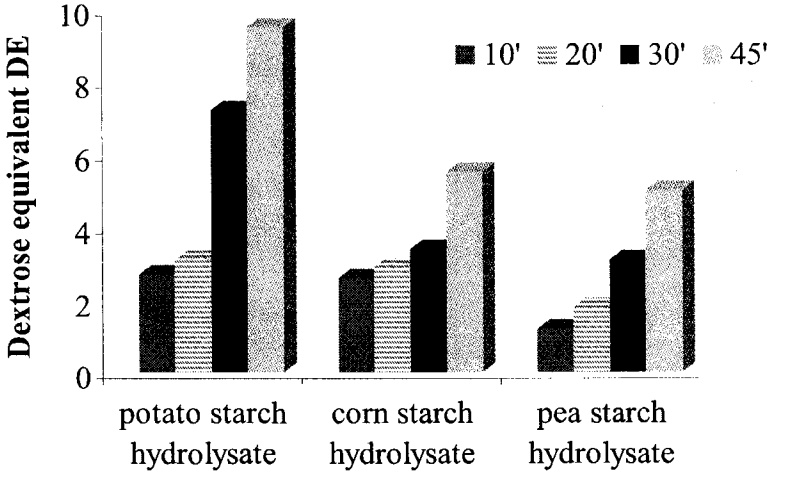


Fig. 1. The influence of enzyme dosage and reaction time on the level of reducing sugars (substrate: 15–20% d.s.; temperature: 95°C; pH 6,5; A – reaction time 20 minutes; B – enzyme dosage: 0.013 KNU/g d.s.).

Obtained results of research confirmed the rule created in literature: the decrease of viscosity followed by the increase of starch depolimerization [5, 22].

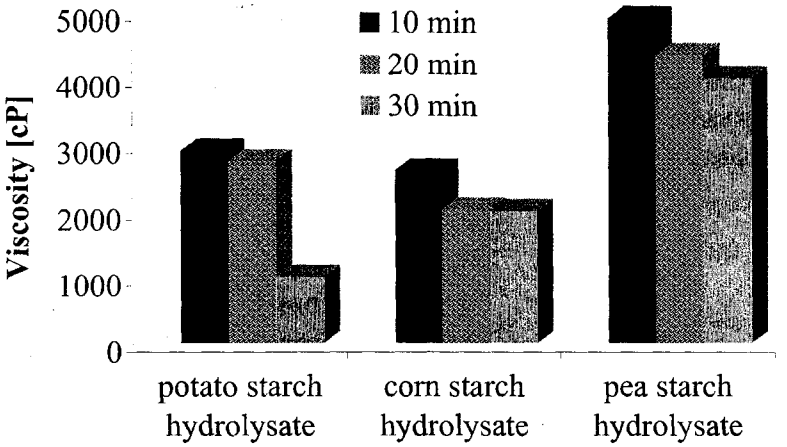


Fig. 2. The influence of reaction time on starch hydrolysate viscosity (substrate: 15–20 –% d.s.; temperature: 95°C; pH 6,5; enzyme dosage 0,013 KNU/g d.s.).

Carbohydrate composition

Carbohydrate composition of the starch hydrolysates changed dependence on the depolimerisation degree of starch. Enhancement of the depolimerisation degree was associated with the increase of simple sugar amount and the decrease in the oligosacharides content [16].

The influence of enzyme dosage and reaction time on carbohydrate composition of tested hydrolysates is presented in Table 2.

Table 2

Carbohydrate composition of tested hydrolysates of starch.

Hydrolysate type	Enzyme dosage KNU/g d.s.	Glucose [%]			Maltose[%]			Higher sugar [%]		
		Reaction time [min]								
		10	20	30	10	20	30	10	20	30
Potato starch hydrolysate	1.3	0.5	1.0	5.9	1.0	1.0	10.0	98.5	98,0	84,1
	2.7	1.0	2.7	6.0	2.7	3.1	10.1	96.3	94.2	83.9
	4.1	1.3	3.0	6.3	3.0	3.6	11.8	95.8	93.4	81.9
Corn starch hydrolysate	1.3	0.2	0.2	0.3	1.8	2.6	4.3	98.1	97.2	95.4
	2.7	1.0	3.4	3.5	2.1	7.0	7.3	97.0	89.6	89.2
Pea starch hydrolysate	1.3	0.6	1.0	2.8	1.9	4.1	9.1	97.5	94.8	88.1
	2.7	1.3	2.8	3.2	2.9	7.6	9.2	95.8	89.7	87.6

Iodine absorbance

Fig. 3. illustrates the correlation between DE of different starch hydrolysates and iodine absorbance value. This hydrolysate property determines the degree of hydrolysate transparency. Potato starch hydrolysates indicated the lowest iodine absorbance whereas pea starch hydrolysates had it the highest.

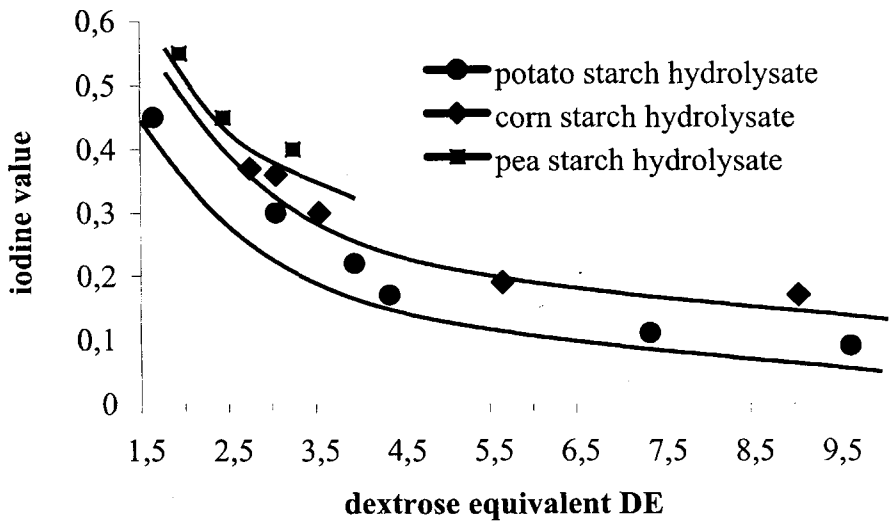


Fig. 3. Correlation between DE of different starch hydrolysates and iodine absorbance (substrate: 15-20% d.s.; temperature: 95°C; pH 6,5; enzyme dosage 0.013 KNU/g d.s.; reaction time 20 min.).

Solubility and swelling

Hydrolysed starches were dried in a spray dryer and subjected to further studies. The influence of dextrose equivalent on solubility and swelling power of the dry hydrolysates is shown in Fig. 4. Results of research indicated that solubility of the dry hydrolysates depended on DE; solubility increased moderately to DE increase. For example: 52% potato starch hydrolysates dissolved in water in the case of hydrolysate with 2.9 DE but hydrolysates of 9.5 DE dissolved already in 91%.

Water solubility of the hydrolysates also depended on type of starch. Solubility of hydrolysates with similar DE (2.9 DE) was as follows:

potato starch hydrolysate – 51%,
corn starch hydrolysate – 47%,
pea starch hydrolysate – 29%.

Swelling power of hydrolysates was associated with DE and type of starch. Corn starch hydrolysates had the highest swelling power whereas pea starch hydrolysates had it the lowest.

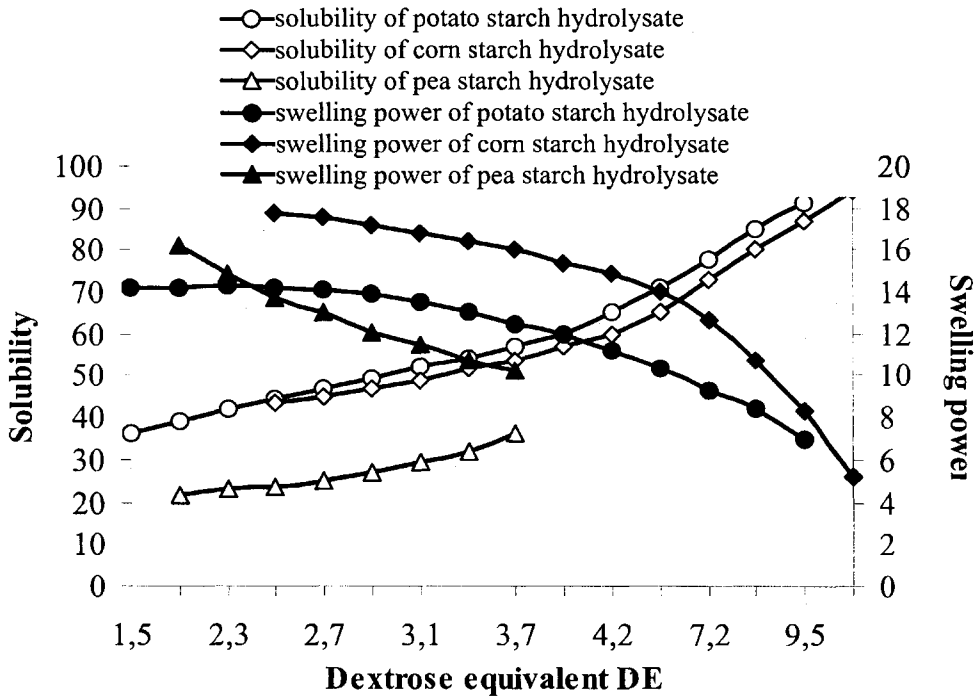


Fig. 4. Solubility and swelling power and DE of tested hydrolysates (substrate: 15-20 % d.s.; temperature: 95°C; pH 6,5; enzyme dosage: 0.013 KNU/g d.s.; reaction time: 20 minutes)

Gelling ability

Corn hydrolysates had the highest gelling ability among hydrolysates with similar DE (Tab. 3). This result fitted suggestion that starches with high amount of amylose form strong gels [22].

Table 3

Characteristics of starch hydrolysates

Hydrolysate type	DE	Gelling ability
Potato starch hydrolysate	3.1	1.614
Corn starch hydrolysate	2.9	3.003
Pea starch hydrolysate	3.1	1.843

Conclusion

The tested starches treated by alpha-amylase show their different enzymatic susceptibility. In production of starch hydrolysates with 3.1-3.4 DE it is necessary to apply different reaction conditions: enzyme dosage – 0.013 KNU/g d.s. of starch and reaction time – 10 min for potato starch, 20 min for corn starch and 30 min for pea starch.

Comparison of starch hydrolysates with similar DE indicate that:

- Potato starch hydrolysates have the lowest iodine absorbance and the highest solubility. They can be used in pasteurised or cold prepared foods.
- Corn starch hydrolysates characterise the highest swelling power and gelling ability – properties compatible with fats and oil. They can be indicated as the best fat replacers.
- Pea starch hydrolysates have the highest viscosity – important factor for improvement emulsion stabilising properties.

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NISKOSCUKRZONE HYDROLIZATY SKROBIOWE

Streszczenie

Badania dotyczyły upłynniania trzech rodzajów skrobi: ziemniaczanej i dwóch amylozowych- kukurydzianej i grochowej. Proces hydrolizy skrobi termostabilną alfa-amylazą (Termamyl 120L) prowadzony był przy zastosowaniu zmiennych parametrów procesu (stężenie skrobi, dawka enzymu, czas reakcji), prowadzących do uzyskania hydrolizatów o zbliżonej wartości DE (DE < 10). Uzyskane hydrolizaty poddawane były ocenie fizykochemicznej przy wykorzystaniu metod analitycznych określających: skład węglowodanowy, zdolność absorbji jodu, rozpuszczalność, zdolność żelowania, lepkość, siłę pęcznienia. Badania wykazały, że hydrolizaty ziemniaczane charakteryzują się najwyższą rozpuszczalnością. Natomiast hydrolizaty wysokoamylozowe kukurydziane cechuje najwyższa siła pęcznienia i zdolność żelowania, a hydrolizaty grochowe wykazują najwyższą lepkość.

[1]ALICA BURISOVÁ, [2]ZDENKA HROMÁDKOVÁ, [2]ANNA EBRINGEROVÁ, [1]EDITA DZIVÁKOVÁ, [1]ALEXANDER DANDÁR

EFFECT OF HEMICELLULOSES ON THE PROPERTIES OF POTATO AND CORN STARCHES

Summary

Cereal hemicelluloses are plant cell wall polysaccharides which have a great influence on the processing and quality of starch-based food products due to the interactions between these polysaccharides. In this paper the influence of hemicelluloses isolated from non-food sources i.e. 4-O-methylglucuronoxylan from beech wood and arabinoglucuronoxylan from corn cobs on the rheological and pasting properties of corn and potato starches. A series of blends was prepared from the two starches in combination with both hemicellulose types added in concentration range of 1.0 to 2.0%. The rheological properties of the blends were characterised by flow curves and the retrogradation of starches/hemicellulose blends were investigated on the refrigeration and freeze-thawing. Both types of hemicellulose exhibited a significant, positive effect on the syneresis of starch at its ~2% concentration.

Introduction

Hemicelluloses are polysaccharides constituting, together with cellulose, the cell walls of higher plant tissues. Among hemicelluloses, the most representative are D-xylans with long chains of (1→4)-β-D-xylopyranose units carrying short side chains composed of L-arabinose, D-glucuronic acid and its 4-O-methyl ether, rarely D-glucose and D-galactose [1]. The average content of hemicelluloses in cereal flours reaches 2–4%. These hemicelluloses are represented mainly by arabinoxylans, have attracted considerable attention because of their important physiological effects incereal products and their effects on baking properties of flours and quality of baked and non-baked products [2]. Positive effects of arabinoxylans have been reported after their

[1]*Dr inž. A. Burisová, mgr inž. E. Dziváková, prof. dr hab. A. Dandár, Department of Food Technology, Slovak Technical University, Faculty of Chemistry and Food Technology, Radlinského 9, 812 37 Bratislava, burisova@chtf.stuba.sk*
[2]*Dr inž. Z. Hromádková, dr hab. inž. A. Ebringerová, Institute of Chemistry Slovak Academy of Science, Dúbravská cesta 9, 842 38 Bratislava*

blending with low-quality flours or starches [3]. There are no reports concerning the effect of hemicelluloses on the quality of other starch-based food products such as soups, souces, puding, etc. In this paper we describe the rheological beahviour of pastes prepared from potato and corn starches with and without addition of model hemicelluloses isolated from beech wood meal and corn cobs.

Material and methods

Corn starch (Gustin) and potato starch (Solamyl) were fine corn starch produced by Dr. Oetker s.r.o. (Bratislava, Slovakia). As model xylans, the arabinoglucurono-xylan from corn cobs (AGX) and 4-O-methylglucuronoxylan (GX) from beech wood meal were produced in the pilot plant of the Institute of Chemistry, Slovak Academy of Sciences (Bratislava, Slovakia). The analytical characteristics of the xylans are summarised in Table 1.

The flow properties of the starch pastes were determined using the coaxial cylinder viscometer Rheotest 2 (2-50 Hz, VEB MLW Prüfgeräte Medingen, Germany) with the S_3 measuring system (r/R = 0.81, D = 0.17-146 s^{-1}). The rheological tests were performed at temperatures 55, 70, and 90°C. Starch pastes were characterised using the Brabender viscograph (Brabender, Duisburg, Germany).

Table 1

Analytical characteristics of the model xylans.

Neutral saccharides composition (x_i /mol. %)	GX	AGX
Xylose	96.8	88.3
Arabinose	1.5	6.1
Glucose	0.8	3.1
Galactose	0.7	2.5
Mannose	0.2	-
Uronic acids (%) [a]	12.4	88.3
N (%)	0	2.5
Lignin (%) [b]	0.3	3.1
Mean molecular weight [c]	30 000	75 000
Solubility in water (%)	75	85

a) Expressed as the anhydro unit of 4-O-methylglucuronic acid.

b) Determined as Klason lignin.

c) Estimated by SEC using pullulan standards.

Preparation of starch pastes: Blends of corn starch with hemicelluloses added in concentrations of 1.0; 1.2; 1.5; 1.7; and 2.0% were prepared. For testing by Rheotest, the blends were suspended in water and the suspensions were heated in a boiling water bath for 10 min and further boiled for 15 min at constant stirring. The pastes were cooled to room temperature and used for rheological measurements. For Brabender experiments, the aqueous suspension of the starch blends were prepared at ambient temperature and then the pasting behaviour was measured at various temperatures within 2 h.

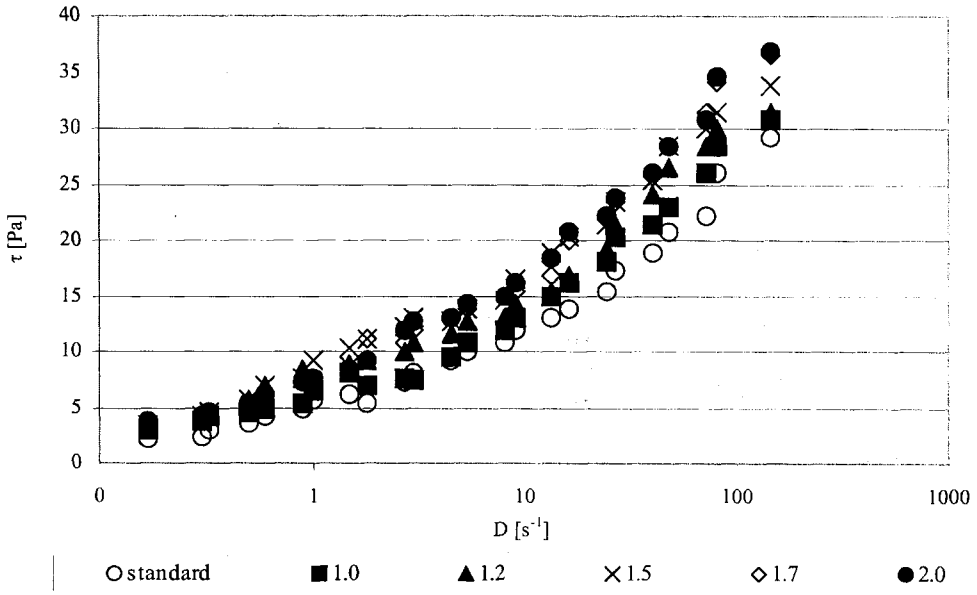
The retrogradation of the obtained starch pastes were measured after refrigeration at 4°C for one week and then heated to 40 °C for 2h (duplicated), and in four freeze-thaw cycles (-18°C for 16 h and 40°C for 2h). The amounts of absorbed and excluded water was determined in relation to the free water content of the starting pastes determined by centrifugation (3000 rpm/min for 10 min) of starch pastes.

Results

The rheological properties of corn starch paste (standard) and pastes prepared from starch/AGX and starch/GX blends were characterised by the flow curves measured at 55, 70, and 90°C using the Rheotest (Figs. 1a, 1b). The curves indicated a pseudoplastic behaviour of all corn starch, significantly affected only by addition of GX at the highest concentration (2%) what indicates a higher resistance against mechanical stress. The effect of GX and AGX addition on the flow behaviour at low and high shear rates is illustrated in Figures 2a and 2b. As seen, after addition of AGX, the apparent viscosity of the pastes increased continuously within the concentration range applied in the low and high shear rate regions and this trend was retained also at higher temperature. In contrast, a similar behaviour was observed for GX only at higher concentrations. At the lowest dose (1%), the viscosity of the pastes decreased in comparison to the standard, but about the same effect was observed at the highest dose applied (2%) with GX and AGX.

The flow behaviour of potato starch/xylan blends are illustrated on Fig. 1c and 1d. The η_{app} of the potato starch pastes are substantially higher in comparison to that of corn starch pastes. As shown in Fig. 3a and 3b, a significant decrease in η_{app} at the lowest dose of added xylans (1%) in comparison to the standard, was observed in the case of potato starch blend pastes with both GX and AGX. With the increasing amount of added xylans, the η_{app} of the influence of GX was also less pronounced in comparison to that of AGX, even at the highest dose applied (2%).

a)



b)

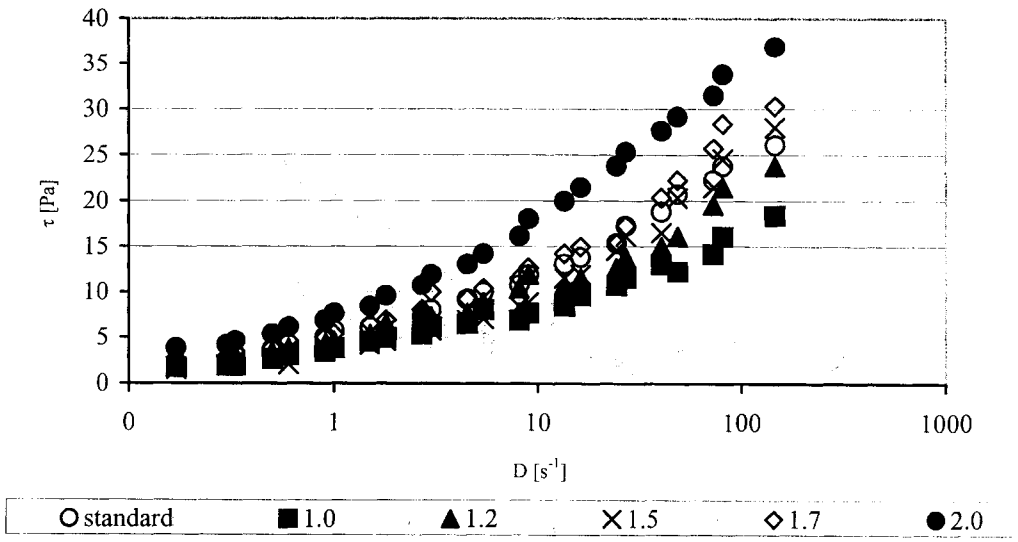
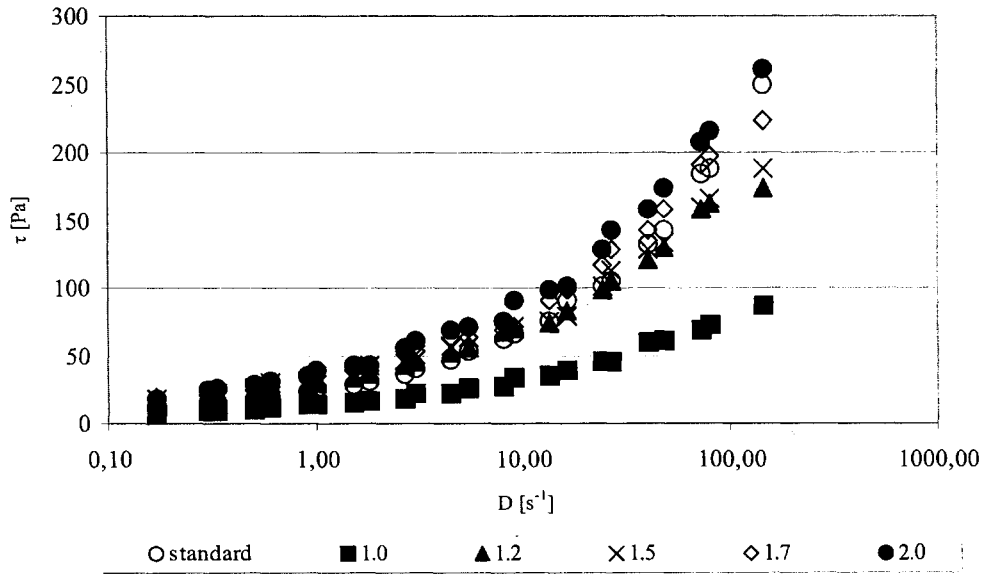


Fig. 1a, b. Flow curves measured at 55°C:
a) corn starch/AGX blends,
b) corn starch/GX blends.

c)



d)

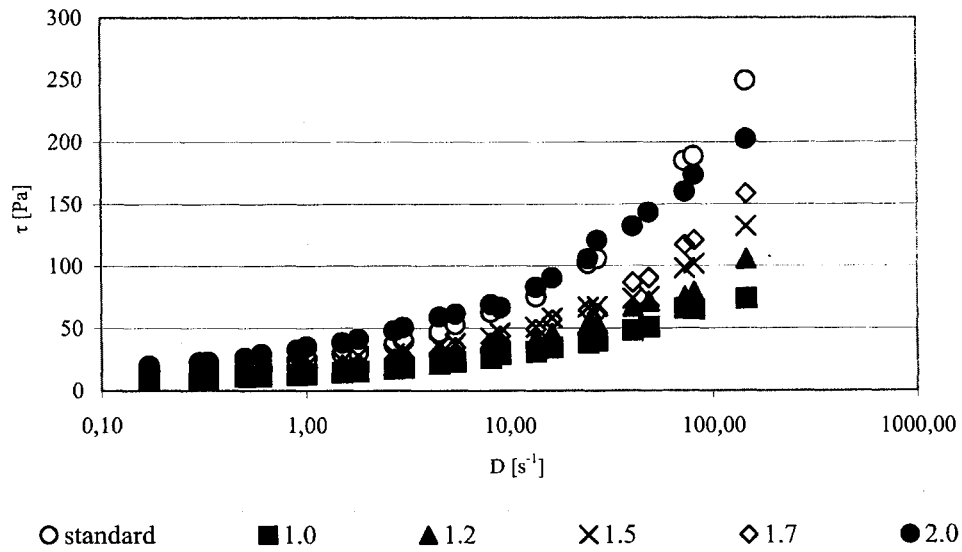


Fig. 1c, d. Flow curves measured at 55°C:
c) potato starch/AGX blends,
d) corn starch/GX blends.

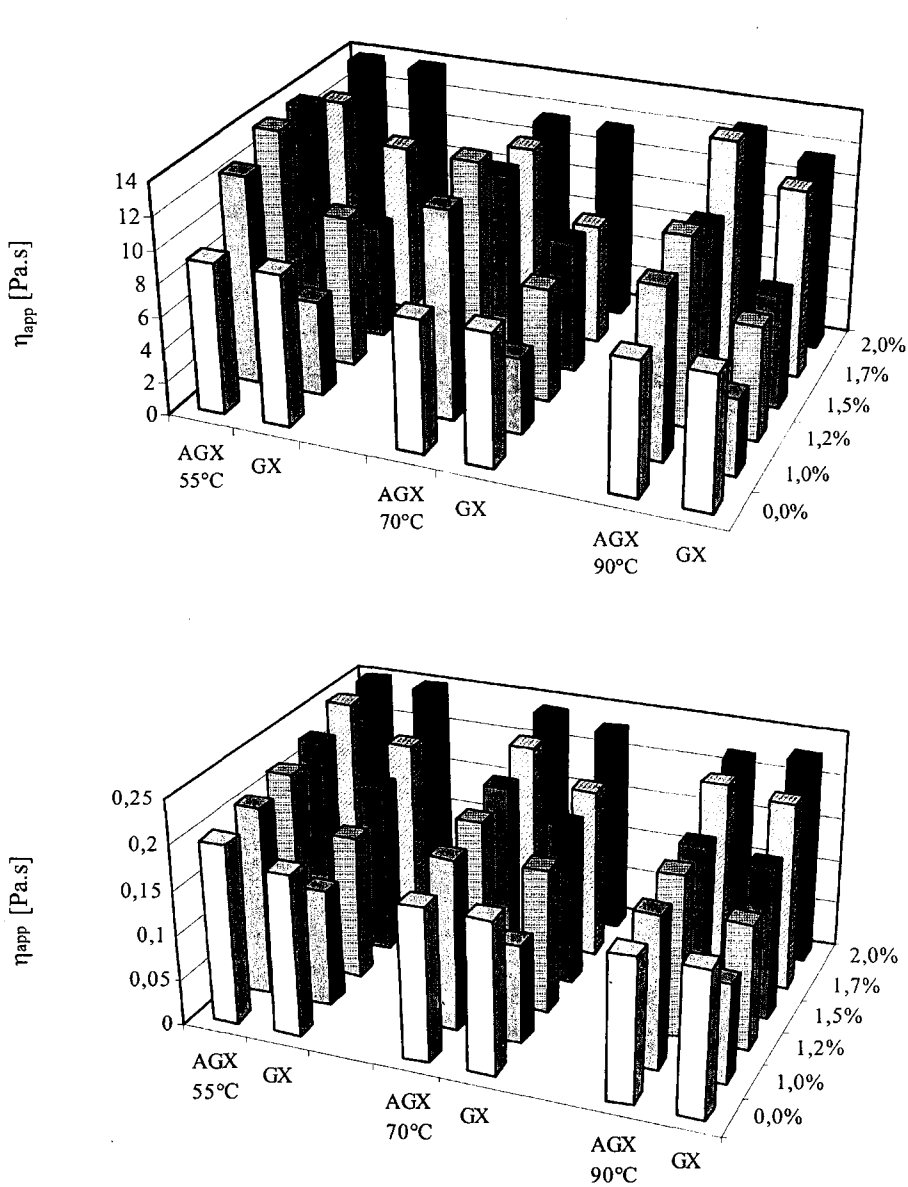


Fig. 2. Influence of AGX and GX on viscosity of corn starch pastes at shear rate;
a) $D = 0.33\ s^{-1}$,
b) $D = 146\ s^{-1}$.

The results of the pasting behaviour of the pastes prepared from blends obtained from corn strach and various amounts of added AGX are presented in Table 2. At 1%

addition of both xylans, the viscosity (P) decreased. In contrast to GX, it did not reach the value of the standard at the highest dose with AGX. The effect of AGX addition is best expressed by the breakdown value, retrogradation ratios C/P and C/H and breakdown ratio (H/P) at 2% of added xylan. Except of the H/P parameter, similar effect were found for GX at the highest concentrations (1.7 and 2%). The low retrogradation parameters (C/P and C/H) indicate high stability of the pastes.

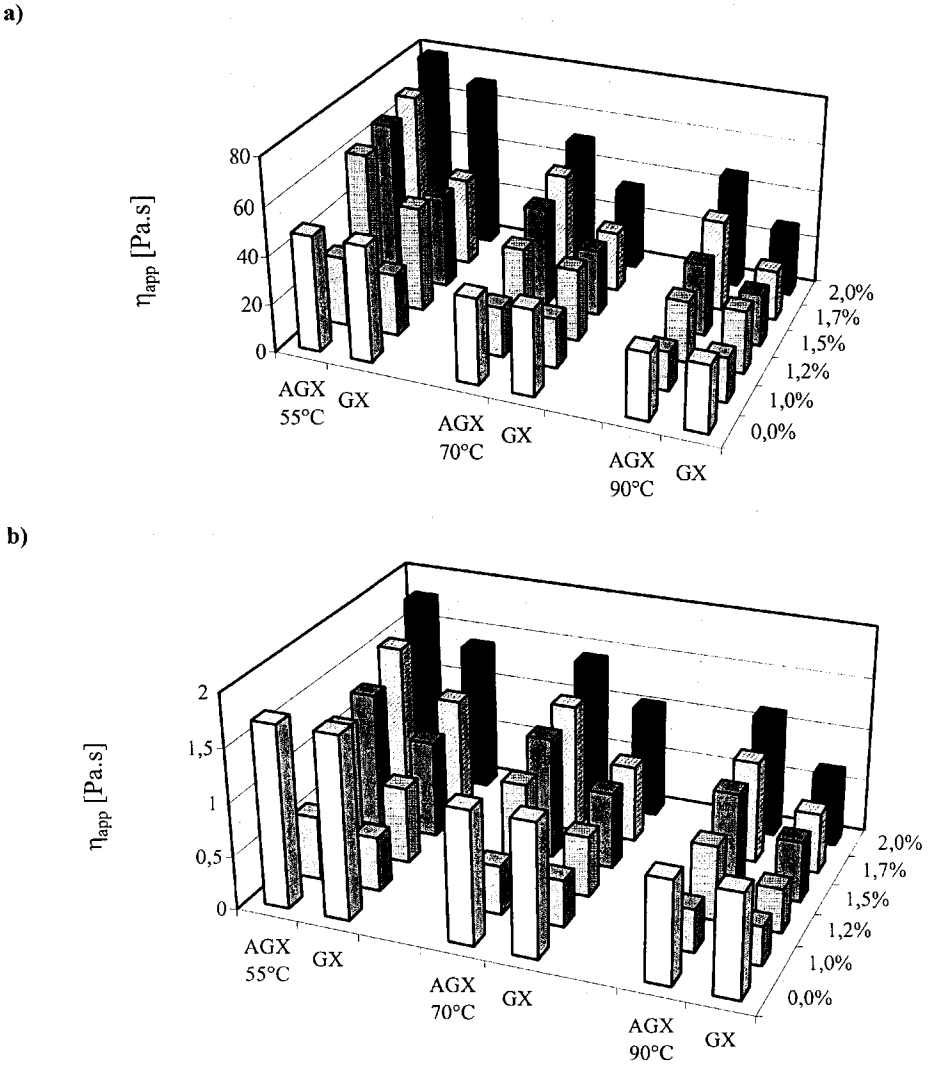


Fig. 3. Influence of AGX and GX on viscosity of potato starch pastes at shear rate:
a) D = 0.33 s^{-1},
b) D = 146 s^{-1}.

Table 2

Pasting data from the viscograms of corn starch/AGX and corn starch/GX blends.

	PT [°C]	P [BU]	H [BU]	C [BU]	BD [BU]	SB [BU]	C/P	C/H	H/P	T_{max} [°C]	P_t [min]
Standard	77.8	410	350	560	60	210	1.37	1.60	0.85	82.6	39.0
AGX											
1.0 %	81.3	290	270	440	20	170	1.52	1.63	0.93	89.0	42.7
1.2 %	79.8	330	310	490	20	180	1.49	1.58	0.94	79.4	36.5
1.5 %	79.4	340	320	510	20	190	1.50	1.59	0.94	85.8	41.5
1.7 %	74.6	340	330	530	10	200	1.56	1.61	0.97	84.2	37.0
2.0 %	76.2	370	350	460	20	110	1.24	1.31	0.95	87.4	38.0
GX											
1.0 %	77.8	260	255	440	5	185	1.69	1.72	0.98	87.4	35.2
1.2 %	79.4	280	275	490	5	215	1.75	1.78	0.98	87.4	36.3
1.5 %	79.4	340	335	570	5	235	1.68	1.70	0.98	84.2	36.3
1.7 %	85.8	340	330	370	10	40	1.09	1.12	0.97	93.8	40.5
2.0 %	85.8	420	290	540	130	250	1.29	1.86	0.69	87.4	40.5

PT: Pasting temperature (temperature at which the viscogram first ascends from the baseline).
P: Maximum viscosity in Brabender units (BU).
P_t: Time of reaching maximim viscosity.
H: Hot paste viscosity.
C: Cooled paste viscosity.
DB: Breakdown (P-H)
SB: Setback (C-H)
C/P: Retrogradation ratio
C/H: Total retrogradation ratio
H/P: Breakdown ratio

As shown in Tab. 3, the addition of GX and AGX to potato starch in the concentration range of 1–1.7% had similar effects as shown for the corn starch/xylan blends, i.e. the viscosity of the blends reached about the value of the standard at the highest dose (2%). At this dose, both GX and AGX improved significantly the retrogradation ratios C/P and C/H.

The final syneresis of the standard corn starch paste is substantially lower than that of the standard potato starch paste. There are differences between the process of syneresis of standard pastes based on corn and potato starch as well on the blends. Whereas, the syneresis with corn starch/AGX blends started in the third freeze-thawing cycle, no syneresis was observed with corn starch/GX blends. In the case of the standard and blends of potato starch with both xylans, syneresis was observed only in the first cycle. The blends containg 2% of GX showed the lowest syneresis.

Table 3

Pasting data from the viscograms of potato starch/AGX and potato starch/GX blends

	PT [°C]	P [BU]	H [BU]	C [BU]	BD [BU]	SB [BU]	C/P	C/H	H/P	T_{max} [°C]	P_t [min]
Standard	87.4	260	260	370	0	110	1.42	1.42	1.000	93.8	41.6
AGX											
1.0 %	85.8	140	140	160	0	20	1.14	1.14	1	93.8	40.5
1.2 %	85.8	200	200	280	0	80	1.40	1.40	1	94.0	40.5
1.5 %	81.0	210	210	330	0	120	1.57	1.57	1	94.0	37.3
1.7 %	73.0	250	250	350	0	100	1.40	1.40	1	93.8	32.0
2.0 %	84.2	260	260	330	0	70	1.26	1.26	1	93.8	39.5
GX											
1.0 %	81.0	150	150	190	0	40	1.26	1.26	1	92.2	37.3
1.2 %	87.4	160	160	220	0	60	1.37	1.37	1	94.0	41.6
1.5 %	82.6	180	180	240	0	60	1.33	1.33	1	92.2	38.4
1.7 %	90.6	200	200	280	0	80	1.40	1.40	1	94.0	43.7
2.0 %	87.4	280	280	280	0	0	1.00	1.00	1	93.8	41.6

Conclusions

The results indicated that both xylans exhibited a pronounced improvement in the flow properties of blends prepared from corn as well as potato starch at higher concentrations (1.7–2%). Despite of the differences in the pasting properties of the parent starches, those of their blends with both xylan types were significantly improved, particularly in the same concentration range. In comparison to AGX, GX had a higher positive effect on the syneresis of blends prepared from both starches. The result suggest that both xylan types represent potential additives used for stabilisation and thickening of starch-based food products.

Acknowledgement

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WPŁYW HEMICELULOZ NA WŁAŚCIWOŚCI SKROBI ZIEMNIACZANEJ I KUKURYDZIANEJ

Streszczenie

Hemiceluozy zbożowe pochodzą z polisacharydowych elementów ścian komórek roślinnych. Wywierają one olbrzymi efekt na sposób przeróbki oraz jakość produktów spożywczych zawierających skrobię, a przyczyną tego są oddziaływania między makrocząsteczkami polisacharydów. W niniejszej pracy opisano wpływ hemiceluloz wyodrębnionych z surowców niespożywczych, tzn. 4-O-metyloglucoronoksylanu z drewna bukowego i arabinoglukoronoksylanu z kolb kukurydzianych, na właściwości reologiczne i żelowanie skrobi ziemniaczanej i kukurydzianej. Przygotowano szereg mieszanek obu skrobi z obydwoma typami hemiceluloz dodanymi w ilości 1,0 do 2,0%. Właściwości reologiczne tych mieszanek charakteryzowano za pomocą krzywych płynięcia i skłonności do retrogradacji w trakcie trzech kolejnych zamrażań i rozmrażań. Oba rodzaje hemiceluloz przy stężeniu 2% bardzo wyraźnie polepszają synerezę skrobi.

[1]ALICA BURISOVÁ, [2]ZDENKA HROMÁDKOVÁ, [2]ANNA EBRINGEROVÁ, [1]KATARÍNA STADLEROVÁ, [1]ALEXANDER DANDÁR

FLOW PROPERTY STUDY OF CORN STARCH AFTER ADDITION OF HEMICELLULOSES FROM BUCKWHEAT HULLS

Summary

Buckwheat seeds are an important pseudocereal used as flour in baking industry. Also hulls, a waste material produced in the de-hulling process is of certain interest. Hemicelluloses were isolated from buckwheat hulls. They were characterised and shown to be of the glucuronoxylan type. Their effect upon rheological properties of gels of corn starch with varying amount of hemicelluloses (0.3%; 0.5%; 0.7% and 1.0%) is described. The rheological properties of the blends were investigated using the Brabender viscograph and Rheotest 2 viscograph. At the same pasting temperature, pastes containing an optimum of hemicelluloses (0.5–0.7%), exhibited the highest viscosity and stability against mechanical stress. At this optimum concentration, hemicelluloses increased substantially the apparent viscosity of the pastes at low and high shear rates and this trend was observed also with increase in temperature. Effect of the hemicellulose addition to corn starch on retrogradation of the pastes was studied in the refrigeration as well freeze-thaw processes. A minimum addition of hemicelluloses (0.3%) significantly affected the syneresis of starch.

Introduction

In the recent years, ground buckwheat has found many applications as flour in bakery due to the higher content of proteins and essential amino acids in comparison to cereal grains. Buckwheat plants are not grasses, but the seeds are usually classified among the cereal grains because of similar use [1]. Except of the flour, the hulls of the seeds produced in the de-hulling process are of great interest because they represent a rich source of hemicelluloses of the glucuronoxylan type [2]. The aim of this contribu-

[1]*Dr inž. A. Burisová, mgr inž. K. Stadlerová, prof. dr hab. A. Dandár, Department of Food Technology, Slovak Technical University, Faculty of Chemistry and Food Technology, Radlinského 9, 812 37 Bratislava, burisova@chtf.stuba.sk*

[2]*Dr inž. Z. Hromádková, dr hab. inž. A. Ebringerová, Institute of Chemistry Slovak Academy of Science, Dúbravská cesta 9, 842 38 Bratislava*

tion was to study the effect of the addition of hemicelluloses of buckwheat hulls on the rheological properties of corn starch pastes.

Materials and methods

Corn starch (Gustin) was a fine corn starch produced by Dr. Oetker s.r.o., Bratislava. Hemicelluloses were isolated from buckwheat hulls in the Institute of Chemistry, Slovak Academy of Sciences, Bratislava.

The flow properties of the starch pastes were determined using the coaxial cylinder viscometer Rheotest 2 (2-50 Hz, VEB MLW Prüfgeräte Medingen, Germany) with the S_3 measuring system (r/R = 0.81, D = 0.17–146 s^{-1}). The rheological tests were performed at temperatures 55, 70, and 90°C. Starch pastes were characterised using the Brabender viscograph (Brabender, Duisburg, Germany) and the deformation of the starch gels was measured by the Penetrometer AP 4/1 (VEB Feinmes, Germany).

Preparation of starch pastes: Blends of corn starch with hemicelluloses added in concentrations of 0.3, 0.5, 0.7, and 1.0% were prepared. For testing by Rheotest, the blends were suspended in water and the suspensions were heated in a boiling water bath for 10 min and further boiled for 15 min at constant stirring. The pastes were cooled to room temperature and used for rheological measurements. For Brabender experiments, the aqueous suspension of the starch blends were prepared at ambient temperature and then the pasting behaviour was measured at various temperatures for 2 h.

The retrogradation of the obtained starch pastes were measured after refrigeration at 4°C for one week and then heated to 40°C for 2h (repeated twice), and in four freeze-thaw cycles (-18°C for 16 h and 40°C for 2h). The amounts of absorbed and excluded water were determined in relation to the free water content of the starting pastes determined by centrifugation (3000 rpm/min for 10 min) of starch pastes.

For the penetration tests, the boiled starch pastes were cooled to room temperature for 20h in cylinders of constant dimensions and penetration was measured the next day at 5, 30 and 60 seconds.

Results and discussion

Analytical characteristics of the buckwheat hemicelluloses (Table 1) indicated that they comprised mainly of 4-O-methylglucuronoxylan (GX).

Rheological properties of the corn starch paste (standard) and pastes prepared from starch/GX blends were characterised with the flow curves measured at 55, 70, and 90°C using Rheotest (Figure 1). The curves indicated a positive effect of GX on the pseudoplastic behaviour of starch paste. In comparison to the standard paste, the increase in shear stress (τ) versus shear rate (D) was most pronounced in pastes con-

taining 0.5–0.7% GX what indicated a higher resistance of against mechanical stress. The effect of GX addition on the flow behaviour at low and high shear rates is illustrated in Figures 2a and 2b. At the same pasting temperature, pastes containing 0.5–0.7% GX, exhibited the highest apparent viscosity values at low and high shear rates and this trend was followed also with an increase in temperature.

Table 1

Analytical characteristics of hemicelluloses from buckwheat hulls

Protein %	Neutral sugar composition $x_i$mol %						
	Rha	Fuc	Ara	Xyl	Man	Glc	Gal
8.87	1.6	0.7	7.8	43.8	1.1	40.3	4.7

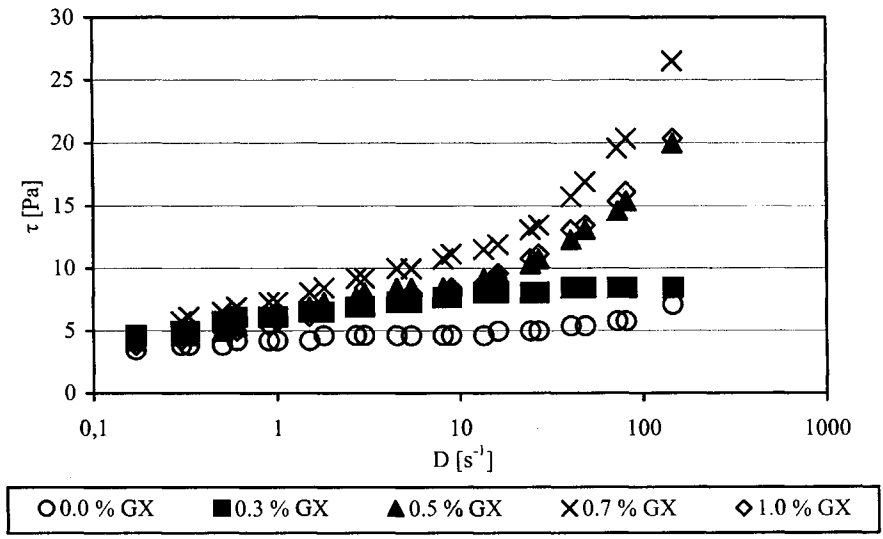
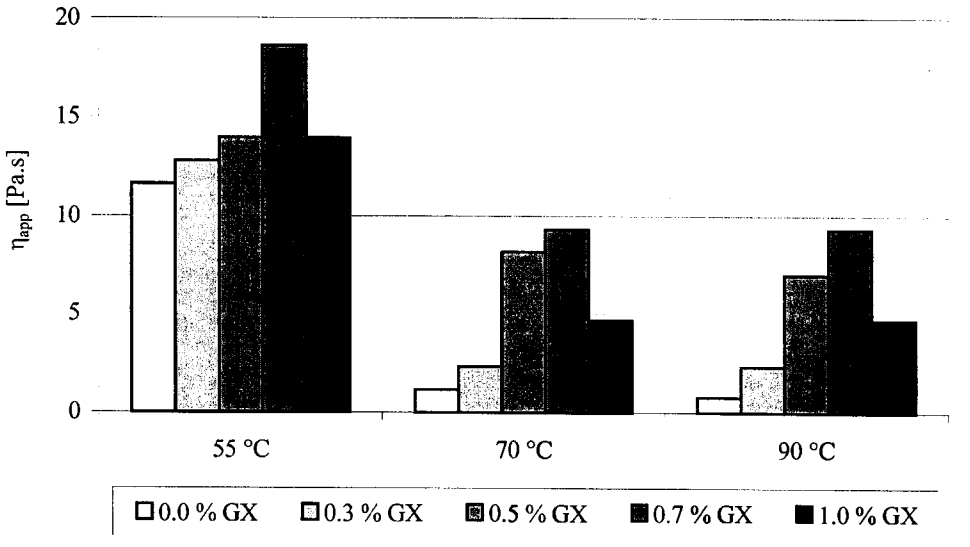


Fig. 1. Flow curves measured at 55°C.

The pasting behaviour of the pastes prepared from blends of corn strach and various amounts of GX are presented in Table 2. As seen, at 0.5% GX addition, the viscosity (P) reached a maximum. However, at higher GX concentrations, the viscosity values decreased but remained were higher as compared to that of pure starch paste (standard). The effect of GX addition is best expressed by the retrogradation ratios C/P and C/H, retrogradation and total retrogradation ratios, respectively. At the optimum of the GX concentration, both ratios showed the lowest value what indicates a high stability of the paste. With increasing GX concentrations, the ratios decreased but the values were lower than of the standard paste.

a)



b)

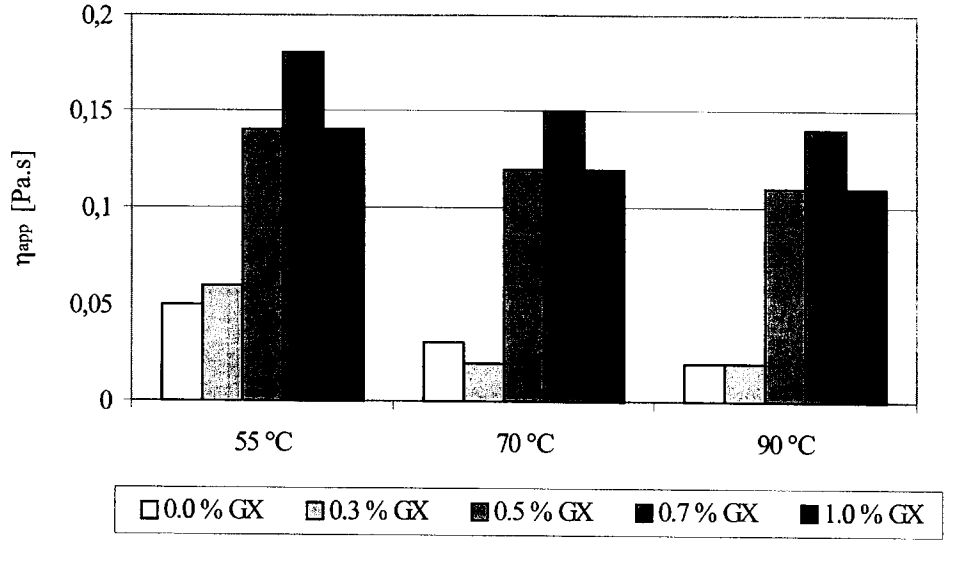


Fig. 2. Influence of hemicelluloses from buckwheat hulls on the viscosity of potato starch pastes at shear rate:
a) D=0.33 s^{-1},
b) D=146 s^{-1}.

Table 2

Pasting characteristics of corn starch/GX blends.

% GX	PT [°C]	P [BU]	H [BU]	C [BU]	BD [BU]	SB [BU]	C/P	C/H	H/P	T_{max}	P_t [min]
0.0%	83	350	340	890	10	550	2.54	2.62	0.97	89	38
0.3%	84	345	340	810	5	470	2.35	2.38	0.99	92	45
0.5%	86	420	410	850	10	440	2.02	2.07	0.98	92	45
0.7%	84	365	360	790	5	430	2.16	2.19	0.99	93	43
1.0%	83	345	340	780	5	440	2.26	2.29	0.99	92	44

PT: Pasting temperature (temperature at which the viscogram first ascends from the baseline).
P: Maximum viscosity in Brabender units (BU).
P_t: Time of reaching maximim viscosity.
H: Hot paste viscosity.
C: Cooled paste viscosity.
DB: Breakdown (P-H)
SB: Setback (C-H)
C/P: Retrogradation ratio
C/H: Total retrogradation ratio
H/P: Breakdown ratio

Retrogradation of the starch pastes prepared in the Brabender viscograph was characterised by means of the volume of excluded water after refrigeration and freeze-thaw cycles, respectively as shown in Table 3. With increasing amount of added GX, the volume of free water of starch/GX pastes decreased from 82 to 17.6% due to the high swelling capacity of GX and ability to bind water molecules [3]. The syneresis of starch was expressed as the volume of water excluded by the recrystallised amylose according to equation: S (%) = (% excluded water + % absorbed water) - % free water. The addition of GX showed non-signifcant effects on the syneresis during the two refrigaration cycles. However, distinct effects with an increasing tendency were observed during the freeze-thaw cycles. As seen in Figure 3, there is an optimum GX concentration influencing the syneresis. It was situated at 1.0% in the first three cycles and at 0.5% in the last cycle. This concentration fitted optimum of added GX found on the studies of the rheological and pasting behaviour.

Effect of the GX addition on the penetration of gels prepared from the boiled pastes is demonstrated in Figure 4. The penetration decreased as the concentration of the GX increased with a discontinuity at the concentration of 0.5–0.7%. The results indicatd that the gels containing GX were more rigid and resistant to deformation by pressure what might be explained by the gel-forming properties of GX-type hemicelluloses [4].

Table 3

Final syneresis [%].

Sample	Free water [%]	Refrigeration cycles		Freeze-thaw cycles			
		I	II	I	II	III	IV
0.0% GX	3.4	0	0.1	17.2	7.8	5.6	3.4
0.3% GX	2.8	0	0.2	11.2	6.0	4.4	2.0
0.5% GX	2.0	0	0.2	7.8	5.0	3.8	0.8
0.7% GX	1.3	0	0.3	6.1	3.9	3.1	1.1
1.0% GX	0.6	0	0	5.2	3.0	2.4	1.2

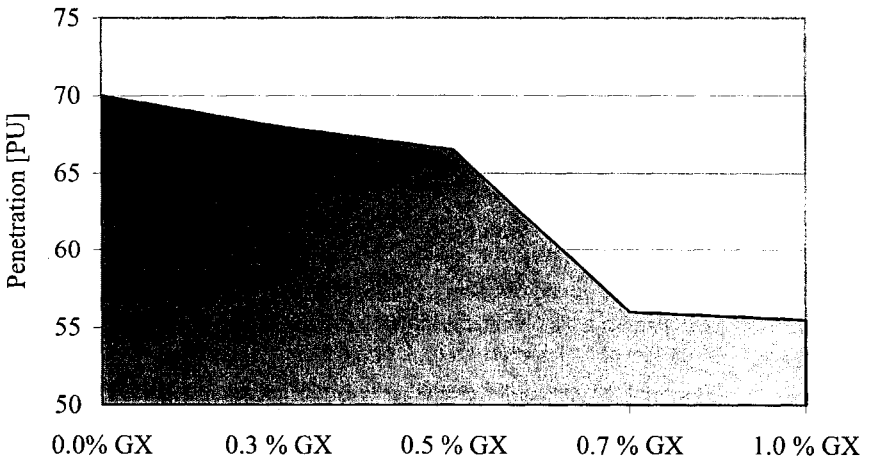


Fig. 4. Penetration of starch/GX gels after 5 sec of pressure deformation.

Conclusions

The results indicated that hemicelluloses of the GX type, isolated from the hulls of buckwheat seeds, significantly affected rheological and pasting properties of corn starch pastes at very low concentrations in comparison to studies [5] where water-insoluble GX from beech pulp were applied in concentrations above 20%. It can be suggested that GX from buckwheat hulls represented a potential additive in various corn starch-based food products due to their ability to force the structure of pastes, prevent retrogradation of starch, and improve staling properties of baked products.

Acknowledgement

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BADANIE PŁYNIĘCIA SKROBI KUKURYDZIANEJ PO DODANIU HEMICELULOZ Z ŁUSEK KASZY GRYCZANEJ

Streszczenie

Kasza gryczana jest ważnym pseudozbożem używanym przez przemysł piekarski. Także łuski kaszy gryczanej stanowiące odpad stają się obiektem zainteresowania. Można z nich wydzielić hemicelulozy glukuronoksylanowe. Zbadano wpływ ich dodatku (0,3, 0,5, 0,7 i 1,0%) na właściwości reologiczne skrobi kukurydzianej. W badaniach posłużono się wiskografami Brabendera i Rheotest-2. Przy tej samej temperaturze żelowania żele zawierające optymalną ilość hemiceuloz (0,5 do 0,7%) miały najwyższą lepkość i odporność na działanie mechaniczne. Przy tym optymalnym stężeniu hemiceuloz wyraźnie wzrastała lepkość pozorna żeli przy niskiej i wysokiej szybkości ścinania i ta tendencja utrzymywała się też w zakresie wyższej temperatury. Wpływ dodatku hemiceluloz do skrobi kukurydzianej na retrogradację żeli badano przez kolejne zamrażanie i rozmrażanie. Już minimalny (0,3%) dodatek hemiceluloz wywoływał znaczne zmiany synerezy żelu.

S. POLISZKO, D. M. NAPIERAŁA, R. REZLER, G. HOFFMANN

MOLECULAR DYNAMICS IN DEHYDRATED STARCH GELS

Summary

We have recently developed a transformation method for representation of different relaxation processes data (DMTA, DETA and 1H-NMR) as a function of free energy of activation in freeze-dried wheat starch gel. In our previous paper [12] the method of transformation was applied for freeze-dried wheat starch gel of density of 0.13 g/cm^3, while in this paper we present results of a similar analysis of relaxation parameters measured in a wide temperature range in dehydrated wheat starch gel of a twice lower density. In the system studied, we observed a decreasing value of the complex rigidity modulus, due to much lower degree of crosslinking. Similar courses of dispersion profile obtained with 1H-NMR relaxation method and DMTA method, at temperature ranging from 100 to 380 K, point to a correlation between the nuclear and mechanical relaxation processes, confirming the results of our previous work, which are related to the dynamics of hydroxymethylene groups in freeze-dried starch gel.

Introduction

Dehydrated starch gels have been commonly met as components of many food products based on starch and obtained in a form of glassy-crystalline extrudates such as corn flakes, crackers or chips. Molecular mobility and physical state of these biopolymers in freeze-drying starch products posses important and informative aspects related to food stability. Characterisation of the structure of freeze-dried product, texture, mechanical and thermal properties might help in understanding of the structure and behaviour of the corresponding frozen food. Several techniques such as DSC, SEM, X-ray diffraction, are used to study food in a glassy state [3-6, 8, 15]. Relaxation methods, DMTA (dynamic-mechanical thermal analysis) [4-6, 8, 10], NMR relaxation [2, 12], DETA (dielectric-thermal analysis) [11] are one of the most powerful tool to probe the structure of such material for determining changes in molecular mobility and dynamic biopolymer interactions. Each technique involves a type of external force

Department of Physics, Agricultural University of Poznań, 60-637 Poznań, Wojska Polskiego 38/42, dnapiera@woodcock.au.poznan.pl

under specified and controlled conditions in order to elicit a response from the material during measurements, thus probing the property of interest. It is expected that a comparison of different relaxation processes will serve to identify and characterise the specific motions responsible for the observed relaxation. The main purpose of this work is to verify formerly described transformation procedure, used for comparison of different relaxation experiments, applied to freeze-dried wheat starch gel of the density of 0.065 g/cm^3.

Theory

The spin-lattice proton relaxation rate (R_1) is a sensitive parameter describing molecular dynamics in a macromolecular system. In order to compare molecular response of a system to an external perturbation of mechanical, magnetic, and dielectric character, a transformation procedure was applied based on the theory of the absolute reaction rate. In the first step temperature changes of R_1 are transformed into its changes as a function of the magnetic field frequency. A NMR dispersion profile was obtained, which could be fitted to an adequate distribution function. As shown by Koenig [7], in a macromolecular system, spin-lattice magnetic relaxation experimental data can be fitted to the Cole-Cole [1] dispersion formula (1):

$$R_1 = A\frac{1+(\omega/\omega_c)^{\beta/2}\cos(\pi\beta/4)}{1+2(\omega/\omega_c)^{\beta/2}\cos(\pi\beta/4)+(\omega/\omega_c)^{\beta}} \tag{1}$$

where A, ω_c, and β are the degree of dispersion, inflection frequency, and steepness of the inflection, respectively.

The distribution parameters obtained by a computer fitting, enabled determination of the course of NMR relaxation time spectrum, using the formula (2) [14]:

$$\Phi(\ln\tau/\tau_c)=\frac{1}{2\pi}\frac{\sin(1-\beta/2)\pi}{ch[(\beta/2)(\ln\tau/\tau_c)]-\cos(1-\beta/2)\pi} \tag{2}$$

where τ and τ_c are the nuclear correlation time and mean correlation time at the maximum of the relaxation rate, respectively.

In the subsequent step the transformation eliminated effect of the difference in the frequency of the measuring force fields used in experiments, on the localisation of dispersion regions. In the case of local relaxation processes, relaxation as well as the correlation times satisfied the relation following from the theory of absolute reaction rate (3):

$$\tau = (\hbar / kT)\exp(\Delta F/RT) \tag{3}$$

where ΔF is the free energy of activation of relaxation process, $\hbar$, k, R are Planck, Boltzmann and gas constants, respectively, and T is a temperature.

Taking into account that angular frequency, $\omega = 1/\tau$, the free energy of activation of the relaxation process can be expressed by (4):

$$\Delta F = - RT \ln (\hbar\omega / kT) \quad (4)$$

This analysis provided normalised curves describing temperature or frequency changes in the relaxation parameters: complex rigidity modulus in mechanical relaxation, complex dielectric permitivity in dielectric relaxation, and function of nuclear correlation in NMR, and representing the spectrum of relaxation as a function of free energy of activation.

Materials and methods

Freeze-dried starch gel was prepared from solutions of *Triticum durum* wheat starch (Int. Grain Products, Canada) gelated on cooking for 1h on continuous stirring and maintaining constant concentration by addition of water. The solutions of the starch concentration of 0.05 g/cm^3 were used to fill cylindrical tubes stored in a dessicator for 24 h in the atmosphere of saturated water vapour at 298 K. After this time the cross-linked starch gel was subjected to sublimation drying in the lyophiliser (the LGA 05 type manufactured by MLW, Leipzig, Germany) at 284 K. As a result of 10% reduction of the sample volume the xerogel of the density of 0.065 g/cm^3 was obtained. The samples for measurements were in the form of rods (11 cm length, 0.55 cm diameter). Measurements were carried out in the temperature range of 100–380 K under nitrogen.

Dynamic mechanical - thermal analysis (DMTA) measurements

DMTA measurements were performed in the free vibration system (Spectra-Spin Poznań, Poland) based on the inverted torsion pendulum. One side of a cylindrical rod in the system was rigidly mounted and the other was attached to an inertia disk providing its free oscillation. The successive oscillation amplitudes decreased in time because of damping. It gradually converted the elastic energy of the system into heat. The mechanical system of the pendulum was fixed on the granite base mounted on a polystyrene table ensuring elimination of external interactions as vibrations of the base and other mechanical interactions. The measuring unit was equipped in an optical-electronic set for vibration periods and vibrating amplitudes reading. The dynamic mechanical technique was based on the analysis of the sinusoidal signals related to the applied stress and resulting strain of the sample. The frequencies of free vibrations and logarithmic decrements of damping were measured in the system with and without the sample. From the displacement – time curve, one could determine both the dynamic shear or torsion modulus G_1 and logarithmic decrement δ. The shear modulus G_1 (real

part of the complex shear modulus G) for a cylindrical rod was given by equation (5) [9]:

$$G_1 = (8 \pi I L / \gamma^4) (1/P^2) \quad (5)$$

where L. I, γ,. and P were the length of specimen, moment of inertia of the inertial member,, the cylinder radius, and period of oscillation in seconds, respectively. The logarithmic decrement Λ was calculated from the logarithm of the ratio of the amplitudes of two successive oscillations (6):

$$\Lambda = \ln \frac{A_1}{A_2} \quad (6)$$

where A_1 and A_2 were the amplitude of the first and the second oscillation, respectively.

It was related to the dissipation factor, tan δ by the relation: $\Lambda = \pi \tan \delta = \pi (G_2/G_1)$.

The data provided calculation of two components of the complex rigidity modulus, real part (G_1), and imaginary part (G_2) of the studied material. The real part G_1 of the complex modulus reflected the capability of the examined material to storage mechanical energy of the strain. The imaginary part G_2 was related to the energy dissipation processes and reflected a capability for exchange of mechanical energy into the heat. The mean frequency of mechanical perturbation was 0.1 Hz.

1HNMR spin-lattice relaxation measurements

The measurements of spin-lattice proton relaxation rate R_1, in freeze-dried wheat starch gel were carried out on the pulse, laboratory made solid-state NMR spectrometer (Institute of Physics, Adam Mickiewicz University, Poznań, Poland) operating at 25 MHz. The sequences of pulses composed of a saturating series were used, which nullifies the transversal component of magnetisation and a $\pi/2$ pulse measuring the recovery of the magnetisation vector. All magnetisation recovery curves were one-exponential functions of time.

Results and discussion

Temperature changes of complex rigidity modulus (both, real G_1 and imaginary part, G_2) and the spin-lattice proton relaxation rate, R_1 in freeze-dried starch gel of the density of 0.065 g/cm^3 are presented in Fig.1 and 2, respectively. Over 6-fold decrease in storage rigidity modulus with temperature increasing from 100 K to 300 K was observed. It is interesting to note that the lowest storage modulus obtained for the freeze-dried wheat starch gel of the density of 0.065 g/cm^3 at 300 K was exactly half of that observed for the freeze-dried starch gel of the density of 0.13 g/cm^3. This concentra-

tion dependence of the storage modulus pointed to a relation between the polymer

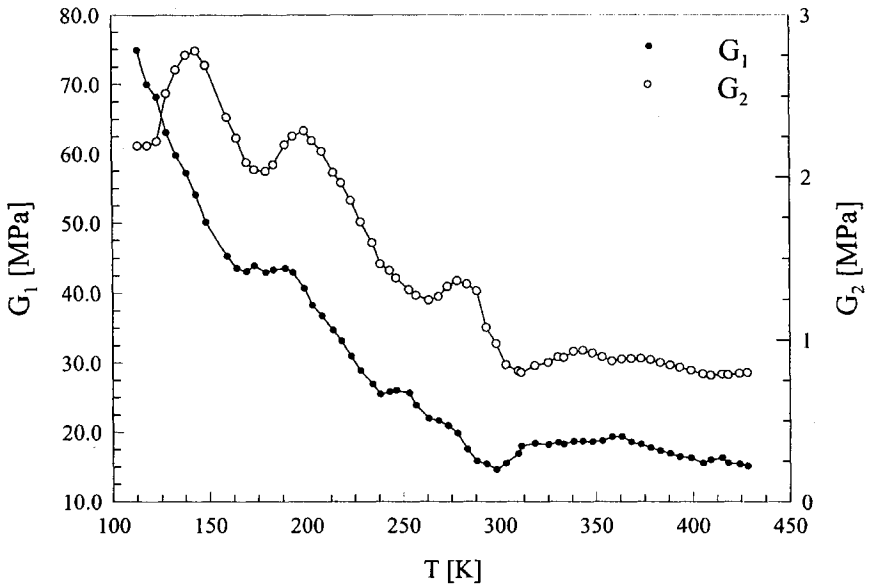

Fig. 1. Temperature dependence of the storage component (G_1) and loss component (G_2) of the complex rigidity modulus in freeze-dried wheat starch gels of the density of 0.065 g/cm^3.

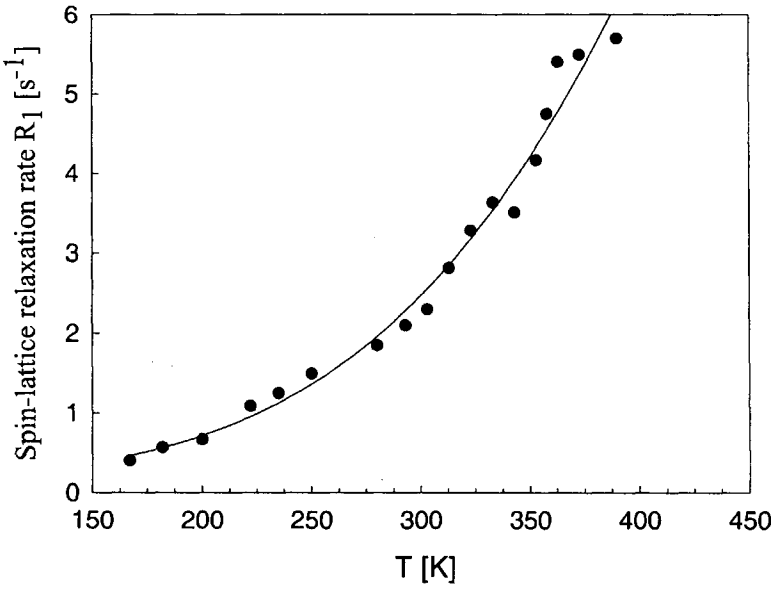

Fig. 2. Temperature course of spin-lattice proton relaxation rate R_1 in freeze-dried wheat starch gel of the density of 0.065 g/cm^3.

concentration in solution and the concentration of the network segments determining mechanical properties of the system after freeze-drying. The freeze-drying of starch gel resulted in fixing of the three-dimensional network of cross-links, forming a starch gel on cooling. The concentration of the network segments determined the mechanical properties of the product and the values of dynamic modulus of hydrogels formed [11].

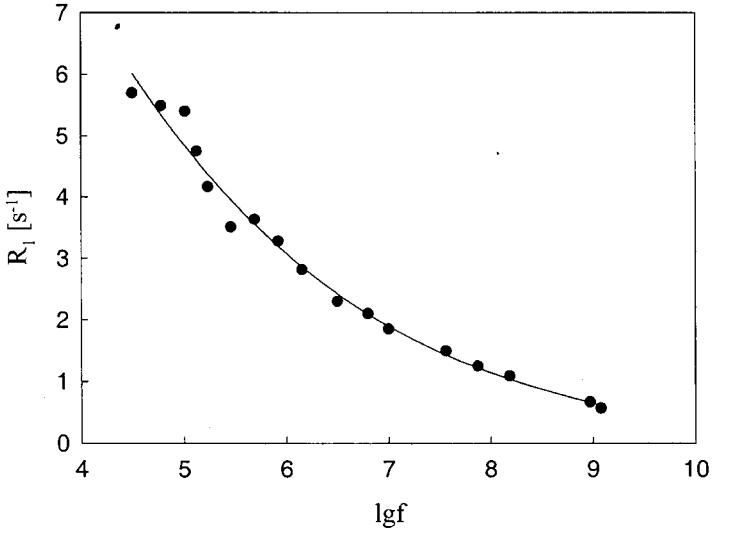

Fig. 3. 1HNMR dispersion profile for the freeze-dried wheat starch gel of the density of 0.065 g/cm^3. The solid line was calculated from the Cole - Cole Eq. (1).

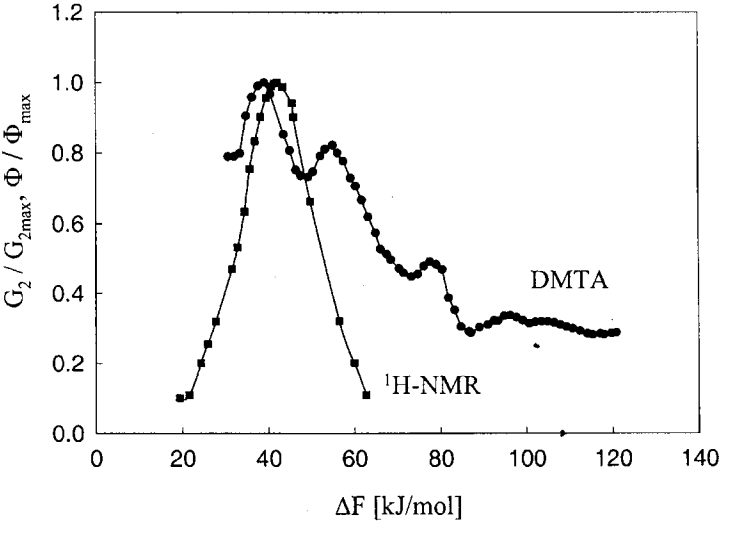

Fig. 4. The normalised curves of mechanical loss, G_2/G_{2max} and 1HNMR relaxation spectrum, $\Phi(\Delta F)/\Phi_{max}$ as a function of the free energy of activation of relaxation in freeze-dried wheat starch gel of the density of 0.065 g/cm^3.

As it is known from earlier study [13], for a given starch concentration, mechanical-rheological properties of freeze-dried starch in amorphous xerogel form were determined by the rigidity of macromolecule chains rather then by the network density. Moreover, the results have shown that complete dehydration of starch chains resulted in an increase of its rigidity by about 10^5. According to the theoretical assumptions, the temperature changes in R_1 were transformed into its changes as a function of the magnetic field frequency. An NMR dispersion profile shown in Fig. 3 was obtained, which was fitted to Eq. (1). The fitting procedure gave the following parameters of distribution: degree of dispersion A = 8.80 s^{-1}, inflection frequency ω_c = 0.65 MHz and steepness of the inflection β = 0.71. These parameters provided determination of the spectrum of correlation times from Eqs. (2) and (3) as a function of the free energy of activation of the relaxation process. The results of this transformation are shown in Fig. 4. The NMR relaxation spectrum was compared to the normalised curve of mechanical loss G_2/G_{2max}, representing the spectrum of mechanical relaxation. The position of the most intense maximum of mechanical relaxation was close to the pattern of the spectrum of magnetic relaxation. This maximum was characterised by the activation energy ΔF of about 40 kJ/mol. Because the proton magnetic relaxation method was sensitive to the dynamics of groups rich in protons, one could assume that the relaxation transition observed is attributed to the polar hydroxymethylene groups in starch polymer chains. The intrachain bonds of hydroxymethylene groups were responsible for the high rigidity of starch chains in the range of low temperatures. With increasing temperature, dissociation of these bonds induces an increase in the flexibility of starch polymer chains. The results of the temperature analysis of the relaxation phenomena in the glassy state of wheat starch gel of the density of 0.065 g/cm^3 showed the same character of the relaxation transitions recorded at 150 K by DMTA and at 320 K by 1HNMR, as in the previously studied freeze-dried starch gel of the density of 0.13 g/cm^3 with the same value of energy of activation.

The results indicated that the proposed method of reduction of the data obtained by different relaxation techniques to the functions of free activation energy could be considered as a very efficient tool in studying molecular dynamics in local dispersion regions of macromolecular system.

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BADANIE DYNAMIKI MOLEKULARNEJ ODWODNIONYCH ŻELÓW SKROBIOWYCH

Streszczenie

W pracy przedstawiono wyniki temperaturowych badań relaksacji mechanicznej (DMTA) i magnetycznej (1H-NMR) w liofilizowanym żelu skrobi pszenicy, otrzymanym w wyniku liofilizacji 5% kleiku skrobiowego. Do analizy danych, modułu sztywności (G_1 i G_2) i szybkości relaksacji spin-sieć protonów (R_1) zastosowano procedurę transformacji, testowaną wcześniej na układach o większej koncentracji sieci, eliminującą efekt różnicy częstości zewnętrznego pola zaburzającego. Procedura ta polega na przekształceniu zależności temperaturowych parametrów dynamiki mechanicznej i jądrowej na zależność od energii swobodnej aktywacji badanego procesu. Spośród trzech przejść relaksacyjnych obserwowanych w temperaturze 150, 210 i 280 K za pomocą zmian modułu sztywności kserożelu skrobi, jedno z nich, zachodzące w temperaturze 150 K związane jest z dynamiką molekularną tych samych grup, które odpowiadają za przejście relaksacyjne obserwowane w widmie 1H-NMR w temperaturze 320 K. Energia swobodna aktywacji tych procesów jest rzędu 40 kJ/mol. Są to najprawdopodobniej grupy hydroksymetylenowe łańcuchów skrobiowych, które jak wskazują wyniki, są także odpowiedzialne za wysoką sztywność sieci dehydratowanego żelu w niskiej temperaturze.

K.-J.APPENROTH[1], R. REIMANN[1], F. KROK[2], J. SZYMOŃSKA[3*]

STARCH GRAIN SURFACE AND STARCH DEGRADATION IN TURIONS OF THE DUCKWEED *SPIRODELA POLYRHIZA (LEMNACEAE)*

Summary

Turions are survival organs of aquatic plants such as the great duckweed (*Spirodela polyrhiza*). They consist of approximately 50% storage starch (per dry weight) used to support the growth of newly formed sprouts following germination. They could be employed as a good model system for investigations of the storage starch degradation in plants. To induce starch degradation in the plant cells turions must be irradiated for a few days with continuous light absorbed by the plant photoreceptor phytochrome. During such treatment changes in the profile of proteins associated with the starch grain surface have been observed. It was shown by *in vitro* binding studies that several proteins (α-amylase, starch dikinase R1, β-amylase) are desorbed from the surface or lose the ability to bind to it. This effect was especially obvious when starch grains from turions irradiated for 4 days (irradiated samples) were compared to those from turions kept in darkness (dark control). A hypothesis was presented that unknown changes in the surface properties of starch grains might be very important in the mechanism of starch degradation, by altering the binding of proteins.

The aim of the study was to investigate these properties immediately before and after the start of the starch degradation. Precise structural analysis of the starch grain surface was performed using a non-contact atomic force microscopy (nc-AFM). The grain surface revealed increasing roughness and a reduced density of the structural elements in the samples after irradiation. Two different kinds of randomly organized surface elements were detected by nc-AFM: the one type of a globular structure and the other one more oblong. They could be considered as the carbohydrate lamellas situated in the different way at the starch granule surface. Both were observed to become larger after irradiation. This might be a result of binding of water molecules to the carbohydrate lamellas or bending the surface carbohydrate helices into superhelices by new inter-carbohydrate hydrogen bonds. Such a modification of the starch granule surface could be a consequence of events started by the photoreceptor phytochrome involving starch phosphorylation / dephosphorylation, perhaps mediated by the newly discovered starch dikinase.

[1]*University of Jena, Plant Physiology, Dornburger Str. 159, D-077743 Jena, Germany*
[2]*Regional Laboratory for Physicochemical Analyses and Structural Research, Jagiellonian University, ul. Ingardena 3, 30-060 Kraków, Poland*
[3]*Department of Chemistry, Agriculture University, Al.Mickiewicza 24/28, 30-059 Kraków, Poland*

Intoduction

Turions are resting fronds of aquatic vascular plants. In *Spirodela polyrhiza* (duckweed), turions have an important function in the survival strategy of the plants as vegetative fronds cannot tolerate low temperatures, and, therefore, usually die during late autumn. These resting fronds overcome unfavourable seasons by sinking to the bottom of the ponds or lakes [6]. Turions contain two meristematic pockets from which new vegetative sprouts can develop following germination [1]. In spring, after rise of the temperature, turions germinate. The main storage compound in turions is starch [4]. It has been shown previously that starch does not seem to contribute to early events in germination. Instead, starch fulfils two distinct functions [7]. Firstly, starch secures the survival of turions during periods of unfavourable germination conditions by very slow degradation lasting for months or even years. Secondly, it supports accelerated growth of the newly formed sprouts following germination, in a faster degradation response lasting for a few days. This second response is regulated by light and this light effect is mediated by the plant photoreceptor phytochrome. Turions could be considered as a model system for investigation of the mobilisation of storage starch in plants. Starch degradation in turions could be induced by repetitive red light pulses (Rp) as reported by Dölger et al. [4]. Whereas one Rp per day, applied for a period of 6 days, shows already a measurable effect, the full response has only been observed after hourly applied Rps [3]. These results were explained in terms of a developing source-sink system and by the existence of two separate steps in the process of starch degradation in turions: formation of a sprout (= sink) during the Rp-induced germination, and starch degradation in the storage tissue (= source) induced by the second light treatment.

Following various light pre-treatments on *Spirodela polyrhiza* turions, native starch granules were isolated and two fractions of starch-related proteins were distinguished: proteins enclosed within the starch particles (starch-internalised proteins) and those attached to the surface (starch-associated proteins). Two starch associated proteins were identified immunochemically as α-amylase (EC 3.2.1.1) and the R1 protein [8, 9]. Continuous illumination with red light induces a rapid degradation of starch. Within the first 24 h of illumination the level of starch-associated α-amylase transiently increased and subsequently decreased rapidly. Similarly, the amount of the starch-associated R1 also decreased during illumination. The dissociation of both α-amylase and R1 from the starch granules preceded the decrease in starch content [9]. However, binding of the two proteins to starch granules remained unchanged when the turions did not perform net starch degradation as observed during continuous darkness. Thus, during net starch degradation so far unidentified changes are postulated to occur at the surface of the starch particles that are relevant for protein binding. This conclusion was supported by *in vitro* studies. The enzyme did bind to starch granules pre-

pared from dark-stored turions (in which starch degradation had not been initiated), but not to those isolated from illuminated (starch degrading) turions [9].

The aim of the study was to investigate the starch granule surface properties immediately before and after the start of the starch degradation and to detect possible changes in the presence of proteins at the starch grain surface.

Material and methods

Material

Formation of the turions

All experiments were performed using etiolated turions of the duckweed *Spirodela polyrhiza* (L.) Schleiden, strain SJ cultivated in the way described elsewhere by Appenroth *et al.* [2]. To obtain non-dormant turions (that are capable of phytochrome-induced germination), cold treatment (5 ± 1°C) was carried out for additional 28 days in continuous darkness. Except the cold period, turions were kept at 25.0 ± 0.1°C.

Isolation of starch granules

Following various light treatments, turions and newly formed sprouts were frozen in liquid nitrogen and stored at – 80°C until use. The samples were prepared according to the method described by Ritte *et al.*(2000). The obtained starch fraction was washed twice with buffer 0.5 M HEPES-KOH, pH 7.0 (5 ml and 1 ml, respectively), and dried to dryness under vacuum for approximately 8 h. The samples were stored at – 80°C until use. Starch was quantified according to Ley *et al.* [7].

Methods

Light sources and irradiation

For the irradiation experiments the following light sources and filters were used: red light pulses (λ_{max} = 683 nm, half-bandwidth 63 nm, 490 μmol m^{-2} s^{-1}, 5 min) were applied using a slide projectors (Diafant 250, Liesegang, Düsseldorf, Germany; 24 V / 250 W) equipped with a glass filter RG645, 3 mm thick (Schott, Mainz, Germany) and a dichroic filter IR7, 3 mm thick (OptoChem, Stromberg, Germany); for illumination with continuous red light (λ_{max} = 658 nm, half-bandwidth 25 nm, 12 μmol m^{-2} s^{-1}) red fluorescence tubes (36 W/ 60; Osram, München, Germany) plus a red Plexiglas (GS501, 3 mm thick; Röhm, Darmstadt, Germany) were used. All manipulations of the turions were carried out in dim green light (λ_{max} = 553 nm, half-band width 8 nm, < 0.2 μmol m^{-2} s^{-1}) as described before by Appenroth *et al.* [2].

Non-contact atomic force microscopy

High-resolution non-contact Atomic Force Microscopy (nc-AFM) was performed using a Park Scientific Instrument Autoprobe CP model (California, USA) of the Regional Laboratory for Physicochemical Analyses and Structural Research at the Jagiellonian University, described elsewhere [5, 12]. Starch granules were spread onto an adhesive tape fixed onto an AFM sample holder, and observed at ambient conditions. The granules were partially embedded in the "sticky tape" to overcome problems with the large height variation in granule topography. For each starch sample images of several starch granules were collected.

Results and discussion

The surface of control starch grains isolated from dark kept turions of *Spirodela polyrhiza* and analyzed by nc-AFM is shown in Fig. 1. The starch grain surface revealed increasing roughness and a reduced density from ca.160/μm^2 to 50/μm^2 of its structural elements after irradiation with continuous red light for 4 days. Two kinds of randomly organized surface species were detected at the starch granule surface by nc-AFM: the one type of a globular structure and the other more oblong (Fig. 1 and Fig. 2). They could be considered as the carbohydrate lamellas situated in different ways at the starch granule surface. Those, densely packed were visible from the top-side (so detected as globular elements), while the other-loosely packed, laying at the surface were side-viewed by the microscope (and detected as oblong species). This observation indicates that the structure elements were not uniformly distributed at the grain surface. After the irradiation the surface species became larger. It was estimated that the granular elements of the dark control samples (no starch degradation) have a diameter of approximately 60 nm whereas the same elements from samples irradiated for 4 days (starch degradation) have a diameter of 100 nm (Fig. 3 and Fig. 4). The oblong elements were approximately 50 nm thick and 120 nm long before irradiation. Following a red light irradiation, the size of these elements increased to approximately 70 nm and 170 nm, respectively (Fig. 5 and Fig. 6). The observed modification of the grain surface is most probably not the result of a physical interaction of starch and light but, more indirectly, a consequence of events started by the plant photoreceptor phytochrome involving starch phosphorylation / dephosphorylation. The function of the so-called R1 protein as starch dikinase, postulated already several years ago [8], was recently revaeled by Ritte *et al.* [11]. The phosphorylation level may enhance the binding of more water molecules to the carbohydrate helices or may induce the formation of new hydrogen bonds between lamellar helices present at the granule surface, which bend together into superhelices. Such a process, involving of some surface OH-groups, supported by the decreasing of the surface element density, might result in lowering of

the granule surface capacity towards proteins. The decreased protein binding under starch degrading conditions was shown in several systems [9, 10]. A number of problems still remains to be investigated to understand the role of the starch grain surface properties in the starch degradation process.

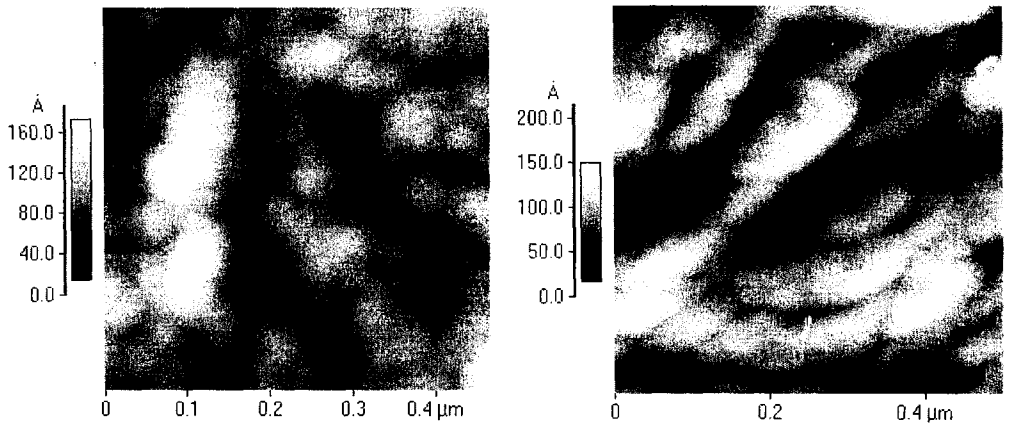


Fig. 1. Nc-AFM images of the surface elements of the dark control (native) starch samples.

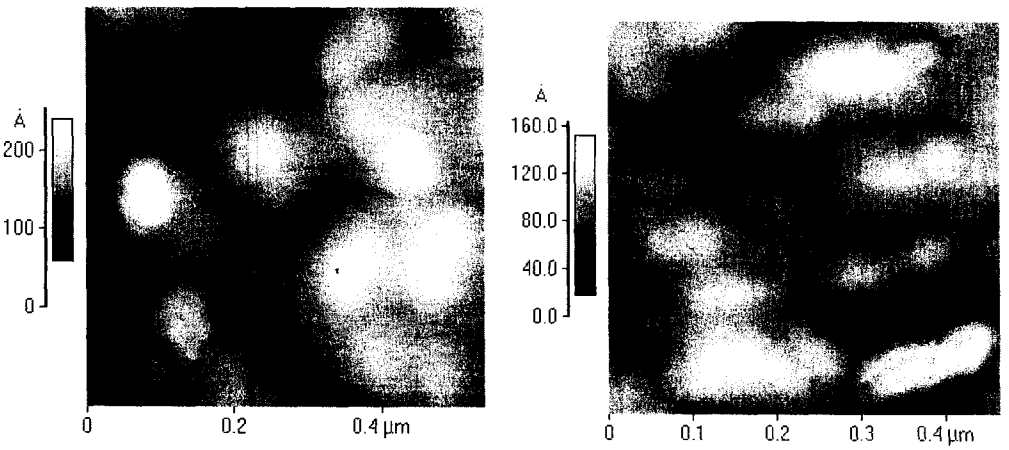
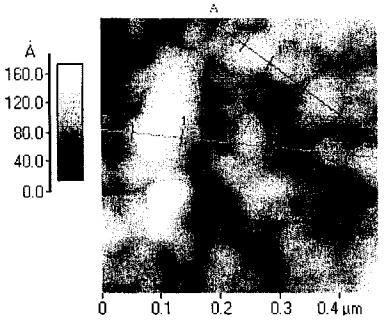
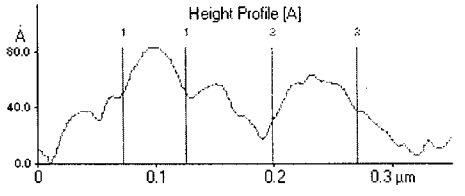


Fig. 2. Nc-AFM images of the surface elements of the irradiated starch samples.



Line	Distance [nm]
[A]	1: 52,2 2: 73,0
[B]	1: 82,7 2: 75,4 3: 60,7



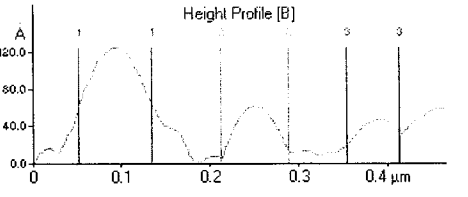
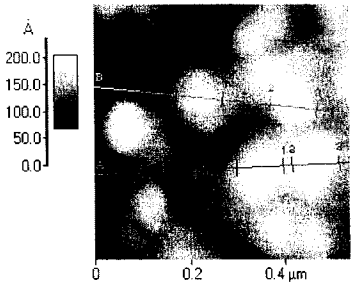
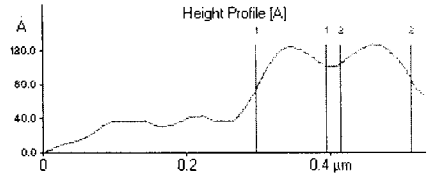


Fig. 3. Dimensions estimated for the granular species observed at the surface of the dark control starch grains.



Line	Distance [nm]
[A]	1: 98,9 2: 98,9
[B]	1: 97,1 2: 99,4



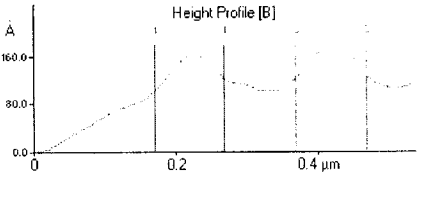
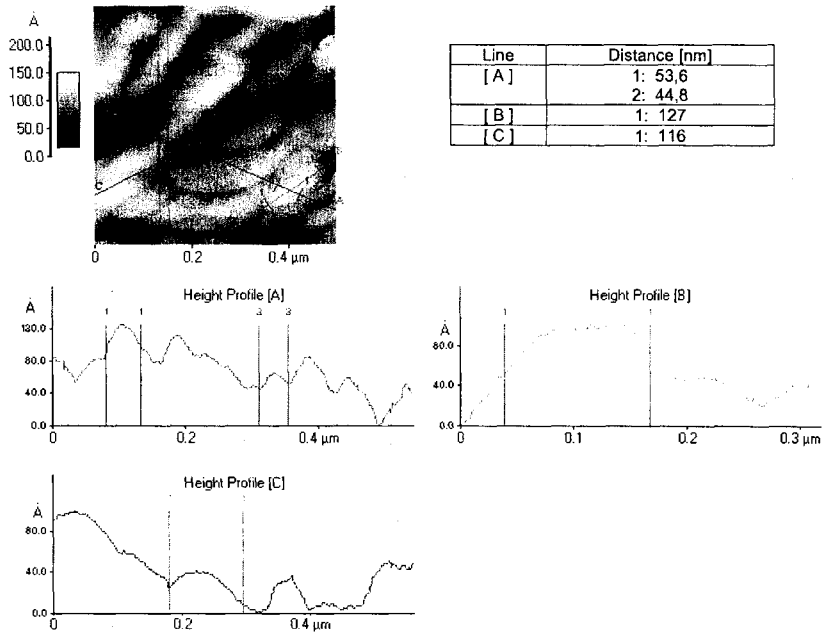
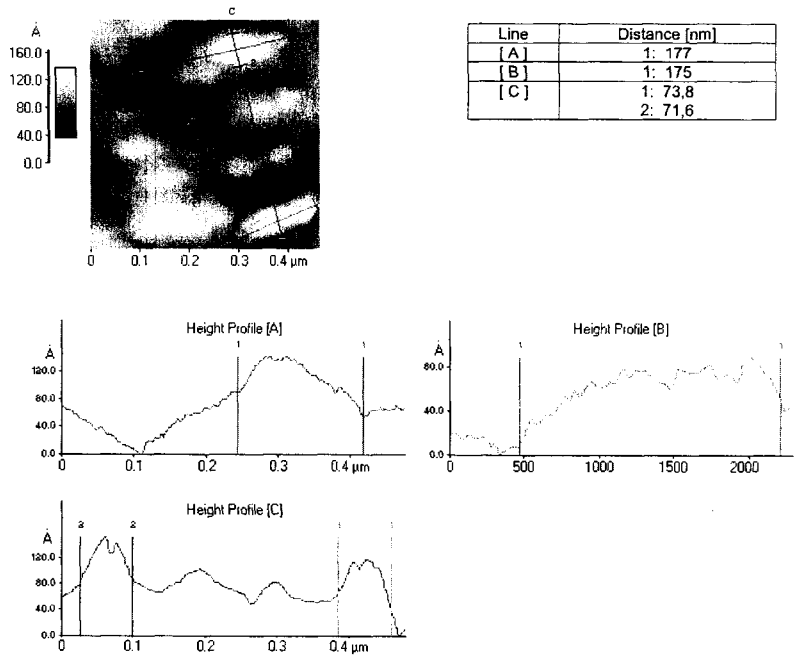


Fig. 4. Dimensions estimated for the granular species revealed at the surface of the irradiated starch grains.



Line	Distance [nm]
[A]	1: 53,6 2: 44,8
[B]	1: 127
[C]	1: 116

Fig. 5. Dimensions estimated for the oblong species revealed at the surface of the dark control starch grains.



Line	Distance [nm]
[A]	1: 177
[B]	1: 175
[C]	1: 73,8 2: 71,6

Fig. 6. Dimensions estimated for the oblong species observed at the surface of the irradiated starch grains.

Conclusions

1. Red light irradiation caused an increasing of the granule surface roughness and reduced density of the structural elements. These structural elements became larger after irradiation probably due to binding of water molecules to the carbohydrate lamellas or bending the surface carbohydrate helices into superhelices by new inter-carbohydrate hydrogen bonds.
2. Observed modification of the starch granule surface could be a consequence of events started by the photoreceptor phytochrome involving phosphorylation / dephosphorylation of starch. This could result in lowering of the granule surface capacity towards binding proteins.
3. A number of problems still remains to be investigated to understand the role of the starch grain surface properties in the starch degradation process.

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ZMIANY POWIERZCHNI GRANULI SKROBIOWEJ A DEGRADACJA SKROBI W TURIONACH ROŚLINY WODNEJ *SPIRODELA POLYRHIZA (LEMNACEAE)*

Streszczenie

Turiony są organami przetrwalnikowymi roślin wodnych, jak *Spirodela polyrhiza* Zawierają ok. 50% skrobi, która jest zużywana na wspomaganie rozwoju nowo powstających kiełków. Turiony mogą służyć jako modelowy system do obserwacji procesu degradacji skrobi w roślinach. Rozpoczęcie tej degradacji, po kilkudniowym naświetleniu turionów światłem ciągłym, absorbowanym przez fotoreceptor roślinny – fitochrom. Początek degradacji skrobi jest związany z desorpcją protein (alfa-amylaza, beta- amylaza czy skrobiowa dikinaza R1) z powierzchni granul skrobiowych. Efekt ten jest szczególnie wyraźny po porównaniu skrobi pochodzącej z turionów roślin naświetlanych przez 4 dni ze skrobią turionów roślin trzymanych w ciemności. Założono więc, że naświetlanie powoduje nieznane dotychczas zmiany na powierzchni granuli skrobiowej, które wpływając na wiązanie protein decydują o mechanizmie degradacji skrobi. Celem badań było obserwowanie powierzchni gałeczek skrobiowych przed i po naświetlaniu, czyli tuż przed i po starcie degradacji skrobi. Precyzyjna analiza powierzchni gałeczek skrobi była wykonana metodą bezkontaktowej mikroskopii sił atomowych (nc-AFM). W próbkach skrobi naświetlanej stwierdzono większą chropowatość powierzchni gałeczki skrobiowej i luźniejsze upakowanie jej elementów. Na badanych powierzchniach zaobserwowano przypadkowo rozmieszczone elementy o dwu rodzajach kształtów: bardziej okrągłe lub podłużne. Można uważać je za węglowodanowe łańcuchy w różny sposób usytuowane na powierzchni granuli. Stwierdzono, że po naświetlaniu powierzchniowe „cegiełki" zwiększają swoje rozmiary. Może to być spowodowane przyłączaniem cząsteczek wody do łańcuchów glukozowych albo też efekt łączenia się tych łańcuchów w większe poprzez międzycząsteczkowe wiązania wodorowe. Obserwowana modyfikacja powierzchni jest prawdopodobnie skutkiem zdarzeń zapoczątkowanych przez fotoreceptor fitochromowy, a obejmujących fosforylację/defosforylację skrobi przy współudziale nowoodkrytej dikinazy skrobiowej.

WRONKOWSKA MAŁGORZATA[1], SORAL-ŚMIETANA MARIA[1],
BIELECKA MARIA[2], BIEDRZYCKA ELŻBIETA[2]

PHYSICALLY-MODIFIED WHEAT OR POTATO STARCHES, THEIR PHYSICO-CHEMICAL PROPERTIES AND METABOLISM BY *BIFIDOBACTERIA*

Summary

The aim was to study the effect of physical processes (autoclaving/cooling cycles and spray drying) on starches having different crystalline structure: wheat (type A) or potato (type B) starch. First, the extent of changes in the physico-chemical properties of these physically-modified starches as the carbon and energy sources for growth in *in vitro* conditions was investigated.

Characteristics of functional properties, e.g. water binding capacity (WBC), indicated that both native starches had low affinity to water, that increased however 4-times after modification. The opposite tendency was observed for fat absorption (FA). Viscosity of water dispersion dramatically decreased after modification of both starches.

The ability of the tested *Bifidobacterium* strains to metabolise native or physically- modified wheat and potato starches was differentiated. *B. pseudolongum* KSI9 and *B. animalis* KSD29a3 isolated from animals utilised the examined starches as easily accessible substrates of fermentation, whereas *B. breve* ATCC 15700 isolated from human did not metabolise or only negligibly fermented starch preparations. The number of bifidobacteria populations as well as their acidifying activity were higher in the media containing wheat starch in comparison to the potato starch, whereas no significant differentiation was observed between the results obtained in media with native or modified starch.

The results suggest that native or experimentally-modified wheat and potato starches with some fraction of resistant starch can be a good substrate for colonic bifidobacteria.

Introduction

Botanical origin of native starch determines different diffraction patterns, i.e. A-type and B-type. When crystallising linear chains in solution, during the *in vitro* studies, the A-type is favoured kinetically and B-type thermodynamically [11]. The struc-

Polish Academy of Sciences, Institute of Animal Reproduction and Food Research, Division of Food Science, 10-747 Olsztyn, ul.Tuwima 10, Poland
[1]Department of Functional Properties of Food, [2] Department of Food Microbiology

ture of the A-type is obtained preferentially under conditions of high crystalisation temperature, high polymer concentration, and short chain length [12]. For native starches, amylopectin molecules from A-type starches have shorter constitutive chains and higher numbers of short-chain fractions than those from B-type starches [15]. It seems that mechanism of the retrogradation process is analogous to that found for many other helix-forming polysaccharides. This stems from the central role of double helix formation in either amylose or amylopectin retrogradation behaviour. For long amylose chains, gelation and related network properties are a direct result of multiple helix formation creating a meshwork of cross-links between chains in an exactly analogous mechanism to e.g. gelatin or agar [13]. For amylopectin, the analogies are fewer due to the unusual clustering of relatively short branches. Complexity increases when mixtures of amylose and amylopectin are being retrograded. As a result of physical modification, the following changes can be observed in the granules of native starches: swelling, gelatinisation, solubilisation, retrogradation. This distributional heterogenity has an impact on functional properties.

The large bowel harbours nutritionally and physiologically diverse range of bacteria, promoting normal intestinal function, and offering the host protection against infections [18]. Bifidobacteria are generally considered beneficial for human health and together with lactobacilli are widely used in probiotic preparations and foods. Several positive effects have been related to bifidobacteria. These include synthesis of vitamins, supplementation in digestion and absorption, inhibition of growth of exogenous organisms, and stimulation of the immune system. Bifidobacterial numbers in the human gut tend to decrease with age [16]. To maintain a high level of bifidobacteria in the gut a two-fold strategy can be applied. Numbers of bifidobacteria can be increased either by continuous ingestion of bifidobacteria-containing preparations or foods, or food can be supplemented with substrates (bifidogenic factors or prebiotics) that specifically promote the growth of endogenous bifidobacteria in the gut [14].

Amylose-resistant starch is considered a very good substrate easily fermented by microflora of the large bowel. This fermentation is of significant importance when it comes to environment and functioning of this part of the alimentary tract. Apart from gas products (hydrogen and methane), short-chain fatty acids (acetic, propionic, and butyric) are produced [7]. Proportion between short chain fatty acids, e.g. acetic, propionic and butyric, produced during 24-hour fermentation *in vitro* has been indicated to depend on the origin of resistant starch [21]. As a substrate for rats intestine microflora, resistant starch from wheat and potato can affect the pH lowering of fermented medium and that of potato origin can be a source of valeric acid during fermentation over 12 hours. It was stated that during fermentation of amylose-resistant starch relatively higher amount of short-chain fatty acids is formed, compared to fermentation of dietary fibre fractions [6, 9, 22]. It has been also suggested that at simul-

taneous fermentation of resistant starch and dietary fibre, the starch undergoes fermentation first [8].

The aims of the study were to determine how the gelatinisation, retrogradation and dehydration in stream of hot air affect the properties of wheat or potato starches, and whether native or modified starches can be a substrate for the growth of certain *Bifidobacterium* strains that inhabit the human or animal intestine.

Material and methods

Material

Commercial wheat and potato starches were investigated. The modification was made in a laboratory scale using such physical processes as autoclaving/cooling cycles and spray-drying for dehydration, according to technological procedure described earlier [24].

Methods

Chemical components of native and modified starches were determined as follows: nitrogen by the Kjeldahl method and ash after mineralisation in muflon oven at 700°C according to the AOAC standard chemical methods [1]. The content of elements was analysed according to AAS method, after wet mineralisation by the mixture of nitric and perchloric acids.

The amylose content was determined according to Morrison et al. [17]. The resistant starch analysis was carried out using the Champ method [4]. Functional properties as water binding capacity (WBC) and fat absorption (FA) were also analysed [19]. The course of gelatinisation was followed with Brabender viscograph procedure under the following conditions: 8% water dispersion was measured in cartridge 700 cmg; heating/cooling (25–95°C) with the rate of 1.5°C/min; thermostating for 30 min.

The following strains of *Bifidobacterium* were tested: reference strain *B. breve* ATCC 15700, and *B. pseudolongum* KSI9 as well as *B. animalis* KS29a3 all isolated in the Department of Food Microbiology. They were selected in the preliminary studies on the basis of the ability to utilise starch using standard API 50CH test (BioMerieux) and commercial potato starch. Starch utilisation was evaluated by the following criteria: growth and acidifying activity of bifidobacteria for the examined substrates, compared with glucose. The ability of *Bifidobacterium* strains to metabolise native or modified, wheat or potato starches was studied in liquid minimal media, which contained meat peptone 1% (w/v), L-cysteine hydrochloride 0.04% (w/v), buffering salts and essential ions, final pH 6.4 [2]. After dispersing 1% (w/v) of glucose or the examined starch preparations in the medium, they were immediately heat-treated (95°C/10 min). The media were inoculated with active bifidobacteria cultures

(~$1 \cdot 10^7$ cfu/mL) and incubated at 7°C for 24 h in anaerobic conditions (pyrogallol stoppers). After incubation, the number of bifidobacteria was determined in modified Garche's medium [2] using the plate technique. The plates were incubated at 37°C for 48 h in anaerobic jars – Oxoid Anaerobic System with Gas Pak H_2+CO_2. Acidifying activity, as pH of fermentation medium, was determined for each strain.

Results and discussion

Analysis of the chemical composition of the investigated material indicated that, as a result of the modification process used, only negligible part of amylose fraction was released (tab. 1). However, the content of resistant starch decreased, compared to natural starches prior to modification. As a result of gelatinisation and retrogradation, the III-type resistant starch was obtained, while in native starch – type II RS is present [20].

Table 1

Chemical composition of native and modified starches.

Sample	Amylose [% d.m.]	Nitrogen [% d.m.]	Ash [% d.m.]	Resistant starch [% d.m.]
Wheat starch:				
native	21,34	0,04	0,25	22,22
modified	19,08	0,12	0,26	7,76
Potato starch:				
native	28,75	0,03	0,45	61,60
modified	26,72	0,15	0,41	8,58

Table 2

Content of microelements in native and modified starches.

Sample	Content of elements [μg/g d.w.]								
	Ca	Mg	Na	K	P	Cu	Mn	Fe	Zn
Wheat starch:									
native	83.45	17.30	220.40	103.37	473.51	0.10	0.29	0.90	0.99
modified	267.13	42.05	271.31	130.04	513.93	4.83	0.49	11.03	5.23
Potato starch:									
native	416.02	63.61	17.21	54.91	581.68	0.15	0.53	1.55	0.42
modified	678.59	98.56	51.99	90.17	663.78	1.16	0.89	8.31	12.13

Therefore, physiological process is connected with a different substrate what is clearly classified from the nutritional point of view [10]. Physically-modified starches

were characterised by a higher content of macroelements: Ca, Mg, and Na, compared to native starches (tab. 2). The content of other elements also increased in starches subjected to modification, what was connected with changes in the granular structure of starch as well as with production of heterogeneous mixture of amylose and amylopectin as a consequence of the technological processes applied.

Modification of the investigated starches caused a change in their character, compared to native starches which gained hydrophilic character (fig. 1). On the basis of Brabender viscosity curves (fig. 2, 3), it was stated that the course for native starches was typical of that kind of starches. In the case of modified wheat and potato starches the curves were typical for pregelatinised starches. As a result of the physically process used, low viscosity obtained for dispersion of modified starches indicated the significant changes of that properties of investigated starches.

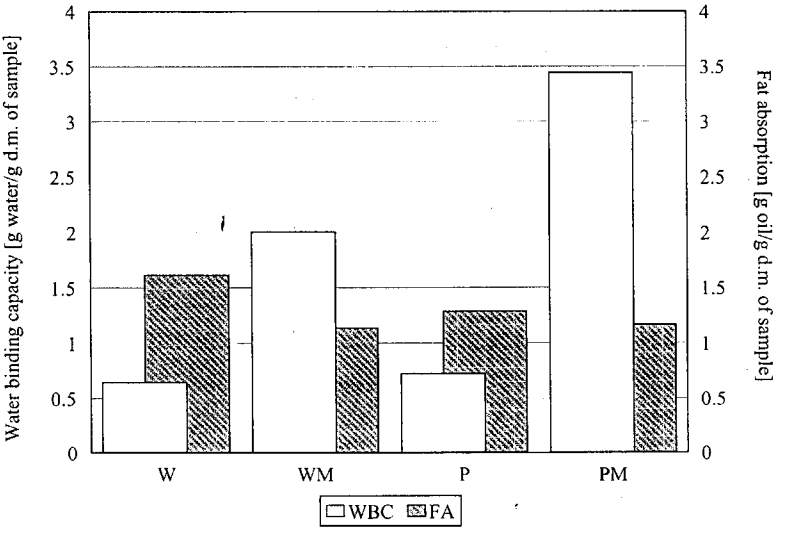

Fig. 1. Functional properties of native and modified starches:
W – native wheat starch, WM – modified wheat starch, P – native potato starch, PM – modified potato starch.

Strains of *Bifidobacterium* were tested *in vitro* for the metabolism of native and modified starches to the growth of population (tab. 3). In the control medium containing glucose as a good source of carbon and energy for bifidobacteria, the growth of the tested strains was differentiated. After 24 h of incubation the number of *B. breve* $1.8 \cdot 10^9$ cfu/ml was the highest and accompanied by the highest reduction of pH to the level of 4.6. The population numbers of the other strains – *B. animalis* and *B. pseudolongum* – were lower, $3.8 \cdot 10^8$ and $1.7 \cdot 10^8$cfu/ml, respectively, and the pH values of these cultures were reduced only to the level of 5.3 and 5.0, respectively.

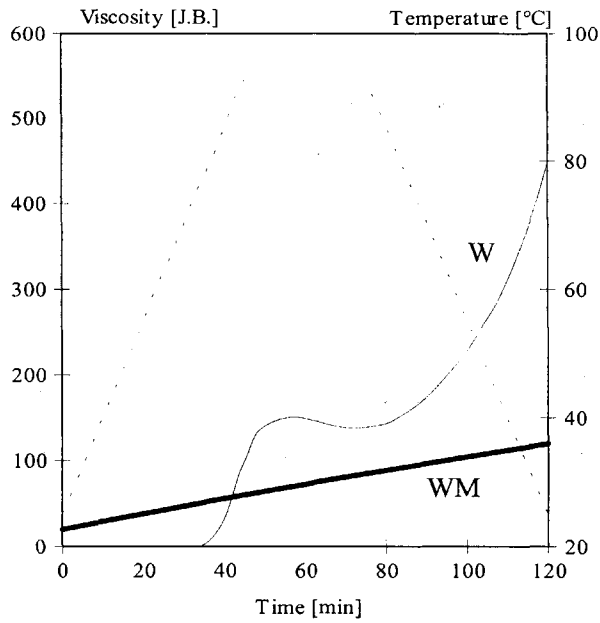

Fig. 2. Brabender viscosity curves of native (W) and modified (WM) wheat starches.

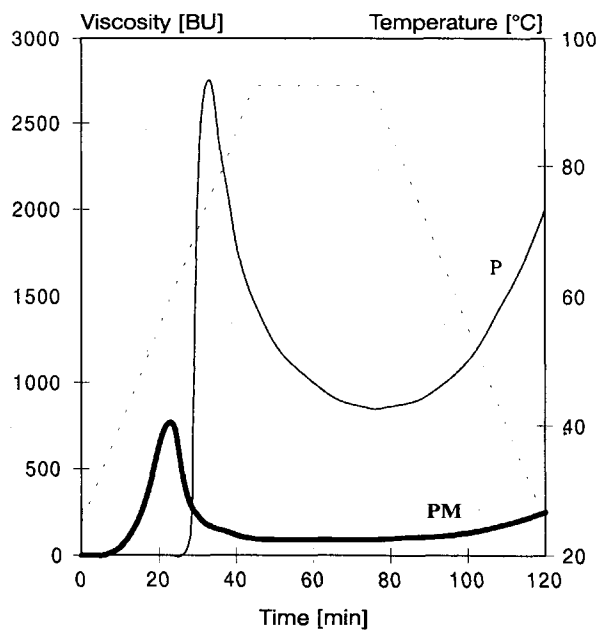

Fig. 3. Brabender viscosity curves of native (P) and modified (PM) potato starches.

Table 3

Growth and acidifying activity of bifidobacteria.

Substrate	*B. breve* ATCC 15700		*B. pseudolongum* KSI9		*B. animalis* KS29a3	
	Number [cfu/ml]	pH	Number [cfu/ml]	pH	Number [cfu/ml]	pH
Glucose	$1.8x10^9$	4.6	$1.7x10^8$	5.0	$3.8x10^8$	5.3
Wheat starch:						
native	$7.5x10^7$	6.0	$2.0x10^8$	5.0	$4.6x10^8$	5.1
modified	$2.2x10^7$	6.0	$1.9x10^8$	4.9	$4.0x10^8$	5.5
Potato starch:						
native	$5.5x10^6$	5.9	$1.4x10^8$	5.0	$3.2x10^8$	5.5
modified	$2.1x10^7$	6.0	$1.1x10^8$	5.1	$2.8x10^8$	5.6

Inoculum of bifidobacteria strains ~$1x10^7$ cfu/ml; after inoculation pH ~6.2; 24-h anaerobic culture with 1% of examined starch substrates or glucose as control.
The results are means of 3 determinations.

In opposite to the control, in the cultures with the examined starches as a substrate instead of glucose, the number of *B. breve* population remained close to the inoculum level. The pH value slightly lowered to the level of ~6.0. The results indicated only negligible or lack of fermentation of the starches by *B. breve* strain isolated from human. The number of *B. pseudolongum* populations in the cultures with starch preparations ranged from $1.1 \cdot 10^8$ to $2.0 \cdot 10^8$ cfu/ml and the pH level was close to 5.0, which was comparable to the results in the control medium with glucose. In the cultures of *B. animalis* with starches as a substrate, the population numbers ranged from $2.8 \cdot 10^8$ to $4.6 \cdot 10^8$ cfu/ml, and were comparable to the control, whereas pH value, ranging from 5.1 to 5.6, was generally lower than in the control. The results indicate that the strains of *B. animalis* and *B. pseudolongum* isolated from animals utilised the examined starches as easily accessible substrates of fermentation.

The ability of starch utilisation seems not to be a common feature within genus *Bifidobacterium*. As a result of selection of 40 *Bifidobacterium* strains to complement resistant starch in a synbiotic yoghurt, Crittenden et al. [5] revealed that only 1 strain *B. lactis* Lafti™ B94 (closely related to *B. animalis*) could hydrolyse resistant starch. Our preliminary results indicated that the starches were metabolised only by several strains isolated from animals and, in lower extent, from infants [data not published]. They belonged entirely to the species *B. animalis*, *B. pseudolongum* and *B. breve*. While examining *in vitro* the utilisation of amylopectin and high-amylose maize starch granules by human colonic bacteria, Wang et al. [23] showed that only 6 out of 36 cultures tested utilised maize-starch amylopectin. The authors demonstrated that *Bifidobacterium* spp., *Bacteroides* spp., *Fusobacterium* spp., and strains of *Eubacterium*,

Clostridium, *Streptococcus* and *Propionibacterium* could hydrolyse the gelatinised maize-starch amylopectin, while only *Bifidobacterium* spp. and *Clostridium butyricum* could efficiently utilise high-amylose maize starch granules.

The use of starch as substrate enhancing bacterial fermentation in the colon is promising but also very difficult area of studies, because the examined material is not uniform, since it contains digestible as well as non-digestible compounds in the upper gastrointestinal tract. Our previous studies *in vivo* on the effect of different non-digestible preparations on the gut microecosystem of rats showed that modified corn starch significantly increased the bifidobacteria number by 1.2 log cfu/g of faeces [3]. Unfortunately, the preparation also increased the values for markers of unhealthy caecal changes ($N\text{-}NH_3$ content and β-glucuronidase activity). The development of starch preparations selectively stimulating the growth of bifidobacteria needs further research.

Conclusions

As a result of physical modification, native wheat and potato starches lost their granular structure and both modified starches were different from the native ones in the affinity to water, viscosity and gelatinisation.

The strains of *B. animalis* and *B. pseudolongum* isolated from animals utilised starch as a fermentation substrate, whereas the strain *B. breve* of human origin fermented only negligibly or not at all the examined preparations of wheat and potato starches. The bifidobacteria population number as well as medium acidification were higher in the media containing wheat starch than the potato starch.

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SKROBIE FIZYCZNIE MODYFIKOWANE PSZENNA I ZIEMNIACZANA, ICH WŁAŚCIWOŚCI FIZYKOCHEMICZNE ORAZ METABOLIZOWANIE PRZEZ BIFIDOBAKTERIE

Streszczenie

Celem badań było określenie wpływu procesów fizycznych (cykle autoklawowania/chłodzenia, suszenie rozpyłowe) na skrobie posiadające odmienną strukturę krystaliczną: pszenna (typ A), ziemniaczana (typ B). Badany był zakres zmian właściwości fizykochemicznych skrobi poddanych modyfikacji fizycznej, które stanowiły źródło węgla i energii do wzrostu bifidobakterii w warunkach *in vitro*.

Charakterystyka właściwości funkcjonanych, takich jak zdolność wiązania wody (WBC), wskazała, że obie naturalne skrobie wykazywały niskie powinowactwo do wody, które po modyfikacji zwiększyło się ok. 4-krotnie. Zauważono przeciwną tendencję w przypadku absorpcji oleju (FA). W wyniku ogrzewania zawiesiny wodnej badanych skrobi w aparacie Brabendera stwierdzono znaczące obniżenie lepkości wskutek modyfikacji obu skrobi.

Zdolność wybranych szczepów *Bifidobacterium* do metabolizowania naturalnych bądź fizycznie modyfikowanych skrobi pszennej i ziemniaczanej była zróżnicowana. *Bifidobacterium pseudolongum* KSI9 i *Bifidobacterium animalis* KS29a3, izolowane od zwierząt, wykorzystywały badane skrobie jako łatwo dostępny substrat do fermentacji, natomiast *Bifidobacterium breve* ATCC 15700, izolowany od człowieka, nie metabolizował, lecz nieznacznie fermentował badane skrobie. Liczebność populacji bifidobakterii, jak również ich aktywność kwasząca, były wyższe na podłożu zawierającym skrobię pszenną w porównaniu ze skrobią ziemniaczaną. Jedynie nieznaczne zróżnicowanie obserwowano w tych wskaźnikach *in vitro* pomiędzy skrobiami naturalnymi i modyfikowanymi.

Uzyskane wyniki sugerują, że naturalne i modyfikowane skrobie, pszenna i ziemniaczana, zawierające frakcję amylazooporną mogą stanowić dobry substrat dla bifidobakterii zasiedlających jelito grube.

G. LEWANDOWICZ[1], W.BŁASZCZAK[2], A.WALKOWSKI[1]

SMALL GRANULE POTATO STARCH, STRUCTURE AND USABILITY

Summary

Small granule potato starch (SGPS) produced by "Wielkopolskie Przedsiębiorstwo Przemysłu Ziemniaczanego" during potato starch production season 1999 was investigated to evaluate its physico-chemical properties, structure and usability. SPGS was used as a raw material for different modification processes typically applied in the Polish starch industry to obtain both food and non-food products. The obtained preparations were compared with industrial products: food grade modified starches E 1403, E 1404, E 1412, E 1414 and E 1422, as well as two types of preparations for paper industry - oxidised starch for wet end application and corrugated board adhesive.

The experimental and reference starch samples were examined by chemical analysis, rheological methods, scanning electron microscopy and X-ray diffractometry. Textural parameters of deserts prepared by means of food grade modified starches as well as some specific functional properties of industrial preparations were investigated.

It was found that SPGS like standard potato starch contained quite small amounts of inorganic impurities as well as crude fibre, revealed similar rheological properties but relatively low crystallinity. SGPS due to its unique physicochemical properties could be recommend as a raw material for the production of corrugated board adhesive. Reactivity of SGPS towards sodium hypochlorite was found lower as compare to standard one. On the contrary susceptibility of SGPS to crosslinking with sodium trimetaphosphate seemed to be higher than of standard starch. The texture of food grade modified starches much differed from standard counterparts, which make possible to extent the assortment of these type products.

Introduction

Small granule fractions of potato, wheat and corn starches are characterised by the largest real surface, volume of pores and their average diameter. Fractions of small starch granules were also characterised by higher content of total phosphorus, crude protein and amylose as well as lower capability of swelling and solubility than large

[1]*Starch and Potato Products Research Laboratory, 62-030 Luboń, ul. Armii Poznań 49, Poland*
[2]*Polish Academy of Sciences, Institute of Animal Reproduction and Food Research, Division of Food Science, 10-747 Olsztyn, ul.Tuwima 10, Poland*

starch granules [1]. Similar observation have been made by Gambuś et al. on their investigations of triticale starch [6]. Small granules of triticale starch are characterised by higher total phosphorus content, gelatinisation temperature and maximum viscosity, but lower water binding, solubility and intrinsic viscosity. Particularly high attention was paid to differences between small and high granule fractions in wheat especially regarding pasting properties and fat content [3, 10]. The amounts of lipid per granule tend to be proportional to the granule surface areas of the larger granules, but proportional to the volumes of the smaller granules [10]. Potato starch reveals especially low fat and protein contents as compared to cereal starches [13]. Consequently these substances located mainly in the surface layer of starch granules probably are not the main factor differentiating properties of starch fractions in relation to granules size. Small granules of potato starch undergo more extensive phosphorylation in reaction with trimethaphoshate than big ones [5].

Materials and methods

Small granule potato starch fraction (SGPS) produced by "Wielkopolskie Przedsiębiorstwo Przemysłu Ziemniaczanego" during potato starch production season 1999 as well as potato starch "Superior Standard" were used as raw materials.

Modification reactions

Food grade modified starches were prepared according to technologies applied in Polish starch industry elaborated by Starch and Potato Products Research Laboratory team i.e.:

- E 1403 – bleached starch, industrial product "Skrobia budyniowa" made by WPPZ Luboń according to [11];
- E 1404 – oxidised starch, industrial product "Skrobia żelująca" made by WPPZ Luboń, according to [11];
- E 1412 – distarch phosphate, industrial product "Lubostat" made by WPPZ Luboń, according to [15];
- E 1414 – acetylated distarch phosphate, industrial product "Lubosol" made by WPPZ Luboń, according to [15];
- E 1422 – acetylated distarch adipate, industrial product "Zagęstnik AD" made by WPPZ Luboń, according to [8].

Industrial starch preparations were prepared according to technologies applied in Polish starch industry elaborated by Starch and Potato Products Research Laboratory team i.e.:

- oxidised starch for wet end application, industrial product "Oxamyl" made by WPPZ Luboń, according to [12].

- corrugated board adhesive, industrial product "Spoiwo do tektury falistej 50S" made by WPPZ Luboń, according to [9]. Corrugated board adhesive was prepared in two types containing different amounts (10% and 12%) of the carrier.

Analytical methods

Granule size distribution

Granule size distribution was measured automatically with Scanning Photo Sedimentograph FRITSCH analystette 20 (Germany) in water as a suspension liquid. The particle size distribution was calculated from the measured sinking speed of solids in suspension. In order to perform this analysis properly, the densities of sample and sedimentation liquid as well as dynamic viscosity of sedimentation liquid had to be exactly known.

Microscopic examinations

The structure of potato starch granules has been studied with SEM. The samples of starch preparations were applied on metal discs on specimen holder and than they were coated with gold in vacuum evaporator JEOL JEE 400. The obtained specimens were observed in JSM 5200 scanning electron microscope.

X-ray diffractometry

X-ray diffractometry was carried out with a TUR 62 Carl Zeiss X-ray diffractometer under the following conditions: X-ray tube CuKα (Ni filter); voltage 30 kV; current 15 mA; scanning from $\Theta = 2°$ to 18°.

Chemical analysis

Ash content was determined according to EN ISO 3593 standard, crude fibre content according to ISO 5498 standard, and carboxyl groups content according to Joint FAO/WHO Expert Committee Recommendations [4].

Rheological properties

The course of gelatinisation was monitored with a Brabender viscograph under the following conditions: measuring cartridge 0.07 Nm; heating/cooling rate 1.5°C/min; thermostating at 95°C – 30 minutes.

The viscosity of oxidised starch preparations were measured with a Brookfield Digital Viscometer Model DVII, at 50 rpm, for the 10% solutions, prepared at 90°C for 20 minutes and then cooled to the temperature of 50°C.

The viscosity of corrugated board adhesive were determined with Ford Cup No 4, for the suspensions containing one part of starch preparation and four parts of water previously stirred for about 30 minutes.

Sedimentation of suspensions of corrugated board adhesive

The suspensions of corrugated board adhesive prepared for the viscosity measurements were allowed to sedimentation for 24 hours. Then the sedimentation rate defined as a ratio of the clear fluid height to the whole fluid height (expressed in percent) was calculated.

Texture analysis

Two types of the desserts were prepared using both standard and small granule preparations i.e.:

- pudding type (according to the Polish standard PN-A-74723) were prepared using starch preparations E 1403, E 1412, E 1414 and E 1422,
- gel type (according to the industrial standard ZN-84/MRiBŻ-I-09/89) – were prepared using E 1404.

The textural parameters of deserts were measured using TA-XT2 texture analyser (Stable Micro System UK) with 35-mm diameter cylinder aluminium probe. The original TPA curves were interpreted in terms of five textural parameters, two measured and three calculated: hardness (N), – adhesiveness (Ns), cohesiveness, springiness and gumminess [N].

Results and discussion

Small granule potato starch revealed significantly different granule size distribution from the standard ones (Fig. 1). Medium diameter of standard potato starch was of about 40 μm whereas small granule type of about 15 μm what made it similar to typical corn starch. In spite of that, pasting properties (Fig. 2) and X-ray diffraction pattern (Fig. 3) made it typical for potato starch. However, remarkable differences in pasting properties of small granules and standard potato starch could be observed (Fig. 2). Small granule potato starch started to paste at the temperature of about 5 centigrade lower than standard starch, and reached higher values of peak viscosity. The viscosity after cooling to 25°C was also higher in case of small granule starch.

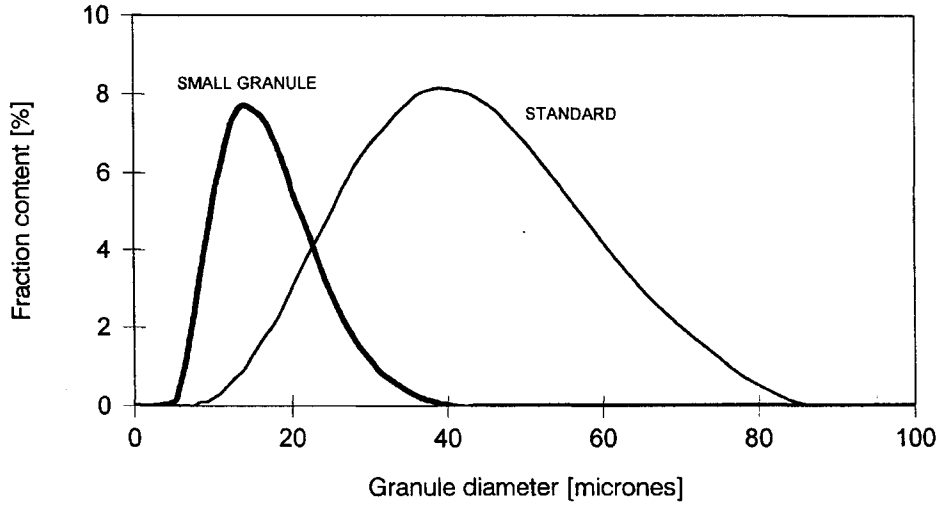

Fig. 1. Granule size distribution of small granule and standard potato starch.

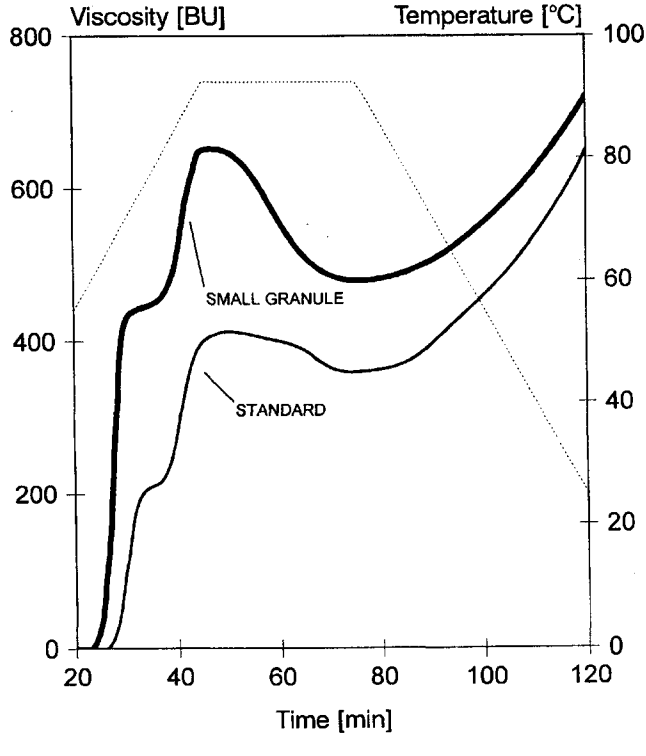

Fig. 2. Brabender viscosity curves (c=3,3%) of potato starch: S – standard; D – small granule.

Small granule and standard starch samples revealed the same B type of X-ray diffraction pattern typical of potato starch (Fig. 3), but a significant difference in their relative crystallinity seemed to be the most important observation. Small granule potato starch exhibited rather low degree of crystallinity what may be associated with a maturity of starch granules. According to Gambuś et al [7] the small starch grains are synthesised in cereal kernel mainly during late-waxy phase. Short time of growing of small granules could be the reason of their chaotic organisation and consequently smaller crystallinity. In spite of that SEM analysis did not pointed to any damages of the granules surface (Fig. 4).

The chemical analysis of standard and small granule potato starch (Table 1) proved that purification processes performed in WPPZ Luboń were satisfactory. Ash

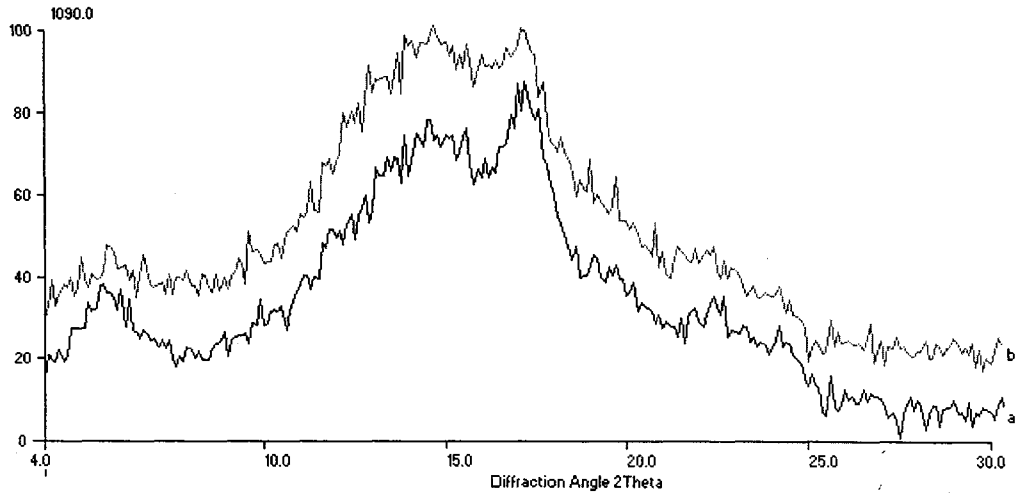

Fig. 3. X-ray diffraction patterns of potato starch: a – standard; b – small granule.

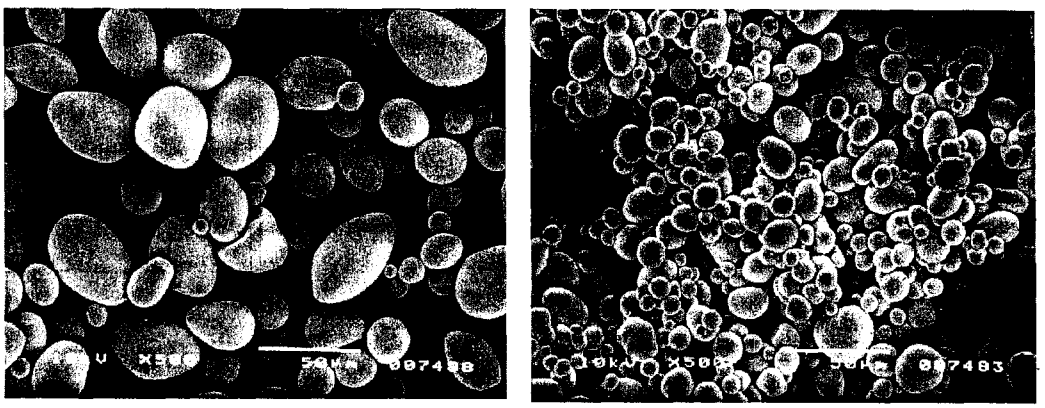

Fig. 4. SEM microphotographs of potato starch: a – standard; b – small granule.

as well as fibre content revealed similar level in both types of starch and were typical for high-grade potato starch. These observations were confirmed by SEM analysis (Fig. 4). Both types of starch did not contain any impurities, which can be visible in microphotographs.

Table 1

Some physicochemical properties of small granule and standard potato starch.

Type of raw material	Chemical analysis of native starch		Parameters of oxidised starch for wet end application	
	Ash content [%]	Crude fibre content [%]	Viscosity of 10% solution of the preparation [mPas]	Carboxyl groups content in the preparation [%]
Standard potato starch	0,30	0,02	56	0,436
Small granule potato starch	0,35	0,02	98	0,406

These differences in physicochemical properties caused significant differences in usability of two types of potato starch. For example, Stein-Hall type corrugated board adhesive contained the same amounts of carrier and different types of potato starch revealed very similar viscosity values, but significantly different tendency to sedimentation (Fig. 5). These made small granule potato starch an excellent raw material for the production of Stein-Hall type corrugated board adhesive. Also reactivity of SGPS towards sodium hypochlorite (Table 1) much differed as compare to standard starch. The difference between degree of substitution with carboxyl groups was found remarkable, but the difference between viscosity was so high that made these two products useful in quite different uses.

The application of native small granule for the preparation of pudding type desserts pointed to significant differences in sensory properties of these products as compare to desserts made from standard potato starch. These sensory observations were confirmed by texture analysis (Fig. 6-9). Small granule potato starch gave pudding type desserts of significantly lower hardness, cohesiveness and guminess but far higher adhesiveness. Consequently using of small granule potato starch as a raw material instead of standard could make possible to obtain the whole range of new food grade starch preparations. This hypothesis was fully confirmed by further investigations. The technologies applied by Polish starch processing factories for the production food grade modified potato starch preparations were simply used in laboratory in aim to obtain small granule counterparts. As the result five new types of food modified potato starch were obtained. All new modified food starches significantly differed in texture

as compare to standard counterparts. It should be emphasised that the way of changes was not simply and was difficult to predict. All types of small granule modified starches gave desserts of higher adhesiveness than standard preparations (Fig. 7). This phenomenon was observed also in case of native starches. Hardness of desserts made from small granule preparations were higher in case of E 1403 and E 1414, but smaller in case of E 1404, E 1412, E 1422 (Fig. 6). It could be not classified in terms of type of modification reaction. Similar statements could be made in case of cohesiveness and guminess. Cohesiveness of desserts made from small granule preparations was higher in case of E 1404 and E 1412 but smaller in case of E 1403, E 1414 and E 1422 (Fig. 8). Guminess of desserts made from small granule preparations was higher in case of E 1403, E 1404 and E 1414 but smaller in case of E 1412 and E 1422 (Fig. 9).

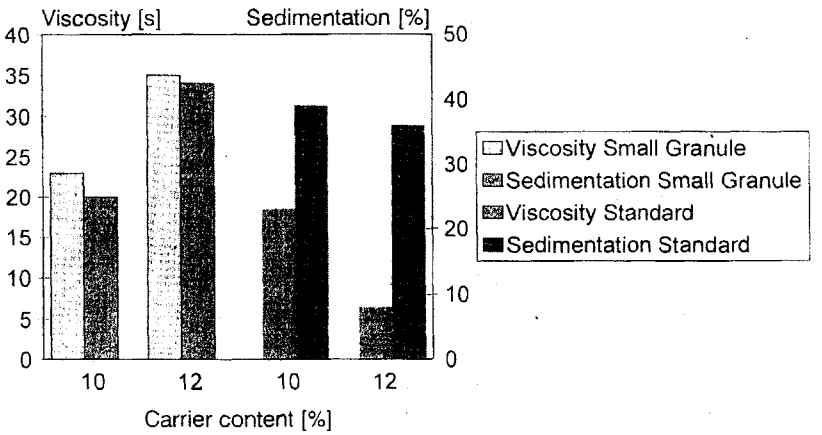

Fig. 5. Viscosity and sedimentation of Stein-Hall type corrugated board adhesive.

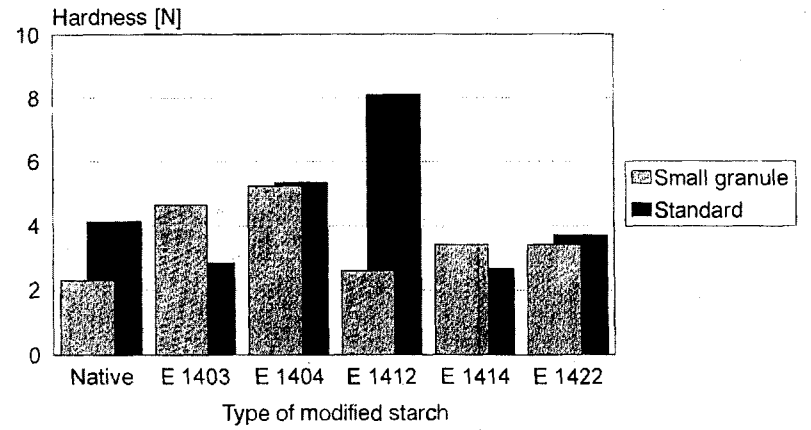

Fig. 6. Hardness of pudding type (raw materials: native starch, E 1403, E 1412, E 1414, E 1422) and gel type (raw material - E 1403) desserts made using standard and small granule raw materials.

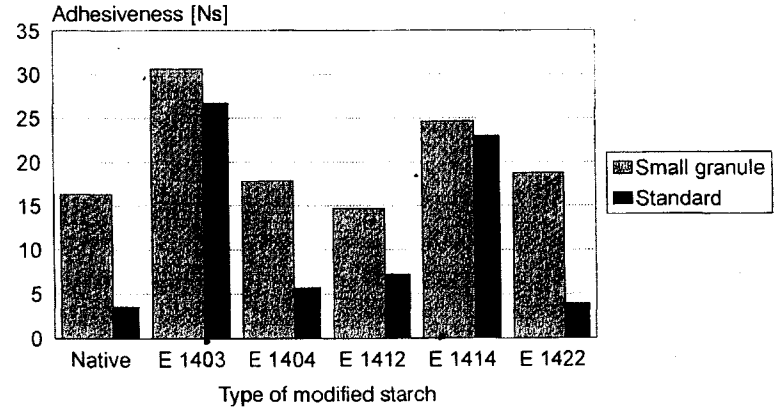

Fig. 7. Adhesiveness of pudding type (raw materials: native starch, E 1403, E 1412, E 1414, E 1422) and gel type (raw material - E 1403) desserts made using standard and small granule raw materials.

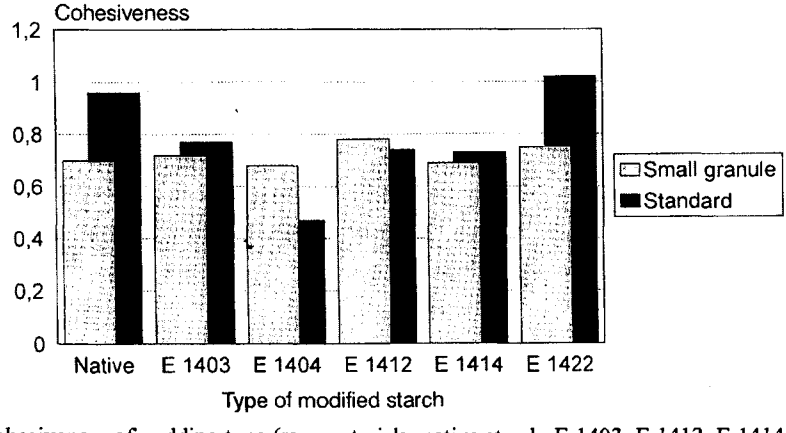

Fig. 8. Cohesiveness of pudding type (raw materials: native starch, E 1403, E 1412, E 1414, E 1422) and gel type (raw material - E 1403) desserts made using standard and small granule raw materials.

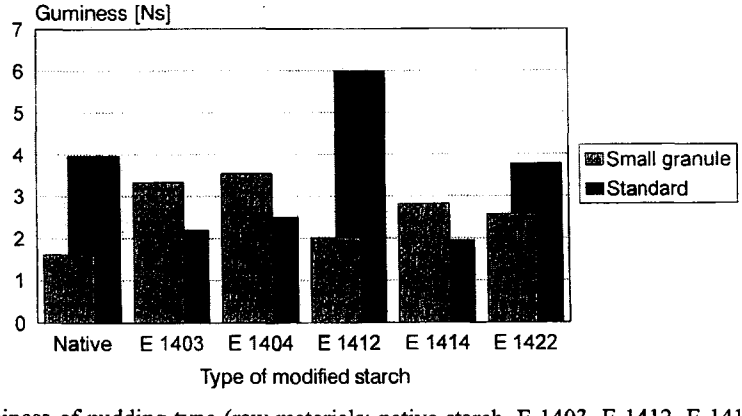

Fig. 9. Guminess of pudding type (raw materials: native starch, E 1403, E 1412, E 1414, E 1422) and gel type (raw material - E 1403) desserts made using standard and small granule raw materials.

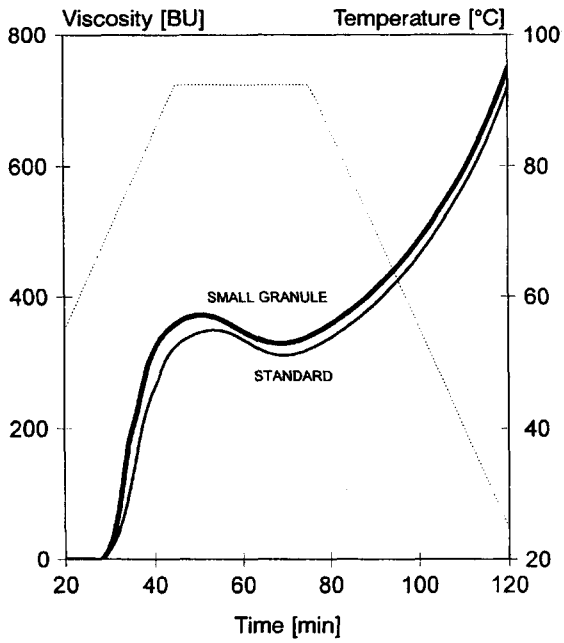

Fig. 10. Brabender viscosity curves (c = 3,3%) of E 1403 preparation made from: S – standard; D – small granule potato starch.

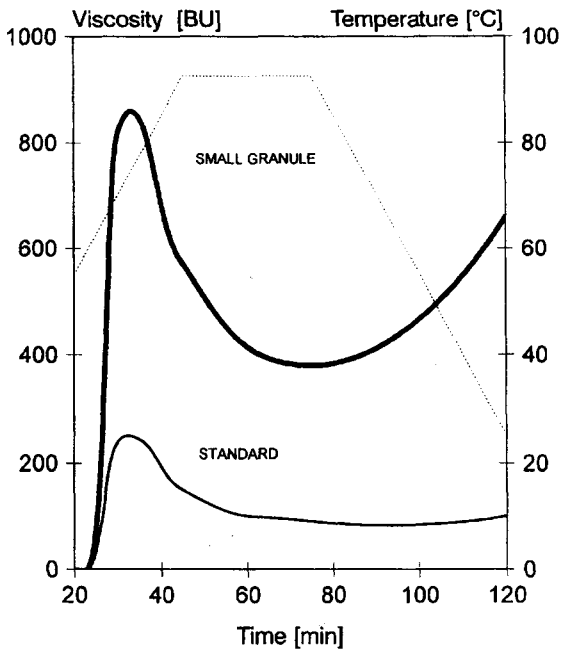

Fig. 11. Brabender viscosity curves (c = 6,0%) of E 1404 preparation made from: S – standard; D – small granule potato starch.

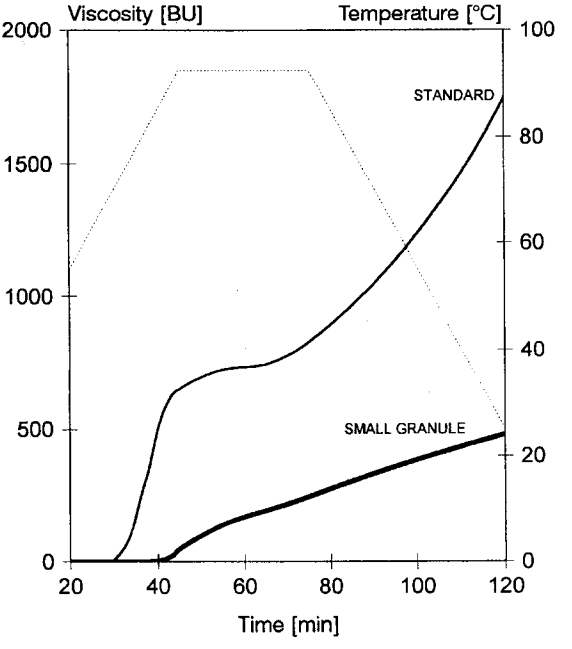

Fig. 12. Brabender viscosity curves (c = 3,3%) of E 1412 preparation made from: S – standard; D – small granule potato starch.

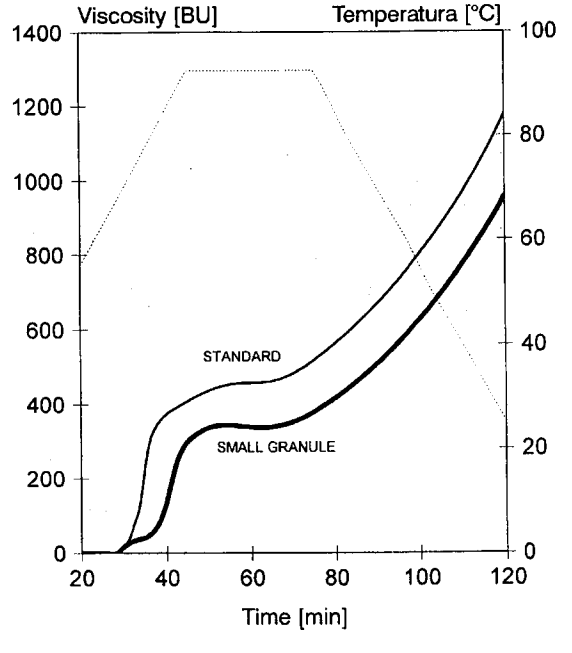

Fig. 13. Brabender viscosity curves (c = 3,3%) of E 1414 preparation made from: S – standard; D – small granule potato starch.

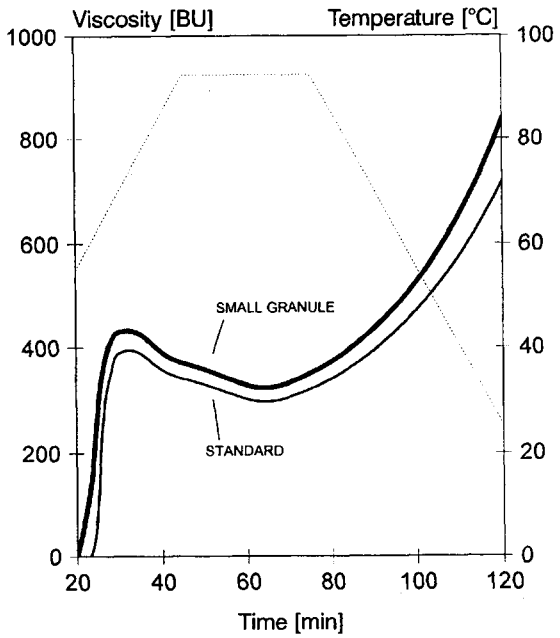

Fig. 14. Brabender viscosity curves (c = 3,3%) of E 1422 preparation made from: S – standard; D – small granule potato starch.

Observation more easily subjected to classification could be made on study of pasting properties (Fig.10-14). SGPS and standard bleached starch E 1403 preparations, which were only slightly chemically changed, revealed almost the same pasting properties (Fig. 10). Brabender viscosity curves of E 1403 preparation only slightly differed from pasting curves of native starches (Fig. 2). Pasting properties of small granule and standard oxidised E 1404 preparations differed significantly, especially in terms of viscosity (Fig. 11). Significant difference in Brabender viscosity of two types E 1404 starches corresponded to the difference in viscosity of oxidised starch preparation for paper industry (Table 1). Both, food grade oxidised starch as well as oxidised starch for wet end application made from SGPS revealed much higher viscosity than standard counterparts. This phenomenon as well as degree of substitution with carboxyl groups pointed to a lower reactivity of small granule potato starch towards sodium hypochlorite than standard preparations. On the contrary, susceptibility of SGPS to crosslinking with sodium trimetaphosphate seemed to be higher than these of standards. Small granule E 1412 preparation revealed significantly higher pasting temperature and more restricted type of swelling characteristic than standard counterpart that unquestionable pointed to higher degree of crosslinking of preparation made from small granule starch as compare to standards (Fig. 12). Similar but less pronounced differences could be observed in case of acetylated distarch phosphate E 1414 prepara-

tions (Fig. 13). That could be caused by stabilising effect of acetyl groups. Acetylated distarch adipate E 1422, preparation of higher acetyl groups content than E 1414, made from small granule raw material revealed even smaller pasting temperature than standard counterpart (Fig. 14). All observations regarding rheological and functional properties of chemically modified starches showed that SGPS due to its physicochemical properties could be used as a raw material for the production of a whole range of new starch preparations. However, simple application of present existing industrial procedures seemed to be impossible due to unforeseeable character of the reactivity of SGPS.

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DROBNOZIARNISTA SKROBIA ZIEMNIACZANA – STRUKTURA I WŁAŚCIWOŚCI UŻYTKOWE

Streszczenie

Zbadano strukturę oraz właściwości fizykochemiczne i użytkowe drobnoziarnistej skrobi ziemniaczanej (SGPS), wyprodukowanej w Wielkopolskim Przedsiębiorstwie Przemysłu Ziemniaczanego w toku kampanii ziemniaczanej w roku 1999. SGPS użyto jako surowca do laboratoryjnej syntezy wybranych spożywczych i niespożywczych skrobi modyfikowanych, produkowanych przez polskie zakłady ziemniaczane. Otrzymane preparaty były porównywane z produktami handlowymi: spożywczymi skrobiami modyfikowanymi E 1403, E 1404, E 1412, E 1414 i E 1422 oraz z dwoma preparatami stosowanymi w przemyśle papierniczym – skrobią utlenioną do powierzchniowego zaklejania papieru oraz klejem do produkcji tektury falistej.

Badane preparaty poddano analizie chemicznej, określono ich właściwości reologiczne, a także przebadano strukturę krystaliczną i mikroskopową. Określono ponadto parametry tekstury a także wybrane, specyficzne właściwości użytkowe preparatów dla przemysłu papierniczego.

Stwierdzono, że SGPS zawiera podobne ilości popiołu i włókna surowego jak standardowa skrobia ziemniaczana, podobne właściwości reologiczne, ale niższą krystaliczność względną. Ze względu na swe unikalne właściwości fizykochemiczne, SGPS może być zalecana do produkcji kleju do tektury falistej. Reaktywność SGPS w stosunku do podchlorynu sodu jest niższa niż skrobi standardowej, natomiast w stosunku do trimetafosforanu sodu – wyższa. Parametry tekstury spożywczych skrobi modyfikowanych, otrzymanych z SGPS jako surowca, różnią się znacznie od standardowych odpowiedników. Stwarza to możliwość produkcji całej gamy nowych produktów dla przemysłu spożywczego.

YUNG-HO CHANG*, KUN-SAN LIN

ACID-ALCOHOL DEGRADATION OF STARCH

Summary

For revealing the degradation effect of acid-alcohol modification on starch, commercial corn and potato starches were hydrolyzed by 0.36% HCl in methanol at 25°C for 1 to 15 days. Results showed the yields of the modified starches ranged from 91 to 100%, and the average granule size of the modified starch was slightly smaller as compared to their counterpart native starches. After 15 days of modification, no significant configuration change was found whereas the solubility of starches obviously increased with the increase in the hydrolysis time. Corn starch showed a slower increasing tendency than that of potato starch. Gelatinization onset temperatures (T_o) of starches after modification showed a decrease tendency in corn starch, and an increase tendency in potato starch. For both corn and potato starches, the peak (T_p) and conclusion (T_c) temperatures of gelatinization increased with the increase in the treatment time.

Consequently, the range of gelatinization increased from 12.2 to 23.9°C for corn starch, and from 12.8 to 19.8°C for potato starch. However, the gelatinization enthalpies (•H) of modified starches showed 1 to 2 J/g lower than their counterpart native starches. For the acid-alcohol treated starches, the area of amylopectin fractions determined by high-performance size exclusion chromatography (HPSEC) decreased with the treatment time. Compared to the gradual degradation pattern of corn starch, potato starch showed a stepwise pattern. The weight average degree of polymerization (DP_w) of starches rapidly decreased within the first 5 days of treatment, and potato starch had a higher rate of decrease than corn starch. The number average degree of polymerization (DP_n) of corn and potato starches after acid-alcohol treated for 15 days were 162 and 183, respectively. It was concluded that the degradation of amylopectin by the acid-alcohol modification might alter the molecular structure of starch, which resulted in the fast disruption of granule and the decrease of viscosity of starch paste during heating.

Introduction

For many years, acid hydrolysis has been used to modify starch granule structure and produce "soluble starch". In industry, acid-modified starch is prepared on treatment of starches with dilute HCl or H_2SO_4 at 25–55°C for various time periods. The product, thin boiling starch, is used extensively in food, textile and paper industries

*Corresponding author, e-mail: yhchang@pu.edu.tw
Address: Department of Food and Nutrition, Providence University, Shalu 43301, TAIWAN

[1]. Although the viscosity or fluidity properties of acid-hydrolyzed starch varies with the conditions used during modification, the yield of the modified starch consistently decreases with the increases of acid concentration and hydrolysis time.

For maximum conversion of raw starch into soluble starch with minimal production of low-molecular-weight dextrins, Small [2] proposed a preparation procedure by refluxing starch granules in 95% ethanol containing 0.2–1.6% (w/v) HCl for 6–15 min. Ma and Robyt [3] showed that treatments of potato and waxy maize starches with different anhydrous alcohols (methanol, ethanol, 2-propanol, and 1-butanol) containing 0.36% HCl at 65°C for 60 min produced starches with different values of average degree of polymerization (DP), with the highest value being obtained in methanol and the lowest value in 1-butanol. The yields of the modified starches were high (ranging from 100 to 88%), and the size distribution of the starch chains was more narrow and homogeneous than that of native starch. It was proposed that the different alcohols produced different concentrations of acid inside the granules. Hydrolysis of the glycosidic linkage took place exclusively inside granules with the granule-bound water. However, the hydrolysis of potato starch granules in the presence of the above alcohols with 0.36% HCl did not proceed indefinitely, the DP values of the modified products rapidly decreased and became constant after 72 h of reaction [4].

Most studies on acid-alcohol modification of starch concerned the effects of acid concentrations, starch types and concentrations, and alcohol types and concentrations on the DP values [4, 5, 6], the particle size and morphology [7] of the modified starches, and the viscosity and stability of the emulsion made from the modified starches [8]. Few reports [3] considered the effect of acid-alcohol modification on the molecular size distribution, and its final results on the physicochemical properties of the modified starch. In this report, commercial corn and potato starches were hydrolyzed by 0.36% HCl in methanol at 25°C for 1 to 15 days. The treatment effects on the granule size, morphology, solubility, and gelatinization properties of starch were investigated. The changes in molecular size distribution of modified starches were also determined by high-performance size exclusion chromatography (HPSEC) and discussed with the change in the physicochemical properties for further elucidating the effects of acid-alcohol modification on starch.

Materials and methods

Starches

Corn starch and potato starch were obtained from National Starch and Chemical Company (Bridgewater, NJ). The moisture content of corn starch was 12.6% (w/w), and potato starch 13.7%.

Acid-alcohol modification

Starch (25g) was suspended in 100 mL methanol (< 0.3% water) in a 250 mL flask. The suspension was stirred at 25°C. Reaction was started by adding of 1 mL of concentrated (36% by weight) hydrochloric acid, and allowed to proceed for 1, 3, 5, 7, 9, 11, 13, and 15 days, respectively. The reaction was stopped by adding of 14 mL of 1M $NaHCO_3$. The starch was centrifuged at 2,500 × g for 5 min and washed four times with 50% ethanol. The precipitate was air-oven dried at 40°C. The yield was calculated by weight of the recovery starch to the initial weight of dry starch.

Size distribution and morphology of starch granule

The size distribution of starch granule was determined by using a laser light scattering based particle size analyzer (Mastersizer Micro, Malvern Instruments, UK.). Granule morphology of starch was studied with a scanning electron microscope (SEM ABT150S, Topcon, Japan). Starch samples were mounted on circular aluminum stubs with double sticky tape, coated with gold, and then examined and photographed at an accelerating potential of 10 kV.

Solubility

Starch (0.1g, dry basis) was heated in 40 ml of water to the desired temperature for 30 min. The formation of lump was prevented by continuously stirring. The mixture was centrifuged at 4 000 × g for 15 min, then the supernatant was decanted and the swollen starch sediment weighed. An aliquot of supernatant was evaporated overnight at 130°C and weighed. The solubility was the ratio in weight of the dried supernatant to the initial weight of the dry starch.

Pasting properties

Pasting properties of starch were determined with a Rapid Visco-Analyzer (RVA 3D°C, Newport Scientific, Australia). Each starch suspension (7%, w/w, dry basis for corn and 6% for potato; 28g total weight) was equilibrated at 50°C for 1 min, heated to 95°C at a rate of 12°C/min, maintained at 95°C for 2.5 min, and then cooled to 50°C at a rate of 12°C/min. Paddle speed was set at 960 rpm for the first 10 sec and then 160 rpm for the rest of the analysis.

Gelatinization properties

Gelatinization properties of starch were determined by using a differential scanning calorimeter (Micro DSC VII, Setaram, France). Starch sample (about 150 mg, dry basis) was weighed in the sample pan, mixed with distilled water (about 450 mg), and

sealed. The samples were heated from 25 to 120°C at a heating rate of 1.2°C /min. Onset (T_o), peak (T_p) and conclusion (T_c) temperatures together with gelatinization enthalpy (ΔH) were quantified.

Molecular weight distribution

The molecular weight distribution of starch was determined by HPSEC. The solution of native starch was prepared by solubilizing 75 mg (dry basis) of starch with 15 mL, 90% dimethyl sulfoxide (DMSO) solution in a boiling water bath for 1 h with constant stirring, and then continuously stirred for 24 h at room temperature. Starch was precipitated from an aliquot of DMSO solution (2.1 mL) with excess absolute ethyl alcohol and centrifuged at 4,000 × g for 10 min. The precipitated amorphous starch pellet was resolubilized in deionized water (15 mL, 95°C) and stirred with a magnetic stirrer in a boiling water bath for 30 min. To the acid-alcohol modified starch, the starch solution was prepared by solubilizing 10 mg (dry basis) of starch with 15 mL deionized water and stirred in a boiling water bath for 1 h.

Each starch solution was filtered through a 5.0 μm syringe filter, and then the filtrate (100 μL) was injected into an HPSEC system. This system consisted of an HP G1310A isocratic pump (Hewlett Packard, USA), refractive index (RI) detector (HP 1047A), and a multiangle laser light-scattering (MALLS) detector (Dawn DSP, Wyatt Tech., USA) with a helium-neon laser light source (λ = 632 nm) and a K-5 flow cell. The columns used were PWH (guard column), G5000PW and G4000PW (TSK-Gel, Tosoh, Japan) HPSEC columns connected in series and kept at 70°C. The mobile phase was 0.1M $NaNO_3$ solution containing 0.02% NaN_3 at a flow rate of 0.5 mL/min.

Results and discussion

The yields of the starches modified by 0.36% HCl in methanol at 25°C for 1 to 15 days were high, ranging from 91 to 100% (Table 1). Compared to their counterpart native starches, the average granule size of the modified starch was slightly smaller. For the potato starch after 15 days of treatment, the average granule size decreased from 47.2 μm of native starch to 44.8 μm (Table 1). The changes of granule size of corn starch after modification were less obvious.

Examined by SEM, the native corn starch (Fig. 1, A and B) showed polygonal, irregular shape, while potato starch (Fig. 1, E and F) had oval or spherical-like shape. The granule surface of the native starches was smooth without obvious fissures or cavities. After 15 days of modification, no significant configuration change such as fragmentation or swelling was found. However, the granule surface of corn starch changed rough, and the naturally-occurred, randomly-distributed surface openings [9]

of some granules became more obvious (Fig. 1, C, and D). The granule surface of potato starch also changed rough with partial protuberances (Fig. 1, G and H).

Table 1

Yields and average granule sizes of native starches and starches treated by 0.36% HCl in methanol at 25°C for 1 to 15 days.

Time (days)	Yield*(%)		Average granule size (μm)	
	Corn	Potato	Corn	Potato
Native	–	–	13.7±0.1	47.2±0.2
1	98.5	98.0	13.4±0.0	47.6±0.1
3	98.4	98.4	13.6±0.0	46.1±0.9
5	98.4	99.8	13.6±0.0	47.5±0.4
7	100.0	100.0	13.6±0.0	47.9±0.1
9	97.1	94.6	12.4±0.2	47.6±0.0
11	97.2	96.4	12.5±0.3	45.4±0.1
13	91.7	92.7	12.8±0.0	43.3±0.2
15	97.7	95.2	13.2±0.0	44.8±0.7

*(Weight of starch after acid-alcohol treatment)/(weight of starch before treatment) x 100%.

Fig. 2 shows the solubility of corn and potato starches measured at different temperatures (65–95°C). In spite of the measurement temperature, the solubility of native corn and potato starch was below 10% and 22%, respectively. After acid-alcohol modification, the solubility of starches obviously increased with the increase in the hydrolysis time. Corn starch showed a slower increasing tendency, and the solubility value stabilized after 11 days of treatment. In contrast, the solubility of potato starch increased rapidly, and reached its maximum value after 3 days of treatment. As the measurement temperature was above 75°C, the solubility of modified potato starches were higher than 90%. This indicated the starch granules were nearly fully dissolved. Results of pasting properties (Fig. 3) of starches measured by RVA confirmed the high solubility of modified starches. Native potato starch had higher peak viscosity and lower pasting temperature than the native cornstarch. After the acid-alcohol treatment the pasting viscosity of the modified starches decreased obviously for both potato and corn starches. Among the modified starches prepared, only the cornstarch after acid-alcohol treated for 1 day showed the entire profile of the RVA amylograph similar to that of the native starch. Other modified starches showed either very lower (less than 100 cps) or undetectable peak viscosity.

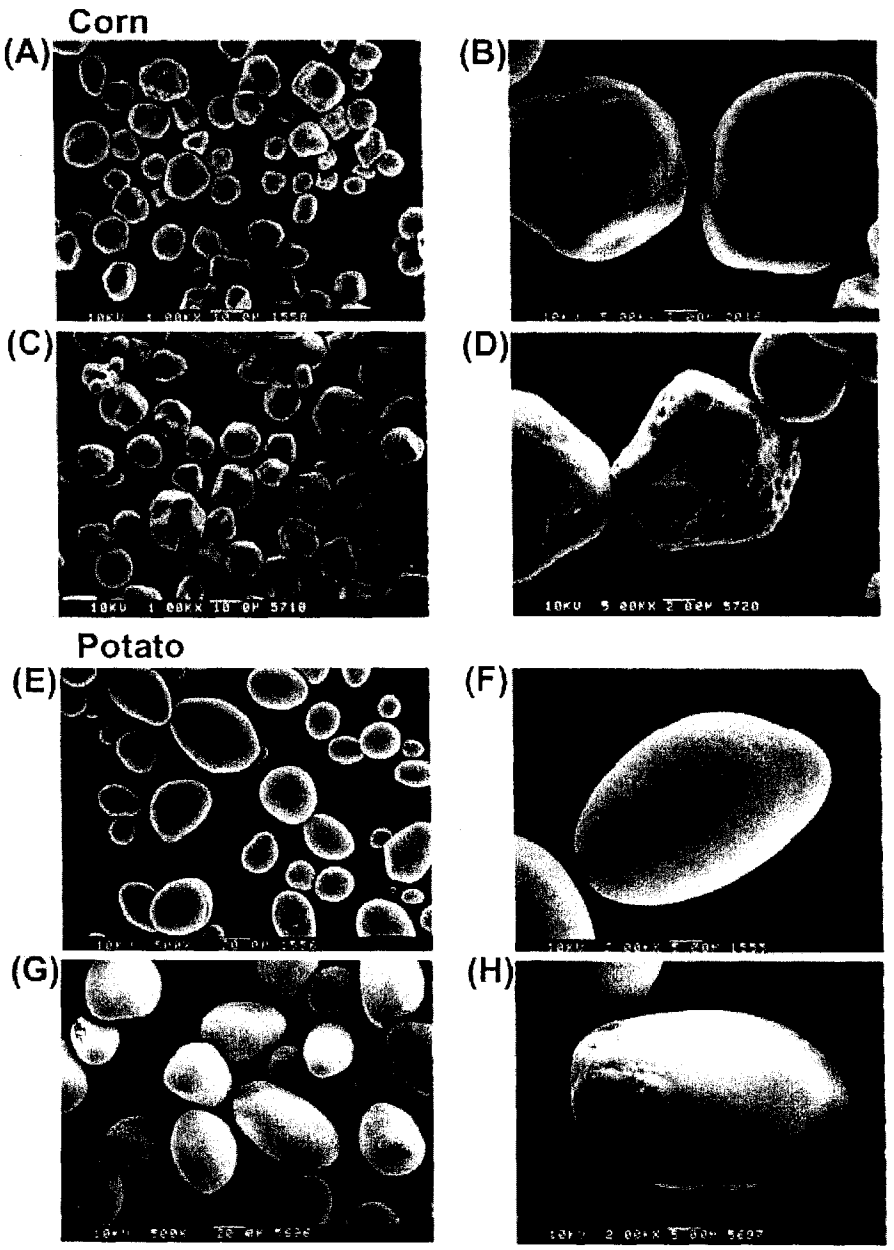

Fig. 1. Scanning electron micrograms of corn and potato starches: native (A, B, E, F), and treated by 0.36% HCl in methanol at 25°C for 15 days (C, D, G, H).

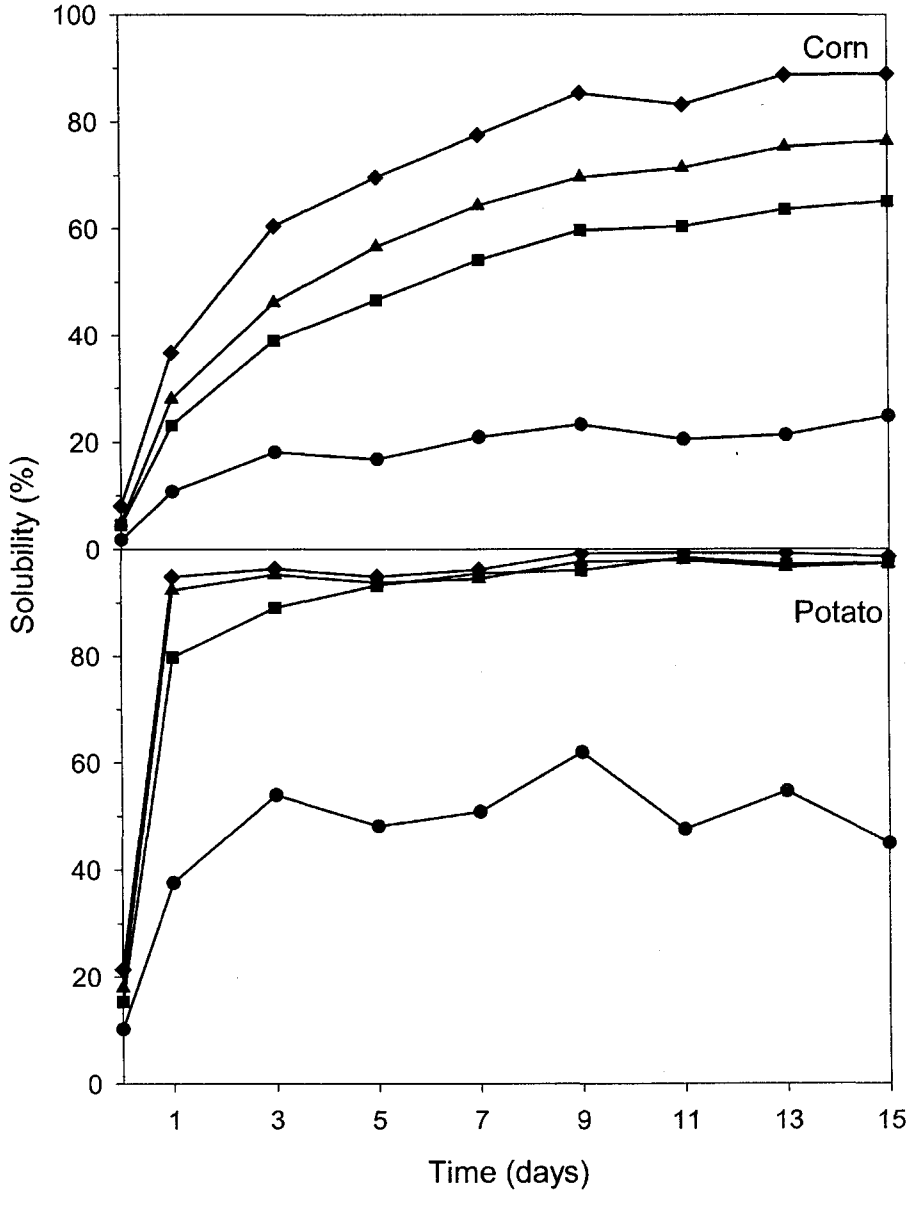

Fig. 2. Solubility of starches measured at 65 (●), 75 (■), 85 (▲), and 95°C (□), respectively. Starches were hydrolyzed by 0.36% HCl in methanol at 25°C for 1 to 15 days.

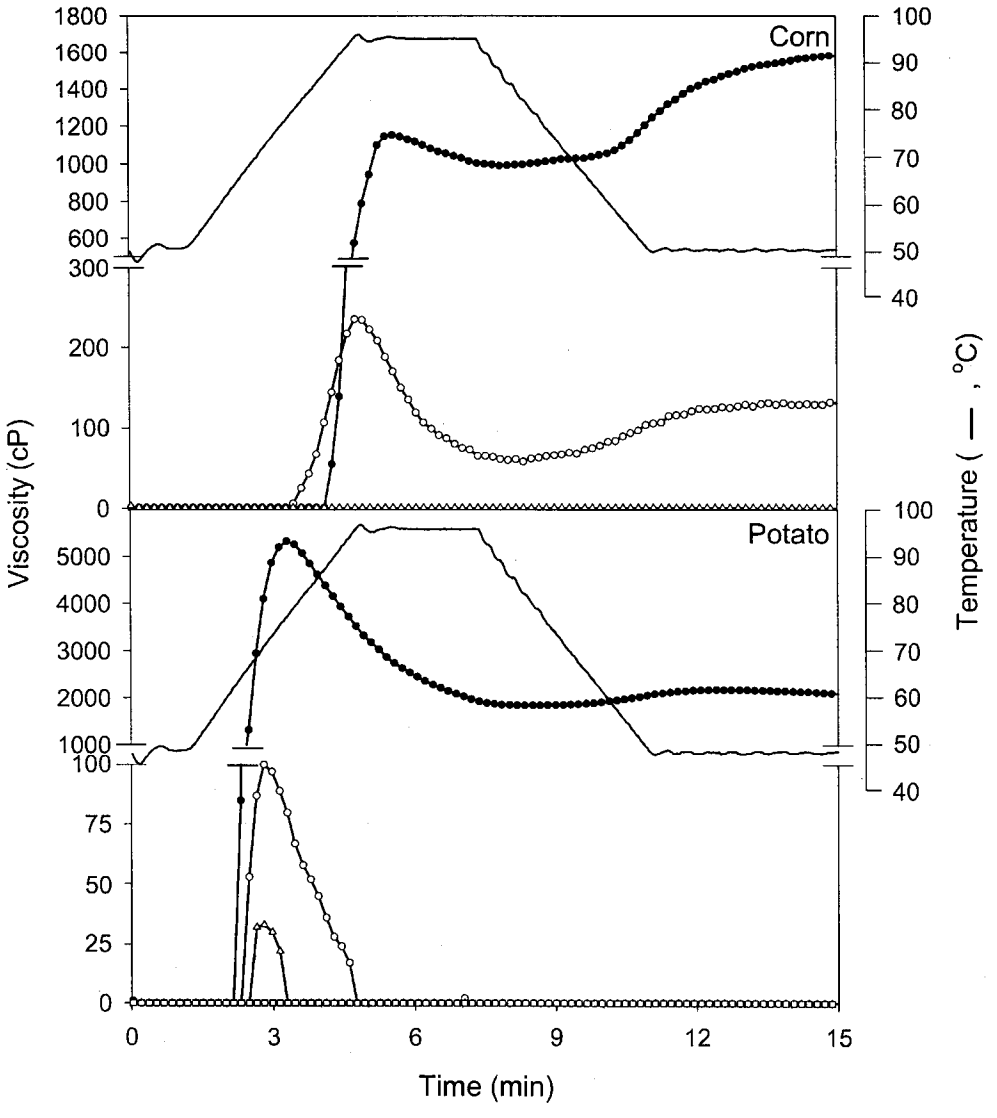

Fig. 3. RVA amylograms of native starches (•) and starches treated by 0.36% HCl in methanol at 25°C for 1 (○), 3 (Δ), and 5 days (□), respectively.

Gelatinization properties of starches determined by DSC are shown in Table 2. The gelatinization onset temperatures (T_o) of corn starches decreased from 61.1°C for native starch to 58.6°C for starch hydrolyzed for 15 days. The decrease tendency of T_o of cornstarch during acid-hydrolysis treatment was the same as that of wheat starch hydrolyzed by 2.2N HCl at 35°C [10]. In contrast, the T_o of potato starches increased

from 56.1°C for native starch to 58.2°C for starch hydrolyzed for 15 days. This was the same tendency as potato starch hydrolyzed by 2.2N HCl at 35°C [10]. For both corn and potato starches, the peak (T_p) and conclusion (T_c) temperatures of gelatinization increased with the increase of treatment time. Accordingly, the range of gelatinization increased from 12.2 to 23.9°C for cornstarch, and from 12.8 to 19.8°C for potato starch. The time courses of T_p and T_c during acid-alcohol hydrolysis were the same as starches hydrolyzed by 2.2N HCl at 35°C [10].

Table 2

Gelatinization temperatures and enthalpies of native starches and starches treated by 0.36% HCl in methanol at 25°C for 1 to 15 days.

Time (days)	Gelatinization temperature[§](°C)			T_o-T_c (°C)	Enthalpy[#] (J/g)
	T_o	T_p	T_c		
Corn Native	61.1	66.6	73.3	12.2	11.9
1	59.4	65.0	72.9	13.5	10.9
3	58.6	66.2	77.6	19.0	9.5
5	58.8	66.8	77.2	18.4	10.6
7	58.4	67.3	79.0	20.6	9.8
9	58.4	68.5	80.9	22.5	9.9
11	59.0	68.6	81.1	22.1	9.6
13	58.8	69.6	82.7	23.9	10.1
15	58.6	69.9	82.5	23.9	10.3
Potato Native	56.1	61.5	68.9	12.8	16.9
1	56.8	63.0	73.1	16.3	15.8
3	55.6	61.3	71.6	16.0	14.7
5	56.2	62.5	73.2	17.0	14.6
7	57.0	63.5	74.6	17.6	15.2
9	56.5	62.8	74.6	18.1	15.1
11	57.4	63.5	74.9	17.5	15.8
13	57.5	63.8	76.8	19.3	15.8
15	58.2	64.5	78.0	19.8	15.4

[§]T_o, T_p, and T_c stands for the onset, peak, and conclusion temperature of gelatinization, respectively. Standard deviations ± 0.6°C.

[#]Standard deviations ± 0.5 J/g.

Although the gelatinization enthalpies (ΔH) of modified starches were 1 to 2 J/g lower than their counterpart native starches, there were no obvious differences among the enthalpies of starches treated with different times. Similar result was found on star-

ches hydrolyzed by 2.2N HCl [10]. Although the crystallinity of starch granule was increased after acid hydrolysis [11–12] and the hydrolysis was preferential on the amorphous regions of the starch granule, the ΔH of acid-hydrolyzed starch did not show consistent correlation with the crystallinity of starch [10].

Molecular weight distributions of starches determined by HPSEC are shown in Fig. 4. The first fractions in the profiles corresponded to amylopectin, and the second fractions to the low molecular weight molecules. For the acid-alcohol treated starches, the areas of F1 fractions decreased with the increase of treatment time, while the areas of F2 fractions increased. This indicated the degradation of amylopectin to low molecular weight molecules due to the acid-alcohol hydrolysis. The degradation of amylopectin could cause the disruption of granular structure and the increase in leaching when starch was heated with water. Consequently, high extents of starch solubility (Fig. 2) and low pasting viscosity (Fig. 3) were observed.

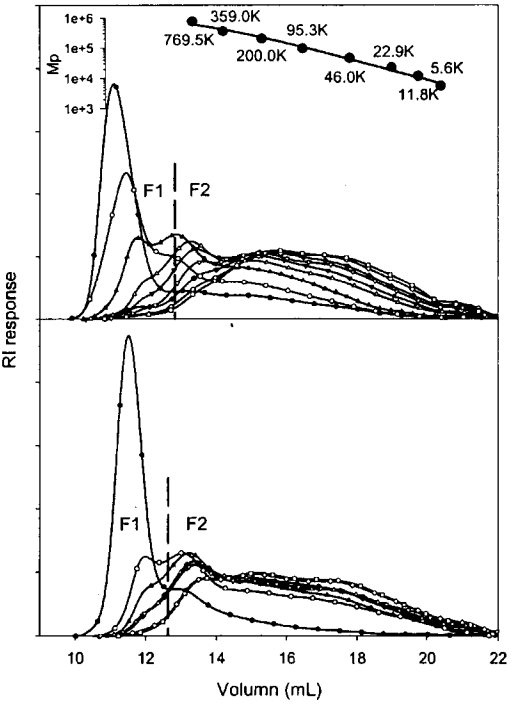

Fig. 4. HPSEC chromatograms of native starches (●) and starches treated by 0.36% HCl in methanol at 25°C for 1 (○), 3 (▲), 5 (Δ), 7 (▼), 9 (▽), 11 (◇), 13 (◆), and 15 days (□), respectively.

Compared to the gradual degradation pattern of cornstarch, potato starch showed a stepwise pattern (Fig. 4). The molecular weight distribution profiles of potato starches treated by 0.36% HCl in methanol at 25°C for 5 to 11 days were overlapped

with each others. The same is true for the distribution profiles of potato starches treated for 13 and 15 days. Figs. 5 and 6 show the changes and the normalized percentage of changes on the weight average degree of polymerization (DP_w) of corn and potato starches as a function of hydrolysis time. The results indicated the decrease in DP_w occurred mostly within the first 5 days of treatment, and potato starch had a higher rate of decrease than cornstarch. While the changes of number average of polymerization (DP_n) of starches (Fig. 7) also showed that the potato starch had higher decreasing rate, the normalized percentage of changes of DP_n (Fig. 8) indicated a relatively higher decrease extent of corn starch.

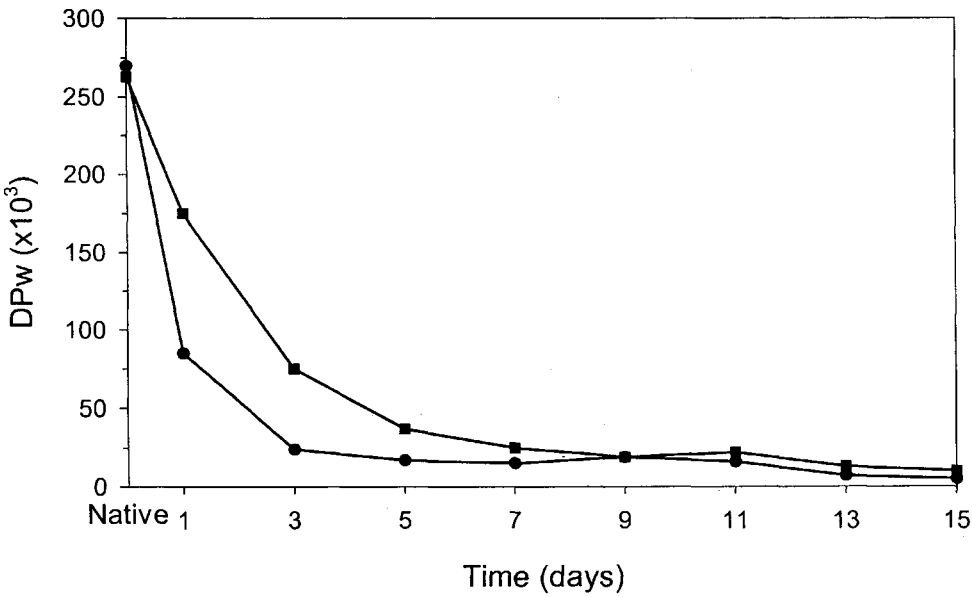

Fig. 5. Changes of weight average degree of polymerization (DP_w) of corn (■) and potato (●) starches as a function of hydrolysis time.

The DP_n of corn and potato starches after acid-alcohol treated for 15 days were 162 and 183, respectively, whereas the residue obtained after acid hydrolysis (2.2N HCl, 35°C, 14 days) of starch showed a bimodal distribution of chains on DP 14 and 28 [13]. The results of higher DP_n and higher yield of starch after acid-alcohol hydrolysis implied that the acid-alcohol treatment hydrolyzed the starch granule according to the mechanism different from that of acid hydrolysis in water. The mechanism of acid-alcohol hydrolysis in the starch granules can be further resolved by comparing the chain length distribution and X-ray patterns of native and acid-alcohol treated starches.

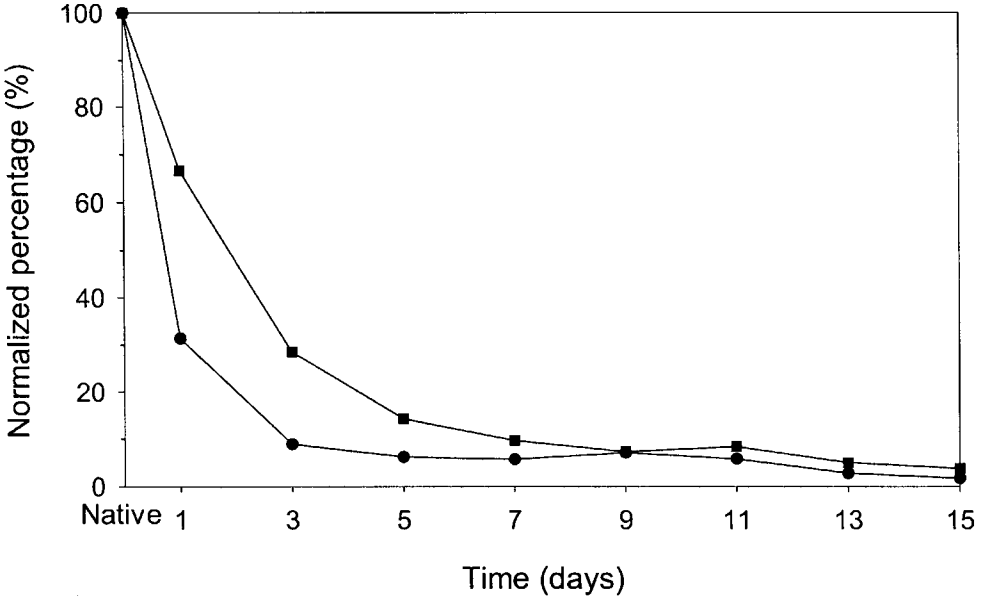

Fig. 6. Normalized changes of weight average degree of polymerization (DP_w) of corn (■) and potato (●) starches as a function of hydrolysis time.

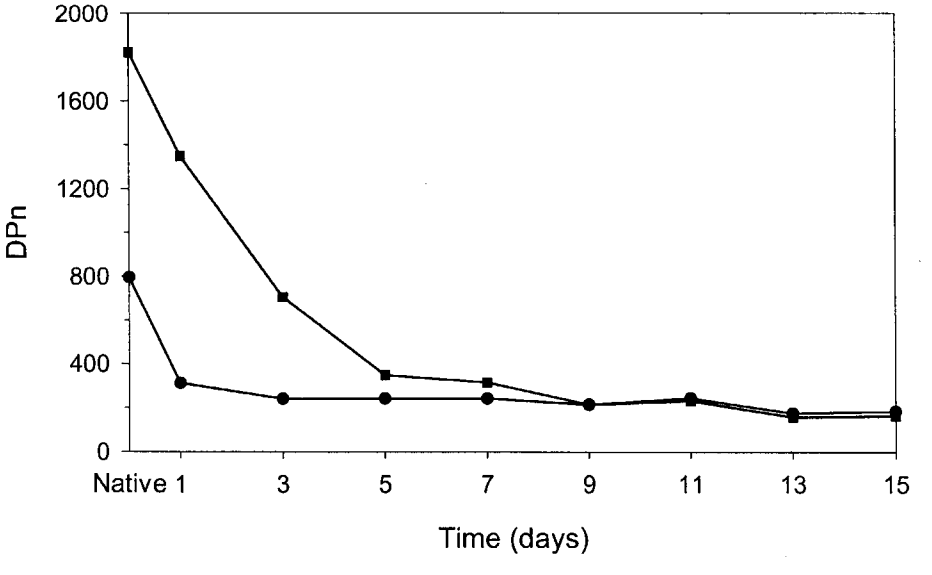

Fig. 7. Changes of number average degree of polymerization (DP_n) of corn (■) and potato (●) starches as a function of hydrolysis time.

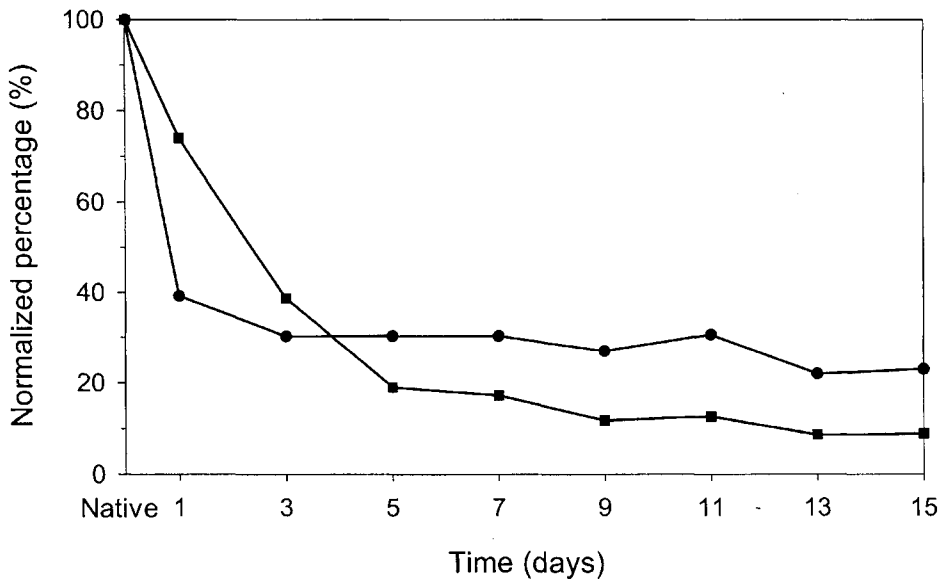

Fig. 8. Normalized changes of number average degree of polymerization (DP_n) of corn (■) and potato (●) starches as a function of hydrolysis time.

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DEGRADACJA SKROBI KWASAMI W ALKOHOLU

Streszczenie

W celu sprawdzenia degradacyjnego efektu modyfikacji skrobi za pomocą kwasów w alkoholu poddano hydrolizie, 0,36% roztworem chlorowodoru w metanolu, handlowe skrobię ziemniaczaną i kukurydzianą. Modyfikacje prowadzono w temperaturze 25°C w trakcie 1 do 15 dni. Wydajności modyfikowanych skrobi wynosiły od 91 do 100% a średni rozmiar tak modyfikowanych gałeczek skrobiowych był nieco mniejszy od rozmiaru gałeczek skrobi natywnych. Po piętnastodniowej modyfikacji nie stwierdzono zmian kształtu gałeczek, natomiast ich rozpuszczalność w wodzie wzrastała z czasem trwania modyfikacji. Zależność ta była wyraźniejsza w przypadku skrobi ziemniaczanej niż skrobi kukurydzianej. Temperatura początku żelowania (T_o) skrobi po modyfikacji malała w przypadku skrobi kukurydzianej i wzrastała w przypadku skrobi ziemniaczanej. W przypadku obu skrobi temperatura w maksimum (T_p) i końca żelowania (T_c) w wyniku tych modyfikacji wzrastała w miarę wydłużania czasu modyfikacji. W następstwie modyfikacji zakres temperaturowy, w którym zachodziło żelowanie rozszerzył się z 12.2 do 23.9°C w przypadku skrobi kukurydzianej i z 12.8 do 19.8°C w przypadku skrobi ziemniaczanej. Jednak entalpie żelowania (ΔH) skrobi modyfikowanych obniżały się zaledwie o 1 do 2 J/g. W tak modyfikowanych skrobiach obszar frakcji amylopektynowej wyznaczony za pomocą wysokosprawnej żelowej chromatografii podziałowej (HPSEC) malał wraz z czasem prowadzonej modyfikacji. Zmiany w przypadku skrobi kukurydzianej odbywały się w sposób ciągły, natomiast w przypadku skrobi etapowo. Średni stopień polimeryzacji (DP_w) gwałtownie obniżał się w ciągu pierwszych pięciu dni prowadzenia procesu, przy czym w przypadku skrobi ziemniaczanej był on gwałtowniejszy. Po 15 dniach obróbki stosowne wartości DP_w skrobi kukurydzianej i ziemniaczanej wynosiły odpowiednio 162 i 183.

Badania te pozwalają stwierdzić, że modyfikacje zmiany struktury molekularnej skrobi odbywają się za sprawą degradacji amylopektyny. Skutkiem modyfikacji jest zniszczenie gałeczek i obniżenie lepkości żeli.

ERIC BERTOFT[1]

THE STRUCTURE OF CLUSTERS FROM POTATO AMYLOPECTIN

Summary

The clusters from size-fractions of hydrolysed amylopectin from amylose-free potato starch (APP) were isolated by controlled α-amylolysis. The external chains were removed by further phosphorolysis and β-amylolysis, thereby transforming the APP and the clusters into ϕ,β-limit dextrins. The DP of the ϕ,β-LD of the clusters was rather uniform around 40-50. The unit chain composition was analysed by HPAEC-PAD and it was shown that the long internal B-chains, that interconnected the clusters in the amylopectin, had preferentially been cut into B-chains with DP 8-23. Smaller clusters possessed also increased amounts of the shortest B-chain with DP 3. The A:B-chain ratio decreased after α-amylolysis, showing that A-chains were not formed. The clusters were built up of only 4–6 chains and the density of branches was 11–13%. Very small, branched building blocks were also isolated from the APP and the clusters by an extensive α-amylolysis and analysed by GPC and HPAEC. The building blocks ranged between DP 5–30 and contained 2–5 very short chains (approx. CL 2-7). The predominating branched building blocks had DP 7–8 and were singly branched. The density of branches within building blocks was high (25%) and the clusters were at average composed of 2 or 3 building blocks.

Introduction

Amylopectin, the branched component of starch, is build up of numerous clusters [1-4] of short chains with a degree of polymerisation (DP) within the range 6–35. These chains are designated B1-chains (short chains carrying other chains through α-(1→6)-linkages) and A-chains (short chains not carrying other chains) [5]. Longer chains with DP approximately 35–60 (B2-chains) or longer (B3-chains) interconnect the clusters [6]. A large number of investigations on the unit chain profiles of different amylopectins have shown that they all possess this general pattern. However, starch granules with a B-crystalline X-ray diffraction pattern (mainly tuber and root starches) have somewhat longer average chain lengths (CL) and a clearly lower ratio of short chains to long chains than granules with A-crystallinity (mainly cereal starches) [7].

[1]*Address: Department of Biochemistry and Pharmacy, Åbo Akademi University, P. O. Box 66, FIN-20521 Turku, Finland.*

When considering the extensive number of investigations on the unit chain profile of amylopectins, surprisingly little work has focussed on the actual branching pattern within the units of clusters and the mode of interconnection of the clusters. In fact, in order to really understand the fine structure of different amylopectins, these latter aspects are at least as important as the knowledge of the unit chain distribution. An enzymatic method, in which the α-amylase of *Bacillus amyloliquefaciens* plays the key role, was developed in my laboratory for the isolation of the units of clusters [8]. Shortly, the amylopectin macromolecule is initially rapidly fragmented into intermediate α-dextrins by the attack of the enzyme on long internal chains between the clusters [9]. When such internal chain segments no longer remain, the nine subsites around the active site on the enzyme will not be fully interacting with the substrate anymore [10]. As a result, the reaction rate decreases and the obtained clusters can be size-fractionated by methanol precipitation [11].

To study the branching pattern within the clusters, the external chains (that also have been partly attacked by the α-amylase) are largely removed with the enzymes phosphorylase and β-amylase [12]. In the remaining ϕ,β-limit dextrins (ϕ,β-LD) of the clusters all A-chains are found as maltosyl-stubs, whereas all B-chains are longer[9]. Thus, after a debranching and chromatographic analysis, the ratio of A- to B-chains can be estimated and the distribution of the internal parts of the B-chains is also obtained [12, 13]. The ϕ,β-LD can also be further hydrolysed by the α-amylase by a 100-fold increased enzyme activity. This extensive hydrolysis results in small building blocks (α-limit dextrins) of very tight branchings [14]. Therefore, it is also possible to study the size distribution of the building blocks found within the clusters.

The branching pattern of the clusters of amylopectin from an amylose-free potato is now reported. The results are compared with earlier studies on the amylopectin from waxy-rice [13, 14] and two maize mutants [15].

Materials and methods

Starch from amylose-free potato (APP) was a kind gift from Lyckeby Stärkelsen, Sweden. α-Amylase of *Bacillus amyloliquefaciens* (EC 3.2.1.1), with an activity of 600 U/mg, was from Boehringer-Mannheim (Germany), β-amylase of barley malt (EC 3.2.1.2) from Megazyme International (Ireland), and phosphorylase *a* of rabbit muscle (EC 2.4.1.1) from Sigma (Germany). The debranching enzymes isoamylase of *Pseudomonas amyloderamosa* (EC 3.2.1.68) and pullulanase of *Klebsiella pneumoniae* (EC 3.2.1.41) were from Hayashibara Shoji Inc. (Japan).

Isolation of clusters. APP (10 g) was dissolved in 90% dimethylsulphoxide (DMSO), diluted in sodium acetate buffer (pH 6.5), and treated at 25°C with diluted α-amylase (0.03 U/mL) at a substrate concentration of 10 mg/mL as previously described

[12]. The reaction was stopped after 1h and branched intermediate α-dextrins were precipitated with five volumes of methanol. The precipitate was then dissolved in water and size-fractionated by the method of Bertoft and Spoof [11]. Four of the fractions, representing large (fraction L), medium (M), small (S), and very small (VS) dextrins, were collected for analyses in this study. Fractions L, M, and S were further treated preparatively with the amylase for 1.5 h as described above, and the products were again collected by precipitation in methanol (5 volumes). Fraction L was finally treated for an additional 3 h with the enzyme. After these treatments all fractions were comparatively resistant to further hydrolysis and were therefore considered as being composed of units of clusters.

Production of ϕ,β-LD. Preparative production of ϕ-LD by phosphorolysis [16] and further into ϕ,β-LD by β-amylolysis [12] was previously described. However, the products glucose 1-phosphate and maltose, respectively, were removed from the fractions of clusters on two PD-10 columns (Pharmacia) coupled in series and from APP by dialysis.

Debranching. APP or intermediate α-dextrins were debranched with isoamylase at pH 3.5, whereas ϕ,β-LD were debranched with pullulanase at pH 5.5 essentially as described elsewhere [13]. All samples were analysed by HPAEC-PAD as described below.

Production and analysis of building blocks. ϕ,β-LD (5 mg/mL) were treated with concentrated α-amylase (6 U/mL) for 3 h at 35°C. The reaction was stopped by boiling and the sample was then lyophilised. The dried sample (4 mg) was dissolved in hot DMSO (0.2 mL). One part of the sample was diluted in water and analysed on a column of Superdex 75 or by HPAEC. Another part (15 μL) was diluted to 1 mL with NaOAc buffer (pH 5.5), treated with pullulanase (1 μL) overnight at room temperature, and finally analysed by HPAEC.

Gel-permeation chromatography (GPC). Products from α-amylolysis of ϕ,β-LD were analysed on a column (1x90 cm) of Sepharose CL 6B (Pharmacia) as described by Bertoft *et al.* [14]. A column (1x90 cm) of Superdex 75 was eluted with 0.01 M KOH and used for the analysis of unit chains and building block profiles. Superdex 30 (1x90 cm) was eluted with water and was used for preparative isolation of size-fractions of building blocks or unit chains from APP. The columns were calibrated with samples of dextrins of known DP [11, 17].

High-performance anion-exchange chromatography (HPAEC). Ion-exchange chromatography was performed on Dionex series 4500i (USA) equipped with a BioLC gradient pump and pulsed amperometric detection (PAD). The main column and the guard column (CarboPac PA-100, Dionex) were eluted at 1 mL/min. The gradient included eluent A (150 mM NaOH) and eluent B (150 mM NaOH containing 500 mM

NaOAc). The sample (25 μL) was applied at 85% A and 15% B. Debranched samples of APP, α-dextrins, and ϕ,β-LD were then eluted with the following gradient: from 0–9 min a linear gradient of eluent B from 15–36%; 9–18 min from 36–45%; and 18–110 min from 45-100%. Building blocks and debranched blocks were eluted with a different gradient: from 0–15 min from 15–34%; 15–26 min from 34–40%; 26–52 min from 40–49%; and from 52–54 min from 49–100% of eluent B. For quantitative analysis the system was calibrated by the method of Koch *et al.*[18] using chains from debranched APP or building blocks from the ϕ,β-LD of APP prepared by fractionation on Superdex 75 or Superdex 30, respectively.

Results and discussion

A selected series of intermediate α-dextrins was obtained after 1 h of hydrolysis of the potato amylopectin and analysed on Sepharose CL-6B. Large dextrins in fraction L possessed an average DP of 279 and represented 7.1% of all branched dextrins from the hydrolysis mixture (Table I).

Table I

Characterisation of amylopectin and fractions of α-dextrins.

Sample	Yield (%)	α-Amyl. time[1] (h)	Total time[2] (h)	DP[3]	CL[4]	ECL[5]	ICL[6]
APP		0	0	-	21.2	13.7	6.5
L	7.1	1.0	1.0	279	15.1	8.3	5.8
L-II		1.5	2.5	111	13.4	6.9	5.5
L-III		3.0	5.5	60	10.5	4.3	5.2
M	10.5	1.0	1.0	104	16.1	8.3	6.8
M-II		1.5	2.5	65	13.9	6.5	6.4
S	3.9	1.0	1.0	87	15.5	7.9	6.5
S-II		1.5	2.5	58	13.9	7.5	5.4
VS	7.2	1.0	1.0	37	16.0	9.0	6.0

[1]Hydrolysis with α-amylase in each successive step.
[2]The sum of the successive steps.
[3]From GPC on Sepharose CL-6B.
[4]From HPAEC after debranching with isoamylase.
[5]ECL = CL x (% ϕ,β-limit/100) + 1.5.
[6]ICL = CL - ECL - 1.

The DP of the other fractions (M, S, and VS) decreased to DP 37. When the fractions were treated further with the α-amylase, the smallest dextrins (fraction VS) were practically resistant to the enzyme (Fig. 1), which suggested that they represented small clusters from the amylopectin. Dextrins in fractions M and S decreased only slightly in

size by a second amylase treatment for 1.5 h (giving fractions M-II and S-II) and clusters were therefore probably to a large extent already present in these fractions after the initial 1 h treatment. The dextrins in fraction L were, however, readily attacked by the second amylase treatment, which showed that they represented groups of interconnected clusters. The dextrins from the second treatment (L-II) could even be hydrolysed a third time for an additional 3 h into fraction L-III before the hydrolysis rate became very low and clusters were obtained (Fig. 1). The average chain length (CL) decreased from 21.2 in the original APP to between 10.5 and 16. As shown below, this was mainly due to the attack at external chains by the enzyme [10], rather than the attack at longer chain segments between the clusters.

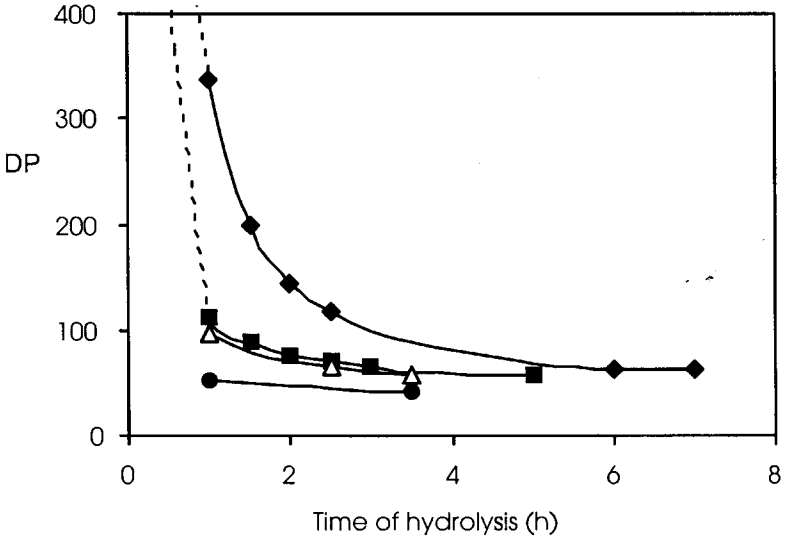

Fig. 1. DP as a function of time during α-amylolysis of fractions L (◆), M (■), S (△), and VS (●). The DP was estimated from GPC on Sepharose CL-6B excluding dextrins of DP < 20.

The external chains were next removed by successive phosphorolysis and β-amylolysis from APP and the fractions containing the clusters (fractions L-III, M-II, S-II, and VS). The remaining ϕ,β-limit dextrins represented therefore the internal structures, or branching zones of the clusters (BZC) [15]. Their DP ranged from 31–48 (Table II) and was comparable to that found for the double mutant *aewx* from maize [15]. As both starches were of B-crystalline type, this suggests a common cluster size of such starches and is in contrast to the several A-crystalline starches earlier investigated [14-16, 19], in which the clusters tend to be larger and the DP-range is much broader.

Table II

Characterisation of the ϕ,β-LD of amylopectin and fractions containing clusters.

Sample	ϕ,β-Limit (%)	DP[1]	CL[2]	NC[3]	Branch density[4]	A:B[5]
APP	57	-	9.1	-	11.0	1.3
L-III	26	40	7.8	5.1	12.8	0.9
M-II	36	46	8.9	5.2	11.2	0.8
S-II	43	48	7.9	6.0	12.7	1.0
VS	47	31	8.5	3.7	11.8	0.8

[1]From GPC on Sepharose CL-6B.
[2]From HPAEC after debranching with pullulanase.
[3]Number of chains = DP/CL.
[4]Density of branches (%) = (1/CL) x 100.
[5]Molar ratio of A-chains to B-chains.

The ϕ,β-LD of the APP and the clusters were debranched and analysed by HPAEC-PAD. From the difference of the CL-values before and after limit dextrin production, the ϕ,β-limit values were calculated (Table II) and finally these were used for estimation of the average lengths of the external and internal chains [9] of the samples (Table I). The APP sample possessed similar values to those we earlier found for amylopectin from a normal potato starch [12], though the CL and ECL were slightly lower compared to other reports [20]. The effect of the α-amylolysis on the ECL-values was clearly seen, but the ICL decreased only little (Table I). This could be explained by the fact that the long internal chain segments between clusters, that the enzyme easily attacks are rather few compared to the very short internal chains found within the clusters. The average number of chains (NC) in the clusters was approx. 4-6 and the branch density 11–13% (Table II), which again was similar to that found for *aewx*-maize starch [15]. In A-crystalline starches from *wxdu*-maize [15] and *wx*-rice [13] both the NC and the density of branches were higher (approx. 6-18 and 13-19%, respectively).

In a ϕ-LD the A-chains have been reduced into maltotetraosyl-stubs [21] and after successive β-amylolysis only a maltosyl-stub remains [9]. This is the case with all normal amylopectins, in which chains shorter than DP 6 are not found [22, 23]. In the α-dextrin samples, however, a part of the A-chains (5–15%) had been reduced into maltotriosyl-stubs, in addition to trace amounts of maltosyl-stubs (not shown). Such very short chains are resistant to both phosphorylase and β-amylase, and the maltotriose formed after the debranching will therefore be a mixture of maltotriose from a part of the A-chains and the shortest possible B-chains. It was therefore necessary to subtract the maltotriose originating from A-chains from the maltotriose peak of the

debranched ϕ,β-LD clusters in order to obtain a correct estimation of the ratio of A:B-chains. In APP, the ratio was 1.3 (Table II) and it was clearly lower (0.8–1.0) in the isolated clusters. This suggested that the long B-chains between clusters mainly were cleaved into two new B-chains by the α-amylase [12]. The result was different from that found earlier for amylopectin from a normal potato starch, for which an increased A:B-ratio was found after α-amylolysis [12]. It remains unclear if this was due to true differences between the two samples or if it was a result of the improved resolution obtained with HPAEC compared to GPC that was used in the earlier investigation.

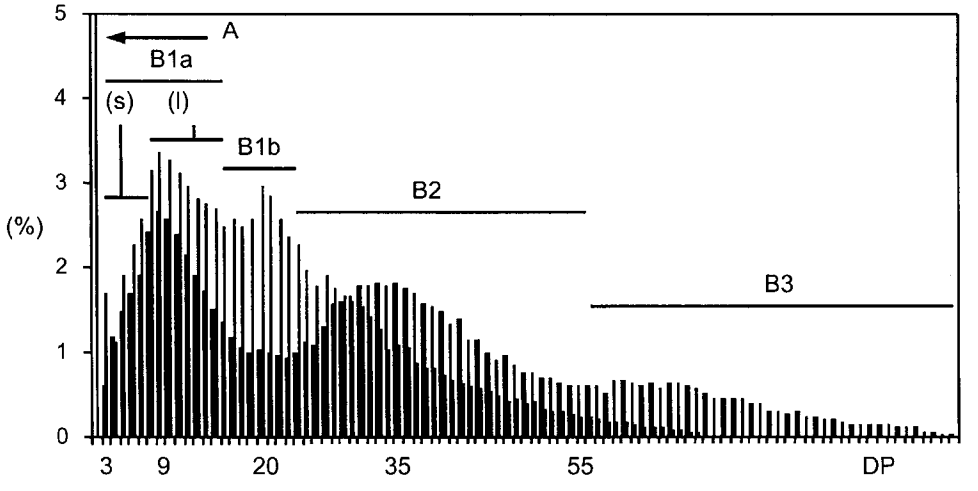

Fig. 2. Bar graph of the chain length distribution of the ϕ,β-LD of APP (black bars) and clusters (sample S-II; light bars). Areas of peaks in HPAEC were corrected to carbohydrates by weight. Groups of different chains are shown. All A-chains are shown as maltose (DP 2). Because peaks at DP >55 were not actually resolved, the bars for B3-chains were only roughly estimated by comparison with GPC of the debranched samples.

The distribution of the internal B-chains of the ϕ,β-LD was analysed by HPAEC (Fig. 2). The long chains of APP were divided into B2- and B3-chains [6] and, on the basis of the division of chains in *wx*-rice starch [13], the short B-chains were sub-divided into B1a- and B1b-chains. The former group was further sub-divided into very short chains [B1a(s)] and somewhat longer chains [B1a(l)]. It was interesting to notice, that the profile of the shortest B1a(s)-chains was different from that of the *wx*-rice starch [13], which possessed a peak at DP 5, rather than at DP 7 (Fig. 2). This indicated that the mode of tightly clustered branching in the two starches was different. From the example given in Fig. 2 (fraction S-II) it is clearly seen that, in order to release the clusters, the α-amylase cleaved the long internal chains, which mainly resulted in the production of B1b-chains together with some B1a(l)-chains. The profiles

of the other clusters were similar (though not identical) to that of fraction S-II. However, the clusters produced from fraction L did not show any increase in maltotriose, whereas increasing amounts were obtained for clusters from fractions M, S, and VS (only S-II shown). This suggested that the clusters, though fairly similar regarding their sizes and unit chain composition, possessed minor differences regarding their mode of interconnection.

The analysis by GPC of the size-distribution of building blocks after an extensive α-amylolysis was first described for *wx*-rice starch and its clusters [14]. Interestingly, the size-profiles could be divided into two types that possibly originated from different structural domains of the starch granules. The DP of the branched building blocks ranged from 5 to approx. 40, and the average DP was 13–17 [14], which was similar to that later found for the amylopectin from *wxdu*-maize [15]. The size-distribution of the building blocks of APP obtained by GPC is shown in Fig. 3.

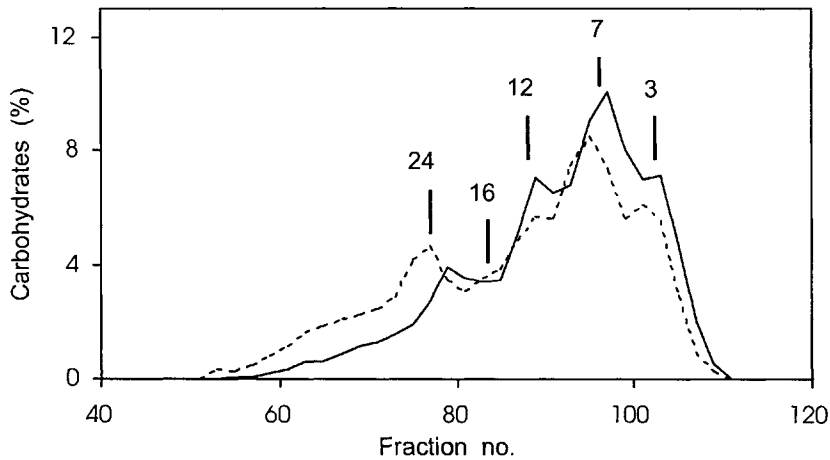

Fig. 3. Fractionation on Superdex 75 of building blocks obtained by extensive α-amylolysis of the ϕ,β-LD of APP (——) and clusters (fraction S-II; ----). DP values are indicated.

The major fraction of blocks had a DP of 6-8 and only small amounts of blocks with DP >14 were found. This was similar to the mainly B-crystalline *aewx*-maize sample [15] and suggested a common, small building block structure for this type of starches. The profile of building blocks was also analysed with HPAEC (Fig. 4), by which a large number of individual peaks representing building blocks up to DP 19 were resolved.

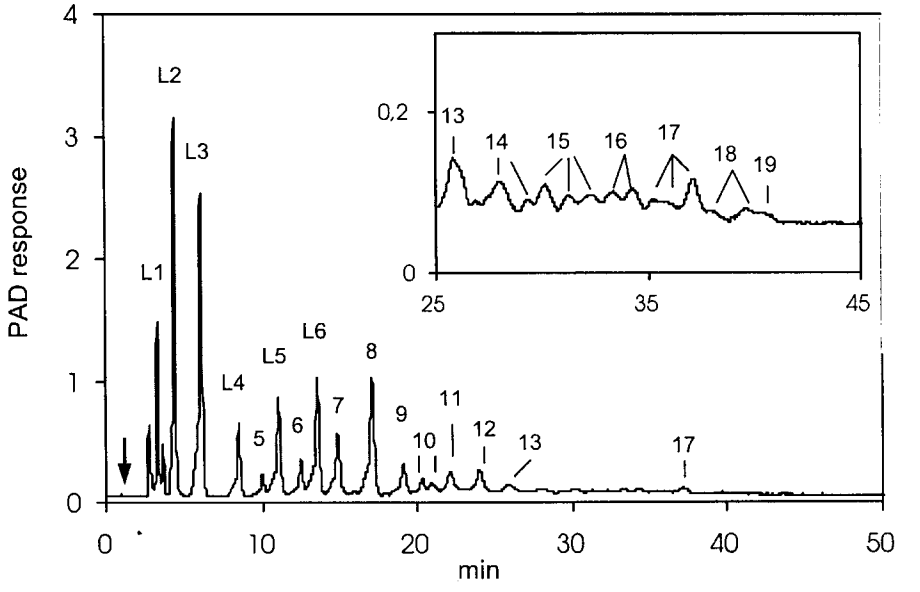

Fig. 4. Fractionation by HPAEC-PAD of building blocks obtained by extensive α-amylolysis of the ϕ,β-LD of APP. DP values are indicated. L indicate linear dextrins. Sample injection and start of the gradient is shown by arrow. Inset is a magnification of the chromatogram.

Linear dextrins of DP 1-6 represented fragments of the internal chains between the clusters of the amylopectin and between the building blocks within the clusters. The smallest branched building block had DP 5-7. Several of the larger blocks were represented by more than one peak, suggesting that they were found as singly and/or multiply branched molecular species. The PAD-response, which decreases with the DP of linear dextrins [22], was found to give a similar response for the branched dextrins. The relative molar amount of the branched building blocks of APP is shown in Fig. 5a. Blocks with DP 8 predominated and the distribution of building blocks of the clusters (not shown) was similar to that of the whole amylopectin sample (with the exception of fraction VS, in which DP 7 predominated). This suggested a rather high structural homogeneity among the clusters of APP, in contrast to the amylopectins of *wx*-rice[14] and maize mutants [15]. The average number of branched blocks within the clusters of APP was only between 2–3.

The mixtures of building blocks were also debranched with pullulanase and analysed by HPAEC. The relative molar amount of the chains from the branched blocks of APP was obtained by subtracting the pre-existing linear chains from the chromatograms (Fig. 5b).

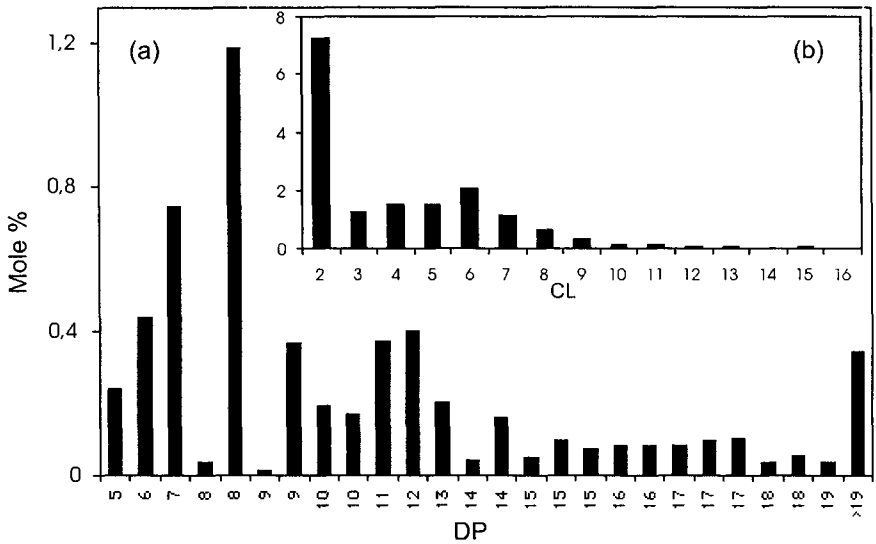

Fig. 5. Bar graph of the molar distribution of (a) branched building blocks from the ϕ,β-LD of APP and inset (b) the chains obtained after debranching. The linear dextrins obtained by the extensive α-amylolysis have been subtracted from the graphs.

The chains of the branched building blocks were very short and corresponded to the shortest chains in APP (A- and B1a(s)-chains). The structural characteristics of the branched building blocks are summarised in Table III. From the DP-values (approx. 12) and the CL (4) an average number of chains near 3 was obtained. If it is assumed that the ECL of the branched blocks (α-LD) was approx. 2, the ICL was also about 2 between the branches inside the blocks. The branching density was approx. 25%.

Table III

Characterisation of branched building blocks in amylopectin and its clusters.

Sample	DP[1]	CL[1]	ICL[2]	NC[3]	Branch density[4]
APP	10.8	4.0	2.2	2.7	24.9
L-III	11.8	3.9	1.9	3.0	25.4
M-II	12.6	4.3	2.5	2.9	23.3
S-II	11.8	4.1	2.1	2.9	24.6
VS	10.3	3.8	1.8	2.7	26.6

[1]From HPAEC.

[2]ICL = [((CL - ECL) x NC) / (NC - 1)] - 1.

[3]Number of chains = DP/CL.

[4]Density of branches (%) = (1/CL) x 100.

To characterise the branched building blocks further, a semi-preparative separation was performed by GPC on Superdex 30. Fractions representing increasing DP were collected, debranched, and finally analysed with the HPAEC-equipment. A linear relation between the DP and the number of chains was obtained (Fig. 6) and could be used to estimate the NC of the individual peaks of building blocks in Fig. 5a.

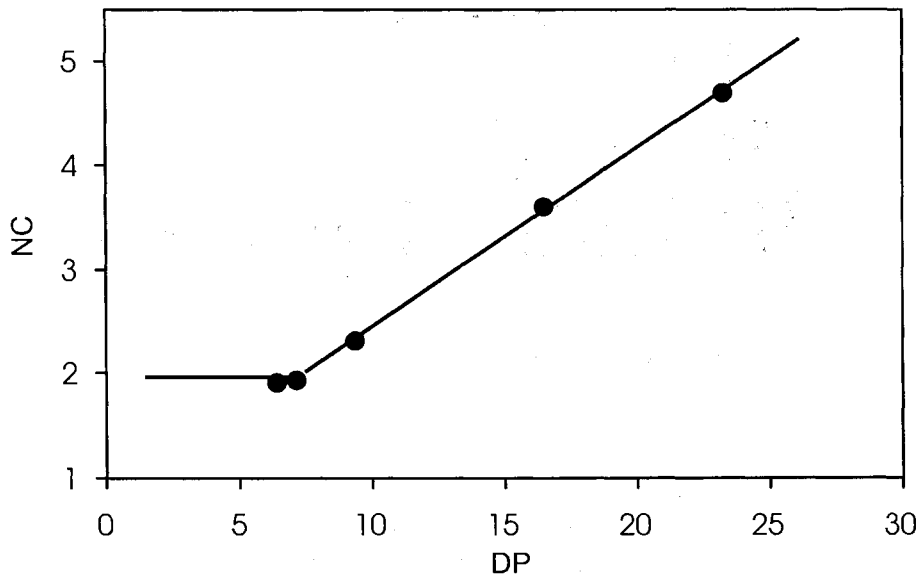

Fig. 6. Number of chains *versus* DP of size-fractions of branched building blocks from APP.

The major blocks of DP 7-8 contained only 2 chains, while the second abundant group of blocks at DP 11-12 mostly was composed of 3 chains. The larger building blocks with DP around 24 (Fig. 3) had 5 chains, whereas blocks with 4 chains were only little represented.

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STRUKTURA KLASTERÓW AMYLOPEKTYNY ZIEMNIACZANEJ

Streszczenie

Za pomocą kontrolowanej α–amylolizy amylopektyny z pozbawionej amylozy skrobi ziemniaczanej (AAP) otrzymano frakcje klasterów o różnych rozmiarach. Zewnętrzne łańcuchy usunięto następnie przez fosforolizę i β-amylolizę przeprowadzając w ten sposób AAP w ϕ,β-dekstryny. DP klasterów tych dekstryn był dość wyrównany mieszcząc się w granicach 40 do 50. Skład poszczególnych łańcuchów analizowano za pomocą HPAEC-PAD pokazując, że długie wewnętrzne łańcuchy B łączące ze sobą poszczególne klastery zostały pocięte głównie na łańcuchy B o DP 8–23. Mniejsze klastery również zawierały większą liczbę krótszych łańcuchów B o DP 3. Stosunek łańcuchów A:B po α-amylolizie obniżał się, co wskazywało, że łańcuchy A nie tworzyły się. Klastery tworzyły się tyko z 4 do 6 łańcuchów, a gęstość odgałęzień wynosiła 11 do 13%. Przez daleko idącą α-amylolizę wydzielono z AAP bardzo małe rozgałęzione fragmenty strukturalne, które zanalizowano za pomocą GPC i HPAEC. DP tych fragmentów wynosił od 5 do 30. Zawierały one 2 do 5 bardzo krótkich łańcuchów (przeciętnie 2–7). Przeważnie fragmenty te miały DP 7 do 8 oraz pojedyncze rozgałęzienia. Gęstość rozgałęzień była wysoka (25%), a klastery przeciętnie zawierały 2 lub 3 takie fragmenty.

LUYBOV A. WASSERMAN[1*], ISRAIL O. ALIEV[2], VLADIMIR P. YURYEV[1]

INTERACTION OF STARCH POLYSACCHARIDES AND THEIR MIXTURE WITH WATER MOLECULES AND MODEL LIPIDS. ESR STUDY

Summary

Electron spin resonance (ESR) was used in order to study interaction of starch polysaccharides (amylose and amylopectin), their mixture and gelatinized potato starch with water molecules and lipids upon cooling. Different spin probes were used, on the one hand spin-labelled stearic acid (5-DSA), which limited lipids, and on the other hand the water soluble probe 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl (Tempol), which was sensitive to changes in dynamic water phase associated with the temperature-induced polysaccharide gel formation. It was shown that interaction between gelatinized starches and lipids related to mainly on presence of amylose macromolecules in the system. On the other hand, interaction between amylopectin macromolecules and lipids takes place also.

Introduction

Depending on starch origin and functional properties gelatinized starches are widely used in complex food systems such as meat products (for example, ham-type products, hamburgers), emulsion sausages, for example, Frankfurter and Bologna types of sausages, and other [1, 2]. Important features of such products, besides sensory attributes (consistency, taste, appearance and juiciness) are water holding and fat holding therefore gelatinized starches are used as gelling or thickening agents. When the treatment leading to the final products implies distruption of natural components, either tissue or cell level, the systems, once sufficiently hydrated at suitable temperatures, display heterogeneity related to phase separations which are mainly driven by the thermodynamic incompatibility between the different polymers components, like

[1]*Institute of Biochemical Physics of Russian Academy of Sciences, 4, ul. Kosygina, 199991 Moscow, Russia; [2]Institute of Chemical Physics of Russian Academy of Sciences, 4, ul. Kosygina, 199991 Moscow, Russia; *Corresponding author: Institute of Biochemical Physics of Russian Academy of Sciences, 4, ul. Kosygina, 199991 Moscow, Russia; e-mail: v.yuryev@sky.chph.ras.ru*

polysaccharides versus proteins [3], or even between different polysaccharides like amylose versus amylopectin [4]. This implies water partition into different phases and different kinds of interactions between water molecules and substrates. Additionally, starch polysaccharides may interact with minor components of complex food systems such as vegetable and animal fats. However, some problems concerning interaction gelatinized starches and starch polysaccharides, in particular, with water, lipids and fatty acids molecules remain not quite discussed till now.

Some methods, such as calorimetry and tradition thermal analyses, that allow to detect the macroscopic properties, like heat capacity and thermodynamic activity, can be sensitive only for the changes occurring in the phases where water has the highest mobility. Others, like Nuclear Magnetic Resonance (NMR) and Electron Spin Resonance (ESR), on the contrary can provide information about many coexisting states of water molecules, which are separated from one another because of the different relaxation times related to the short-range mobility. In particular, as was shown by ESR study the rotational diffusion coefficient (D_{rot}) decreased monotonically with decreasing temperature in the system of gelatinized potato starch-water [5]. Investigating of starch gels, Baster and Lechert [6] established that the coefficient of self-diffusion of water molecules is roughly proportional to the square of the water fraction. At present it is known that spin-lattice relaxation times ($1/T_2$) measured as a function of water contents indicate a wide distribution of correlation times in the processed starch-water systems [7]. As has been shown earlier complex relaxation of water molecules is observed due to formation of amylose aggregates at starch concentrations close to critical gelation concentration or close to critical gelation temperature [8, 9]. Lifetime of these aggregates was comparable or exceeded the relaxation time of water molecules, which formed during gelatinization of starch and dissolution of maltodextrin. However up to now it is not quite understood what of polysaccharides (amylose or amylopectin) is determined the mobility of water molecules in starch-water systems. Additionally, it is not quite clear whether mobility of water molecules in real starch systems can be described using additive scheme assessing of mobility of water molecules and content of starch polysaccharides in the simple polysaccharides systems (amylose – water, amylopectin – water).

In contrast to investigation devoted to interaction of native starches with lipids model (spin probes such as spin-labelled stearic acids), the data concerning interaction in systems of gelatinized starch – spin probe, amylose (amylopectin) – spin probe are not enough. It is known that upon cooling of the potato (maize) starch – water systems the sharp decrease of mobility of spin probe was observed close to the critical gelation temperature [5, 10]. Additionally, it is known that amylose macromolecules can form inclusion complexes with low molecular substances such as fatty acids, lipids and aroma compounds [11, 12]. It is suggested that side chains of amylopectin macromole-

cules can form inclusion complexes also however directly evidence of existing such complexes we didn't find at the analysis of the published data [13, 14]. For better undestanding of features of interactions of the gelatinised starches with water, fatty acids and lipids in complex food systems we study interactions amylose, amylopectin, their mixture and potato starch with these low molecular substances by ESR. Spin-labelled stearic acid (5-DSA) was chosen as lipid model. A water-soluble stable radical (Tempol) was also used in order to probe changes in the properties of aqueous continuous phase.

Materials and methods

Potato starch (20–25% amylose according to literature data [15]) was obtained from Paille (France). The content of proteins and lipids in commercial potato starch was very low and constitutes 0.06% w/w (proteins) and 0.05%w/w (lipids) on dry substance [16]. Amylose (EC N 232-685-9) and amylopectin (EC N 232-911-6) from potato were purchased from Sigma, USA.

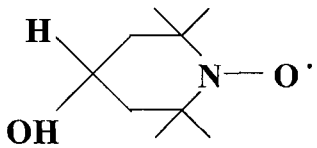
Paramagnetic homologue of stearic acid (5-Doxyl stearic acid (5-DSA)) used as lipid model and Tempol (4-hydroxy-2,2,6,6-tetra-methylpiperidine-1-oxyl) were obtained from Sigma (USA).

The chemical structures of these spin probes are shown on Fig. 1. 40% (w/w) dispersions of amylose, amylopectin, their mixture and potato starch were studied. Spin-labelled stearic acids were poorly soluble in water and were introduced to water as acetone solutions (as described in [17, 18]) The acetone concentration in water did not exceed 1 w/w %. The system was sonicated and mixed intensively for 1 hour at 50–55°C to evaporate the acetone. The concentration of the spin probe in water was $2.6 \cdot 10^{-4}$ M. Potato starch with moisture contents of 14.9% (w/w) was used for preparation of dispersions. Aqueous solutions of spin-probe (0.53 g) were added to investigated polymer systems (0.47 g) for preparation of dispersions (40% w/w) at room temperature. As Tempol was more soluble in water, an aqueous Tempol solution was prepared and added to the starch dispersion in order to reach a 3.6×10^{-4} M concentration of Tempol in the investigated dispersions.

After 24 hours incubation, the samples of starch preparations with different water contents were placed into glass tubes ($d \approx 1$ mm), which were sealed to prevent dehydration during heating. For preparation of a macromolecule dispersion of investigated systems the samples were heated up to 115°C [19]. ESR spectra were recorded with a controlled temperature EPR spectrometer (Radiopan, Poland), using cooling cycles over a temperature range of 115–25°C by stepwise fashion at interval 10°C. Samples were allowed to come to thermal equilibrium for three minutes at each temperature before spectra recording. The microwave power was below saturation. ESR spectra

were recorded three times per sample. No differences were observed between spectra recorded at the same temperature at a given starch content.

a) Tempol



b) 5- Doxyl stearic acid (model of lipid)

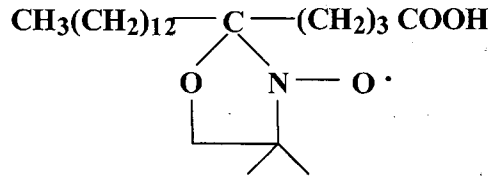


Fig. 1. Chemical structures of spin probes Tempol (a) and 5-doxyl stearic acid (model lipid) (b).

For the description of the spin probe mobility, the rotational correlation time (τ_c) and the rotational diffusion coefficient (D_{rot}) were usually used [20]. The rotational correlation time was determined from features of the spectra obtained with fast isotropic rotation according to the Freed and Fraenkel theory [18, 20].

The following equation was used:

$$\tau_c = 6.65\Delta H_{(+1)}(\sqrt{I_{(+1)}/I_{(-1)}} - 1)10^{-10}, \text{ sec} \quad (1)$$

where: $\Delta H_{(+1)}$ is the peak-to-peak width of the low field line (Gs);

$I_{(+1)}$ and $I_{(-1)}$ are the heights of the low and high field lines, respectively.

The rotational diffusion coefficient was calculated from the following relation:

$$D_{rot} = 1/(6\tau_c), \text{ sec}^{-1} \quad (2)$$

The program of Freed [20] modified by V. Timofeev [21, 22] was used for the simulation of ESR spectra in the region of slow motions. The calculations were made according to the model of isotropic rotation, and the following values for the electron-spin parameters of radicals in the presence of amylopectin were used:

$g_{xx} = 2.088$; $g_{yy} = 2.061$; $g_{zz} = 2.0027$; $A_{xx} = 6.3$ Gs; $A_{yy} = 5.8$ Gs; $A_{zz} = 33.6$ Gs [23], where g_{xx}, g_{yy}, g_{zz} – are the main components of Zeeman interaction tensor (g-tensor), and A_{xx}, A_{yy}, A_{zz} are the main components of hyperfine interaction tensor. A better agreement between experimental and calculated ESR spectra in the presence of amylose was observed when used the following values for electron-spin parameters of radicals:

$g_{xx} = 2.088$; $g_{yy} = 2.061$; $g_{zz} = 2.0027$; $A_{xx} = 6.5$ Gs; $A_{yy} = 6.0$ Gs; $A_{zz} = 33.8$ Gs.

Calorimetric investigations of amylose and amylopectin dispersions in 5-DSA aqueous solution were performed using a high sensitivity differential scanning microcalorimeter DASM-4 (Moscow, Russia) from 10–130°C with a heating rate of 2 Kmin^{-1} and pressure of 2.5 bar. Deionised water was used as a reference material. Calorimetric investigations were made upon different molar ratio [radical]/[polymer]. For deter-

mination molar ratio of radical/polymer there were used the values of molecular weights from [16]. The molar ratio radical/polymer was 1:25 for system 5DSA-amylose, for system 5DSA – amylopectin there were used the following molar ratio radical/polymer: 6290:1, 50649:1. Measurements were obtained both for dispersions prepared at the same day, and after 6 days storage (for one dispersion of 5DSA-amylopectin at molar ratio 50649:1).

Results and discussion

Mobility of the water-soluble spin probe

The ESP spectra of Tempol in water and 40% aqueous (w/w) molecular systems of amylose, amylopectin, their mixture as well as gelatinized potato starches at 25°C after cooling are shown in Fig. 2. Symmetrical triplet signals characteristic of spin probes with a "fast rotation" were obtained for all investigated samples, it'd be like to mark that the same ESR spectra of Tempol were observed in the presence of native maize and potato starches [5, 10, 12].

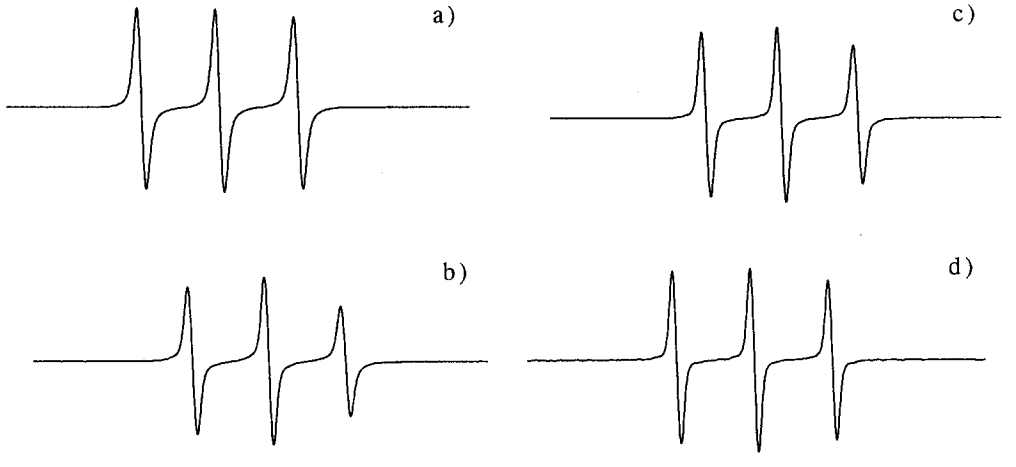


Fig. 2. ESR spectra of Tempol in water (a), in the presence of 40% (w/w) dispersions of amylose (b), amylopectin (c) and potato starch (d) at 25°C after cooling.

Tempol was chosen, since behaviour of this radical simulated the behaviour of water molecules in the system. Only one probe population was observed, suggesting a homogeneous distribution of probes in water phase of the molecular dispersions. This also demonstrates the absence of any direct interaction between the spin probe and starch polysaccharides, i.e. the presented spectra characterize the mobility of water molecules in the dispersion. It should like to note that D_{rot} values for Tempol in bio-

polymer systems were found to be smaller than the values for the probe in bulk water at the same temperature. The differences in D_{rot} could be attributed to water microviscosity in polymer systems.

The dependences of the rotational diffusion coefficient (D_{rot}) on temperature for all investigated systems upon their cooling are shown in Fig. 3. As shown in Fig. 3, the mobility of spin probe Tempol in amylopectin-water system is higher than in amylose-water system. It is known, that upon cooling the amylose macromolecules, in contrast with amylopectin macromolecules, form aggregates followed by its organized three dimensional gel network [8, 9]. That is the reason of the lower spin probe mobility in the amylose-water system as compared with amylopectin-water system.

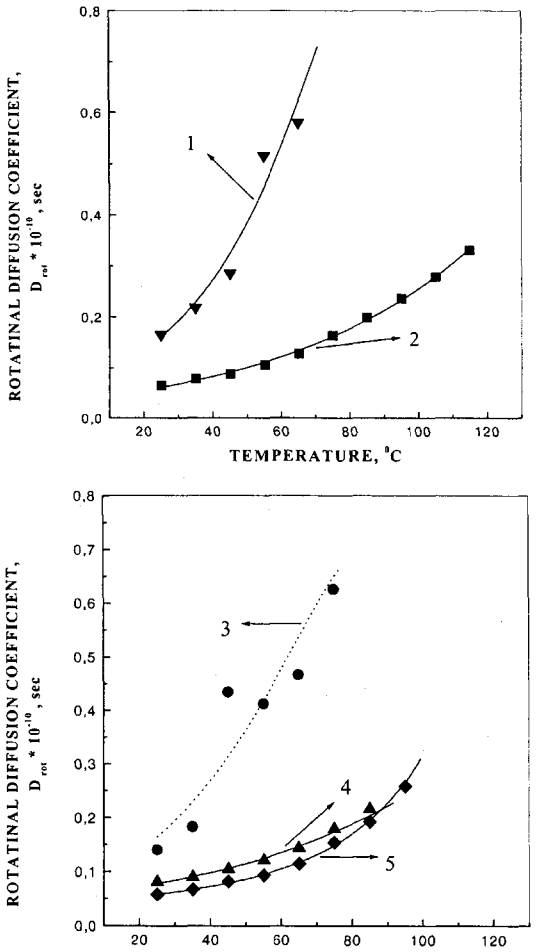


Fig. 3. Dependences of rotational diffusion coeffient on temperature for 40% (w/w) dispersions of amylopectin (1), amylose (2), theoretical (3) and experimental (4) obtained dependence for mixture of amylose and amylopectin (25 w/w % amylose and 75% (w/w) amylopectin), potato starch (5).

Since amylose and amylopectin macromolecules were incompatible in aqueous medium [4] it could be suggested that the observed changes of D_{rot} for model systems (amylose-amylopectin-water) (Fig. 3), can be described by means of additive scheme (3):

$$D_{rot} = D_{rot\ aml}\ \alpha_{aml} + D_{rot\ alp}\ \alpha_{amp} \quad (3)$$

where $D_{rot\ aml}$ and $D_{rot\ alp}$ are the rotational diffusion coefficient for the systems of amylose-water and amylopectin-water, respectively, α_{aml} and α_{amp} were amylose and amylopectin content (%) in model system. To check this assumption, the rotation mobility of so-called "model" system and real potato starch were investigated. "Model" system was realised mixture of amylose and amylopectin, at that weight content of each biopolymer in the mixture was the same as in real potato starch, i.e. 25% (w/w) amylose and 75% (w/w) amylopectin. The comparison of the data obtained shows (Fig. 3b) that generally the experimental and calculated values for model systems differed from one another. So the rotational mobility of system could not be described using additive scheme. The differences between experimental and calculated data for model system could be due to next reasons: (i) the lack of assessment of parameter characterizing mobility of water molecules in interface, since amylose-amylopectin-water is incompatible system [4]; (ii) formation of aggregates and formation of three dimensional gel network upon cooling [8, 9].

At the same time the rotational mobility of spin probe Tempol, which was simulated the behaviour of water molecules in investigated systems, was practically the same in real gelatinized potato starch-water system and in "model" amylose-amylopectin-water system. Moreover the spin probe mobility in the "model' system and in real gelatinizated potato starch-water system was close to spin probe mobility in amylose-water system. From these results it was possible to conclude that the water mobility in real gelatinizated potato starch-water system was mainly related to the presence of amylose macromolecules

Mobility of the spin - labelled stearic acid (5-DSA)

5-DSA was a spin labelled fatty acid with the nitroxide moiety close to the lipid polar head (Fig. 1b). The spectra of 5-DSA in water at 25°C and evolution of ESR spectra in the presence of amylose and in the presence of amylopectin during cooling are shown in Fig. 4. Similar spectra were observed for 40%, 50% and 60% aqueous dispersions of gelatinized potato starch [5]. The ESR spectra of 5DSA in water corresponded to a spin probe with a "fast rotation" (rotation correlation time $\tau_c = 3 \cdot 10^{-10}$ sec; $D_{rot} = 5.5 \cdot 108\ sec^{-1}$) (Fig. 4(a)). After addition of amylose, amylopectin and gelatinized potato starch to the 5-DSA aqueous solution, the motional behaviour of the spin labelled fatty acid drastically decreased. Spectra became characteristic of low-mobility

radicals (i.e. powder-like spectrum). The dominant feature of spectra was the broadened anisotropic line pattern (Fig. 4) indicating greatly slowed down motions as compared with spectra of 5-DSA in water. It could be concluded that interactions between 5-DSA and starch polysaccharides or gelatinized potato starch took place. It was necessary to note that if interaction of amylose macromolecules with 5-DSA could be expected [10, 11], the fact of the interaction of amylopectin macromolecules with 5-DSA, which was considered as model for starch lipids [5, 12],was observed first of all.

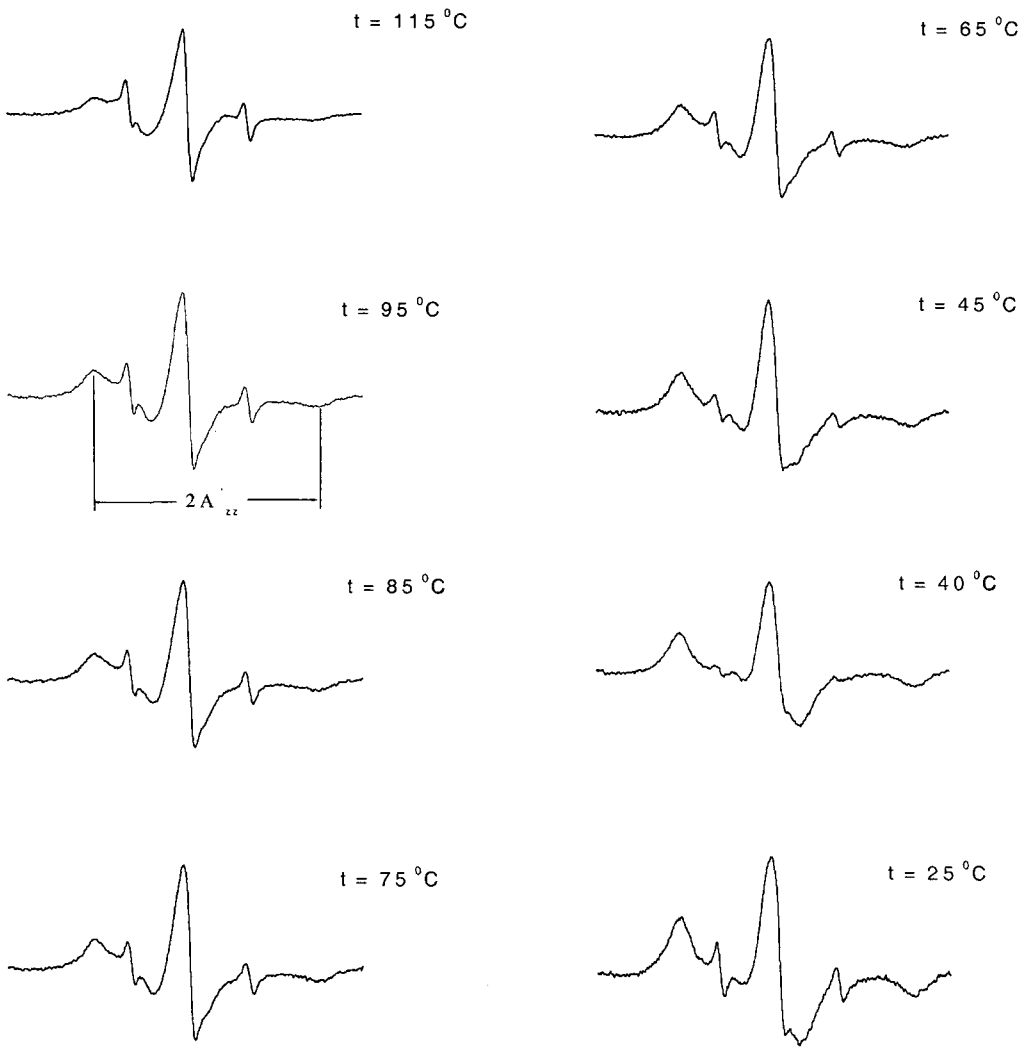


Fig. 4a. ESR spectra of 5-DSA in water at 25°C and evolution ESR spectra of 5-DSA upon cooling in the presence of 40% (w/w) dispersions of amylose.

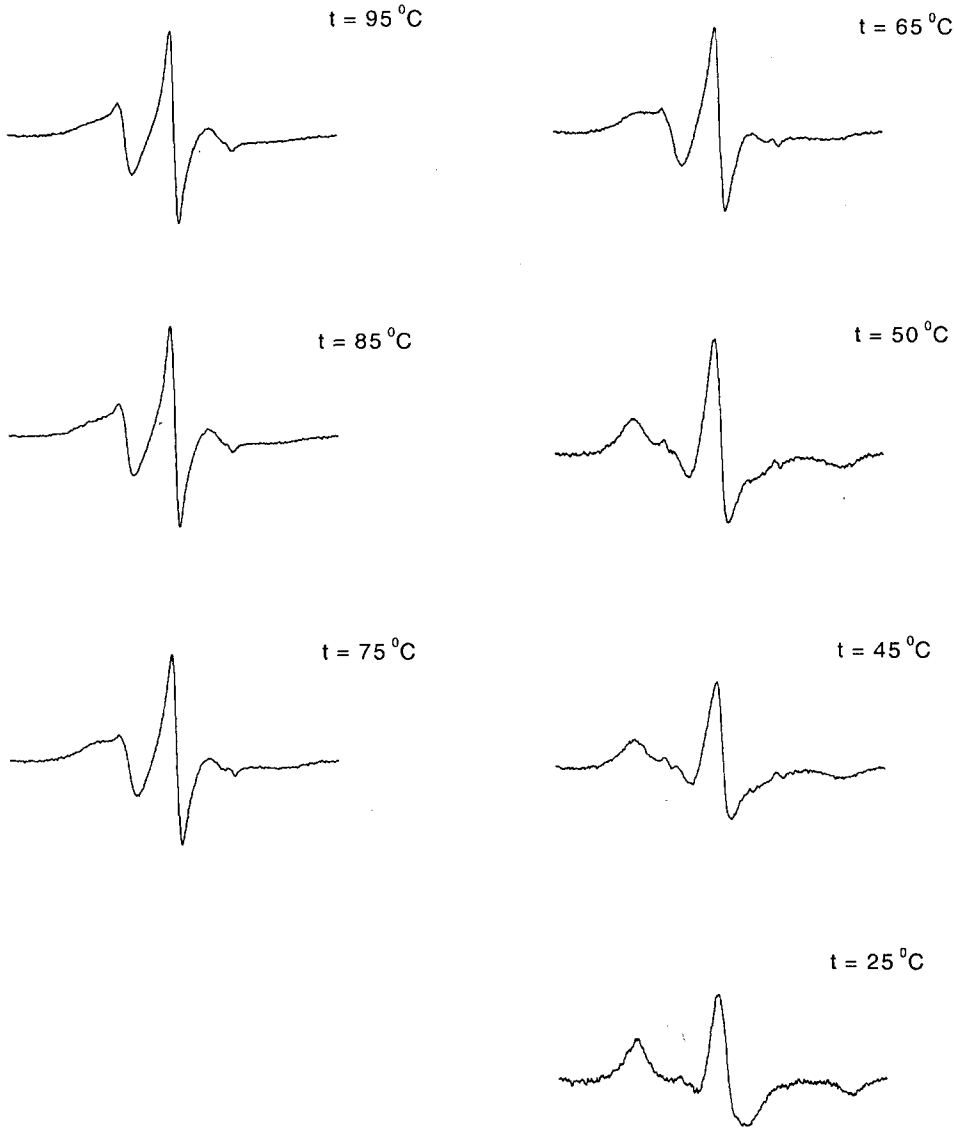


Fig. 4b. ESR spectra of 5-DSA in water at 25°C and evolution ESR spectra of 5-DSA upon cooling in the presence of 40% (w/w) dispersions of amylopectin.

Upon cooling, the powder-like spectra appeared indicating that the mobility of the probe decreased. This meant that more immobilised spectra of 5-DSA were observed. In investigated temperature range two components with "fast" (narrow line spectrum) and "slow" (broad line spectrum, see, for example, Fig. 4(b) at 95°C) motions respectively were superimposed. Fig. 5 presents the evolution of hyperfine extreme separa-

tion ($2A'_{zz}$) as a function of temperature for 5-DSA for all studied systems. The splitting (the value of extrema separation) was higher, the mobility of radical was smaller in the system. Taking into consideration that value of extrema separation was higher in the presence of amylose than in the presence of amylopectin it could be supposed that the interaction between lipids and amylose was stronger than that in the case of amylopectin.

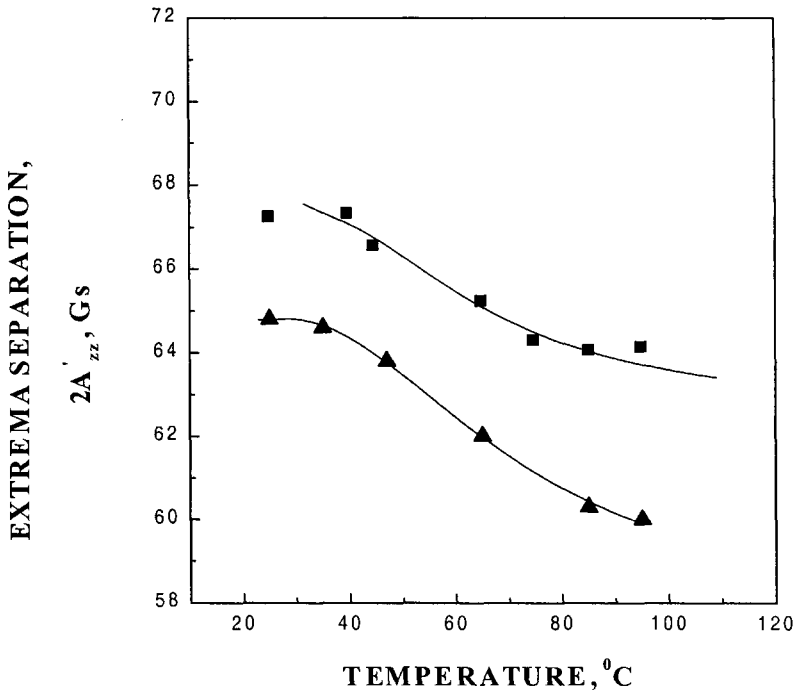


Fig. 5. Dependence extrema separartion for 5-DSA on temperature in the presence of 40% (w/w) dispersions of amylose (■) and amylopectin (▲).

Calculations were carried out to simulate spectra of 5-DSA, in the presence of 40% amylose and amylopectin at 95°C, when apparently, two populations of spin labelled lipids with different mobilities coexisted: "fast" rotating radicals and radicals with slow motions. For both populations isotropic rotation was assumed. The results of this calculation are shown in Fig. 6. Although our model was relatively rough, a relatively good correlation between calculated and experimental spectra was obtained. The simultaneous presence of two different populations suggested a heterogeneous distribution of the labelled fatty acids in different environments. These two populations could be associated to relativelly "free" spin labelled lipids for the most mobile population, whereas the "slow" rotating radicals could be associated to spin-labelled lipids forming of inclusion complexes.

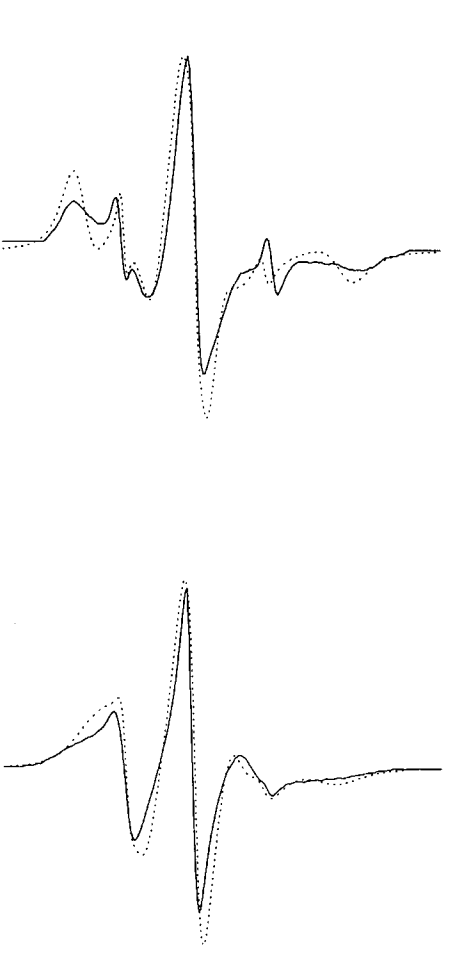


Fig. 6. Experimental (——) and theoretical (------)ESR spectra of 5-DSA in the presence of 40% (w/w) dispersions of amylose (a) (theoretical spectrum was calculated as superposion of spectra with correlation times $\tau_1 = 1.5\ 10^{-8}$ sec (molar part is 98%) and $\tau_2 = 5\ 10^{-10}$ sec (molar part is 2%)) and amylopectin (b) ($\tau_1 = 5\ 10^{-9}$ sec (molar part is 93%) and $\tau_2 = 1.2\ 10^{-9}$ sec (molar part is 7%) at 95°C.

In order to confirm this assumption 5DSA-amylose and 5DSA-amylopectin systems were studied by DSC technique. DSC-thermograms of investigated systems are shown in Fig. 7. The phase transition of first kind for system of 5DSA-amylose at the temperature 90°C was observed (Fig. 7(a)) inspite of that molar raio 5DSA/amylose was lower in DSC measurements as compared with ESR measurements. It is well known that the melting of amylose-lipid inclusion complexes takes place at this temperature. Therefore, populations of radicals associated with "slow" rotating radicals in

the presence of amylose could be associated to spin-labelled lipids forming of inclusion complexes.

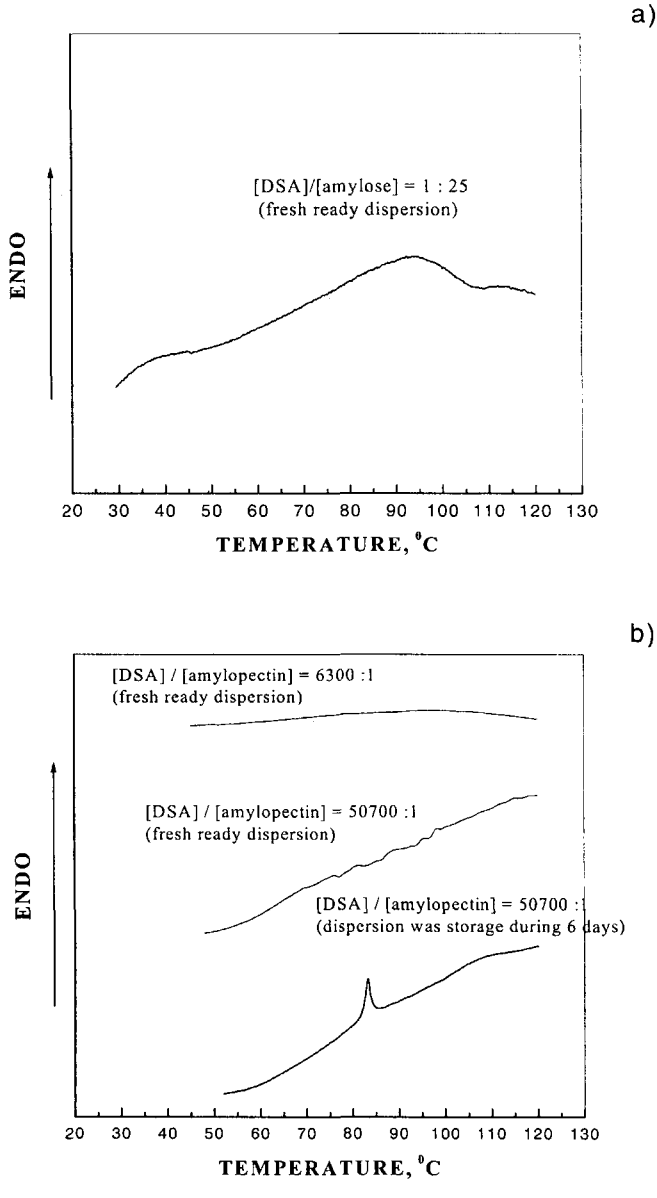


Fig. 7. DSC traces for amylose dispersion in aqueous solution of 5DSA (a) and for amylopectin dispersions (b).

In contrast to 5DSA-amylose system, the phase transitions for 5DSA-amylopectin system with different molar ratio radical/ amylopectin even upon higher than one in

ESR measurements could not be observed on DSC-traces. This meant that the formation of inclusion complexes in the 5DSA-amylopectin system was not observed. Hence, population of radicals with "slow" rotating in the presence of amylopectin was characterized probably formation of complexes other nature. Apparently such complexes were stabilized by adsorption interactions. Therefore the value of correlation time of 5DSA characterizing "slow" radicals in the presence of amylopectin was less than one in the presence of amylose. This meant that interaction amylose with lipids was stronger than amylopectin with lipids. This fact was confirmed our supposition which was discussed above. Although the phase transition of first kind on DSC-traces for 5DSA-amylopectin system after 6 days of storage at 85°C is observed, i.e. was most likely that the formation of amylopectin-inclusion complexes is kinetic process.

Conclusion

Our investigations show that in real three components systems (gelatinized starch-water) interactions the starch polysaccharides macromolecules with water molecules and lipids were related to amylose macromolecules. The interaction of amylose macromolecules with water was caused by formation of aggregates and three dimensional gel network upon cooling. The formation of lipid inclusion complexes was caused by main contribution of amylose macromolecules. On other hand the interaction between amylopectin and lipids also took place.

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ODDZIAŁYWANIE MIĘDZY POLISACHARYDAMI SKROBIOWYMI I CZĄSTECZKAMI WODY ORAZ MODELOWYMI LIPIDAMI. BADANIA ESR

Streszczenie

Za pomocą elektronowego rezonansu spinowego (ESR) zbadano, powstające po chłodzeniu, oddziaływania między polisacharydami skrobiowymi (amylozą i amylopektyną), skrobią i zżelowaną skrobią ziemniaczaną a wodą i lipidami. Zastosowano różne wskaźniki spinowe pozwalające badać zmiany w dynamice fazy wodnej związane z powstawaniem indukowanych temperaturą żeli. Pokazano, że w oddziaływaniach między zżelowaną skrobią i lipidami uczestniczy głównie amyloza chociaż obserwuje się też oddziaływania między lipidami i amylopektyną.

A. CHOSTENKO[1], S. TRUSZKOWSKI[1], A. BUCHALSKI[1], A. CHURKIN[2]

INFLUENCE OF γ–IRRADIATION ON L– AND D–MONOSACCHARIDES

Summary

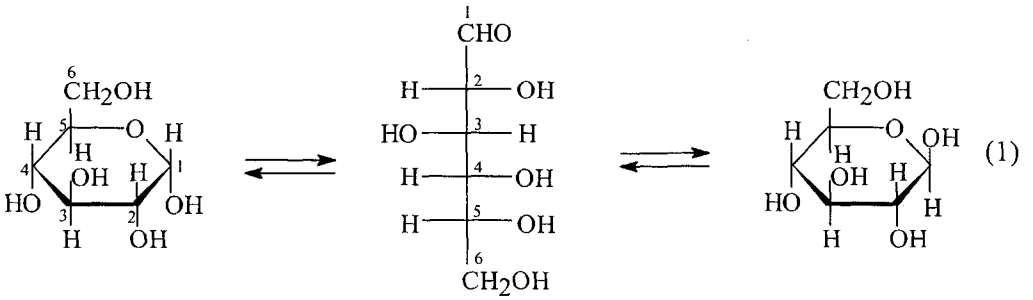
The influence of γ–irradiation on D– and L–glucose, D– and L–galactose, D– and L–mannose was examined in the solid polycrystalline state. Hydrogen was the principal gas product of radiolysis of hexoses. Radiation yield (G) of H_2 changed from 2.2 for galactose to 3.2 (1/100 eV) for glucose and mannose. The ESR spectra of radicals formed in irradiated hexoses were similar and indicated the presence of secondary radical mixture. Integral G-value of radicals decreased with dose. Process of radical disappearance could be described with equations of polychronic kinetics. Radiation stability of galactose was one and a half times higher, than radiation stability of glucose and mannose. This fact rationalised essential contribution of the C_4–H bond breaking process in formation of radical products and H_2. Radiation stability of D– and L–isomers of monosaccharides under non-polarised irradiation was identical. Radicals formed in irradiated monosaccharides lived at room temperatures for several months and it should be taken into account on application of radiation-sterilized pills.

Introduction

Radiolysis of carbohydrates in general and monosaccharides in particular always presents certain scientific interest. Sterilization of medicines in a number of countries is mainly conducted by means of radiating methods. Bearers of pill-forms consist usually of carbohydrates. A large amount of long-life radicals accumulate in carbohydrates at sterilization doses (up to 50 kGy). It can be dangerous for the users of these pills. On the other hand, a high number of isomeric forms of carbohydrates provides study of the axial and equatorial C–H bonds reactivity. The availability of L– and D–isomers gives a chance to check experimentally the hypothesis of enantiomeric excess origin under the influence of polarising radiation on saccharides.

It is known, that pyranoses can exist in one of three forms (D–glucose, as an example):

[1] *Copernicus University, Toruń, ul. Gagarina 13 Poland;* [2] *Mendeleev University, Moscow, Russia*



α–D–glucose D–glucose β–D–glucose

In aqueous solution mutarotation generates predominanly the β-form. There are 8 α-D, 8 β-D, 8 α-L, 8 β-L isomers of pyranoses and 16 linear isomers, however, the latter are of a little importance (< 1%). Crystalline forms of pyranoses had been studied showing that angles between bonds only slightly deviate from tetrahedral [1].

Radiolysis of glucose has been widely studied in various phases (polycrystalline, frozen solutions, syrups) [2–5].

Materials and methods

Irradiations of preliminary evacuated samples of hexapyranoses ("Fluka" > 99%) were performed at 25°C using a ^{60}Co source. Dose rates determined by a Fricke dosimeter were 0.25 and 2.4 kGy/h. The analysis of products was carried out on the chromatograph "Chromatron" GCHF–18.3 with a block for the analysis of gas-products (column: 3m x 4mm, carbon activated 35/50 mesh ASTM, carrier gas – N_2, heat conductivity detector).

The ESR spectra were recorded on PS/X–70 spectrometer system after irradiation immediately and in post-effect. The process of radical disappearance was investigated at 50–130°C.

Results and discussion

Hydrogen was the principal gas product of radiolysis of hexoses. Radiation chemical yield of other products was below 3% of the H_2 yield. For all hexoses the concentration of H_2 increased linearly with the applied dose (Figure 1–3). Small inductive period could be observed only in case of D–glucose. The radiation chemical yield (G-value) of H_2 changed from 2.2 for galactose to 3.2 for glucose and mannose (Table 1).

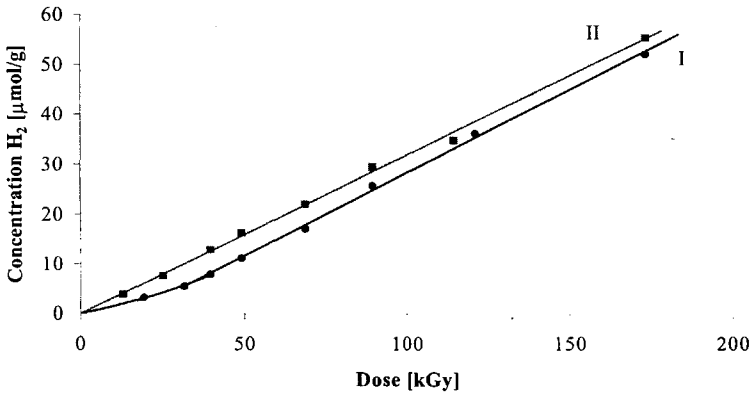


Fig. 1. Hydrogen concentration vs. the absorbed dose: I — D–glucose, II — L–glucose.

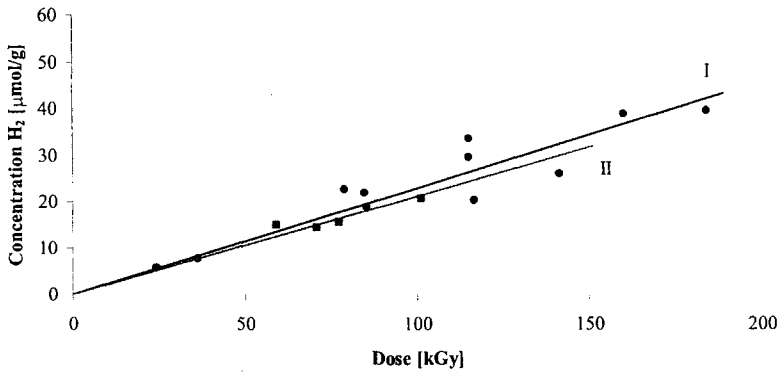


Fig. 2. Hydrogen concentration vs. the absorbed dose: I — D–galactose, II — L–galactose.

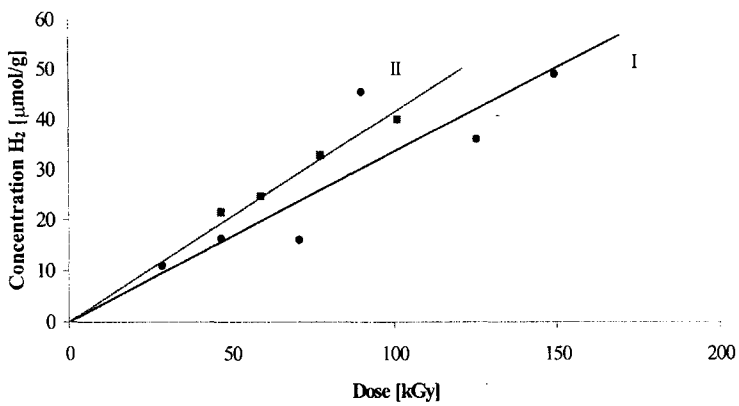


Fig. 3. Hydrogen concentration vs. the absorbed dose: I — D–mannose, II — L–mannose.

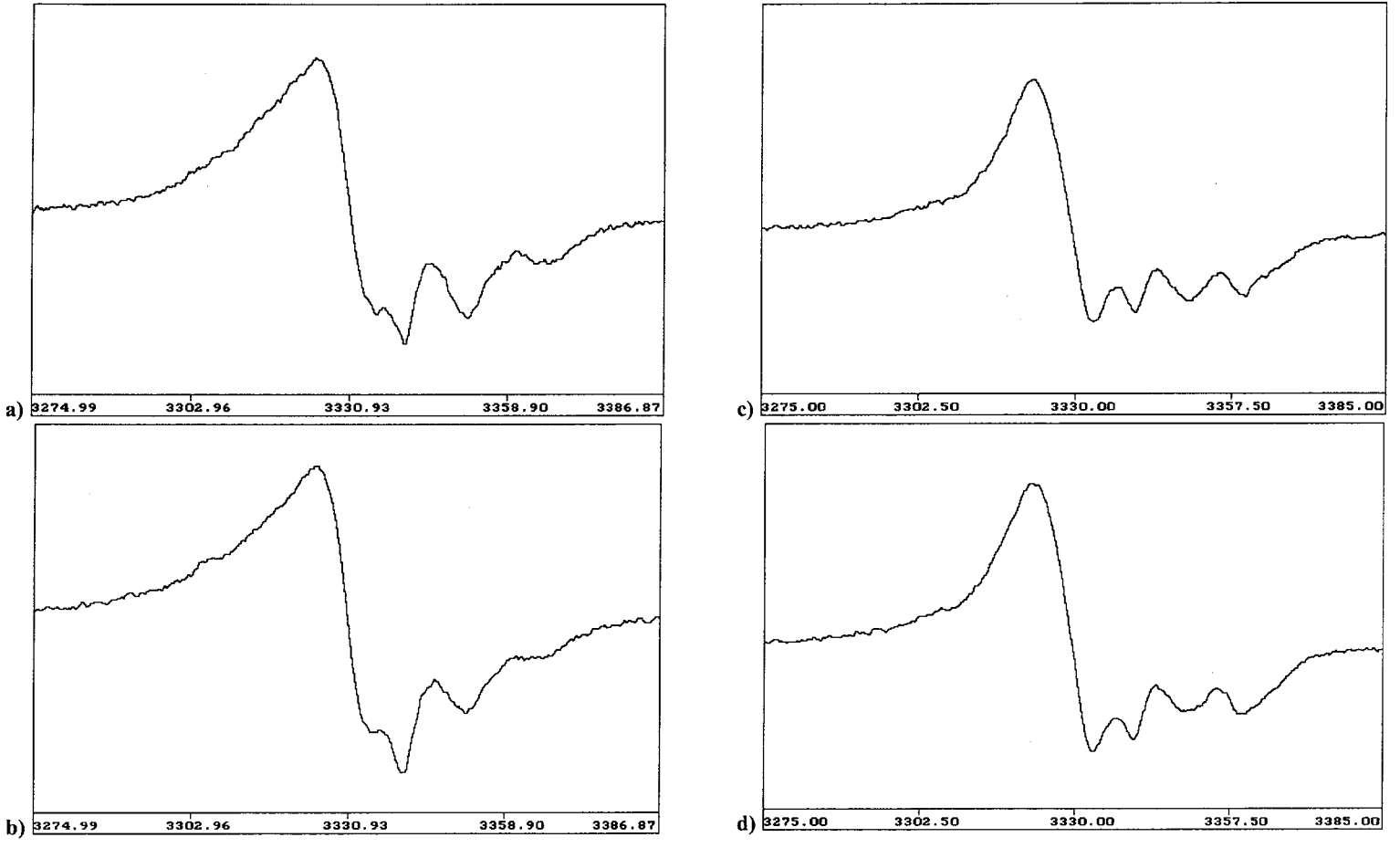


Fig. 4. ESR spectra of irradiated hexoses: a) D–galactose, b) L–galactose, c) D–mannose, d) L–mannose.

Table 1

Radiation-chemical yield (G) of hydrogen in irradiated hexoses.

Hexose	G_{H_2} (1/100eV)
D-Glucose	3,1 ± 0,2
L-Glucose	3,0 ± 1,0
D-Galactose	2,2 ± 0,3
L-Galactose	2,1 ± 0,6
D-Mannose	3,2 ± 0,9
L-Mannose	4,0 ± 0,5

ESR spectra of radicals formed in irradiated monosaccharides were similar. Samples of hexoses which were irradiated and investigated at 25°C gave poorly resolved signals (Figure 4) from secondary radicals mixture.

Formation of radicals in crystalline D–glucose, D–galactose (Figure 5) was similar to curves for irradiated ionic crystals. Rate of radicals disappearance in irradiated examples depended on temperature (Table 2). Radicals lived at 25°C for many months. Considerable acceleration of radicals destruction took place at the temperatures above 130°C (Figure 6).

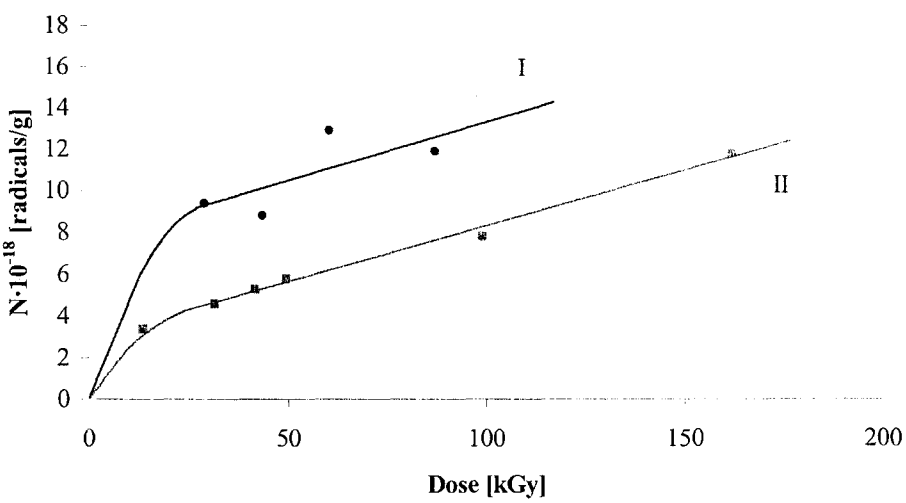


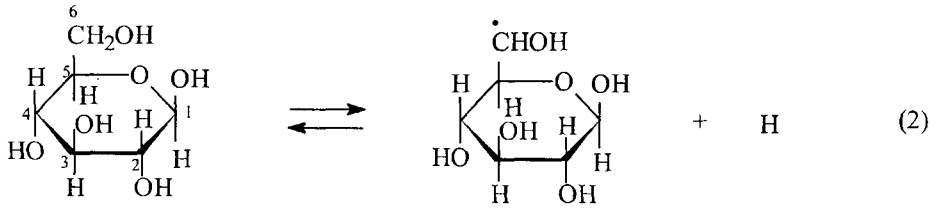
Fig. 5. Free radicals accumulation during γ-irradiation: I — D–glucose, II — L–glucose.

Table 2

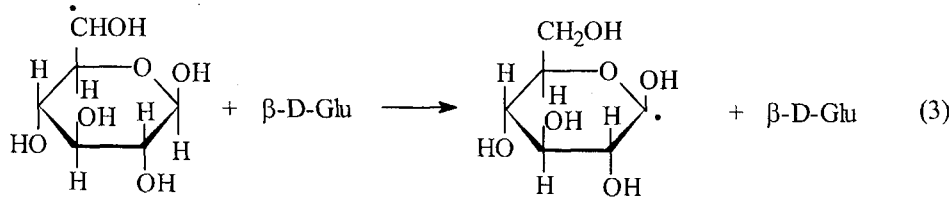
Dependence of relative quantity of living radicals on temperature after irradiation of D–galactose (dose 51.5 kGy, holding time 10 min).

T, °C	50	70	90	100	110	120	130
N/N_0	1.00	0.985	0.926	0.893	0.884	0.754	0.317

The nature of radicals in irradiated hexoses was not univocally determined [2, 5]. Based on [2] initial radicals which formed under irradiation, C_6 – primary hydroxyalkil radicals (I) could be observed. These radicals transformed at room temperature into secondary radicals (II) with unpaired electron at 1,2,3,4,5 C-atoms.



β-D-Glu (I) (2)



(I) (II) (3)

The radicals II might eliminate carbon monoxide and H_2O, producing small amounts of arabinose and 2-deoxyribose (G = 0.25) [2, 4].

There were two different portions on radicals accumulation curve (Figure 5). First of them characterized high G-value of radicals and finished at doses below 10 kGy. Accordingly, the integral G-values of radicals decreased with dose (Table 3).

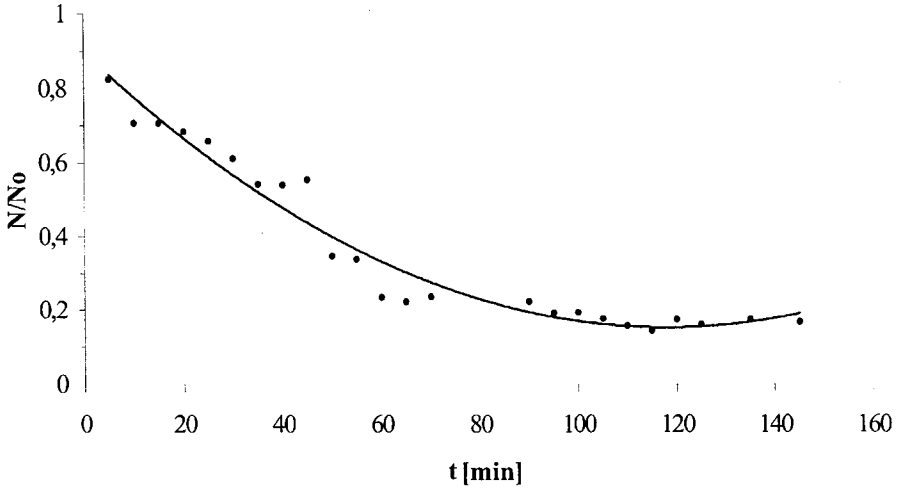


Fig. 6. Relative quantity of radicals vs. holding time of D–galactose.

Table 3

Radiation-chemical yield of radicals in irradiated saccharides.

Dose (kGy)	13,5	29	32	43	50	61	87	103	169
$G_{\alpha\text{-D-glucose}}$	–	5,2	–	3,3	–	3,4	2,2	–	–
$G_{\alpha\text{-D-galactose}}$	4,0	–	2,4	2,0	1,9	–	–	1,3	1,2

It was likely that, as in the case of ionic crystals, in the initial stage of radiolysis the inborn defects of crystal structure played an important role.

Process of radical disappearance at high temperature (Figure 6) could be described with a formal kinetics equation. However, strongly marked steps on curve suggested that radicals disappearance proceeded on polychronic kinetics law [6].

It was important to admit that alteration of OH-group location at C_2-atom did not change the G-value of radicals and H_2 (glucose and mannose). However, galactose was considerably more stable than glucose (Table 1 and 3). This fact rationalised an essential deposit of the C_4-H bond breaking process in formation of radical products and H_2.

Thus, radiation stability of D– and L–isomers of monosaccharides under action of non-polarised irradiation was identical. Radiation stability of galactose was one and a half times higher, than radiation stability of glucose and mannose. This fact proved a big role of a C_4-H bond breaking in process of radiolysis. Radicals, which form under irradiation of saccharides, left for several months, and it had to be taken into consideration on application of radiation-sterilized pills.

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WPŁYW PROMIENIOWANIA γ NA L- I D-MONOSACHARYDY

Streszczenie

Prześledzono wpływ promieniowania γ na D- i L-glukozę, D- i L-galaktozę oraz D- i L-mannozę w stanie polikrystalicznym. Głównym produktem gazowym radiolizy był wodór. Wydajność radiacyjna (G) wodoru zmieniała się od 2,2 (galaktoza) do 3,2 (1/100eV) (glukoza i mannoza). Widma ESR rodników powstające przez naświetlanie wszystkich heksoz były do siebie podobne i wskazywały na obecność mieszaniny wtórnych rodników. Scałkowana wartość G rodników obniżała się wraz z dawką promieniowania. Proces zaniku rodników można opisać równaniami kinetyki polichronicznej. Trwałość radiacyjna galaktozy była półtorakrotnie wyższa od radiacyjnej trwałości glukozy i mannozy. Usprawiedliwiało to istotny udział zrywania wiazań C_4-H w tworzeniu wodoru. Radiacyjna trwałość izomerów L- i D-monosacharydów przy zastosowaniu promieniowania niespolaryzowanego była identyczna.

Rodniki powstające z monosacharydów żyły w temperaturze pokojowej przez wiele miesięcy i fakt ten należy brać pod uwagę przy stosowaniu sterylizacji radiacyjnej.

YOSHINORI UTSUMI*, D. V. GLOVER**, NAOYOSHI INOUCHI*, HIDETSUGU FUWA*

STRUCTURE AND THERMAL PROPERTIES OF STARCHES OF ENDOSPERMS POSSESSING DIFFERENT ALLELES AT THE AMYLOSE-EXTENDER (*ae*) LOCI IN MAIZE

Summary

TSK gel permeation chromatography of non-granular starches, amylopectin chain-length distributions measured by HPAEC-PAD, and DSC characteristics of starches of maize endosperms possessing different alleles at the amylose-extender (ae) loci were studied. GPC of non-granular starches through Toyopearl columns showed that elution profiles for 5 ae mutants, Oh43 inbred line ae (standard ae), ae-RWB-2 and ae-RWB-3, and W23xL317 hybrid line ae-PP and ae-Bol 561, were similar to a commercial ae starch, Hylon VII and different from Hylon ae starch and normal maize starches. The elution profile for W23xL317 ae-emll was similar to Hylon and different from Hylon and normal maize starches. HPAEC-PAD of isoamylase-debranched starches showed that the 5 ae mutants were uniquely ae type similar to Hylon VII and different from Hylon V. W23xL137 ae-emll had the amylopectin chain-length distribution similar to Hylon . Gelatinization temperatures (Tp) of the ae starches measured by a Setaram Micro DSC III were high compared with the normal counterpart starches except for Oh43 ae-RWB-1 starch. Oh43 ae-RWB-1 starch had structure and thermal characteristics similar to the normal maize starch.

Introduction

Recently, there has been increasing interest in modifying the starch composition and content in plants, producing novel starches, from the standpoints of fundamental and applied researches [1-4]. For the purpose evaluation of genetic resources for the novel starch is very important as well as creation of new mutants producing the starch by biotechnology and genetic engineering.

Several maize endosperm genes are known to have mutant alleles at the some loci, for example, the amylose-extender (ae), waxy (wx), and sugary-1 (su-1) genes. A

*Department of Applied Biological Science, Fukuyama University, Fukuyama 729-0292, Japan
**Department of Agronomy, Purdue University, West Lafayette, IN 47907-1150 USA

potential exists for kernels homozygous for any one of these alleles to have a starch with unique structure and physicochemcial properties. We reported that starches of six ae mutants, Oh43 inbred line ae (standard ae), ae-RWB-2 and ae-RWB-3, and W23xL317 hybrid line ae-PP, ae-Bol 561 and ae-emll were uniquely ae type by B type X-ray diffractograms and high gelatinization temperatures by differential scanning calorimetry (DSC), together with poor starch-granule digestibility to amylase, and high apparent amylose (37-45%) and high intermediate fraction (13-18%) contents and low ratios (about 1) of long chains to short chains of amylopectin determined by gel permeation chromatography (GPC) of isoamylase-debranched starches [5].

This paper describes TSK gel permeation chromatography of non-granular starches, the amylopectin chain-length distribution measured by high performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) and thermal properties measured by high performance DSC of starches obtained from mature kernels of standard ae, ae-RWB-1, ae-RWB-2 and ae-RWB-3 mutants in Oh43 inbred line of maize (Zea mays L.), and ae-PP, ae-Bol 561, and ae-emll mutants in a maize hybrid W23xL317, and their normal counterparts.

Materials and methods

Maize Seeds and Preparation of Starches

Several independently occurring alleles at the ae locus were used. Namely, ae–RWB-1, ae–RWB-2, and ae–RWB-3 are different mutational events found several years ago by R. W. Briggs (formally of Funk Seeds International, Inc.) in different hybrid backgrounds by chemical mutagenesis. The ae-emll allele is a mutation found by D.V. Glover by inducing with the Ds-Ac controlling element system. The ae-PP and ae-Bol 561 alleles are ae mutational events in the Pastrostrum Pearl and Bolivian strains of maize, respectively. All of these ae alleles are allelic to the standard ae locus (unpublished data, D.V. Glover). An inbred Oh43, and a hybrid, W23xL317, served as the basis for development of near-isogenic series, ae–RWB-1, ae–RWB-2, and ae–RWB-3, and ae-PP, ae-Bol 561, and ae-emll, respectively. Maize materials of single mutants and their normal counterparts were grown at the Purdue Agronomy Research Center.

Endosperm starches were prepared from mature maize kernels according to the Schoch's method [6]. Commercial ae starches, Hylon V (ae V) and Hylon VII (ae VII) (National Starch and Chemical Company, Bridgewater, NJ, USA) and a commercial normal maize starch were gift from Nihon Shokuhin Kako Company, Fuji, Japan.

General Methods

GPC of non-granular starch (starch samples gelatinized in 1 N NaOH at 5°C for overnight) were performed through 4 TSK gel columns connected in series, Toyopearl HW75S (300×20 mm)×2, Toyopearl HW65S (300×20 mm), and Toyopearl HW55S (300×20 mm). The method for recording absorption spectra of starch-iodine complexes and the phenol-sulfuric acid method for carbohydrate determination were reported previously [5]. The procedure for Dionex chromatography (HPAEC-PAD) were reported earlier [7, 8] except for the following minor change in the procedure. Namely, PAD-SC cell was used instead of PAD-standard cell and 0.1 M $NaNO_3$ was used in the elution solution instead of 0.5 M CH_3COONa. The starch samples (10–15 mg) were solubilized in 100 µl of 90% (v/v) DMSO (H_2O) at 100°C for 10 min. Differential scanning calorimetry (DSC) has been described elsewhere [8, 9].

Results and discussion

TSK gel permeation chromatography of ae maize starches and normal maize starch

Figure 1A and 1B show elution profiles of non-granular starches through Toyopearl columns. Elution profiles for the Oh43 and commercial normal maize starches were similar ones started at the elution volume (EV) 120 ml with the first fraction (in general, amylopectin) near the void volume with iodine absorption λ_{max} 550 nm followed by the second fraction (in general, amylose) with iodine absorption λ_{max} 650 nm at the peak. These size exclusion patterns were similar to those for rice [10] and maize [11] normal starches through Sepharose CL-2B columns. The elution profiles for the ae starches through Toyopearl columns were different from those of the normal maize starches. Namely the profiles started with EV 125 ml and over with the first fraction with iodine absorption λ_{max} 560 nm followed by the second fraction with iodine absorption λ_{max} 630–640 nm at the peak. These size exclusion patterns were different from those for the normal maize starches, moreover, different from those for ae starches through Sepharose column [11]. The results suggest smaller molecular sizes for the ae amylopectins comparing with the normal amylopectins. The elusion profiles for 5 ae mutants, Oh43 ae, ae-RWB-2 and ae-RWB-3, and W23xL317 ae-PP and ae-Bol 561, were similar to ae and different from ae V and normal maize starches. The elution profile for W23xL317 ae-emII was similar to ae and different from ae and normal maize starches.

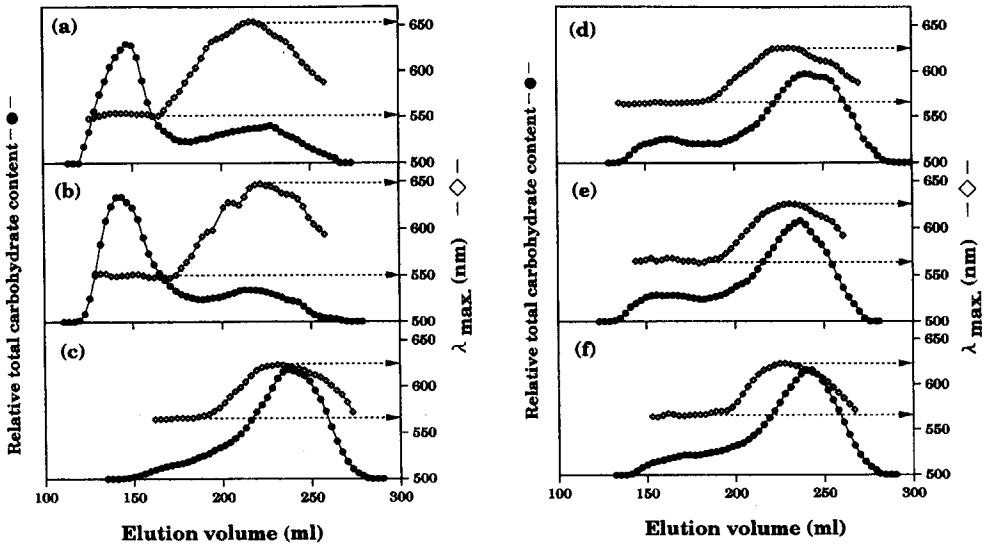

Fig. 1a. Elution profiles of ae starches and normal maize starches separated by TSK gel permeation chromatography: (a) Oh43 normal, (b) Commercial normal maize, (c) Hylon VII, (d) Oh43 ae, (e) Oh43 ae-RWB-2, and (f) Oh43 ae-RWB-3.

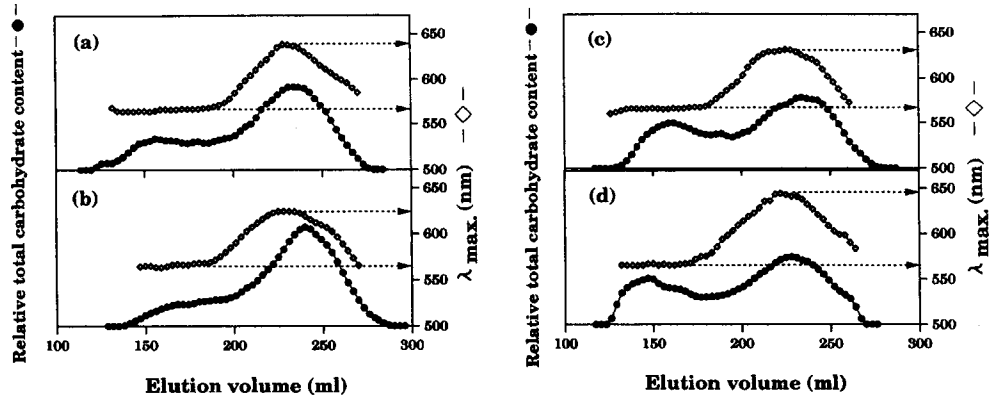

Fig. 1b. Elution profiles of ae starches and normal maize starches separated by TSK gel permeation chromatography: (a) W23 x L317 ae-PP, (b) W23 x L317 ae-Bol 561, (c) W23 x L317 ae-emll, (d) Hylon V.

Amylopectin chain-length distributions measured by HPAEC-PAD of isoamylase-debranched materials of ae maize starches

Amylopectin chain-length distributions were measured by HPAEC-PAD of isoamylase-debranched starches. We calculated the peak area percentage of each unit chains with DP 6–48 for total area percentage assigned to unit chains with DP 6–48 as

100%. The ae starch had decreased amounts of chains with DP 9-17 and increasing amounts of chains with DP 20 and over compared with a commercial normal maize starch. Fig. 2 shows comparison of unit chain-length distributions of isoamylase-debranched 10 sample maize starches to the ae starch. The starches of 5 ae mutants, Oh43 ae, ae-RWB-2 and ae-RWB-3, and W23xL317 ae-PP and ae-Bol 561 were uniquely ae type similar to ae starch, and different from ae starch. W23xL317 ae-emll had the amylopectin chain-length distribution similar to aeV. Mature kernels of Oh43 ae-RWB-1 mutant showed tarnished and translucent phenotype characteristics of the ae genotype but contained endosperm starch having 21–22% of apparent amylose which was lower than that of Oh43 normal counterpart [5]. The ae-RWB-1 starch showed similar amylopectin chain-length distribution to the normal counterpart.

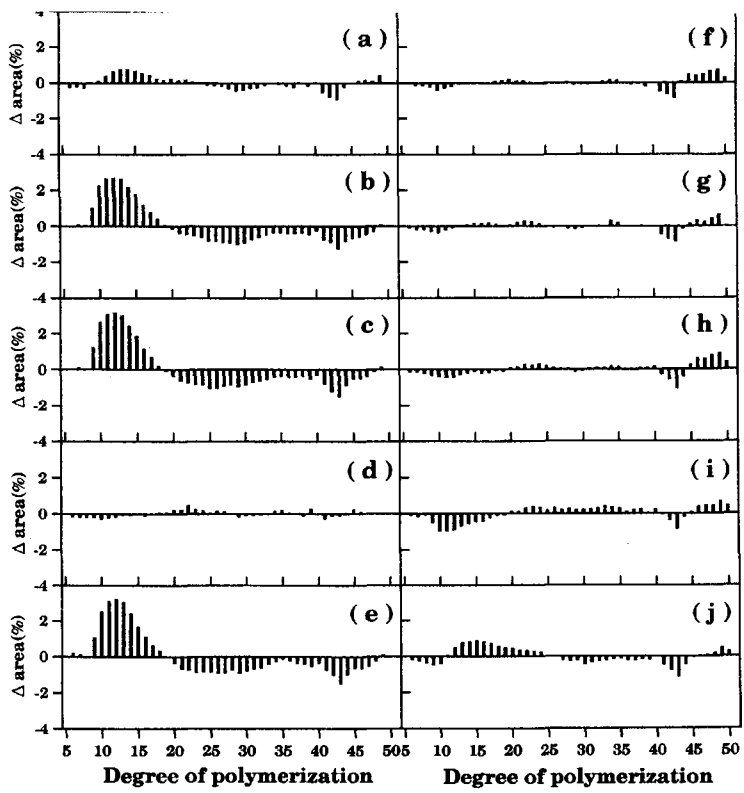

Fig. 2. Differences in chain-length distributions measured by HPAEC-PAD of isoamylase-debranched starches of ae mutants and their normal counterparts of maize with comparison to Hylon VII starch: (a) Hylon V, (b) Oh43 normal, (c) W23 x L317 normal, (d) Oh43 ae, (e) Oh43 ae-RWB-2, (f) Oh43 ae-RWB-3, (g) W23 x L3317 ae-PP, (h) W23 x L317 ae-Bol 561, and (i) W23 x L317 ae-emll.

DSC characteristics of ae maize starches

Fig. 3 and Table 1 show DSC characteristics of the ae starches and their normal counterparts. DSC thermograms for the normal counterparts and Oh43 ae-RWB-1 starches shown in Fig. 3 had 2 separate endothermic peaks, namely native starch gelatinization peak (68–72°C) and amylose-lipid complex melting peak (105°C), however, those for other ae starches had incomplete separation of endothermic peaks. Accordingly, we compared top temperatures of gelatinization (Tp) for these starches shown in Table 1. The ae starches except for Oh43 ae-RWB-1 had higher Tp (78–88°C) compared with their normal counterparts as reported previously [5]. The Oh43 ae-RWB-1 starch had similar thermal properties to those of the Oh43 normal. Thermal properties of retrograded starches of the ae starches are shown in Fig. 4. It was very difficult to estimate Tp from the DSC thermograms for the retrograded ae starches except for Oh43 ae-RWB-1. Accordingly, we could not compare thermal properties of the retrograded ae starches with those of the retrograded normal counterparts. Here again the retrograded Oh43 ae-RWB-1 starch had similar thermal properties to those of the retrograded Oh43 normal counterpart. DSC characteristics of starches were affected by amylopectin properties and not by amylose contents [11, 12].

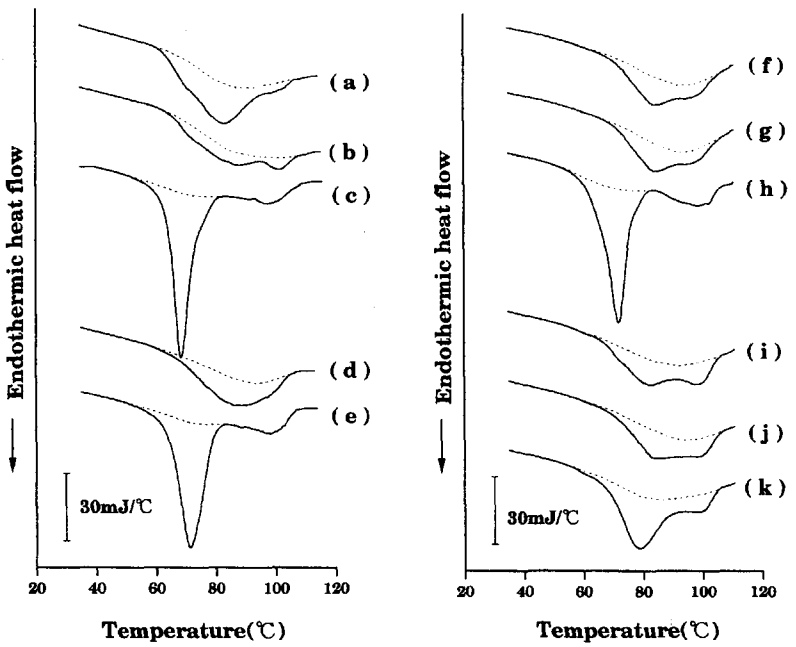

Fig. 3. DSC thermograms of native starches of ae mutants and their normal counterparts of maize: (a) Defatted Hylon V, (b) Defatted Hylon VII, (c) Oh43 normal, (d) Oh43 ae, (e) Oh43 ae-RWB-1, (f) Oh43 ae-RWB-2, (g) Oh43 ae-RWB-3, (h) W23 x L317 normal, (i) W23 x L317 ae-PP, (j) W23 x L317 ae-Bol 561, and (k) W23 x L317 ae-emll.

Table 1

Peak temperatures of gela.tinization *(Tp)* measured by DSC of *ae* and normal maize starches.

Starch sample	Tp (°C)
Nylon V *(ae V)*	81.8
Nylon VII *(ae* VII)	87.6
Normal maize	68.7
Oh43 *ae*	87.8
Oh43 *ae* - RWB-1	71.3
Oh43 *ae* - RWB-1	84.1
Oh43 *ae* - RWB-1	83.3
W23 x L317 normal	72.0
W23 x L317 *ae* - PP	81.7
W23 x L317.*ae* - Bo1.561	84.1
W23 x L317 *ae* - emll	78.0

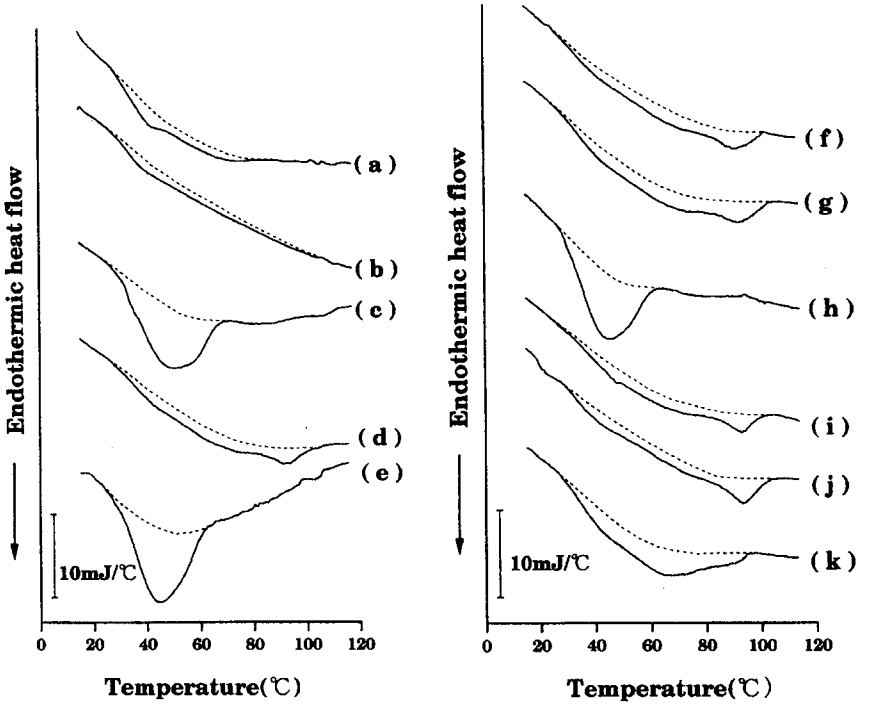

Fig. 4. DSC thermograms of retrograded starches of ae mutants and their normal counterparts of maize: (a) Defatted Hylon V, (b) Defatted Hylon VII, (c) Oh43 normal, (d) Oh43 ae, (e) Oh43 ae-RWB-1, (f) Oh43 ae-RWB-2, (g) Oh43 ae-RWB-3, (h) W23 x L317 normal, (i) W23 x L317 ae-PP, (j) W23 x L317 ae-Bol 561, and (k) W23 x L317 ae-emll.

Conclusions

GPC of non-granular starches showed that elution profiles for 5 ae mutants, Oh43 ae, ae-RWB-2 and ae-RWB-3, and W23xL317 ae-PP and ae-Bol 561, were similar to Hylon and different from Hylon V and normal maize starches. The elution profile for W23xL317 ae-emll was similar to Hylon and different from Hylon and normal maize starches.

HPAEC-PAD of isoamylase-debranched starches showed that 5 ae mutants, Oh43 ae, ae-RWB-2 and ae-RWB-3, and W23xL317 ae-PP and ae-Bol 561 were uniquely ae type similar to Hylon, and W23xL317 ae-emll had the amylopectin chain-length distribution similar to Hylon V.

Gelatinization temperatures (Tp) measured by DSC of the ae starches were high compared with the normal counterpart starches.

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STRUKTURA I WŁAŚCIWOŚCI TERMICZNE SKROBI KUKURYDZIANYCH Z ENDOSPERM POSIADAJĄCYCH RÓŻNE ALLELE W ae-loci

Streszczenie

Skrobie nieziarniste badano za pomocą żelowej chromatografii podziałowej (TSK), zaś udział długich łańcuchów w amylopektynie oznaczono przy użyciu wysokosprawnej chromatografii cieczowej, a skaningową kalorymetrię różnicową wykorzystano do scharakteryzowania skrobi z endosperm ziaren kukurydy, posiadających różne allele w ae-loci. Żelowa chromatografia podziałowa nieziarnistych skrobi na kolumnach Toyopearl pokazała, że profile elucyjne pięciu mutantów ae tj. skrobi z linii ae Oh43 (standard ae), ae-RWB-2 i ae-RWB-3, linia hybrydowa ae-Ppi ae-Bo 561 W23xL317 były podobne do tychże w han-

dlowej skrobi ae Hylon VII i różne od tychże w skrobi Hylon VII i normalnych skrobi kukurydzianych. Wysokosprawna chromatografia cieczowa skrobi pozbawionych odgałęzień za pomocą izoamylazy pokazała, że pięć mutantów ae było wyjątkowo podobnych do skrobi Hylon VII i zarazem różnych od tychże w skrobi Hylon V. W23xL137 ae-emll miała rozkład długich łańcuchów podobny to amylopektyny ze skrobi Hylon V. Temperatura żelowania (T_p) skrobi ae mierzone za pomocą mikrokalorymetru Setram Micro DSC III była wyższe od tychże normalnych skrobi, wyjąwszy skrobię Oh43 ae-RWB-1. Ta ostatnia skrobia miała budowę i termiczną charakterystykę podobne do normalnych skrobi kukurydzianych.

VLADIMIR P. YURYEV*, NATALIA K. GENKINA, LUYBOV A. WASSERMAN

THE INFLUENCE OF THE GROWTH TEMPERATURE ON STRUCTURAL AND THERMODYNAMIC PROPERTIES OF STARCHES

Summary

Effects of environmental factors on composition, structure, and physicochemical properties of native starches from different plants have been extensively studied. Among parameters to which attention was paid were environmental factors there is a growth (soil) temperature during plants development (growth temperature) that plays one of the most important role. The application of different physical approaches for a description of the thermodynamic melting parameters of starches allows to determine the cooperative melting unit, the thickness of crystalline lamellae and to evaluate the role of defects in the structural organization of native granules at changes of growth temperature. As example it is considered the influence of growth temperature of plants on the properties of normal potato and sweet potato starches as well as waxy, normal and high-amylose barley starches, i.e. starches with different polymorphous structures of B-, C- and A- types, correspondingly. The nature of calorimetric peaks doubled for some normal and high-amylose barley starches at a decrease in growth temperature is discussed.

Introduction

The processing of native starches and/or starch containing raw materials is accompanied as a rule, by a gelatinization of starches, i.e. a transformation of granular starch to a viscous-flow state. Since native starch is a semicrystalline substance, from the point of view of modern physics this process can be presented as a melting, this is as the first kind phase transition. The melting temperature of starches extracted even from the same origin can vary from one another [1-5]. Hence, on processing of starches even extracted from the same origins temperature of starch processing should not be constant, otherwise the functional properties of gelatinised starches could vary

*Institute of Biochemical Physics of Russian Academy of Sciences, 4, ul. Kosygina, 199991 Moscow, Russia; *Corresponding author: Institute of Biochemical Physics of Russian Academy of Sciences, 4, ul. Kosygina, 199991 Moscow, Russia; e-mail: v.yuryev@sky.chph.ras.ru*

from each other. It implies that melting temperatures of starches and temperatures of their processing must be mutually coordinated.

Differences in melting temperature of starches have complex origin. For instance, genetically controlled factors might be involved, environmental conditions (soil composition, soil temperature (growth temperature), water regime, duration of light day and some others) as well as the reasons caused by varieties in methods of land cultivation might also pay an essential role. This research focuses on the influence of environmental conditions, primarily on the soil temperature which influences the melting temperature of starches and on the relationship between the structure of native granules and their thermodynamic properties.

Starch is one of the most abundant substances on the earth. It is found in the leaves of green plants, in the amyloplasts of seeds, grains, roots, and tubers of many crops where it is synthesized in a very condensed and granular form depending on genes and enzymatic activities. The comparison of starch produced in plastids with glycogen in prokaryotes suggests that the biosynthetic pathway has changed during evolution because of selection pressure favoring a semicrystalline product. This property allows plants to store massive amount of fixed carbon generated by photosynthesis [6].

The effects of environmental factors on composition, structure and physicochemical properties of native starches from different plants have attracted progressively more attention in recent years. Among environmental factors of particular interest there is a soil temperature during plants development (growth temperature). Perhaps, it plays the most important role [7-20]. At the present it has been established that an increase in the growth temperature of cereal and potato starches, irrespectively of the amylose content, leads to increase in melting temperature [7-21].

It is supposed that an increase of melting temperature of starches with increasing of growth temperature may be caused by changes in the double helix length, optimization of registration within crystalline lamellae and/or amorphous region rigidity [8, 10, 21]. It has been hypothesized that elevated growth temperatures directly enhance *in vivo* "annealing" and this is comparable to annealing *in vitro* [8-10, 21]. Until now any firm evidence to support these hypotheses are lacking. The analysis of the works recently published and devoted to a study of the structure formation in starches during their biosynthesis and to an influence of growth temperature on these processes and starch structure, shows that changes in the melting temperatures (T_m) for native starches [13-16, 22] as well as for semicrystalline synthetic polymers [23] can be described with the Thomson-Gibbs' equation and equations linking fundamental thermodynamic functions characterizing properties of the surface of starch lamellae. It allows to estimate a role of defects in the structural organization of starch granule. Additionally using the "two-state" model [24] for a description of the melting process for waxy and normal starches, melting cooperative units and the thickness of crystal-

and normal starches, melting cooperative units and the thickness of crystalline lamellae can be determined [13-16, 22]. Collection of the data obtained in these and some other works provides estimation of the influence of growth temperature on the structure and thermodynamic properties of native starches of different polymorphous structure. Besides of it, these data allow to predict properties of new starches as well as to develop better understanding of starch biosynthesis phenomenon, and the relationship between structural and thermodynamic parameters of native starches.

The influence of the growth temperature on structure and composition of native granules. A general presentation

It is known [6, 12] that the biosynthesis of two starch polysaccharides (amylose and amylopectin) is under genetic control. Synthases controlling a biosynthesis process are subdivided into two major classes: soluble starch synthases (SSS) which produce amylopectin, and granule bound starch synthases (GBSS) which produce amylose. The ratio of these enzymes during biosynthesis determines the amylose/amylopectin ratio in native starch granules. Since all synthases have individual temperature for their optimal activity, the polymerization degree of synthesized starch polysaccharides and, correspondingly, the structural organization of starch granules can differently depend on environmental conditions, particularly the growth temperature. Furthermore it is well known that temperature and water content in the system are the thermodynamic parameters which control crystallization.

Macromolecules of amylose and amylopectin form starch granules which consist of crystalline lamellae formed by double helices of amylopectin A- chains with polymerization degree of 14 anhydroglucose residue, amorphous lamellae formed by amylopectin B- chains, and an amorphous background formed by amylopectin and amylose macromolecules in unordered conformation. Amylose and amylopectin macromolecules can also form defects located both in crystalline (amylose "tie" chains string type; amylopectin molecular ordered structure) and in amorphous (amylose "tie" and amylopectin chains in unordered conformation) lamellae. For potato and barley starches with B- and A-type polymorphous structure, respectively, an increase in growth temperature does not lead to significant changes in X-ray diffraction patterns irrespectively of amylose content in starches, this is, improvement of the macromolecular packing in the crystalline unit is not observed [7, 13, 16]. For sweet potato starches [7, 14, 15], the transition of crystalline structure from C(A+B)- to A-type is observed at a change of growth temperature from 15°C to 21°C. However, the annealing of C- type sweet potato starches does not induce such temperature [14, 15]. This means that the hypothesis proposed in the works [8–10, 21] according to which elevated growth temperatures directly enhance *in vivo* "annealing" and this is comparable

to annealing *in vitro* is disputable. According to Hizukuri [7], low temperature yields B- type crystalline structure and high temperature favours the A- type.

The question concerning the variations in the thickness of crystalline lamellae remains open and will be consider later.

The analysis of the published data on the amylose content in native granules shows that for maize and rice starches an increase in growth temperature leads to a decrease in the amylose content [17, 25-27] whereas for wheat starch the amylose content slightly increases with temperature [18, 20]. The investigation of barley starches has shown that irrespectively of the starch variety (waxy, normal, high amylose) growth temperature insignificantly changes the amylose content [28] and, similar effect is noted for tuber starches (potato, microtuber) [9, 10]. Environmental influence on the amylose content in sweet potato starch appears to be variable [29].

In contrast to the amylose/amylopectin ratio, level of starch lipids is very sensitive to environmental effects. For example, it is well known that for barley and wheat starches elevating growth temperature results in increase in the amount of starch lipids [8, 12, 18, 20, 28, 30]. For cereal starches insignificant portion of amylose macromolecules complexed with lipids can form both amorphous and crystalline V-type structures from single helices while other portion of starch lipids is free.

The analysis of the data concerning thermodynamic melting parameters of amylose-lipid complexes as well as the results for barley starches published in the works [8, 16, 28] allow to do some remarks about the influence of growth temperature on the formation of amylose-lipid complexes. Taking into consideration that:

(i) an increase in growth temperature leads to an increase of lipid content in some starches and to decrease in the amylose content in barley starches [8, 28],
(ii) the magnitude of melting enthalpy for amylose-lipid complexes is proportional to the content of amylose-lipid complexes in starches,
(iii) the magnitude of melting temperature of amylose-lipid complexes is characteristic for thermostability for amylose-lipid complexes,

one can supposed that in spite of increase in total lipid content in starches, decrease in growth temperature leads to increase in the content of amylose-lipid complexes in starches, at least partly due to a decrease in the amylose content, and decrease in the thermostability of complexes. Since the melting temperature of amylose-lipid complexes depends on nature of fatty acids [31] and its value decreases in the order: linoleic acid < myristic acid < oleic acid < stearic acid < palmitic acid, it can be supposed that at lower growth temperature some fatty acids do not form complexes.

The growth temperature and the relationship between structure and thermodynamic parameters of crystalline lamellae in waxy, normal and high amylose native starches

Environmental factors not always remain constant during plants development so it is very difficult to compare precisely available data on the influence of growth temperature on structure and properties of starches. Changes in structural parameters of starches reflect physicochemical and biochemical processes developing in granules during their biosynthesis. Some suggestions on nature of these changes in starches have been formulated in numerous reports. The differences in structural organization of granules during their biosynthesis can be attributed to the changes in:

1. length of amylose double helix [9],
2. distribution of amylopectin chains length [18, 27, 32-34],
3. ratio between small and large granules, because it is well known that in bimodal starches the melting temperature for small fraction is higher than that for large fraction [35].

The DSC study of native starches under quasi-equilibrium has shown that there is the correlation between the growth temperature of plants and the melting temperature of starches [13-15].

As formerly noted, the melting temperature (T^{exp}_m) of semicrystalline synthetic polymers as well as native waxy and normal starches is determined by Thomson-Gibbs equation [13-16, 22, 23]:

$$T^{exp}_m = T^{o}_m\{1 - 2\gamma_i/(\Delta H^{o}_m \rho_{crl} L_{crl})\} \quad (1)$$

where T^{o}_m and ΔH^{o}_m are the melting temperature and the melting enthalpy of a hypothetical crystal with unlimited size (a perfect crystal), ρ_{crl}, and L_{crl} are the density and the thickness of the crystal, respectively and γ_i is the free surface energy of formation of the crystal. Since the values of T^{o}_m and ΔH^{o}_m for starches are not available, the data reported for the A- and B- type spherolitic crystals can be used instead for estimation of T^{exp}_m.

The analysis of the equation (1) shows that T^{exp}_m is the function of three variables: the type of polymorphous structure in crystal, the thickness of crystal, and the free surface energy of its formation. The polymorphous structure in native starches can be determined with the X-ray method while the thickness of crystalline lamellae is available from DSC data and equations (2 and 3) [13–16]:

$$\nu = \Delta H^{vH}/\Delta H_m \quad (2)$$

$$L_{crl.} = 0.35\nu \quad (3)$$

where ν is the melting cooperative unit, ΔH^{vH} is the van't Hoff enthalpy, ΔH_m is the experimental melting enthalpy of starches, 0.35 nm is the pitch height per anhydroglucose residue in a double helix. According to [13-16, 23], the magnitude of the γ_i could be estimate using equations (1, 4, and 5):

$$q_i = [(\Delta H^\circ_m - \Delta H_{exp})\rho_{crl} L_{crl}]/2.5 \quad (4)$$

$$\gamma_i = q_i - T_m s_i \quad (5)$$

where q_i and s_i are surface enthalpy and entropy of crystalline lamellae, respectively. It is well known that the main contribution in the γ_i is caused by the magnitude of the q_i which is proportional to the content of defects in crystal. Since the melting of crystal begins from its defects, their increase leads to a formation of crystals with a more "mellow" surface and, respectively, to a decrease in T^{exp}_m.

Therefore, taking into consideration mentioned above, i.e. that with the exception of sweet potato starches an increase of growth temperature does not lead to the change in starch polymorphous structure [7, 13-16], some characteristics of crystalline lamellae such as thickness and surface free energy, and entropy, and their changes on varying of the growth temperature should be considered for a better understanding of the mechanism underlying the correlation between melting and growth temperatures.

Table 1

The values of the melting cooperative unit (ν_{crl}), the thickness (L_{crl}), the free surface energy (γ_i), enthalpy (q_i) and entropy (s_i) of face sides for crystalline lamellae of different starches [10,13-16]. (Thermodynamic parameters characterizing surface faces for crystalline lamellae in starches with asymmetric or doubling DSC-peaks as well as for C-type starches weren't calculated.)

Genotypes	Polymorphous structure	Growth temperature, °C	T_m, K	ν, anhydroglucose residues	L_{crl}, nm	γ_i (J/cm^2) 10^7	q_i (J/cm^2) 10^7	s_i (J/cm^{2}K)10^7
Potato (*Maris Piper*)	B	10	333.4	15.7	5.5	4.6	53.9	0.147
	B	16	336.0	14.1	4.9	3.7	39.0	0.105
	B	20	338.6	14.1	4.9	2.8	44.0	0.121
	B	25	343.4	11.6	4.1	1.2	22.4	0.062
Average 13.8±1.7 4.8±0.6								
Waxy barley (*Oderbrucker*)	A	7	316.8	16.0	5.6	17.8	71.3	0.169
	A	10	324.3	13.6	4.7	15.1	59.4	0.137
	A	13	327.5	13.1	4.6	14.0	55.7	0.127
	A	15	328.0	14.7	5.1	13.8	57.6	0.133
	A	16	329.7	14.5	5.1	13.2	53.7	0.123
	A	20	333.0	14.1	4.9	12.1	54.4	0.127
Average 14.6±0.5 5.1±0.4								
Normal barley (*Golden Promise*)	A	7	314,7	18,7	6,6	21,2	94,8	0,234
	A	10	320,9	14,0	4,9	18,6	80,2	0,192
	A	13	323,5	14,2	5,0	17,6	76,7	0,183
	A	15	325,5	15,9	5,6	16,8	79,3	0,192
	A	16	327,2	16,9	5,9	16,1	73,8	0,176
	A	20	330,9	17,5	6,1	14,5	75,5	0,184

c.d. tab. 1

Geno-types	Polymo-rphous structure	Growth tempe-rature, °C	T_m, K	ν, anhydroglu-cose residues	L_{crl}, nm	γ_i (J/cm^2) 10^7	q_i (J/cm^2) 10^7	s_i (J/cm^2 K)10^7
Average 16,2±0,8 5,7±0,3								
Normal barley (*Triumph*)	A	7	315,0	-	-	-	-	-
	A	10	320,8	12,0	4,2	14,7	55,1	0,126
	A	13	325,0	12,2	4,3	13,4	48,1	0,107
	A	15	326,1	13,7	4,8	13,0	51,8	0,119
	A	16	327,7	13,1	4,6	12,5	45,2	0,099
	A	20	332,0	13,4	4,7	11,1	41,6	0,092
Average 12,9±0,3 4,5±0,1								
High amylose Barley (*Pent-land-field*)	A	7	---	---	---	---	---	---
	A	10	320,7	---	---	---	---	---
	A	13	323,6	---	---	---	---	---
	A	15	325,0	---	---	---	---	---
	A	16	332,1	11,5	4,0	10,1	55,4	0,136
	A	20	334,3	12,1	4,2	9,5	51,7	0,126
Avarage 11,8±0,3 4.1±0,1								
Sweet potato (*Sun-nyred*)	C(A+B)	15	327.6	11.9	4.2	---	---	---
	A	21	336.6	14.9	5.2	11.0	63.7	0.161
	A	27	345.2	15.7	5.5	8.7	58.5	0.143
	A	33	351.5	17.0	6.0	6.4	65.9	0.170
Sweet potato (*Ayamu-rasaki*)	C(A+B)	15	329.7	12.4	4.3	---	---	---
	A	21	337.5	14.8	5.2	10.9	55.9	0.133
	A	27	346.6	15.8	5.5	7.8	52.0	0.127
	A	33	352.3	15.9	5.6	5.7	38.2	0.092

As can be seen from Table 1 [13-16], the elevation of growth temperature does not lead to any cnange in cooperative melting unit and the thickness of crystalline lamellae in native potato and barley starches, i.e. in starches related to B- and A- type, respectively, while for sweet potato starches an increase of growth temperature is accompanied by positive correlation with the thickness of crystalline lamellae. According to [13, 16] observed changes in the melting temperatures for potato and barley starches can be caused by accumulation of defects while for sweet potato starches the changes in T^{exp}_m can be due to accumulation of defects and increase in the thickness of crystalline lamellae [14, 15]. Indeed, calculations have shown that the decrease in growth temperature leads to the increase in the magnitude of surface entropy for starch crystalline lamellae (Table 1). This phenomenon is evident for potato and barley starches irrespectively of the amylose content in granules but it is insignificant for sweet potato starches. One may suppose that such defects in starch granules are amy-

lose "tie" chains, amylopectin molecular ordered structures, F_2 or F_1 amylopectin subfraction [13].

In case of sweet potato starches increase in the thickness of crystalline lamellae can result from: (i) increase in the length of double helixes and (ii) change in polymorphous structure in sweet potato starches at an increase of growth temperature, i.e. C (A+B) $\Rightarrow$ A- type transition in the range of growth temperature between 15°C–20°C [14, 15]. The changes in the structure of crystalline lamellae are presented in Figure 1. As can be seen from Figure 1, an increase in number of defects leads to formation of crystalline lamellae with more "mellow" structure. Apparently due to this reason namely α-amylase susceptibility of native granules increase with decreasing in growth temperature [7].

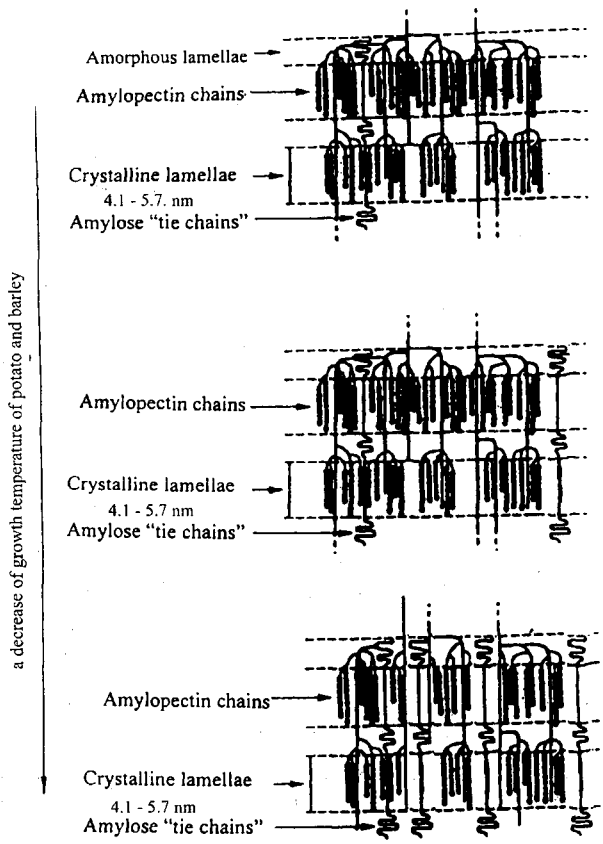

Fig. 1. Schematic presentation of the effects of growth temperature on the amorphous and crystalline lamellae of potato and barley (normal and waxy) starches.

On elevation of growth temperature for some normal and high amylose barley cultivars some structural features in behaviour of their starches can be observed. As

can be seen from Figures 2 and 3 [16], at relative low values of growth temperature the asymmetry on DSC-traces or their doubling is observed. Taking into consideration that the DSC-study of these starches was carried out under large water excess (0.3% d.m. dispersions) such shape of DSC-traces is unusual. Usually the same DSC-traces are observed at intermediate water content in a system of native starch-water [35, 36]. At the present nature of this phenomenon remains unexplained.

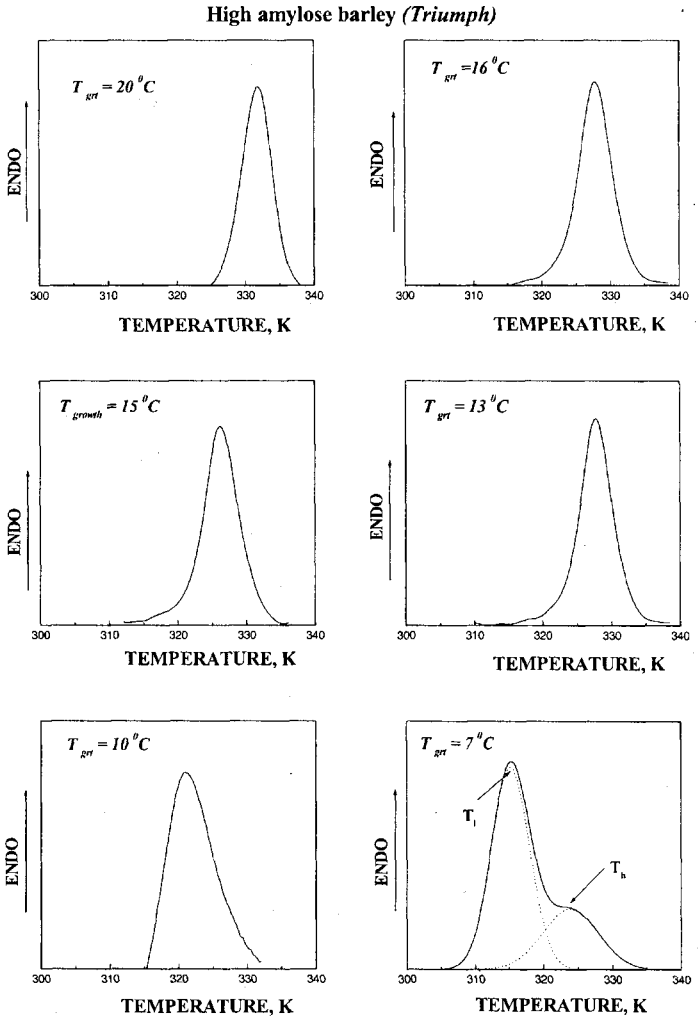

Fig. 2. Excess DSC-traces (—) and results of their deconvolution (------) for starches (*"Triumph"* variety) grown at different growth temperatures. T_l and T_h are the melting temperature of low- and high- temperature structures, correspondingly.

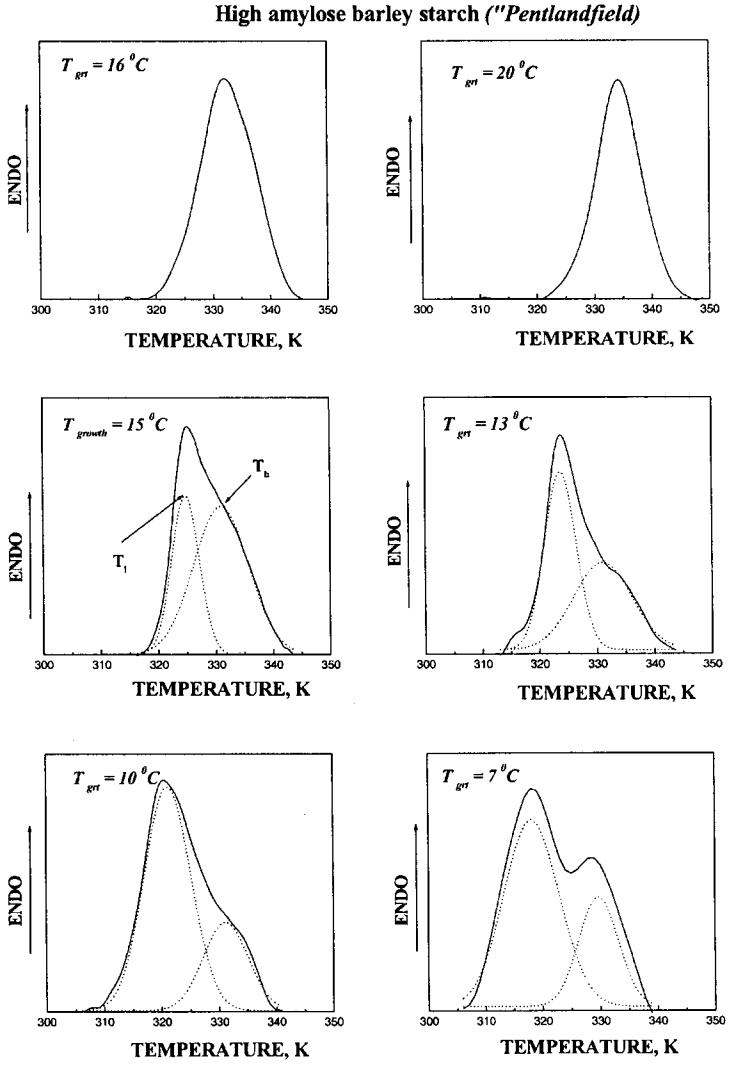

Fig. 3. Excess DSC-traces (—) and results of their deconvolution (------) for starches (*Pentlandfield"* variety) grown at different growth temperatures. T_l and T_h are the melting temperature of low- and high- temperature structures, correspondingly.

Generally, change in DSC-trace shapes on decrease in growth temperature could be caused by three reasons:

1) change in the ratio of small and large granular size fractions in starches because it is well known that granular size distribution of starches influences the width of starch calorimetric peaks [37]. However, as was supposed in the work [22, 23], this reason is apparently unlikely;

2) change in polymorphous structure of starches. However, the X-ray study of the samples showed that the polymorphous structure remains constant irrespective of growth temperature [16];
3) accumulation of defects in starch crystalline lamellae on decrease in growth temperature [13, 16].

At the present one assumes that starch granules contain two types of crystallites, which melt at the different temperatures when water content in a system is insufficient [35, 36]. According to [16, 28] a decrease in growth temperature for the barley starches leads to increase in swelling factor and number of defects in granules (Table 2). According to Donovan [35] starch granules contain two types of crystallites distinguishing in the hydration/swelling properties. Supposedly, differences in the hydration/swelling properties result from structural features in starches which are related on DSC- thermograms to B- and A- type starches at intermediate water content [35, 36]. However, the asymmetric or doubling calorimetric peaks are observed also under the water excess (Figures 2 and 3) as starch grew at low temperature [16]. A hypothesis explaining this phenomenon has recently been put forward in the work [16].

Table 2

Thermodynamic characteristics of low- and high- temperature structures in barley starches ("*Triumph*" and "*Pentlandfield*" varieties) and relevant proportions[a]

Barley variety	Growth temperature, °C	Low temperature structure			High temperature structure		
		T_l, K	ΔH_l, kJ/mol	Proportion, %	T_h, K	ΔH_h, kJ/mol	Proportion, %
Triumph	7	315.2	1.6	69.9	323.9	0.7	30.1
Pentlandfield	7	314.9	0.8	63.7	326.6	0.5	36.3
	10	321.1	1.3	72.2	331.1	0.5	27.8
	13	323.6	1.1	52.6	330.99	1.0	47.4
	15	324.7	0.7	34.8	330.9	1.3	65.2

[a]The low- and high- temperature structure content in starches were determined as relative enthalpic contributions (%) of each structure to the overall melting enthalpy of the samples.

Taking into consideration that

(i) an amount of defects in crystalline growth rings located closerr to a surface of granules is apparently larger than in crystalline growth rings located nearer to hilum [22, 38, 39],
(ii) such defects are accumulated at low growth temperature and induce a decrease of melting temperature [13, 22],

one can assume that observed asymmetry or doubling of calorimetric peaks results from increase in crystalline lamellae with large defect content. At the first approximation the melting of crystalline lamellae containing defects and crystalline lamellae "without" defects can be considered as the melting of two independent structures. When differences in the content of these structures are not too significant the peak doubling can be observed only under the intermediate water content in the starch-water systems. While these differences became significant the peak doubling can be observed under the water excess. At this cas, using deconvolution procedure for asymmetric calorimetric peaks, determination of the content of structures in native granules becomes available.

Results of deconvolution are presented in Figures 2 and 3. The low-temperature endotherms can be attributed to the melting of the crystalline lamellae containing the largest number of defects and having the largest swelling factor while the high-temperature endotherms can be related to the crystalline lamellae with the smallest number of defects and swelling factors. The analysis of the results shows that the asymmetry of calorimetric peaks on the DSC- thermograms of barley starches under the water excess is observed when the difference in the content of the low- and high-temperature crystalline structures is approximately of 30-40%. Certainly such explanation of results is only hypothetical and requires additional structural investigations.

Concluding remarks

The application of different physico-chemical approach for the description of melting process of starches, grown under various temperature provides estimation of the thickness of crystalline lamellae, thermodynamic parameters characterizing the surface of starch crystalline lamellae as well as an influence of growth temperature and the amylose content on number of defects in starches with different polymorphous structure. It is apparent that decrease in growth temperature on development of starch granules containing of B-, A- and C (A+B) -type crystalline structures leads to the formation of starch crystals with decreased melting temperature whereas the thickness of crystalline lamellae remains constant. Such behaviour can be explained by the accumulation of defects located in crystalline and amorphous lamellae. The increase in number of defects can be due to decrease in growth temperature and increase in the amylose content this is it can lead to appearance of peak doubling on DSC-traces .

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WPŁYW TEMPERATURY WZROSTU NA STRUKTURALNE I TERMODYNAMICZNE WŁAŚCIWOŚCI SKROBI

Streszczenie

Badano wpływ czynników środowiskowych na skład, strukturę i właściwości fizykochemiczne natywnych skrobi z różnych roślin. Wśród parametrów, na które zwrócono szczególną uwagę była temperatura gleby w trakcie wzrostu roślin i ona wydaje się być najistotniejszym parametrem. Różne sposoby opisu termodynamicznych parametrów topnienia skrobi pozwalają wyznaczyć kooperatywne jednostki topnienia, grubość lamelli krystalicznych i oszacować rolę defektów strukturalnych w organizacji natywnych gałeczek spowodowanych zmianami temperatury wzrostu. Przykładem może być wpływ temperatury wzrostu roślin na właściwości skrobi ze zwykłych i słodkich ziemniaków oraz woskowych, zwykłych i wysokoamylozowych odmian jęczmienia, tzn. skrobi o różnych polimorficznych strukturach odpowiednio typu B, C i A. Omówiono naturę pików kalorymetrycznych skrobi z niektórych normalnych i wysokoamylozowych odmian jęczmienia rosnących w obniżonych temperaturach.

CATHERINE LOISEL[1], ALBERTO TECANTE[2], JEAN-LOUIS DOUBLIER[3]

RHEOLOGY AND STRUCTURE OF CROSS-LINKED STARCH DISPERSIONS

Summary

The rheological properties of chemically cross-linked waxy maize starch (CWMS) dispersed in water has been studied in relation to its swelling behaviour. The main parameters that were considered were starch concentration (2–4%), pasting temperature (96°C–136°C) and shear conditions (~10^5 s^{-1}). The swelling behaviour was assessed by means of swelling experiments and by a measure of the size distribution of the swollen particles. The rheological study was performed by means of steady shear measurements (viscometry) and in oscillatory shear (viscoelasticity). In all conditions, starch dispersions exhibited the behaviour of suspensions of swollen particles as assessed from viscosity measurements. The flow behaviour of the dispersions was shear-thinning and a yield stress was clearly evidenced when the concentration was high enough. The viscoelastic behaviour became measurable as soon as the volume fraction of starch swollen particles was high enough for them to fill a large part of the available volume. This was typical of a gel-like system with G' > G" and a flat frequency dependence of G'. All these properties strongly depended upon the pasting temperature with an optimum determined by the degree of cross-linking of starch granules. Sensitivity of starch granules to shear also was strongly dependent upon the pasting temperature. When starch granules were undercooked, their swelling properties, and hence their rheological properties, were reinforced by high shearing. In contrast, when the starch granules were overcooked the rheological properties were depressed by shearing as a result of their high fragility. These overall results allow ways to evaluate the swelling behaviour of crosslinked starch in the formulation of starchy products according to processing conditions.

Introduction

Cross-linked starch is a widely used food ingredient because chemical modification makes it resistant to thermal and mechanical treatment. Therefore, it is possible to use conditions that promote granule swelling without destruction and it can be reasonably considered that swelled granules are dispersed in a phase constituted only by

[1] *LGPA-ENITIA, BP 82225, 44322 Nantes Cedex 3 (France)*
[2] *Depto. Alimentos y Biotechnologia, FQ-" E ", UNAM, México, D.F., 04510, México*
[3] *INRA-UPCM, BP 71627, 41316 Nantes Cedex 3 (France)*

water. Rheologically speaking, such a system can be regarded as consisting of swollen gelled particles, the properties of which are mostly governed by the phase volume occupied by the swollen starch granules and their deformability. This has been clearly evidenced at temperature lower than 100°C and under moderate shearing [1, 3, 6, 11]. However, these conditions are far removed from the usual industrial conditions in terms of temperature and shear treatments. Actually, quite few studies have been devoted to the rheological description of dispersions of cross-linked starch in conditions that are close to the industrial ones, that is at temperature higher than 100°C and under strong shearing [7]. The present paper reports some recent investigations dealing with the properties of cross-linked waxy maize starch (CWMS) with the pasting temperature ranging from 96°C and 136°C and in different shearing conditions. The aim was to describe how the extent of swelling as well as the 'fragility' of starch granules influence the properties of the suspensions.

Materials and methods

Materials

Two CWMS samples were adipate/acetate starch supplied by Roquette Frères : Clearam® CH10, lot E9887 and Clearam® CH20, lot 61546). Both samples are devised to resist to high temperature treatment without being disrupted. They differ by their degree of cross-linking. CH 10 being less reticulated than CH 20 swells to a larger extent but is more susceptible to shearing under heat treatment.

Pasting procedure

Starch at 3, 3.5 or 4% (w/w) was slurried in water at room temperature under mechanical stirring to avoid settling. Then the suspension was poured into a jacketed vessel (capacity 2l, stirring rate 500 rpm) inside which gradual heating (5°C/min) was applied from 20°C to the upper pasting temperature (98 to 136°C); this temperature was maintained for 20 minutes and was followed by a cooling step (1.5°C/min) down to 70°C before sampling. Three pasting temperatures were compared to define three swelling degrees of the starch granule: 'undercooked' (90 and 110°C), 'well cooked' (120–125°C) and 'overcooked' (130°C).

Particle size determination of swollen starch granules

Particle size determination was performed at room temperature using a Malvern Master Sizer (Malvern Instruments, Ltd) laser scattering analyser with a 300 mm Fourier cell (range 0.05 to 879μm) as described in Loisel et al. [9]. The starch dispersion was first diluted (1/10) with water at 20°C immediately after the pasting procedure,

then dispersed in the sample dispersion unit (1 ml/100 ml water) and fed into the measuring cell. Volume distribution was obtained using the Mie scattering theory which requires refractive index of the media to be specified: we used, 1.529 and 1.33, respectively for starch and liquid phase and 0.1 for the starch granule absorption. From each distribution a median volume diameter D(v, 0.5) was calculated.

Swelling determination

Swelling was estimated after centrifugation of a starch suspension diluted at 0.5% at 700 g for 15 min [8] using the dye exclusion technique as described by Dengate et al [5] with Blue Dextran as the dying agent. From the absorbance at 630 nm of the supernatant, after dilution of starch suspension by a Blue Dextran solution (0.1%) the amount of water absorbed by the swollen starch granules is given by:

$$W_{abs}=W_0(1-C_1/C_2) \quad (1)$$

with:

W_{abs}, amount of water absorbed by starch granules (%, w/w),
W_0, total amount of water in the starch suspension (%, w/w),
C_1, concentration of the added Blue Dextran solution,
C_2, concentration of the solution in Blue Dextran in the supernatant.

Considering that the solubility of starch can be neglected, the swelling capacity (Q) is given by:

$$Q=\left(\frac{W_{abs}+(100-W_0)}{100-W_0}\right) \quad (2)$$

Shearing of starch suspensions

The heated starch suspension was sheared directly out of the reactor after cooling to 70°C by flowing it through a conical contractor (contraction angle = 30°, 10^{-3} m internal diameter). The upper wall shear rate applied was estimated to 10^5 s^{-1}, for a feed rate of 25 L/h.

Rheological measurements

Steady shear tests were carried out at 60°C using a cone-plate measuring device (6 cm/4°) with a controlled stress rheometer (TA Instruments AR 1000). Two consecutive shear scans were performed by programming linearly the shear rate from 0 to 660 s^{-1} and then back to zero. Each cycle was performed for 4 minutes. This was followed by stepwise measurements with a logarithmic programmation of shear rate from 660 to 0.1 s^{-1}.The same device was used to perform oscillatory shear tests on a new aliquot (unsheared sample). A 4% strain amplitude was fixed after determination of the linear

viscoelastic range. Each sample was analysed through a three steps protocol: (1) mechanical spectrum (G' and G'' as a function of frequency) at 60°C to characterize the hot paste, (2) gelling kinetics (at 1 Hz) after rapid cooling of the sample from 60 to 25°C using Peltier effect, (3) mechanical spectrum at 25°C of the gelled system.

Results and and discussion

From the swelling capacity (Q), the volume fraction (Φ) occupied by the swollen particles is obtained by the expression ($\Phi = c\ Q$). Some values are given in Table 1 corresponding to the concentrations and temperatures that have been studied in the present work. Clearly, the swelling capacity of CH20 was much lower than that of CH10 due to its higher degree of crosslinking. For the examples chosen here, the volume fraction ranged from ~0.50 to ~0.75 depending upon the type of starch, the concentration and, but to a lower extent, the heating temperature. As a result of the different degree of crosslinking, a volume fraction of ~0.50 required a 3% starch concentration in the case of CH10 instead of 3.5% in the case of CH20.

Table 1

Swelling capacity (Q) of two CWMS at different treatment temperatures and corresponding volume fractions (Φ) at different concentrations.

Temperature (°C)	CH10			CH20	
	Q (g/g)	Φ3%)	Φ4%)	Q (g/g)	Φ3.5%)
96	17.0	0.51	0.68	15.1	0.53
112	17.5	0.52	0.70	15.7	0.55
125	18.2	0.55	0.73	16.0	0.56

Note: volume fractions (Φ are obtained from the expression $\Phi = cQ$

Figure 1 shows the typical flow behaviour of a CWMS (CH10) dispersion heated to 96°C. At 3% a shear-thinning behaviour was clearly evidenced. The 'up' curve being superimposed to the 'down' curve, no shear dependency was exhibited. At 4%, the CWMS dispersions exhibited an anticlockwise loop in the first cycle between 0 to 660 s^{-1}. The second cycle then was superimposed to the return curve of the first cycle. Such antithixotropic behaviour has been reported by several authors in the case of crosslinked starches [4, 11, 12].This is indication of a flow-induced structure the origin of which has been postulated from granule rupture and partial leaching of amylopectin [11]. However, as it will be evidenced in the following, starch granules heated at such a low temperature (96°C) are not prone to break down easily and this assumption is far from being proven. As an alternative explanation, it can be suggested that such a be-

haviour in concentrated suspensions is to be ascribed to a rearrangement of the close-packed particles thus yielding a higher organization of the system [2].

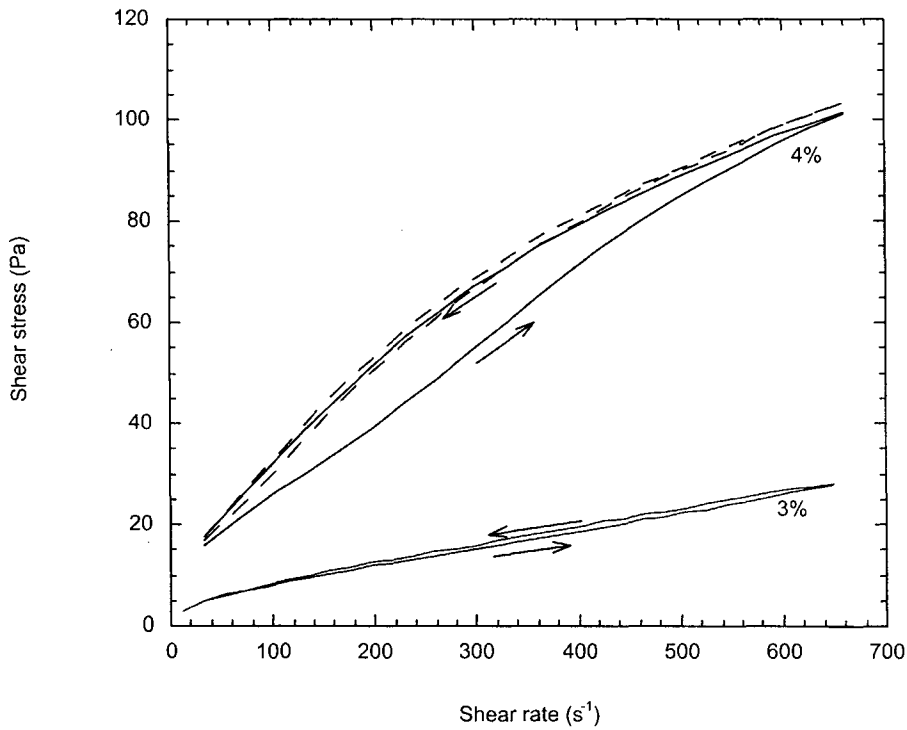

Fig. 1. Flow curves of 3% and 4% CWMS (CH10) dispersions heated to 96°C. Measurement temperature: 60°C. For the 4% dispersion, two successive cycles are plotted: 1st cycle: continuous line; 2nd cycle: dashed line.

The effect of heating temperature at 96, 112 and 125°C, respectively, on the flow behaviour of a 3% CH10 dispersion is illustrated in Figure 2. As was expected, increasing this temperature above 100°C resulted in an increase of the viscosity. Furthermore, the behaviour was 'antithixotropic' at 112°C and 125°C while not at 96°C. This may be ascribed to an increase of the size of the particles and hence of the phase volume occupied by the swollen particles.

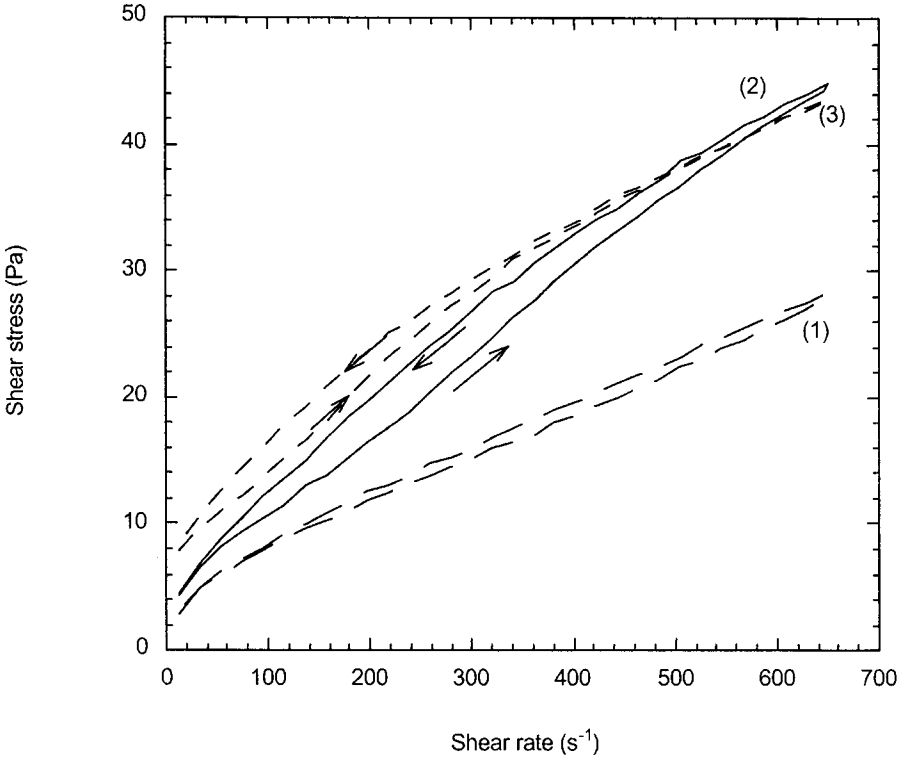

Fig. 2. Flow curves of 3% (CH10° dispersions heated to 96°C (curve 1), 112°C (curve 2) and 125°C (curve 3). Measurement temperature: 60°C.

The viscoelastic behaviour of these dispersions is illustrated in Figure 3. In the present case, measurements were performed at 25°C. Although displaying visually a liquid aspect, all these systems exhibited a solid-like behaviour with G' > G" and G' almost independent of frequency. Since the dispersion is composed of swollen starch granules, this clearly indicates that the dispersion is concentrated enough to develop viscoelastic properties. In other terms, the swollen particles are close-packed which makes them to govern the rheological properties. In these experiments, Φ ranged from 0.50 to 0.55. These observations are consistent with those of Evans et Lips [6] who found that a volume fraction of 0.4–0.5 is required for noticeable elastic properties to be developed in the case of waxy cross-linked maize starch. Although the moduli differed quite significantly between the different temperatures, the shape of the curves were similar suggesting a comparable structure. It can also be noticed that a higher G' value was obtained after treatment at 112°C than at 125°C and 96°C. The optimum swelling temperature should be close to 120°C, starch granules being 'overcooked' at 125°C and 'undercooked' at 96°C.

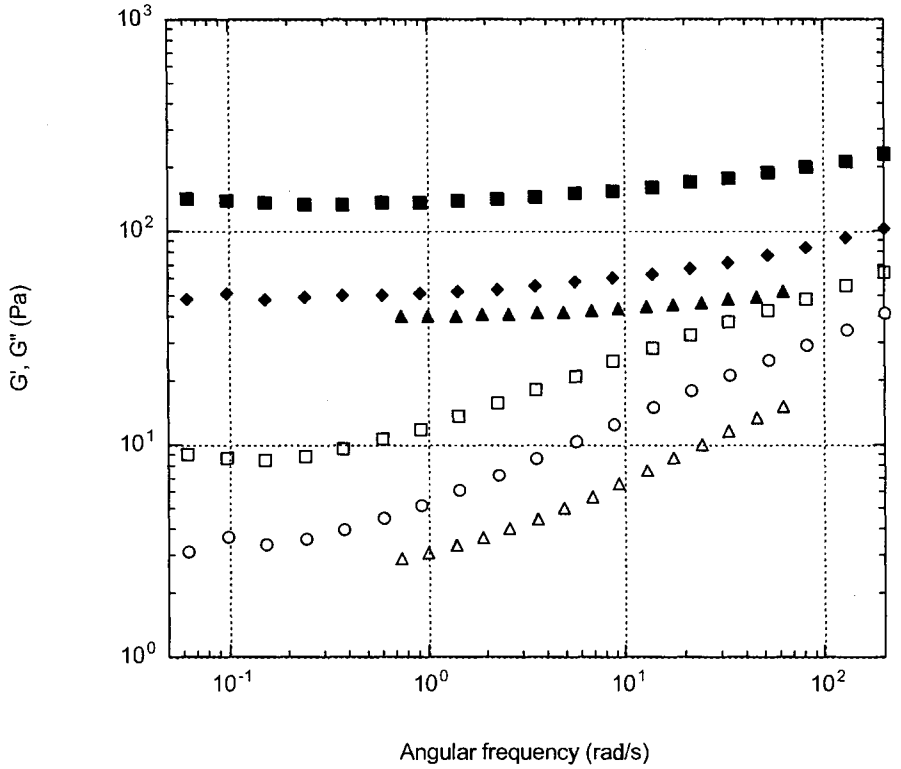

Fig. 3. Mechanical spectra (G' and G" as a function of frequency) of 3% dispersions (CH 10). Filled symbols: storage modulus (G'); empty symbols: loss modulus (G"). Triangles, heating temperature: 96°C; squares, heating temperature: 112°C; lozenges, heating temperature: 125°C. Measurement temperature: 25°C.

The flow curves of 3.5% WMCS dispersions (CH20) at different heating temperatures in the range 98–136°C are illustrated in Figure 4. Again, viscosity measurements were performed at 60°C. In the present case, the curves were plotted in log scales allowing one to visualise the low shear range. Clearly, the curves displayed a shear-thinning behaviour with a yield stress at low shear rate. This is the expected behaviour of suspensions of deformable particles and has already been reported for starch dispersions. The yield stress was strongly dependent on the pasting temperature with a maximum at 129°C: around 0.7 Pa at 98°C, 1.2 Pa at 115°C, 2 Pa at 122°C, 3 Pa at 129°C and less than 1 Pa at 136°C. These overall results clearly illustrate the effect of the pasting temperature around the optimum swelling temperature. In the present case, the optimum was close to 130°C to which the maximum viscosity the highest yield stress were reached. The shape of the curve for a treatment at 136°C differed significantly from the other results. This is clear illustration that the structure of

the suspension is noticeably different since starch granules are overcooked and probably more easily broken down.

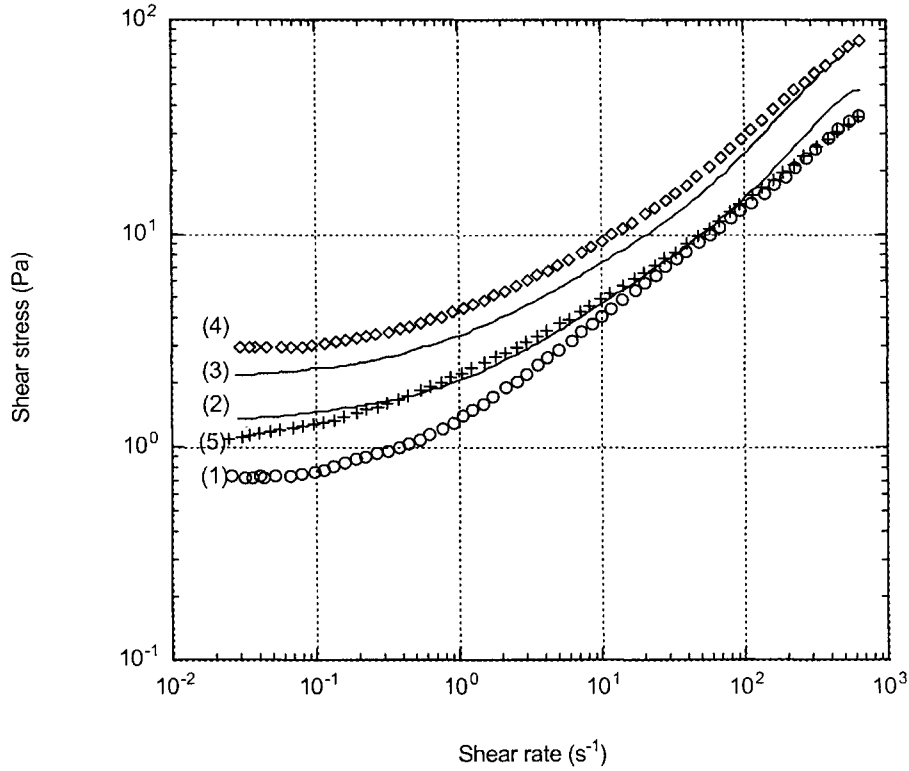

Fig. 4. Flow curves of 3.5% CWMS (CH20) dispersions. Heating temperatures: 98°C (curve 1), 115°C (curve 2), 122°C (curve 3), 129°C (curve 4) and 136°C (curve 5). Measurement temperature: 60°C.

Sensitivity of these starch dispersions to shearing as a function of treatment temperature is illustrated in Figure 5. The dispersions have been prepared in the same way as in Figure 4 but an additional shearing step has been applied at 70°C (see methods). For treatments between 98°C and 129°C, the flow curves were almost superimposed. The yield stress was of the order of 2 Pa. From the comparison with Figure 4, the curve at 122°C was the same while those at 98°C, and 115°C were shifted towards higher shear stress values. The curve for 129°C was slightly shifted downwards (the shear stress was depressed from 3 to 2 Pa) but was superposed to the previous curves. Inversely, the dispersion heated to 136°C experienced a dramatic downward shift. This indicates that the additional shear process imposed to starch granules can either increase the overall viscosity (< 120°C), probably by improving the swelling pattern of

the starch granules, or decrease the overall viscosity (at 136°C) suggesting their shear sensitivity in these conditions. At 122°C, the shearing process did not yield any significant change in the properties. These observations suggest that the optimum swelling can be achieved by combining treatment temperature and high shearing, with the provision that starch granules have not been 'overcooked'. If the optimum temperature is exceeded the swollen particles become susceptible to shearing; as a result, the granules can be broken down and the viscosity is depressed.

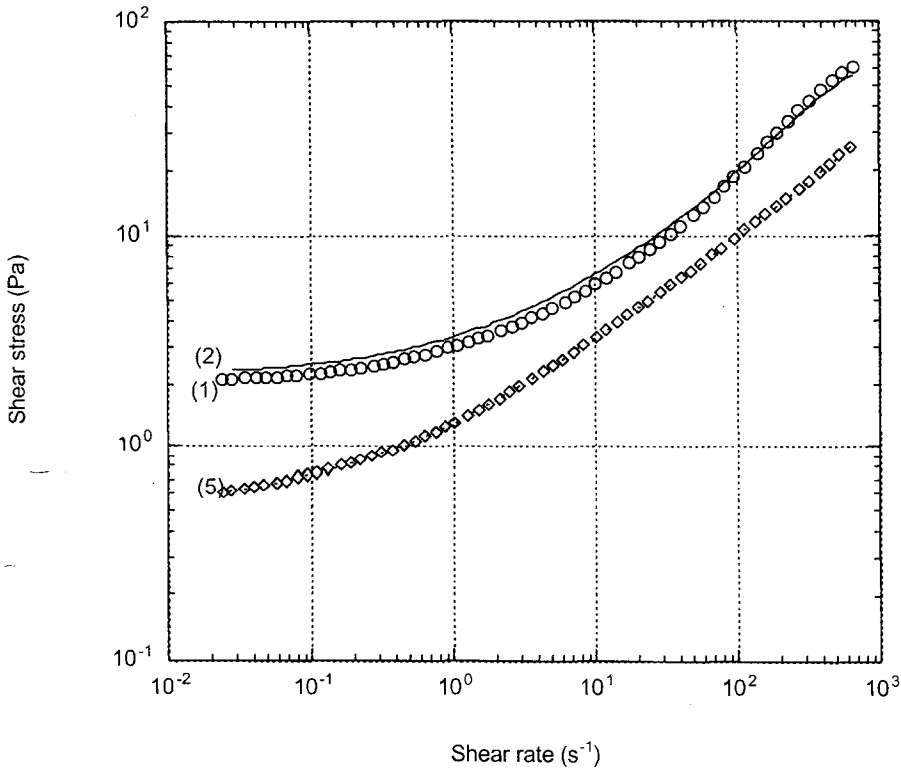

Fig. 5. Flow curves of 3.5% CWMS (CH20) dispersions submitted to strong shearing. The numbers correspond to the same heating temperature as in the previous figure. The results for 122°C (curve 3) and 129°C (curve 4) were superimposed to curve 2 and are not showed.

In order to better understand these effects, we determined the particle size distribution after heat treatment. Figure 6 shows the distribution as a function of the diameter. The median volume diameter at 98°C was increased from around 39 μm without shearing up to ~43 μm upon shearing. Also a slight increase was experienced at 117°C from ~42 μm down to ~44 μm. Figure 7 shows the overall results obtained between 98°C and 136°C. While the median diameter increased continuously from 39 at 98°C to 47

μm at 127°C and then slightly decreased down to 44 μm at 136°C for the unsheared samples, the variations were different when shearing was applied: the diameter was almost constant (at ~43–44 μm) up to 122°C and then dramatically dropped to reach ~26 μm at 136°C. This is clear indication that swollen starch granules of CH20 CWMS become highly susceptible to shearing if treated at temperature higher than 122°C but are highly resistant if the treatment temperature is lower than 122°C. Moreover, the effect of shear on the size of swollen starch granules is beneficial if the treatment is applied at temperature lower than ~125°C. These overall observations are fully consistent with the rheological data (Figures 4 and 5).

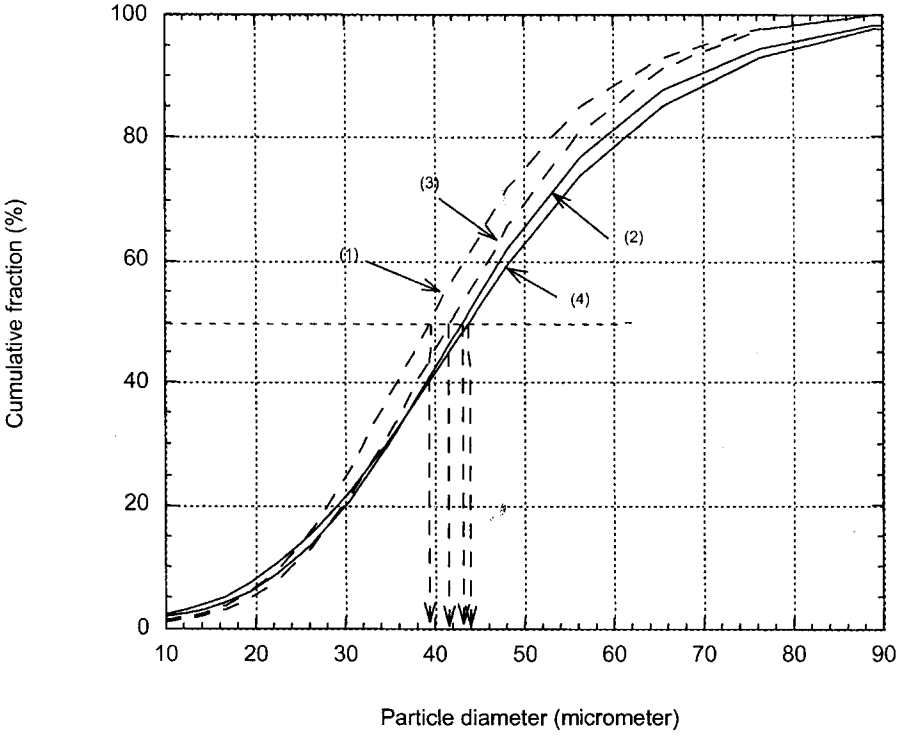

Fig. 6. Size distribution of swollen starch granules (CH20). Heating temperature 98°C; curve 1: unsheared; curve 2: sheared. Heating temperature: 117°C; curve 3: unsheared; curve 4: sheared.

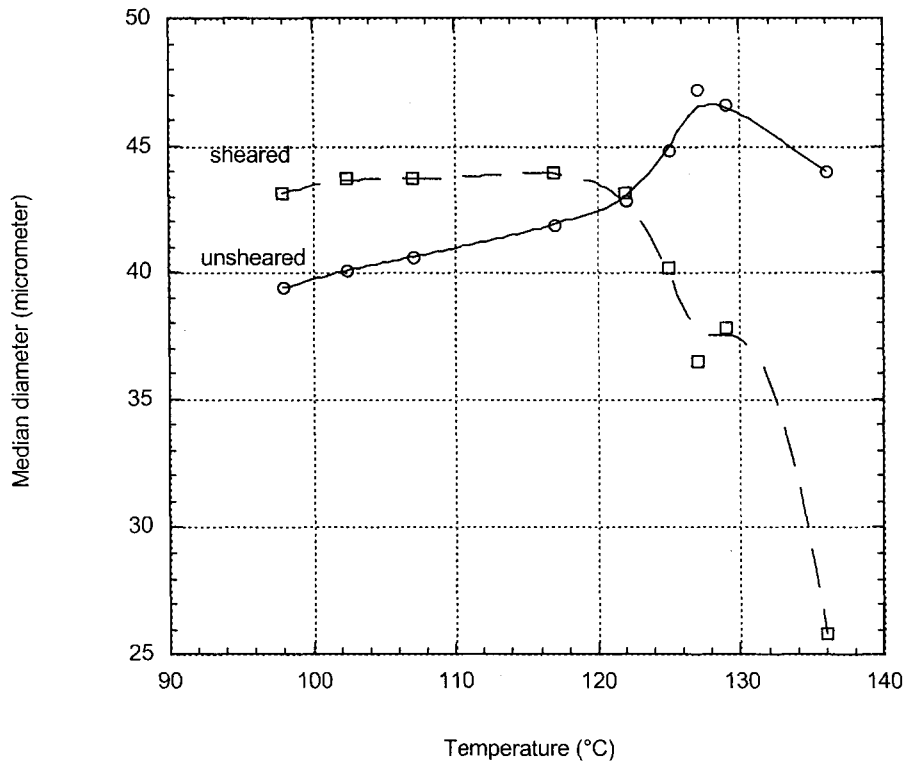

Fig. 7. Median diameter as a function of heating temperature. Comparison of sheared and unsheared samples (CH20).

Conclusions

All these rheological results correspond to starch suspensions with a volume fraction higher than 0.50 (Table 1). The present observations are consistent with those of Steeneken [10] on the basis of viscosity measurements who found that a volume fraction of 0.4–0.5 is required for viscosity development. This author also reported that within the concentration range 0.50–0.70, it is the volume fraction which determines the viscosity of the suspension. However, it is likely that the deformability of the particles should also be taken into account. This is shown by the solid-like behaviour as evidenced by the viscoelastic properties ($G' > G''$) as well as by the flow behaviour at low shear rate (yield stress). A decrease in G' or in the yield stress would reflect an increase in the deformability of the particles. This is clearly illustrated in Figures 3 and 4 which show large differences as a function of the heating temperature while the volume fraction remains almost constant.

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REOLOGIA I STRUKTURA ZAWIESIN SIECIOWANYCH SKROBI

Streszczenie

Badano pęcznienie chemicznie sieciowanych woskowych skrobi kukurydzianych (CWMS) zawieszonych w wodzie. Głównymi parametrami branymi pod uwagę były stężenie skrobi (2–4%), temperatura żelowania (96–136°C) i warunki ścinania (~10^5 s^{-1}). Pęcznienie obserwowano doświadczalnie oraz przez pomiar rozkładu rozmiaru spęczniałych gałeczek. Pomiary reologiczne wykonano wiskozymetrycznie i metodą oscylacyjną (lepkosprężystość). W każdym przypadku zawiesiny skrobi wykazywały pęcznienie z rozrzedzaniem spowodowanym ścinaniem. Zachowanie się zawiesin spęcznionej skrobi było typowe dla pseudożelowanych układów z G' > G" i płaskim przebiegiem zależności częstość – G'. Właściwości te zdecydowanie zależały od temperatury kleikowania z maksimum zależnym od stopnia usieciowania gałeczek skrobiowych. Przy niezupełnym skleikowaniu gałeczek obserwowano wysoką wartość ścinania. Po nadmiernym gotowaniu lepkość kleików spadała.

QIANG LIU[1], ELIZABETH WEBER[1], MING Z. FAN[2], RICKEY YADA[3]

PHYSICOCHEMICAL PROPERTIES OF POTATO DRY MATTER ISOLATED FROM VARIOUS CULTIVARS AT DIFFERENT TIMES DURING GROWTH

Summary

Potato dry matter was isolated from three cultivars of potato tubers at different times during growth. The physicochemical properties of these potato dry matters were characterized for starch and protein content, thermal properties by differential scanning calorimtery (DSC) and paste properties by rapid viscosity analysis (RVA). Dry matter content of potato tubers increased to the highest level and then decreased as growth processed. Superior cultivar potato had a lowest dry matter content and the highest protein content as compared to Snowden and Shepody potatoes. Gelatinization enthalpy and temperature of dry matter varied with growth times and potato cultivars. Immature potato tubers (the earliest harvest) resulted in the highest temperatures for gelatinization, pasting and retrogradation of dry matter, indicating the molecular structure of starch plays an important role in the functional properties of potato dry matter. The quality of table and processing potato could be affected by growth times and cultivars.

Introduction

Fresh potato tubers contain 13 to 37% dry matter which includes starch, cellulose, hemicellulose and ash, and 63 to 87% of water and water-soluble components such as carbohydrates, proteins, phenolic substances, mineral components and organic acids [10]. Chemical composition and structure of components such as starch, non-starch polysaccharide, sugars, organic and inorganic compounds, and proteins influence the properties of potatoes and potato products [6, 7, 8]. The yield of potato chips and French fries, and the texture of French fries and canned and reconstituted dehydrated potatoes are directly related to the dry matter content of the potatoes [3].

However, no information is available on the physicochemical properties of dry matter due to the complexity of the potato. In this paper, we report the physicochemi-

[1]*Food Research Program, Agriculture & Agri-Food Canada, Guelph, Ontario, Canada N1G 5C9*
[2]*Dept. of Animal and Poultry Science, University of Guelph, Guelph, Ontario, Canada N1G 2W1*
[3]*Dept. of Food Science, University of Guelph, Guelph, Ontario, Canada N1G 2W1*

cal properties of dry matter isolated from various cultivars at different times during growth. The objective of this study was to provide the physicochemical analysis tools for characterizing the functional properties of potato dry matter and for predicting the quality of potato tubers. The effect of major components of potato dry matter on the functional properties is also discussed.

Materials and methods

Materials

Three potato varieties (Superior, Shepody and Snowden) grown at the Cambridge Research Station, University of Guelph, Cambridge, Ontario (Canada) were harvested at different growth times in the year 2000 season and were stored at room temperature for one day before isolation of dry matter and starch.

Potato dry matter was isolated according to the method of Liu et al [5].

Methods

Dry matter content

Dry matter content was determined from the difference in the weight of potato samples before and after freeze-drying in a Freeze Dryer 8 (Labconco®, Kansas City, MO, USA). Moisture content of dry matter was measured by weighing samples (triplicate) before and after drying at 85°C and 710–740 mm Hg vacuum for 7 hr.

Starch content

Starch content of dry matter isolated from various cultivars at different times during growth was determined based on AACC method 76.13 [1] with modification [5]. To 100 mg potato dry matter, 100 μL (300U) α-amylase (Sigma A-6380, St. Louis, MO) solution, and 2.9 mL 45 mM MOPS buffer (pH 7.0) were added. The sample was heated in a boiling water bath for 6 min with constant stirring, was then cooled to below 50°C. 100 μL (20U) amyloglucosidase (Sigma A-7255, St. Louis, MO) and 3.9 mL 200 mM sodium acetate buffer (pH 4.5) were added to the sample. The sample was mixed well and incubated at 50°C for 30 min with constant stirring. The glucose content of supernatants was measured by YSI 2700 Select Biochemistry Analyzer (Yellow Springs, Ohio, USA). Pure starch from different potatoes was employed as a standard in every batch experiment to verify enzyme activity. Starch content of potato dry matter was expressed as a ratio of glucose content in the dry matter to glucose content in pure starch following starch hydrolysis. Blank samples (without enzymes) were also measured using the same protocol. Finally, starch content in potato tubers was ex-

pressed by multiplying dry matter content by starch content in dry matter. The reported values are the means of triplicate measurements.

Protein content

Protein content of dry matter was determined by using a ThermoQuest CE Instruments NA 2100 Protein Analyzer (ThermoQuest Italia S.P.A., MI) according to AACC method 46-30 (AACC). The nitrogen content was determined using software (Eager 200 for Windows™, Version 1.02, ThermoQuest Italia S.P.A., MI). Atropine, DL-methionine, acetanilide and nicotinamide were used as standards. Protein content was calculated by multiplying nitrogen content by the factor 6.25. The reported values are means of triplicate measurements.

Differential Scanning Calorimetry (DSC)

Thermal analyses were performed using a differential scanning calorimeter (2920 modulated DSC; TA Instruments, New Castle, DE, USA) for dry matter gelatinization and retrogradation. Samples of dry matter were weighed into high-volume pans (Part number: 900825-902; TA Instruments, New Castle, DE, USA). Distilled water was added to make suspensions with 70% moisture content. Pans were sealed and equilibrated for 2–4 h at room temperature before heating in the DSC. The measurements were carried out at a heating rate of 10°C/min from 5 to 180°C. Sample weights were about 20 mg. The instrument was calibrated using indium and an empty pan as reference. The enthalpy (ΔH) of phase transitions was measured from the endotherm of DSC thermograms using software (Universal Analysis, Version 2.6D, TA Instruments) based on the mass of dry solid. Peak temperature (Tp) of endotherms was also measured from DSC thermograms.

After heating to 180°C, samples were air-cooled to 5°C. Once the temperature reached 5°C, the sample was immediately removed from the DSC and stored in a refrigerator (5°C). After 14 days, the sample pan was removed from the refrigerator and placed into the sample holder of the DSC. Stored samples were heated from 5°C to 180°C at 10°C/min. The enthalpy (ΔH) and peak temperature (Tp) of the endotherm was measured from DSC thermograms based on dry solid mass. The reported values are means of duplicate measurements.

Rapid Viscosity Analysis (RVA)

A Rapid Visco™ Analyser RVA-4 (Newport Scientific Pty. Ltd, Warriewood, NSW, Australia) was employed to measure the pasting properties of potato dry matters (8% dsb, 28 g total weight). Experiment was performed using STD 2 profile (AACC method 76–21), in which the sample was equilibrated at 50°C for 1 min, heated at

6°C/min to 95°C, held at 95°C for 5 min, cooled at 6°C/min to 50°C, and held at 50°C for 2 min. The speed was 960 rpm for the first 10 s, then 160 rpm for the remainder of the experiment. Peak viscosity, final viscosity and pasting temperature of starches were compared from pasting curve.

Results and discussions

Dry matter, starch and protein contents of potato dry matter isolated from various cultivars at different times during growth

Due to the differences among cultivars in tuber growth rates and the harvest dates were different for the selected cultivars. Superior was planted earlier than Shepody and Snowden seed potatoes. Dry matter, starch, protein and moisture contents were measured for samples isolated from various cultivars at different times during growth. The results are presented in Table 1. Dry matter content was 16.6, 21.0 and 18.6% (w/w) in the fresh potato tubers for Superior, Shepody and Snowden cultivars, respectively, at the earliest harvest time. As growth time increased, dry matter content in the tubers increased to its highest level between 64 and 71 days, then decreased. The highest dry matter content for Superior potato was 19.2% at 64 days, 24.2% for Shepody potato at 71 days, and 24.0% for Snowden potato at 71 days. Superior tubers had a lower dry matter content than Shepody and Snowden tubers at all harvest days. These results were consistent with previous studies [2, 9] on dry matter content as a function of tuber growth time. Potato tubers high in dry matter may be suitable for the manufacture of dehydrated food products and they may also be suitable for storage [3].

Starch content of dry matter varied with potato cultivar and growth time and ranged from 66 to 80%. Starch content was 66.0, 67.2 and 71.1% at the earliest time and was 79.7, 74.3 and 75.6% in the dry matter of potato harvested at the longest growth time for Superior, Shepody and Snowden cultivars, respectively. The highest starch content in potato dry matter was 80.4% for Superior potato at 84 days, 78.1% for Shepody potato at 91 days and 78.4% for Snowden at 91 days. Based on this study, potatoes with higher dry matter and starch content could be obtained by selecting specific potato cultivars and harvesting at specific times.

The protein content in the dry matter ranged from 9.9 to 15.2%. It was 11.6, 13.9 and 15.2% harvested at the shortest growth time and was 11.3, 11.8 and 13.8% in the dry matter of potato harvested at the longest growth time for Shepody, Snowden and Superior cultivars, respectively. The change of protein content in the dry matter was small when the growth time increased. However, protein content was dependent on the potato cultivar with Superior having higher protein content than either Shepody or Snowden.

Table 1

Dry matter, starch and protein content of tubers at different growth times.

Cultivar	Growth time (day)	Dry matter content of tuber (%, w/w)*	Moisture content of dry matter (%, w/w)*	Starch content of dry matter (%, w/w)*	Protein content of dry matter (%, w/w)*
Shepody	55	21.0 ± 0.6	6.0 ± 0.1	67.2 ± 1.4	11.6 ± 0.0
	71	24.2 ± 0.9	6.4 ± 0.1	77.6 ± 1.3	11.2 ± 0.1
	91	20.1 ± 0.9	5.1 ± 0.1	78.1 ± 1.4	11.8 ± 0.1
	112	18.8 ± 1.0	6.3 ± 0.0	74.8 ± 1.7	13.9 ± 0.1
	124	19.8 ± 0.4	4.0 ± 0.0	74.3 ± 5.2	11.3 ± 0.2
Snowden	55	18.6 ± 1.6	6.7 ± 0.2	71.1 ± 1.2	13.9 ± 0.2
	71	24.0 ± 1.3	6.1 ± 0.3	77.2 ± 3.0	9.9 ± 0.2
	91	20.9 ± 0.6	5.1 ± 0.1	78.4 ± 2.4	10.0 ± 0.1
	112	17.8 ± 1.5	7.5 ± 0.2	75.8 ± 2.8	12.8 ± 0.2
	124	19.8 ± 1.0	3.9 ± 0.0	75.6 ± 1.4	11.8 ± 0.3
Superior	48	16.6 ± 0.2	6.1 ± 0.0	66.0 ± 1.2	15.2 ± 0.1
	56	16.2 ± 0.4	6.0 ± 0.1	72.7 ± 0.7	13.1 ± 0.0
	64	19.2 ± 1.0	5.8 ± 0.0	77.5 ± 1.0	13.8 ± 0.3
	84	17.3 ± 1.0	5.8 ± 0.0	80.4 ± 0.4	13.1 ± 0.2
	117	16.9 ± 1.1	3.8 ∀ 0.1	79.7 ± 0.9	13.8 ∀ 0.2

*Value denotes mean ± standard deviation

Thermal properties of dry matter from potato with different growth times and cultivars

When potato dry matters were heated in the presence of excess water (70%), a single symmetrical endothermic transition was observed between 70.9 and 79.1°C as shown in Figure 1. A similar behavior was observed for the dry matters' corresponding starch [5]. In that study, an endothermic transition was observed between 70.0 and 74.8°C. Table 2 lists the gelatinization and retrogradation properties of potato dry matters. The thermal properties of potato dry matter were influenced by growth times and cultivar of tubers. At the earliest harvest time, the gelatinization temperature (both Tp and To) of potato dry matter was the highest for all three potato cultivars. Similar results were found for their pure starch [5]. As the harvest time increased, gelatinization temperature of dry matter decreased and then remained at the same range. Due to the other components in the sample, gelatinization temperature of dry matter was about 4°C higher than corresponding starch.

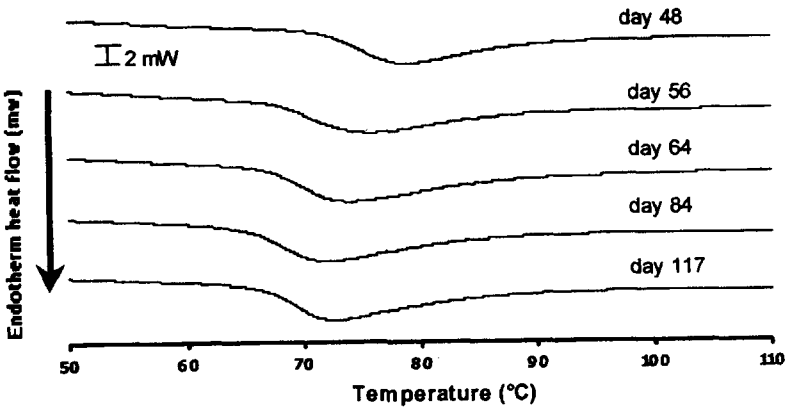

Fig. 1. DSC thermograms of potato dry matter with 70% moisture content during heating (gelatinization). Labeling refers to growth time of Superior potato tuber.

Table 2

Thermal properties of potato dry matter as determined by DSC.

Cultivar	Growth time (days)	Gelatinization			Retrogradation		
		ΔH (J/g)	T_o (°C)	T_p (°C)	ΔH (J/g)	T_o(°C)*	T_p (°C)
Shepody	55	10.0 ± 0.8	71.8 ± 0.1	79.1 ± 0.1	4.0 ± 0.1	-	71.3 ± 0.1
	71	13.5 ± 0.4	65.4 ± 0.3	73.9 ± 1.0	3.4 ± 0.0	-	68.2 ± 0.1
	91	13.7 ± 0.0	66.7 ± 0.1	74.2 ± 0.5	3.6 ± 0.0	-	68.6 ± 0.1
	112	14.2 ± 0.3	66.8 ± 0.2	73.9 ± 0.3	3.7 ± 0.4	-	68.4 ± 0.8
	124	14.3 ± 0.3	66.6 ± 0.1	73.7 ± 0.0	4.5 ± 1.2	-	68.9 ± 1.1
Snowden	55	12.6 ± 0.1	70.0 ± 0.2	77.1 ± 0.0	3.5 ± 0.3	-	70.6 ± 0.8
	71	12.3 ± 0.0	63.7 ± 0.0	70.9 ± 0.1	4.3 ± 0.0	-	67.1 ± 0.0
	91	12.0 ± 0.2	65.0 ± 0.1	72.3 ± 0.0	3.7 ± 0.2	-	68.7 ± 0.2
	112	11.0 ± 0.1	64.9 ± 0.1	73.0 ± 0.1	3.6 ± 0.7	-	69.3 ± 0.3
	124	12.9 ± 0.0	64.8 ± 0.5	72.5 ± 0.5	3.6 ± 0.2	-	68.8 ± 0.9
Superior	48	9.1 ± 0.2	71.3 ± 0.3	78.7 ± 0.2	4.5 ± 0.2	-	70.9 ± 0.3
	56	12.4 ± 0.3	66.8 ± 0.1	75.2 ± 0.3	2.8 ± 1.2	-	71.0 ± 1.8
	64	13.0 ± 0.1	66.2 ± 0.1	73.6 ± 0.2	4.1 ± 0.1	-	68.8 ± 1.1
	84	13.2 ± 0.1	64.9 ± 0.2	72.2 ± 0.1	3.9 ± 0.4	-	68.7 ± 0.4
	117	11.3 ± 1.0	66.4 ± 0.4	72.6 ± 0.0	4.7 ± 0.0	-	69.2 ± 0.2

*not enough slope for software to calculate onset temperature (T_o) for retrograded samples

The gelatinization enthalpy of dry matter was the lowest at the earliest time, i.e. 10, 12.6 and 9.1 J/g for Shepody, Snowden and Superior dry matter, respectively. The enthalpy increased slightly when tuber growth time increased for Shepody and Superior potato. In our previous study [5], the gelatinization enthalpy ranged from 15.6 to 18.1 J/g for starch isolated from various cultivars at different times during growth. The

lower gelatinization enthalpy and higher gelatinization temperature of potato dry matter might be due to the following: lower starch content in the sample compared to pure starch, and/or the interference in starch gelatinization by the non-starch components.

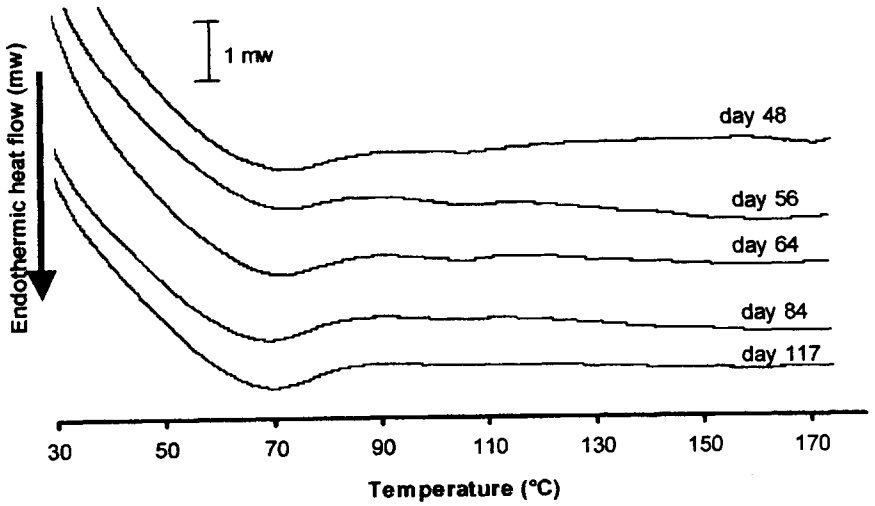

Fig. 2. DSC thermograms of cooked potato dry matter with 70% moisture content after 2-week storage at 5°C. Labeling refers to growth time of Superior potato tuber.

When gelatinized dry matter was heated after it was stored at 5°C for two weeks, an endothermic transition was observed between 68.2 and 71.3°C as shown in Figure 2, indicating starch retrogradation had taken place during storage. Retrogradation endothermic peak temperature was higher for dry matter with the shortest growth time, i.e. 71.3, 70.6 and 70.9°C for Shepody, Snowden and Superior dry matter, respectively. Under the same storage temperature and times, the retrogradation endothermic peak temperature was 69.2, 68.2 and 66.2°C for Shepody, Snowden and Superior starch, respectively, at the earliest harvest. However, the retrogradation enthalpy of gelatinized dry matter was much lower as compared to the corresponding starch. The retrogradation enthalpy was between 2.8 and 4.7 J/g for potato dry matter isolated from various cultivars at different times during growth. It was between 8.4 and 10.2 J/g for starch from potato with different growth times and cultivars. The non-starch components might have inhibited starch retrogradation in the dry matter, resulting in the lower retrogradation enthalpy and higher endothermic peak temperature of gelatinized dry matter compared to the corresponding starch.

Pasting properties of potato dry matter

Table 3 shows the pasting properties of potato dry matter isolated from various cultivars at different times during growth. The pasting curves of Superior potato dry

matter during potato growth are presented in Figure 3. The peak viscosity increased slightly, peaked and then decreased slightly as growth time increased. The final viscosity increased slightly as growth time increased for most dry matters. The pasting temperature of dry matter decreased as growth time increased for Superior potato.

Table 3

Pasting properties of potato dry matter by RVA.

Cultivar	Growth time (days)	Peak Viscosity (cP)	Final Viscosity (cP)	Pasting Temperature (°C)
Shepody	55	N/a		
	71	1224	838	72.0
	91	1242	983	70.3
	112	1104	917	70.3
	124	1184	971	69.1
Snowden	55	N/a		
	71	1364	1019	66.7
	91	1499	1076	66.3
	112	1346	1148	67.6
	124	1187	1156	66.8
Superior	48	541	605	92.6
	56	1198	972	72.3
	64	1369	1027	68.7
	84	1338	1040	67.9
	117	1275	1081	67.9

N/a = not analyzed due to insufficient sample

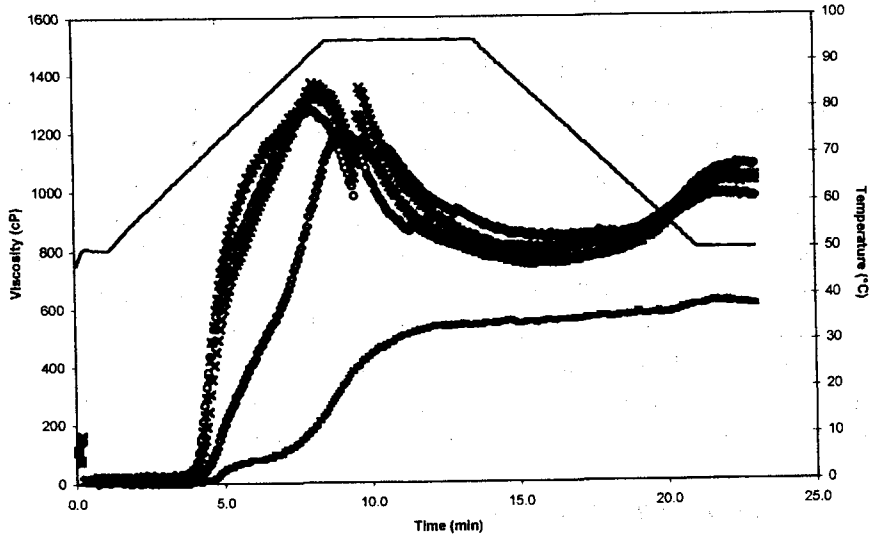

Fig. 3. The RVA pasting curves of Superior potato dry matter during potato growth at 48 (□), 56 (◊), 64 (×), 84 (□) and 117 (○) days as a function of temperature (—).

Among the three cultivars, Shepody potato dry matter had the lowest peak viscosity and final viscosity, and the highest pasting temperatures compared to the dry matter from Snowden and Superior potatoes. For starch isolated from potatoes with the longest growth time, the peak viscosity was 6858, 7217 and 7567 cP for Superior, Shepody and Snowden, respectively. The final viscosity of pasting was 2047, 2738 and 1959 cP from starch isolated from the longest growth time for Superior, Shepody and Snowden potatoes, respectively [5]. However, the peak viscosity was 1275, 1184 and 1187 cP from dry matter isolated from the longest growth time for Superior, Shepody and Snowden potato, respectively. The final viscosity was 1081, 971 and 1156 cP from dry matter isolated from the longest growth time for Superior, Shepody and Snowden potato, respectively.

The lower value of both peak and final viscosity for potato dry matter paste as compared to potato starch paste indicates a weak gel network was formed during the heating of potato dry matter with excess water. Other components such as non-starch polysaccharides, protein, organic and inorganic compounds in the potato dry matter greatly influenced the strength of dry matter gel. The lower starch concentration (about < 6%) [4] might be another factor that produced a lower paste viscosity. The pasting properties of dry matter were almost independent of growth time. However, the peak and final viscosity of dry matter isolated from Superior potatoes with the shortest growth time was much lower (about 50% lower) than that of dry matters isolated from potatoes with longer growth time. From the previous study [5], the lowest molecular weight of starch isolated from the potatoes with the shortest growth time was observed. The peak and final viscosity of its corresponding starch was dependent of growth times and cultivars of potato tubers. Thus, the molecular characteristics of starch and interference of other components might play very important role in the pasting properties of potato dry matter.

Conclusions

Potato dry matter content varied with cultivars and tuber growth times. Starch content in the potato dry matter was the lowest from tubers with the shortest growth time, peaked and then decreased slightly as a function of growth time. Protein content in the potato dry matter was the highest from the tubers with the shortest growth time (< 2 months) for Snowden and Superior potatoes. However, protein content was independent of growth time for Superior potato after 2 months growth. Superior potato dry matter had the highest protein content compared to the other potatoes. The thermal properties of potato dry matter were influenced by the starch as well as non starch components. The shorter growth time of potato tuber resulted in higher gelatinization and retrogradation temperatures and lower gelatinization enthalpies for potato dry matters. The paste made from potato dry matter had much lower viscosity than that from

the corresponding starch, indicating starch concentration, interference of non-starch components in the gel formation, and starch molecular characteristics play important roles in the functional properties of potato dry matter. The pasting properties of potato dry matter were independent of growth time after two months.

Acknowledgements

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FIZYKOCHEMICZNE WŁAŚCIWOŚCI SUCHEJ MASY ZIEMNIAKÓW Z RÓŻNYCH ODMIAN I RÓŻNYCH OKRESÓW ICH WZROSTU

Streszczenie

Sucha masa ziemniaków pochodziła z bulw trzech odmian zebranych w różnych okresach ich wzrostu. Fizykochemiczne właściwości suchej masy obejmowały oznaczenie zawartości skrobi i białek, właściwości termiczne (DSC) i reologiczne właściwości kleików (RVA). Zawartość suchej masy w bulwach ziemniaków wzrastała do pewnego poziomu, a potem w miarę opóźniania zbioru obniżała się. Odmiana Superior cechowała się najniższą zawartością suchej masy i najwyższą zawartością białek w porównaniu z ziemniakami odmian Snowder i Shepody. Entalpia kleikowania i temperatura suchej masy zmieniały się w miarę wzrostu, w różny sposób specyficzny w poszczególnych odmianach. Niedojrzałe bulwy (wczesny zbiór) dawały materiał o najwyższej temperaturze kleikowania i najłatwiejszej retrogradacji co wskazywało, że struktura molekularna skrobi ma istotny wpływ na funkcjonalne właściwości suchej masy. Odmiana ziemniaków i czas ich zbioru mogą mieć wpływ na jakość ziemniaków spożywczych i przemysłowych.

KUNRUEDEE SANGSEETHONG[1], KLANARONG SRIROTH[2,3]

EFFECT OF HYPOCHLORITE LEVELS ON THE MODIFICATION OF CASSAVA STARCH

Summary

The effect of oxidant levels on the structure and physico-chemical properties of modified cassava starch was investigated. Cassava starch was treated with various amounts of sodium hypochlorite (1,000-20,000 ppm of active chlorine on dry starch basis) at 30°C and pH 10.5. The substantial amount of carboxyl groups was formed only when more than 5,000 ppm of hypochlorite was used and it increased with increasing hypochorite levels. With these high levels of hypochlorite, the resulting modified starches exhibited the pasting properties of a typical oxidized starch with low peak viscosity, little breakdown and low setback. With the lower levels of hypochlorite (1,000 and 2,500 ppm), the modified starches showed high peak viscosity, reduced breakdown and high setback. Results from high-performance size-exclusion chromatography reveals that both amylose and amylopectin were degraded and the extent of degradation increase with the increasing levels of the oxidant. Starch modified with hypochlorite concentration lower than 2,500 ppm showed a pronounced reduction in the paste clarity indicating that the starch granule becomes more resistant to disintegration after the modification.

Introduction

Hypochlorite is the most commonly used chemical for the production of oxidized starch. The desired properties from such modification are low viscosity and improved stability of starch paste. Oxidation alters properties of native starch through the introduction of carbonyl and carboxyl groups, which minimizes retrogradation, and depolymerization, which causes drastic decrease in hot paste viscosity [1, 2]. Oxidized starch is widely used in the paper industry as surface sizing and coating binder where

[1]*Casssava and Starch Technology Research Unit/National Center for Genetic Engineering and Biotechnology, Bangkok, Thailand*
[2]*Department of Biotechnology, Faculty of Agro-Industry, Kasetsart University, Bangkok, Thailand*
[3]*Kasetsart Agricultural and Agro-Industrial Product Improvement Institute, Kasetsart University, Bangkok, Thailand*

low paste viscosity at relatively high starch concentration and viscosity stability of starch paste during storage are most desired.

In addition to pH, temperature, time, starch concentration and starch origin, hypochlorite concentration seems to be an important factor affecting the properties of modified starch. For the production of oxidized starch of commerce, hypochlorite is normally used at relatively high level. The Code of Federal Regulation (CFR) of the U.S. Food and Drug Administration [3], classifies the process of treating starch with sodium hypochorite not to exceed 8,200 ppm as bleaching whereas the process when using higher level of the chemical but not more than 55,000 ppm is classified as oxidation. The bleaching process aims to improve whiteness and remove impurities from starch but oxidation intends to alter the rheological properties of starch as previously mentioned. Most studies on hypochlorite modification of starch are pertinent to the oxidation process [4-7]. Very few studies have reported on the effect of low levels of hypochlorite [8-9]. The systematic study covering the range of extremely low and high levels of hypochlorite has not been reported.

The present work aims to study the effect of hypochlorite concentration (1,000-20,000 ppm of active chlorine, dry starch basis) on the physico-chemical properties of cassava starch.

Materials and methods

Materials

Cassava starch was from Chor Chaiwatana Tapioca Co., Ltd. (Thailand). Sodium hypochlorite was from Carlo Erba Reagenti (Italy). The amount of active chlorine was determined according to Iodometric procedure as described in the Standard Methods [10] prior to used. All other chemicals used were of reagent grade.

Preparation of hypochlorite-modified starch

Cassava starch was modified by sodium hypochlorite as described by Kettlitz and Coppin [9]. A 40% starch slurry was prepared by adding 750 g of distilled water to 500 g starch (dry basis) and pH was adjusted to 10.5 with 3% NaOH solution. The temperature of the slurry was brought to 30°C and NaOCl was added slowly to the stirred slurry to reach different final concentrations (1,000, 2,500, 5,000, 10,000, 20,000 ppm). After 3 hours, the pH of slurry was adjusted to 6.5 with sodium bisulfite, filtered, washed three times with distilled water and oven dried at 45°C.

Determination of carboxyl content

The carboxyl content of hypochlorite-modified cassava starch was determined by the ISO method [11]. Starch sample (5 g) was stirred in 0.1 M HCl for 30 min. The slurry was then filtered and washed with distilled water until free of chloride ions. The filtered cake was transferred to 300 mL water and the starch slurry was heated in a boiling water bath with continuous stirring until gelatinized and continue stirring at that temperature for another 15 min. The hot sample was titrated with 0.1 M NaOH using phenolphthalein as an indicator.

Determination of carbonyl content

The cabonyl content was determined as described by Kuakpetoon and Wang [7]. Starch sample (4 g) was slurried in 100 mL of distilled water. The slurry was gelatinized in a boiling water bath for 20 min, cooled to 40°C, adjusted to pH 3.2 with 0.1 M HCl, and 15 mL of hydroxylamine reagent was added. The flask was stopped and placed in a water bath at 40°C. After 4 hour, the excess hydroxylamine was determined by rapid titration to pH 3.2 with 0.1 M HCl.

Pasting properties of starch

The pasting properties of starch were determined with a Rapid Visco Analyser (RVA-4, Newport Scientific, Australia) using standard program Number 1. The starch sample was 3.00 g (on 14% moisture basis). The starch suspension was held at 50°C for 1 min and subsequently heated to 95°C at 12.2°C/min. Holding time at 95°C was 2.5 min. The sample was then cooled to 50°C at 12.2°C/min, and kept at that temperature for 2.1 min. A rotation speed of the paddle was at 160 rpm.

Molecualr weight distribution

The molecular weight distributions of starch samples were determined by High Performance Size Exclusion Chromatography (HPSEC) using one Ultrahydrogel linear and two Ultrahydrogel 120 columns connected in series (Waters Corporation, MS) according to the method of Govindasamy et al. [12].

Light transmittance of starch paste

The light transmittance of a starch paste was determined by the procedure of Lim and Seib [13]. Starch suspension (1%) was heated in a boiling water bath for 30 min with occasional shaking. After the suspension was cooled to room temperature for 60 min, the percent transmittance was determined at 650 nm.

Results and discussion

The carboxyl and carbonyl contents of NaOCl-modified cassava starch are presented in Table 1. Carboxyl content increased as the hypochlorite concentration increased whereas the amount of carbonyl groups formed did not show any dependence on the concentration of the oxidant.

Table 1

Carboxyl and carbonyl group contents of NaOCl-modified starch

NaOCl level (ppm)	Carboxyl content (%)	Carbonyl content (%)
1,000	0.005	0.08
2,500	0.005	0.13
5,000	0.03	0.08
10,000	0.14	0.10
20,000	0.33	0.06

The pasting profiles of native and modified cassava starches using various NaOCl levels are shown in Figure 1. Native cassava starch is characterized by high peak viscosity with drastic breakdown during a heating cycle followed by low setback during cooling. After modification with hypochlorite in the levels of higher than 5,000 ppm, the modified starch exhibited pasting properties of a typical oxidized starch, which has low peak viscosity, little breakdown and low setback.

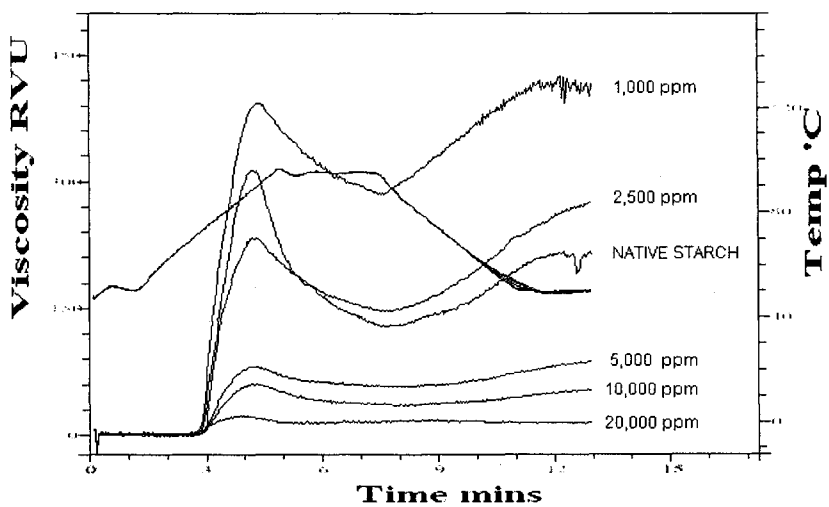

Fig. 1. Pasting profiles of native and NaOCl-modified cassava starches determined by a Rapid Visco Analyser. The levels of active chlorine employed in the reaction are labeled on individual curves.

The reduction in peak viscosity was due to partial degradation of starch molecules, which caused a decrease in the molecular weight. As a result, starch granules of these modified samples fragmented before reaching maximum swelling. These modified starches also showed relatively low setback signifying that they had less tendency for retrogradation. Wurzburg [2] suggested that carboxyl groups introduced into the starch molecules during oxidation were bulkier than hydroxyl groups; they are more effective in preventing the reassociation of amylose molecules thus minimizing retrogradation phenomena. The formation of carboxyl content observed in this work also supports this explanation.

Starch modified with lower levels of hypochlorite showed different pattern of pasting curves. With 1,000 ppm of oxidant, modified sample exhibited higher peak viscosity, lower breakdown and higher setback when compared to native starch. Kettlitz and Coppin [9] also observed similar phenomena when employing this low level of hypochlorite to waxy starch. The increase in peak viscosity suggests that after modification starch granules were easier to swell and they swelled to a greater extent than the native starch. This could be due to the introduction of a small amount of negatively charged carboxyl groups to the starch, which weakens the association forces between starch molecules. However, the significant decrease in breakdown and the high final viscosity suggests that the increase in swelling of starch granules occurred without loss of granule structure. Even though the mechanism for such phenomena is not clear, the results demonstrate that modification with low level of sodium hypochlorite somehow strengthens the structure of cassava starch granule. The pasting properties of starch obtained from this modification are similar to that of lightly crosslinked starch.

The HPSEC chromatograms of native and modified cassava starches are showed in Figure 2. Fraction eluted at 13 min was mainly high molecular weight amylopectin; the lower molecular weight fraction of amylose was eluted at about 22 min. The molecules with intermediate size were eluted at 17 min. The extent of depolymerization of starch during modification depended on the concentration of NaOCl. At 1,000 ppm of active chlorine, no change in the molecular weight distribution can be detected on the chromatogram. The shift to the longer retention time of amylose fraction and the appearance of a new peak at 16 min in the sample treated with 2,500 ppm of hypochlorite indicated that both amylose and amylopectin began to degrade at this condition. With the higher levels of the oxidant, both fractions were degraded to a greater extent and the amylose fraction disappeared.

The paste clarity of native and NaOCl-modified cassava starch is shown in Table 2. NaOCl – modification had a dramatic effect on the paste clarity of the resulting starch pastes. Native cassava starch produced a translucent paste with 62% light transmittance. The results demonstrate that modification with high levels of oxidant (5,000–20,000 ppm) increased light transmittance. The negatively charged carboxyl

groups that formed in these samples caused the repulsion between adjacent starch molecules and reduced interchain association leading to a high clarity paste. On the other hand, starch modified with low level of hypochlorite showed a pronounced decrease in the paste clarity when compared to native starch. It has been reported that crosslinking caused the reduction in the paste clarity [13] possibly due to the presence of undisrupted swollen granules. The decrease in the light transmittance of the resulting modified starch in this study could be due to the remaining of such undisrupted swollen granules.

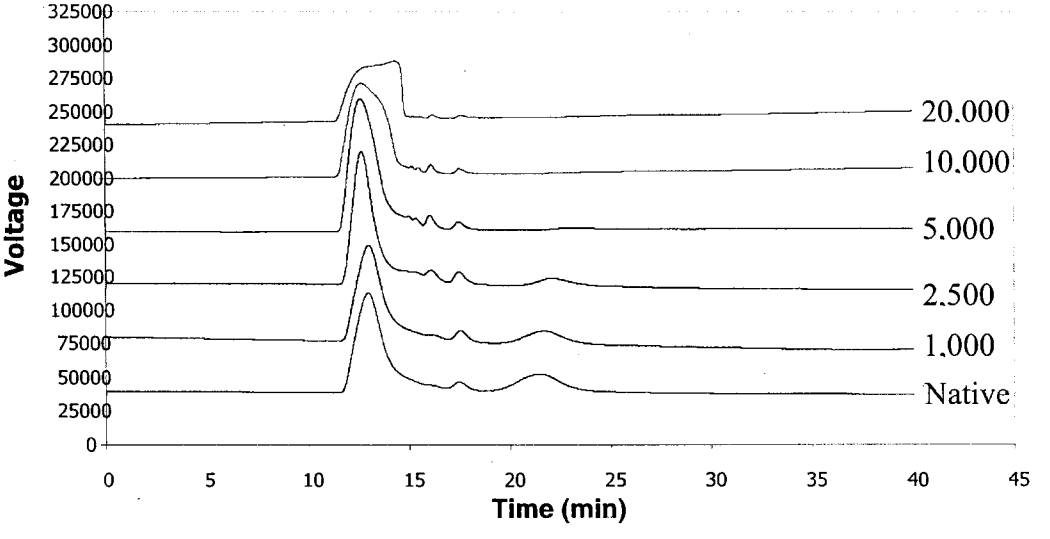

Fig. 2. Molecular weight distribution of native and NaOCl-modified cassava starch determined by high-performance size-exclusion chromatography.

Table 2

Light transmittance of pastes from native and NaOCl-modified cassava starches.

Sample/NaOCl level (ppm)	Transmittance (%)
Native starch	62.3 ± 0.87
1,000	18.4 ± 1.39
2,500	24.5 ± 0.61
5,000	80.1 ± 1.39
10,000	95.0 ± 0.70
20,000	98.9 ± 0.12

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WPŁYW POZIOMU PODCHLORYNU NA MODYFIKACJĘ SKROBI TAPIOKOWEJ

Streszczenie

Zbadano wpływ pozimu podchlorynu jako utleniacza na strukturę i właściwości fizykochemiczne modyfikowanej skrobi tapiokowej. Skrobię tę utleniono w 30°C przy pH 10.5 podchlorynu sodu zawierającego 1000 do 20 000 ppm aktywnego chloru w suchej masie. Znaczące ilości grup karboksylowych powstawały dopiero wtedy, gdy ilość aktywnego chloru wynosiła powyżej 5 000 ppm i wzrastały one w miarę wzrostu stężenia utleniacza. Tak utleniona skrobia wykazywała właściwości kleikujące typowe dla utlenionych skrobi o niskiej lepkości maksymalnej. Gdy stężenie utleniacza wahało się między 1000 i 2500 ppm aktywnego chloru, utlenione skrobie miały wysoką lepkość maksymalną. Badania za pomocą wysokosprawnej chromatografii żelowej pokazały, że zarówno amyloza jak i amylopektyna uległy degradacji i stopień degradacji wzrastał ze stężeniem użytego utleniacza. Skrobia utleniana podchlorynem o stężeniu poniżej 2 500 ppm wykazywała daleko idące zmniejszenie przejrzystości kleików, wskazujące na odporność gałeczek na zniszczenie przez kleikowanie.

KUAKOON PIYACHOMWAN[1], SUNEE CHOTINEERANAT[1], RUNGTIVA WANSUKSRI[1], KLANARONG SRIROTH[2,3], CHRISTOPHER OATES[4]

COMPARATIVE STUDY ON COMPOSITIONAL AND FUNCTIONAL PROPERTIES OF CASSAVA- AND CORN-BASED MALTODEXTRIN

Summary

Cassava-based maltodextrins with different dextrose equivalent (DE = 5, 10, 15 and 20) were prepared using two different enzymes, namely Termamyl 120L and Ban 480L (Novo Nordisk, Denmark). The molecular distribution oligosaccharide component (DP 1 – 7) was characterized and compared with commercial corn-based maltodextrin of the same DE. For all DE's, cassava-based maltodextrins prepared by Termamyl enzyme comprised more high molecular weight saccharides than corn-based maltodextrins. The profiles of saccharide component of cassava-based maltodextrin from Ban were comparable to those of corn-based ones. The shape and size of maltodextrins from two starch bases were different, presumably due to different processing. Corn-based maltodextrins were bigger in size. However, most properties including moisture content, water sorption and viscosity of both cassava- and corn-based maltodextrins were similar.

Introduction

Maltodextrin is a starch hydrolysis product and is usually classified by the extent of hydrolysis as described by the dextrose equivalent (DE), i.e. the percentage of the total reducing sugars, expressed as dextrose, present in the sample to the total dry substance. According to the U.S. Food and Drug Administration, maltodextrin is a non-sweet nutritive saccharide polymer that consists of D-glucose units linked primarily by α-1,4-bonds and has a dextrose equivalent of less than 20. It is prepared as a white powder or concentrated solution by partial hydrolysis of corn or potato starch with safe and suitable acids and enzymes [4]. Maltodextrin is found to be useful for many applications including spray-drying aids for flavors and seasonings, carriers for synthetic

[1]*National Center for Genetic Engineering and Biotechnology, Bangkok, Thailand;* [2]*Department of Biotechnology, Faculty of Agro- Industry, Kasetsart University, Bangkok, Thailand;* [3]*Kasetsart Agricultural and Agro-Industrial Product Improvement Institute, Kasetsart University, Bangkok, Thailand;* [4]*Agro Food Resources (Thailand) Co., Ltd., Bangkok, Thailand*

sweeteners, flavor enhancers, fat replacers, and bulking agent [5]. Typically, the food industry regards maltodextrin to be corn-based products. However, maltodextrin products can also be produced from other starchy sources, such as rice and cassava.

Maltodextrin of the same DE may have different functional properties, depending to some extent on its molecular characteristics. The products of the same DE may contain a different distribution of molecules; most are medium size. Large molecules are few and undesirable as they can precipitate after dissolved. Small molecules do not precipitate but they provide the product sweetness. The molecular composition of maltodextrin can be varied depending on the process (acid or enzyme, type of enzyme used etc.), processing condition (enzyme and starch concentration, temperature, pH) and starch types. Since starches of different sources have different molecular structure (amylose/amylopectin ratio, degree of branching, chain length distribution etc.), maltodextrin can also be expected to have different characteristics.

This paper aims to investigate the molecular composition and describe some functional properties of maltodextrin prepared from cassava starch using two enzymes and to compare the products with commercial corn-based maltodextrins of a comparable dextrose equivalent.

Materials and methods

Materials: Cassava starch was obtained from the factory in Thailand. Two types of enzyme were used including Termamyl 120L (from *Bacillus licheniformis* with the activity of 120 KNU; 1 KNU is the amount of enzyme used to hydrolyze the starch 5.26g per hour according to the standard method of Novo Nordisk) and Ban 480L (from *Bacillus amyloliquefaciens* with the activity of 480 KNU; Novo Nordisk Co., Bagsvaerd, Denmark). Corn-based maltodextrins including Maltrin M040, M100, N150 and M200 were obtained commercially from Grain Processing Corp. (USA) [2].

Preparation of cassava-based maltodextrin: Starch slurry (30% by weight) containing 400 ppm of calcium ion was cooked in the presence of enzyme and incubated at the controlled temperature (100 and 75°C for Termamyl 120L and Ban 480L, respectively) for different times to produce maltodextrin with different DE (Table 1). The reaction was terminated by adjusting the pH to 3 and boiling for 5 min. The hydrolysate was then filtered and spray-dried (BUCHI 190 Mini Spary Dryer, Germany).

Dextrose equivalent: DE values of samples were determined by the Lane and Eynon titration with Fehling's solution [3].

Compositional analysis: The macromolecular components of maltodextrins were determined by High Performance Size Exclusion Chromatography (HPSEC) using one Ultrahydrogel linear and two Ultrahydrogel 120 columns connected in series (Waters Corporation, MS) according to the method of Govindasamy et al. [1]. Oligosaccharide components of maltodextrins were quantified by the High Performance Anion Ex-

change Chromatography with Pulse Amperometric Detector (HPAEC-PAD, Dionex BioLC, Dionex, CA, USA) using CarboPac PA-100 (2 x 250 mm) and two mobile phases including 5mM and 0.5M sodium acetate in 100mM sodium hydroxide. The descriptive ratio, the ratio of the sum of the percentages of saccharides (dry basis), having a DP1 – 6 devided by DE values was also reported.

Table 1

Conditions (enzyme concentration and hydrolysis time) used for preparing cassava-based maltodextrin with different dextrose equivalent values.

Condition	Dextrose equivalent (DE)			
	5	10	15	20
*Termamyl 120L**				
Enzyme concentration (% by weight)	0.1	0.1	0.5	0.5
Hydrolysis time (min)	60	360	30	60
*Ban 480L***				
Enzyme concentration (% by weight)	0.1	0.1	0.5	0.5
Hydrolysis time (min)	10	30	10	30

*Starch concentration was 30% by weight at 100°C, pH 6.0.

** Starch concentration was 30% by weight at 75°C, pH 6.0.

General properties: The scanning electron micrographs of maltodextrin samples was observed under JEOL scanning electron microscopy (JSM-5310, England) at 10-KV acceleration and magnified at 3,000x. The content of moisture was determined by drying the sample at 105°C until constant weight. The pH value of 20% (by weight) solution was recorded. The bulk density of maltodextrin was measured according to the method of Wang and Wang [5]. The sample was filled into a graduated cylinder with an exact volume and the weight of the sample was recorded as the loose-filled weight. The cylinder was then subjected to mixer vibration and the volume of packed sample recorded. The water sorption isotherm of maltodextrin was obtained by incubating the samples under the different relative humidity conditions (21, 31, 51, 67, 80 and 92%) according to Wang and Wang [5]. The viscosity of maltodextrin solution (20% dry weight) was measured at 25°C using a Brookfield viscometer (Model DV-III, spindle # 0 at 10 to 240 min^{-1}, Brookfield Engineering Lab, inc.) and the shear rate and shear stress were recorded.

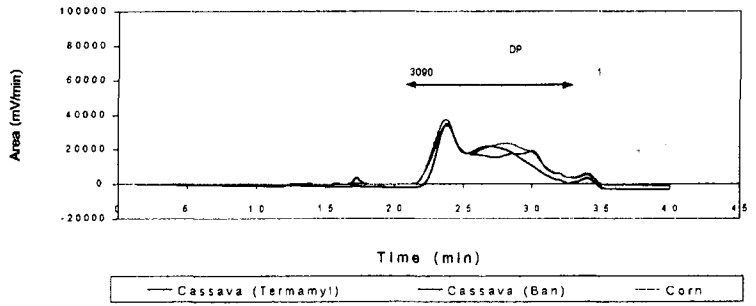
Results and discussion

Cassava-based maltodextrins of DE 5, 10, 15 and 20 were prepared by two bacterial enzymes, namely Termamyl 120L and Ban 480L. The measured DE of all prepared samples was in the range of ±1 except maltodextrin DE 10 prepared by enzyme

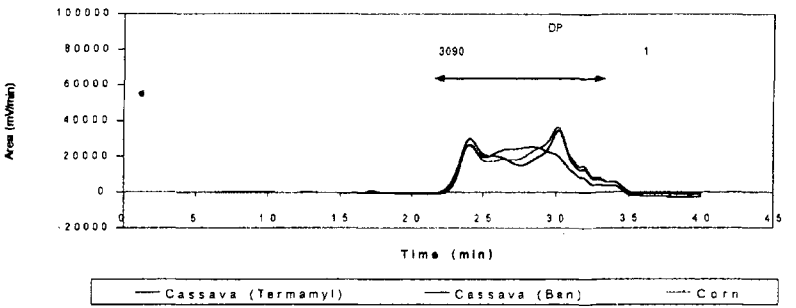
Termamyl and DE 20 prepared by enzyme Ban (Table 2). All corn-based maltodextrin had the measured DE within the range of product specification (GPC, 1999). Figure 1 presents the molecular distribution of cassava- and corn-based maltodextrins with different DE. For all DE's, cassava-based maltodextrins prepared by Termamyl enzyme comprised of more high molecular weight saccharides than corn-based maltodextrins. The profiles of saccharide component of cassava-based maltodextrin from Ban were comparable to those of the corn-based ones (Figure 1). This trend was still observed when the quantity of oligosaccharides (DP 1–7) was investigated (Figure 2). Mainly, cassava-based maltodextrin from Termamyl contained a lower amount of oligosaccharides than the corn-based samples having the same DE. However, the descriptive ratio of corn-based samples were not higher than the cassava-based as the DE values were slightly higher.

General properties of casasva- and corn-based maltodextrins were investigated. All samples were dry with very low moisture contents (less than 8%; Table 2). The pH of cassava- and corn-based samples was different. This assumed to different processes. For all DE's, cassava-based samples, regardless of the enzyme type, had the lower loose density than the corn-based ones, but the packed density were close. Therefore, the percentage of compressibility, the ratio of packed density to loose density, of cassava-based sampels were higher than the corn-based maltodextrins. This was presumably due to the shape and size of maltodextrin products. Corn-based maltodextrins for all DE's were bigger sizes than the cassava-based samples. Moreover, the cassava-based maltodextrins were sphere-like (Figure 3), so they could pack properly and readily. These different starch-based maltodextrin could expect to have different flowability.

The water adsorption capacity is also an important characteristics of maltodextrin. As the dry powder, maltodextrin can uptake water to some extent when placed in a very humid atmosphere. Maltodextrin of high DE can uptake more water (Figure 4). No significant difference in water sorption of cassava- and corn-based maltodextrin was observed. When maltodextrin was dissolved, the solution of all samples, regardless of enzyme used and starch based, had similar viscosity (Table 2)and shear stress (Figure 5).



(a)



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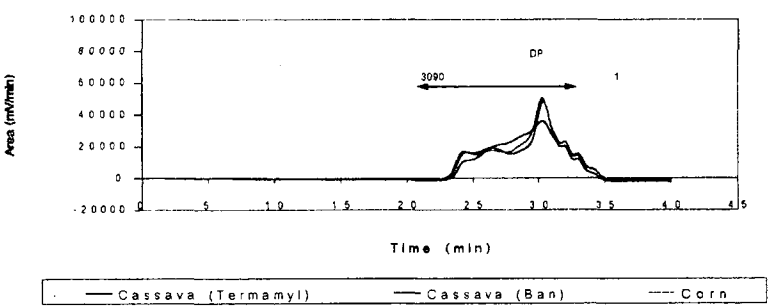

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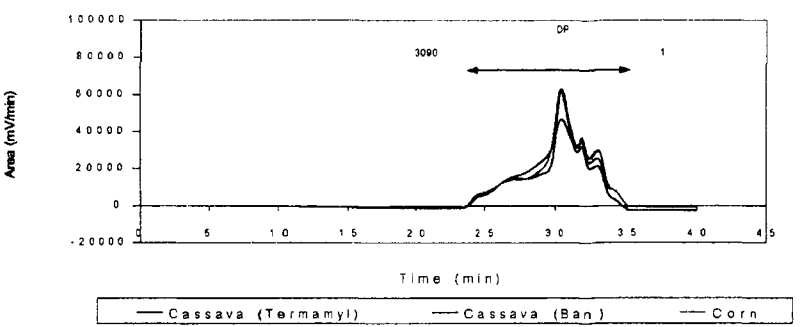

(d)

Fig. 1. Molecular distribution of cassava-based maltodextrin prepared by two enzymes (Termamyl 120L and Ban 480L) and commercial corn-bas ed maltodextrin with different dextrose equivalent (a) DE=5 (b) DE=10 (c) DE=15 and (d) DE=20.

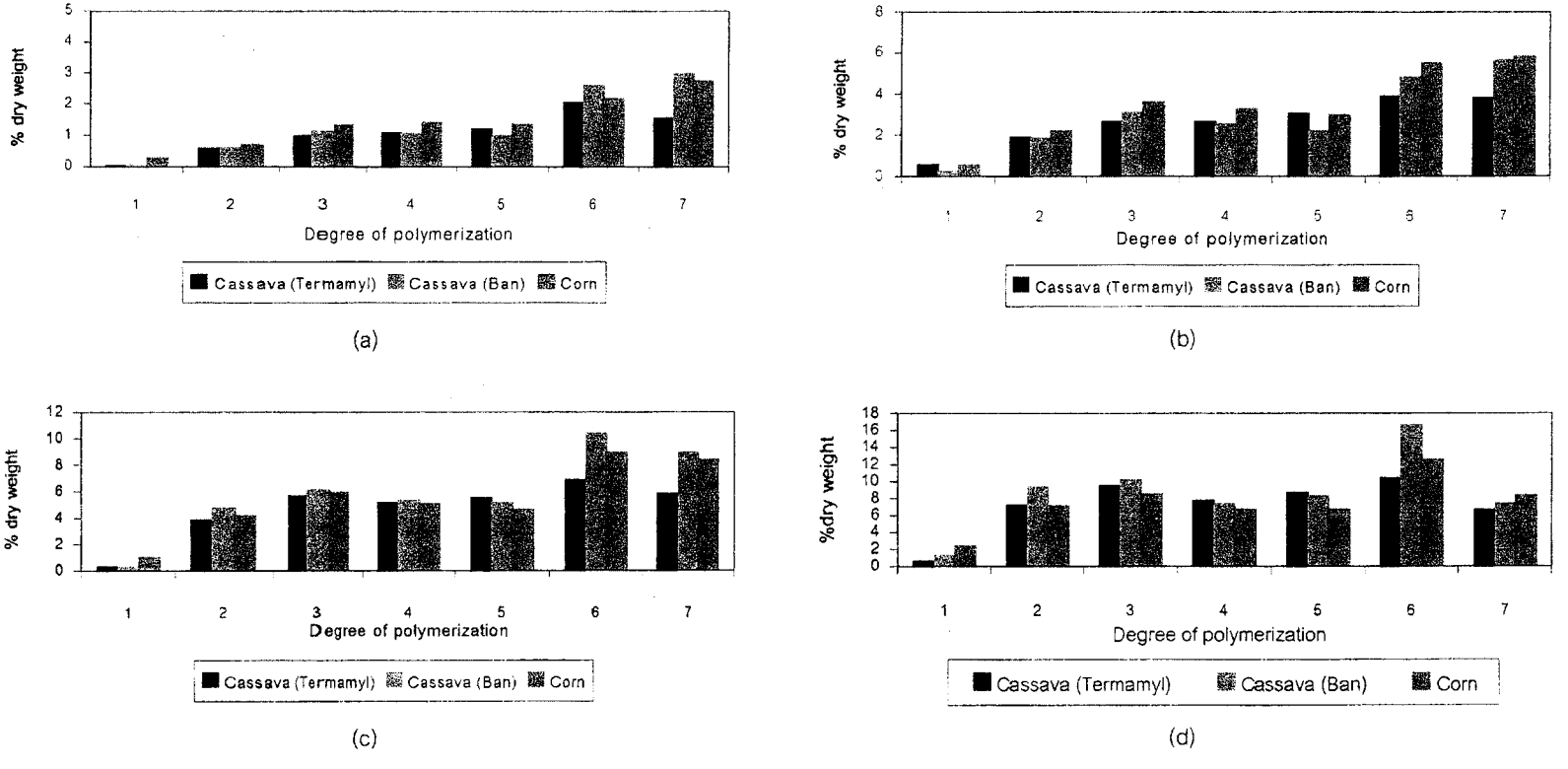

Fig. 2. Oligosaccharide component of cassava-based maltodextrin prepared by two enzymes (Termamyl 120L and Ban 480L) and commercial corn-based maltodextrin with different dextrose equivalent (a) DE=5 (b) DE=10 (c) DE=15 and (d) DE=20.

Table 2

Properties of cassava-based maltodextrin prepared by two enzymes (Termamyl 120L and Ban 480L) and commercial corn-based maltodextrin with different dextrose equivalent (DE = 5, 10, 15 and 20).

Property	Cassava (Termamyl 120L)				Cassava (Ban 480L)				Corn			
	5	10	15	20	5	10	15	20	5	10	15	20
Measured DE	5.37± 0.25	8.43± 0.24	15.08± 0.47	20.62±0. 40	5.70± 0.33	9.83± 0.08	15.63±0. 11	22.58±0. 10	6.65± 0.36	11.76± 0.34	16.26± 0.10	22.46± 0.27
Average DP	21.5	9.5	7.0	5.0	20.7	12.3	7.0	4.7	17.9	10.1	7.4	5.0
Descriptive ratio[1]	1.11	1.74	1.81	2.15	1.14	1.51	2.06	2.36	1.09	1.54	1.83	1.96
Moisture content (%)	7.89± 0.44	6.70± 0.05	5.25± 0.06	6.21±0.0 6	4.87± 0.05	4.88± 0.04	3.83± 0.11	3.58± 0.04	8.71± 0.11	6.88± 0.26	8.57± 0.13	5.38± 0.03
pH	7.45± 0.01	6.97± 0.13	7.12± 0.08	7.04±0.0 6	8.37± 0.06	7.58± 0.03	7.59± 0.07	7.59± 0.01	4.25± 0.00	4.30± 0.01	4.42± 0.01	4.54± 0.01
Loose density (g/l)	0.26± 0.01	0.36± 0.01	0.33± 0.01	0.37±0.0 1	0.27± 0.01	0.29± 0.01	0.35± 0.01	0.43± 0.01	0.35± 0.01	0.45± 0.00	0.45± 0.01	0.46± 0.00
Packed density (g/l)	0.52± 0.02	0.64± 0.01	0.62± 0.01	0.64±0.0 1	0.55± 0.02	0.54± 0.02	0.67± 0.02	0.76± 0.03	0.53± 0.00	0.63± 0.00	0.63± 0.00	0.66± 0.01
Viscosity at 20% solid (cP)	8.03± 0.23	5.09± 0.04	3.10± 0.05	2.72±0.0 4	8.48± 0.31	5.12± 0.08	2.94± 0.53	2.60± 0.13	7.33± 0.23	4.20± 0.13	3.26± 0.00	2.62± 0.00

[1]Descriptive ratio is the ratio of the sum of the percentages of saccharides (dry basis), having a DP1 – 6 devided by DE values.

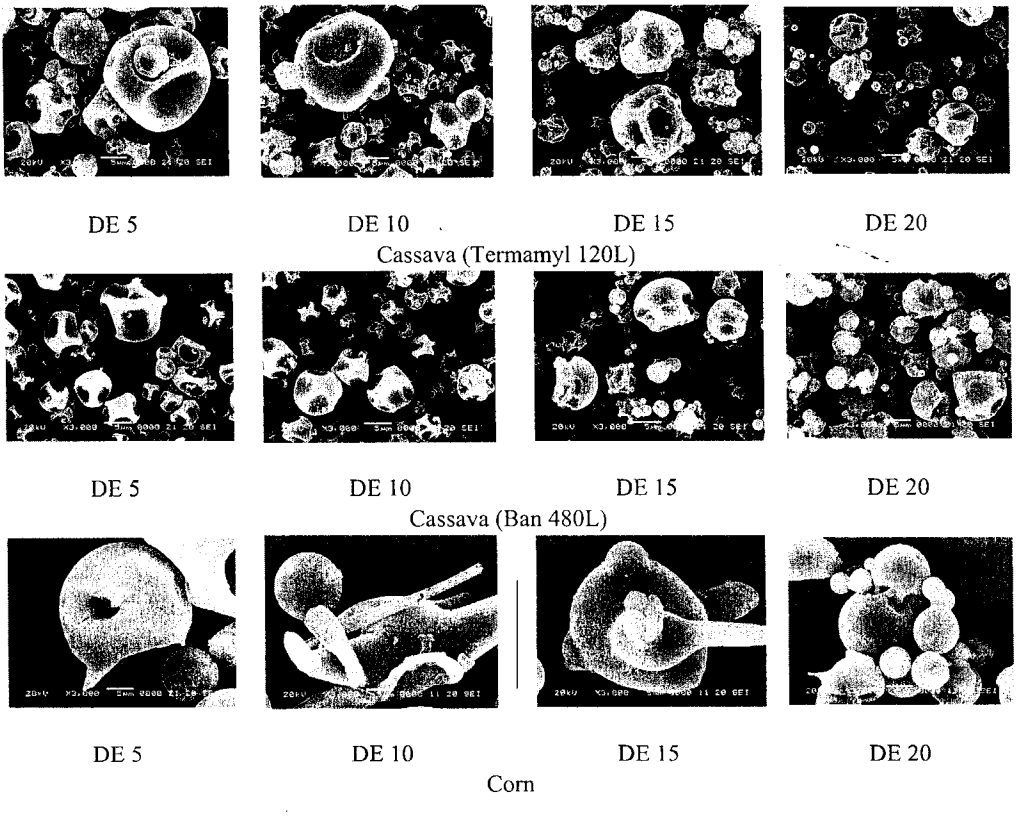

Fig. 3. Scanning electron micropgraph at 3,000x of cassava-based maltodextrin prepared by two enzymes (Termamyl 120L and Ban 480L) and commercial corn-based maltodextrin with different dextrose equivalent (DE = 5, 10, 15 and 20).

Conclusion

This work suggests that although cassava- and corn-based maltodextrin had slightly different molecular profiles, most properties were similar. However, other physical and biological properties such as sweetness, osmolality, gelation and adsorption by humans of these products should be further investigated and compared to support the use of different starch-based maltodextrins.

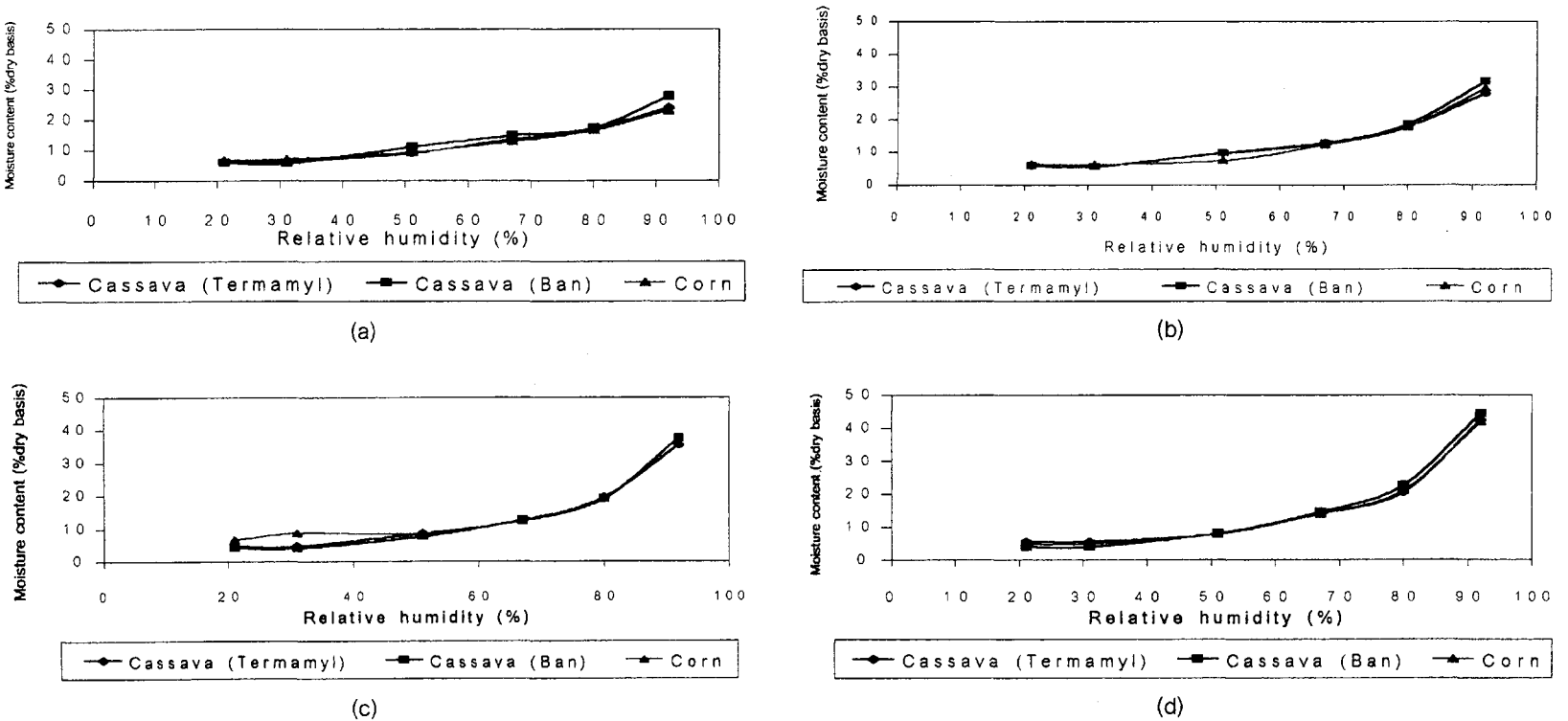

Fig. 4. Water sorption isotherm of cassava-based maltodextrin prepared by two enzymes (Termamyl 120L and Ban 480L) and commercial corn-based maltodextrin with different dextrose equivalent (a) DE=5 (b) DE=10 (c) DE=15 and (d) DE=20.

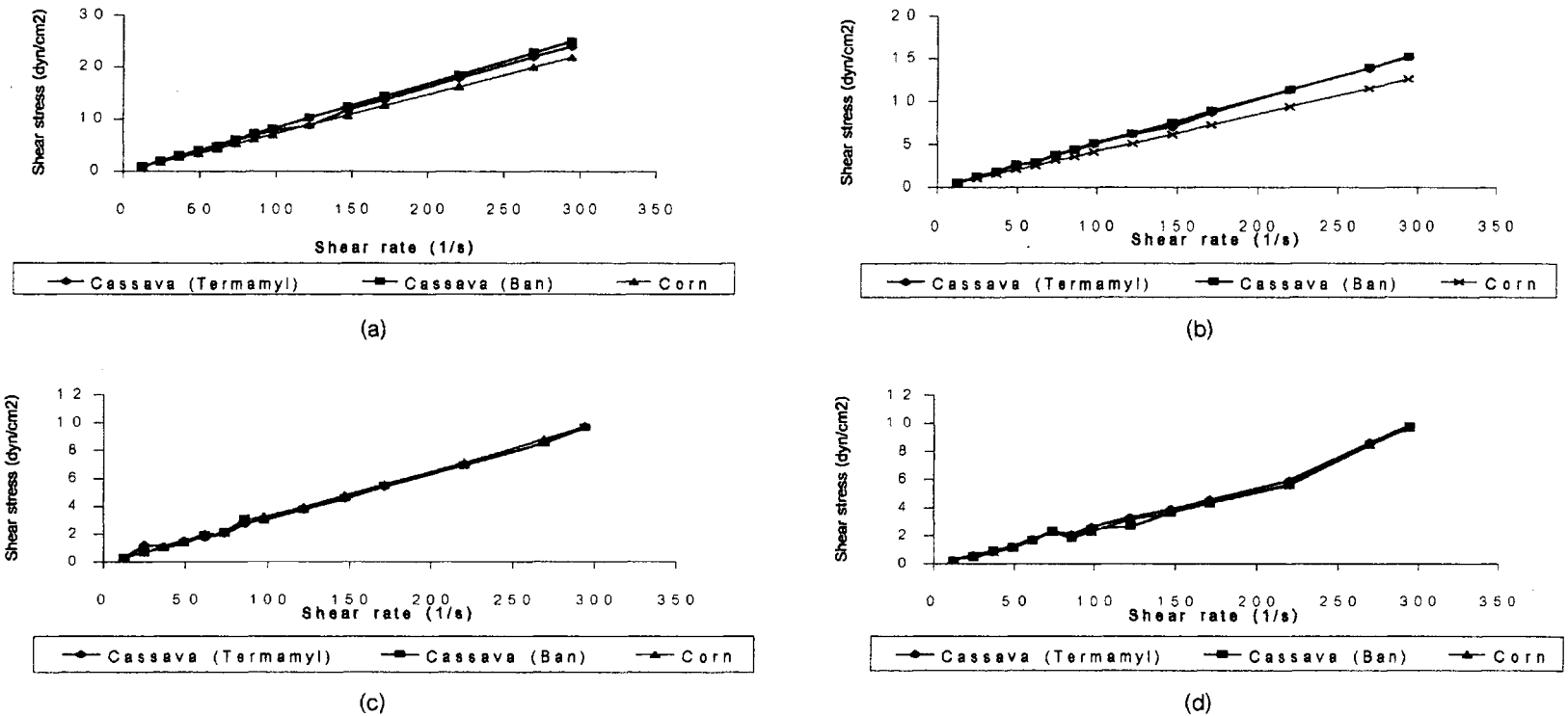

Fig. 5. Shear rate and shear stress of cassava-based maltodextrin prepared by two enzymes (Termamyl 120L and Ban 480L) and commercial corn-based maltodextrin with different dextrose equivalent (a) DE=5 (b) DE=10 (c) DE=15 and (d) DE=20, measured at 20% solutin at 25°C.

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PORÓWNANIE SKŁADU I WŁAŚCIWOŚCI FUNKCJONALNYCH MALTODEKSTRYN TAPIOKOWYCH I KUKURYDZIANYCH

Streszczenie

Maltodekstryny tapiokowe o różnym równoważniku dekstrozowym (DE = 5, 10, 15 i 20) otrzymano przy użyciu dwu różnych enzymów duńskiej firmy Novo Nordisk. Rozkład frakcji i składniki oligosacharydowe (DP = 1–7) zostały scharakteryzowane i porównane z handlowymi maltodekstrynami kukurydzianymi o tym samym DE. Maltodekstryny tapiokowe otrzymane przy użyciu enzymu Termamyl 120L w całym zakresie DE zawierały sacharydy o wyższym ciężarze cząsteczkowym niż odpowiednie maltodekstryny kukurydziane. Profile składowych sacharydowych maltodekstryn topiokowych otrzymanych enzymem Ban 480L były porównywalne z profilami maltodekstryn kukurydzianych. Kształt i rozmiar maltodekstryn z obu skrobi różniły sie od siebie zapewne z powodu odmiennej obróbki. Maltodekstryny kukurydziane miały większe cząsteczki. Jednak większość właściwości maltodekstryn kukurydzianych i topiokowych włącznie z zawartością wilgoci, sorpcją wody i lepkością była podobna.

THONGCHAI SUWONSICHON[1]

TEXTURAL ASSESSMENT OF THAI RICE NOODLES

Summary

Mechanical tensile and fracture test were used in this study for assessing textural properties of five Thai rice noodles having the different levels of substituted tapioca flour (0%, 10%, 20%, 30% and 40% of composite rice flour and tapioca flour). Before testing tensile measurement, all specimens were shaped like dumbbell. The results showed that the maximum force, the extension and the total work significantly decreased from 0.55 to 0.43 N, 82.3 to 59.4 mm, and 0.030 to 0.017 J, respectively, when rice noodles contained tapioca flour 0% up to 40%. For fracture measurement, a single-edge-notched test, the results showed a similar pattern to tensile test. Adding the tapioca flour up to 40% decreased the maximum force, the extension and the total work from 0.8 to 0.6 N, 54.1 to 32.2 mm and 0.042 to 0.025 J, respectively. When correlated with sensory hardness, the maximum tensile force and maximum fracture force, respectively, gave a good correlation of 0.77 ($p < 0.05$) and 0.90 ($p < 0.05$). The relationship between maximum force and sensory hardness can be explained by the quadratic polynomial equation having an R^2 greater than 0.9.

Introduction

Rice noodle was originated in China over two thousand years ago. It is one of popular staple foods in China, Japan and some countries in South East Asia. It is called Mi *fen* in Chinese, *Harusame* in Japanese *and Bihon* in Filipino [1, 2]. In Thailand, it is called *Kwoy Tuel* and is relatively simple to prepare. It is always available in restaurant, supermarket, at roadside stalls, from street vendors or at home. It is served with the addition of meatballs, fish ball, pork-chop, seafood, fish or soy sauce as well as vegetable such as bean-sprout and cabbage. Nowadays, there are many types of noodles offering for consumers not only a choice of shape, sizes but also a variety of mixed ingredients between rice flour with others. The process of making rice noodle varies form the conventional method, being limited to sun drying for small industry, to advanced technology, automatic machines producing fresh rice noodle.

[1]*Dept. of Product Development, Faculty of Agro-Industry, Kasetsart University, 50 Phahonyothin Road, Jatujak, Bangkok, Thailand, 10900*

Quality of rice flour or starch is one of major factors of rice noodle production. The rice noodle products can be varied from hard texture and easy fragile to quite soft, elastic and sticky after cooking with hot water or soups. They depend on rice varieties and their amylose contents. Occasionally, the industries may lack of raw material or would like to reformulate rice noodle to have a better texture or would like to reduce their cost of production. For their reformulation and new product development, manufactures may add other flours like root crop flours such as cassava, potato or arrowroot to get highly attractive in sensory quality such as the specific soft texture, good aroma and taste. Since there is no standard or recommended method for Thai rice noodle industries to assess their rice noodle products, they mostly use their experience to judge their quality. According to this, they will not get much information from their traditional technique for product development and quality control, especially, the cause of textural changes.

The objective of this work was to evaluate the methods of textural measurement as a tool for Thai rice noodle industries to assess the textural quality of their rice noodles.

Materials and methods

Preparation of Rice noodles

Rice flour and various tapioca flour levels of substitution (0%, 10%, 20%, 30% and 40% of composite rice starch and tapioca flour) were mixed together before making five rice noodles (five treatments). The process of rice noodle production for this experiment was shown in Figure 1. All rice noodles of each experiment were cooled down at room temperature (about 28–30°C) for 1 h before testing.

Tensile Test

All of five Thai rice noodles were shaped like dumbbell. Using Texture Analyzer TA 500 of LLOYD Instrument Ltd., England, performed tensile test of each noodle. Before testing, both ends of Rice noodle were sealed with sticky cloth tape and gripped with TG 33, Lightweight grip produced from LLOYD Instrument. All tests were performed at a constant crosshead speed 3 mm/s until noodle was broken down. To obtain the maximum tensile force (Newton: N), the extension (mm) before breaking, the total work (J) and the stiffness (gradient of the initial linear region of the graph), the raw data were imported to and processed by using Nexygen ® software version 3.0. Each rice noodle was performed in eighteen replicates.

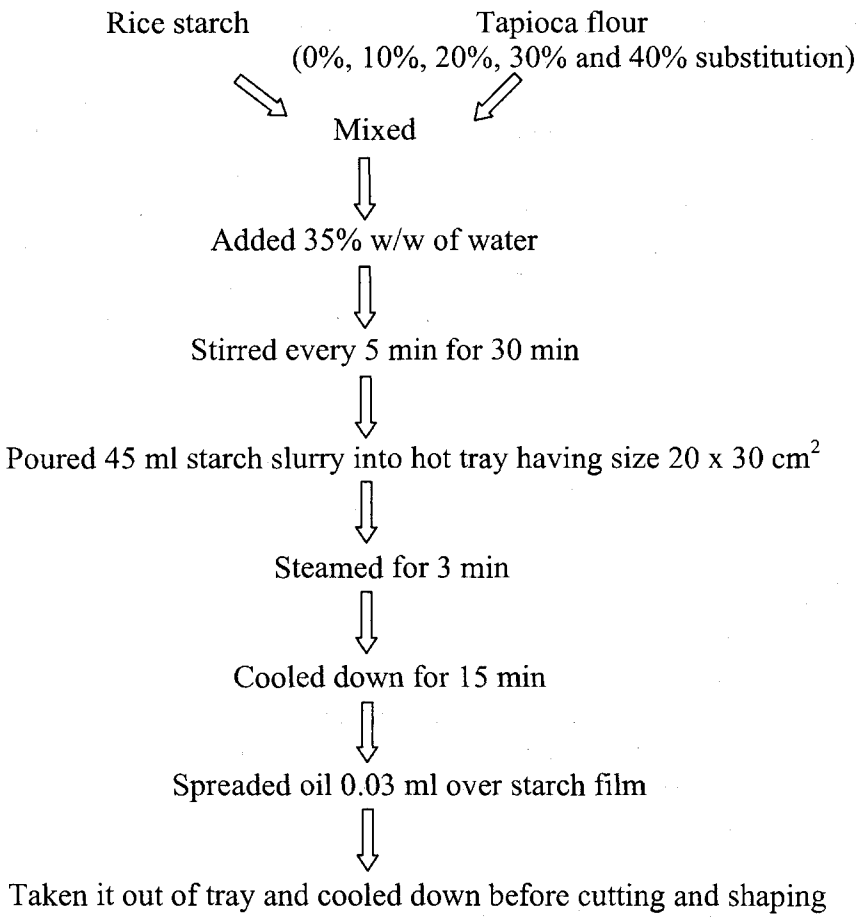

Fig. 1. Flow diagram of rice noodle process

Fracture test

Each rice noodle was cut into rectangles. At the center of noodle, one side was single edge notched about 1 mm before gripping. The condition of fracture testing and data processing were performed as well as tensile testing.

Sensory analysis

Panel of eleven students evaluated hardness and springiness. They are all senior students from Department of product development, Kasetsart University, Thailand. They were selected from 30 students and intensively trained every three days for a month about the definitions of those textural attributes of rice noodles as follows:

- Hardness is the force to pull a rice noodle apart by hands,
- Springiness is the degree to which a rice noodle returns to its original shape once it has been pull away between right and left hands.

After training, they evaluated five rice noodles from this experiment by using Magnitude of estimation, ratio-scaling [3]. Commercial rice noodles, bought from the market, were always served in the first sample and were rated as 100. Consequently, other five noodles were randomly served and were rated in relation to the first commercial rice noodle. For example, if the subsequent sample was half as hard as the first sample, the panel should assign it the value of 50. All data form magnitude estimation were transformed to logarithms before carrying out the analysis of variance (ANOVA).

Statistical analysis

Five rice noodle data from tensile test, fracture test and sensory test were subjected to SPSS version ® 9.05 for analysis of variance, the Pearson correlation and non linear regression. A significance level of $p < 0.05$ were used throughout this study.

Results and discussion

The Characteristic of Tensile force-extension and Fracture force-extension relationships of different five rice noodles

Typical tensile force and extension relationships as well as fracture test of five rice noodles having various percentage tapioca flour levels of substitution were shown in Figure 2 and 3, respectively. Additionally, the results of mechanically tensile tests and fracture tests are listed in Table 1 and 2, respectively.

Table 1

Tensile attribute means[1] and their standard deviations of five Thai rice noodles having different percentage tapioca flour levels of substitution.

Tensile attributes	% tapioca flour				
	0	10	20	30	40
Maximum force(N)	0.55 ± 0.078 a	0.57 ± 0.049 a	0.59 ± 0.119 a	0.50 ± 0.062 b	0.43 ± 0.094 c
Extension (mm)	82.8 ± 23.8 a	84.5± 19.32 a	91.0 ± 15.4 a	85.1 ± 13.9 a	59.4 ± 21.4 b
Total work (J)	0.030 ± 0.012a	0.033 ± 0.010a	0.0037±0.008a	0.031 ± 0.007a	0.017 ± 0.008b
Stiffness (-)	0.019 ± 0.01 a	0.017± 0.01 a	0.017± 0.01 a	0.018 ± 0.01 a	0.015± 0.01 a

Note:[1] Means values are calculated from eighteen replicates samples.
a-c Means within the same row followed by the different letters are significantly different ($p < 0.05$).

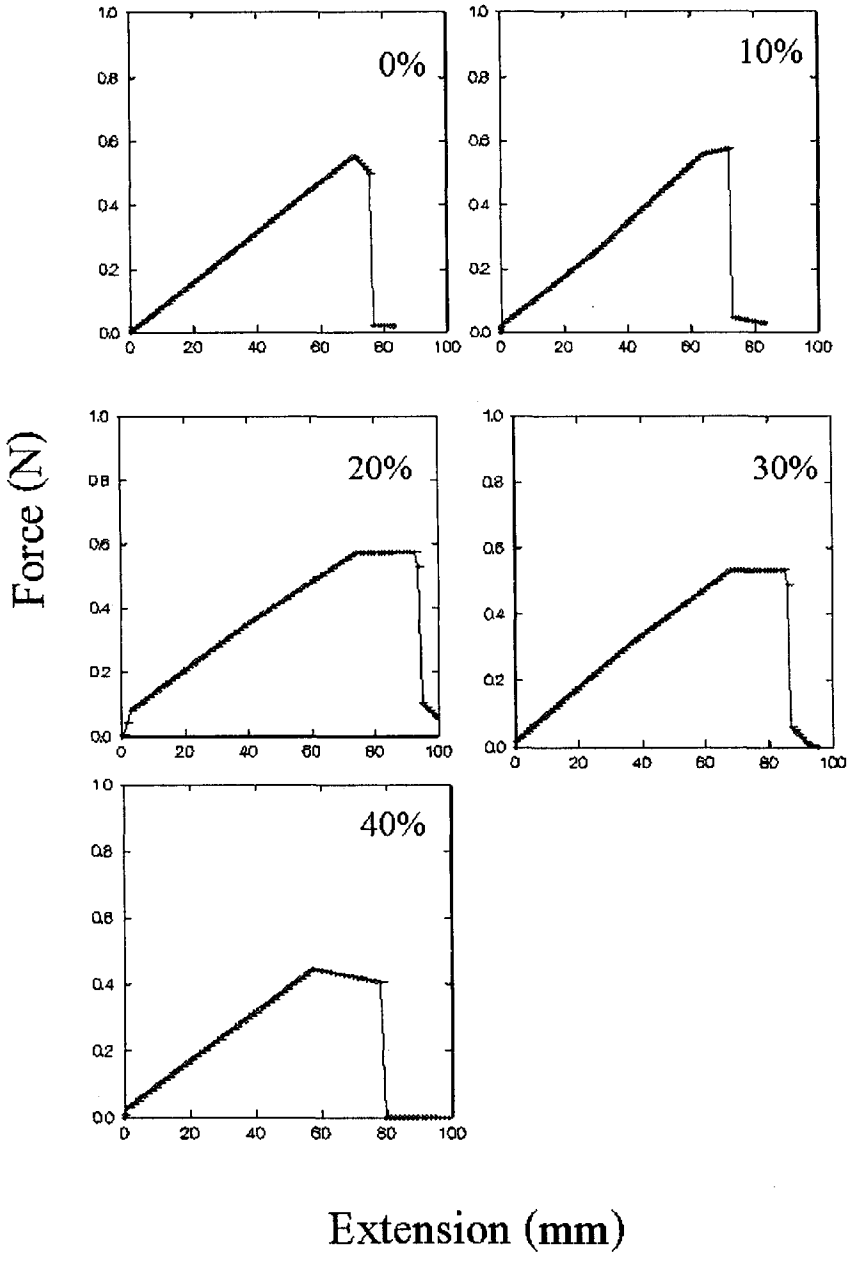

Fig. 2. Typical tensile force-extension of five rice noodles having different percentage tapioca flour levels of substitution.

From Figure 2 and 3, they show that rice noodle without addition of tapioca flour provides a hard texture because it has the highest maximum force. However, it is easy fragile because the force is suddenly droping into the origin after reaching the maxi-

mum force value. Conversely, rice noodle having 40% tapioca flour providing a soft and sticky texture shows the lowest maximum force and is quite ductile and does not suddenly ruptured after getting the maximum force. Moreover, table 1 and 2 also show that additional tapioca flour into rice noodle up to 20% will not provide any statistical significant different ($p > 0.05$) in the maximum force, the extension, the total work and the stiffness of tensile test and fracture test. From these results, it is quite clear that starch paste has an influence on texture of rice noodle product. Rice starch [4], and

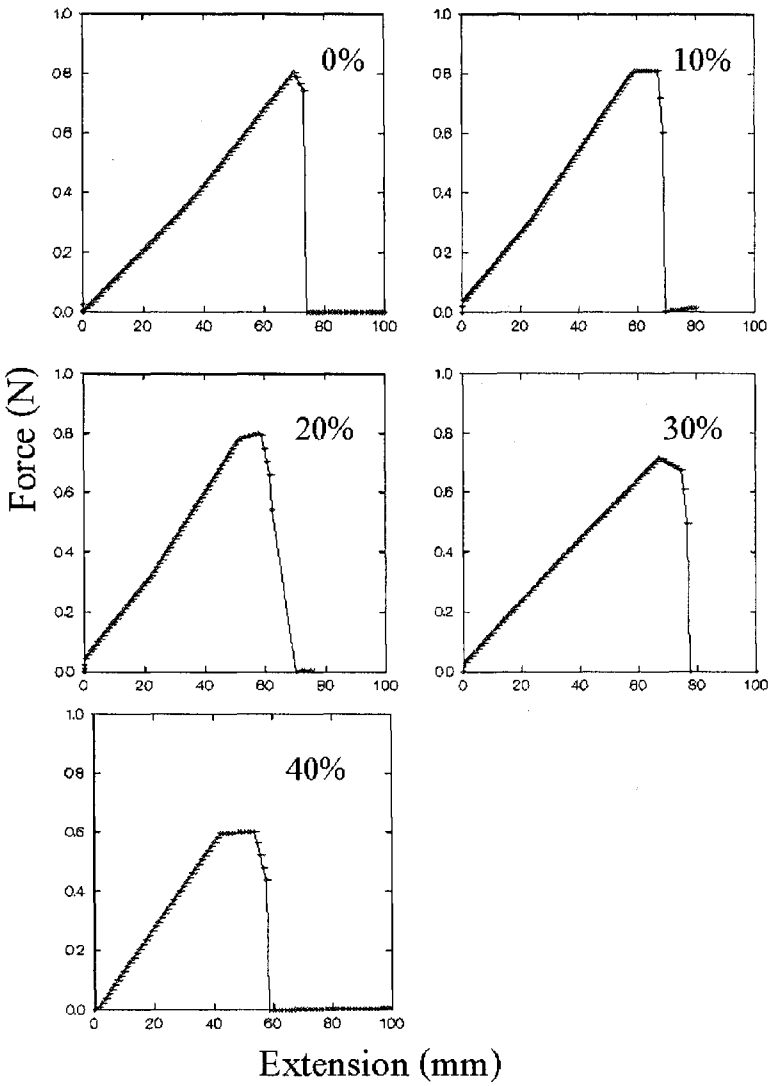

Fig. 3. Typical fracture force-extension of five rice noodles having different percentage tapioca flour levels of substitution.

cereal starch form viscous short bodied pastes and set to opaque gel on cooling. While tapioca starch, root and tuber starch, form highly viscous long bodied pastes, being clear and forming only a weak gel on cooling. In this study, tensile test shows a very good assessment to follow up the effect of starch paste mixture on the rice noodle quality. Rice noodle containing higher-level tapioca flour changes its texture from hard to soft gel and it is also quite sticky, not suddenly rupture.

Table 2

Fracture attribute means [1] and their standard deviations of five Thai rice noodles having different percentage tapioca flour levels of substitution.

Fracture attributes	% tapioca flour				
	0	10	20	30	40
Maximum force(N)	0.80 ± 0.18 a	0.78 ± 0.10 a	0.80 ± 0.06 a	0.62 ± 0.06 b	0.60 ± 0.09 c
Extension (mm)	54.14 ± 18.3 a	42.7± 10.3 a	42.2 ± 16.7 a	38.8 ± 12.2 a	32.2 ± 14.3 b
Total work (J)	0.042 ± 0.019 a	0.035 ± 0.019 a	0.037 ± 0.018 a	0.031 ± 0.008 a	0.025 ± 0.011 b
Stiffness (-)	0.021 ± 0.01 a	0.011± 0.006 a	0.011± 0.005 a	0.017 ± 0.01 a	0.017± 0.01 a

Note:[1] Means values are calculated from eighteen replicates samples.
a-c Means within the same row followed by the different letters are significantly different ($p<0.05$).

Sensory qualities

The panel responses to five rice noodles are presented in Table 3.

Table 3

Magnitude estimation as logarithmic means[1] and their standard deviations of five Thai rice noodles having different percentage tapioca levels of substitution.

Sensory attributes	% tapioca flour				
	0	10	20	30	40
Hardness	4.98 ± 0.13 a	4.78 ± 0.10 ab	4.77 ± 0.2 ab	4.6 ± 0.06 b	4.5 ± 0.68 b
Springiness	4.93 ± 0.25 a	4.66± 0.4 a	4.62 ± 0.46 a	4.71 ± 0.39 a	4.62 ± 0.65 a

Note:[1] Means values are calculated from eighteen replicates samples
a-c Means within the same row followed by the different letters are significantly different ($p < 0.05$).

Table 3 shows that hardness of rice noodles is significantly lessen ($p < 0.05$) as the increment of substituted tapioca flour. None the less, panelists cannot detect any

significant difference in springiness. The results of sensory assessment follow a similar pattern in mechanical testing as described in previous section. There is a significant correlation between maximum force of tensile test or fracture test and sensory hardness as presented in Table 4. These relationships can be predicted by using a nonlinear regression model, the quadratic polynomial equation, having an R^2 greater than 0.9 as shown in Figure 5 and 6.

Table 4

Pearson's correlation coefficients ($p<0.005$) between mechanical and sensory attributes.

Attributes	Maximum Tensile force	Maximum Fracture force	Hardness
Maximum Tensile force	1	0.918	0.765
Maximum Fracture force	0.918	1	0.896
Hardness	0.765	0.896	1

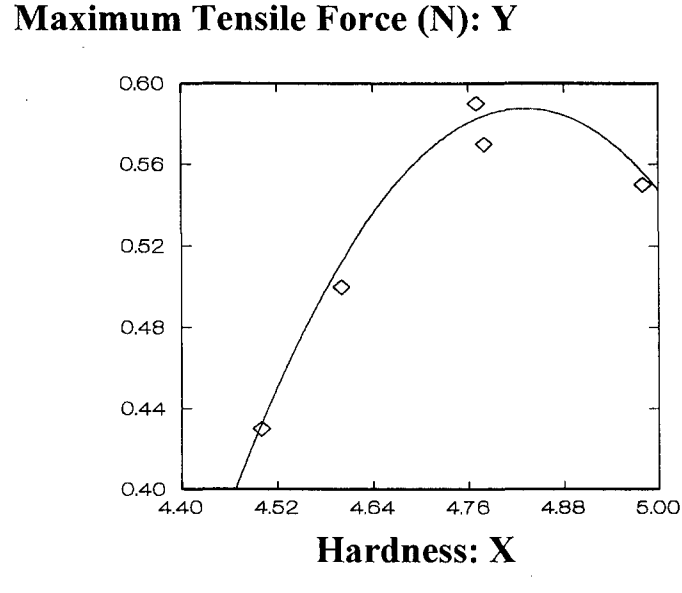

Fig. 5. Relationship between sensory hardness evaluated by Magnitude estimation and maximum tensile force. The solid line is the fit curve of nonlinear equation : $Y = -32.528+13.71*x-1.419*x^2$, $R^2 = 0.98$.

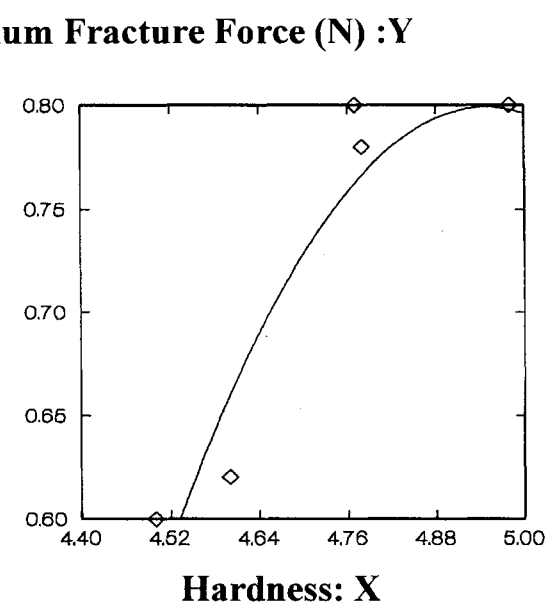

Fig. 6. Relationship between sensory hardness evaluated by Magnitude estimation and maximum fracture force. The solid line is the fit curve of nonlinear equation: $Y = -27.384+11.391*x-1.151*x^2$, $R^2 = 0.91$.

Conclusion

Tensile test and Fracture test are very applicable to assess the textural quality of Thai rice noodles. Form this study, those two methods not only can followed up the effect of substituted tapioca flour in rice noodles but also have a highly correlation with sensory evaluation using ratio scale, Magnitude estimation. The relationship between mechanical test, maximum force, and sensory test, hardness, in this study can be predicted by using non-linear regress providing an R^2 greater than 0.9.

Acknowledgment

The authors gratefully acknowledge Assoc. Prof. Vichai Haruthiathanasan, the director of Kasetsart Agricultural and Agro-Industrial Product Improvement Institue (KAPI), Kasetsart University, Thailand and Assoc. Prof. Klanarong Sriroth, Head of Cassava and Strach Technology Research Unit, KAPI for all of their suggestion and support of this project.

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OCENA TEKSTURY TAJLANDZKIEGO MAKARONU RYŻOWEGO

Streszczenie

Oceniono teksturalne właściwości pięciu tajlandzkich makaronów ryżowych, zawierających zróżnicowaną zawartość mąki tapiokowej (0, 10, 20, 30 i 40%). Podstawą oceny były próby wytrzymałości mechanicznej na rozciąganie i łamanie. Przed pomiarami wytrzymałości na rozciąganie próbkom makaronów nadawano kształt wiosełka. Wykazano, że maksymalna siła, rozciąganie i praca całkowita malały, odpowiednio z 0,55 do 0,43 N, z 82,3 do 59,4 mm i z 0,030 do 0,017 J, w miarę jak zawartość mąki tapiokowej w makaronie wzrastała do 40%. Podobnie, z zawartością mąki tapiokowej pogorszyła się kruchość makaronów. Zaobserwowano wysoką korelację pomiędzy sensoryczną twardością i maksymalną siłą zrywania (r = 0,77) oraz maksymalną siłą kruszącą (r = 0,90).

KLANARONG SRIROTH[1, 2] KUAKOON PIYACHOMWAN[3],
KUNRUEDEE SANGSEETHONG[3], CHRISTOPHER OATES[4]

MODIFICATION OF CASSAVA STARCH

Summary

Cassava (*Manihot esculenta* Crantz) is an important food crop in many tropical countries in Africa, South America and Asia. However, in Thailand, this crop has been well recognized as more than a subsistence crop. It is important commercially as the raw material for a large and complex industrial system that has a significant impact to the country's economics. The roots of this crop contain high a starch content and approximately half of the total roots produced (20 million tons) are used for the starch industry. Cassava starch has many remarkable characteristics including high paste viscosity, high paste clarity and high freeze-thaw stability, which are advantageous to many industries. In particular, the native starch with high purity can be readily modified by physical, chemical and enzyme process to many diversified products to improve the starch functionality and, consequently, encourage more industrial application. This paper aims to describe the unique modification of cassava starch produced at the industrial level in Thailand with respect to technological aspect and product quality.

Introduction

Cassava (*Manihot esculenta*) is an important food crop in tropical countries such as Brazil, Nigeria, Indonesia and Thailand. The roots of cassava are rich in starch and consumed as human food or animal feed. Only a small amount of roots is converted into other industrial products. Thailand is the only country where most of the roots are processed into chips, pellets and starch. Against the total world root production of 175 million tons (Table 1), Thailand produces about 18 million tons. Ten million tons are converted to starch, producing approximately 2 million tons starch/year, and the rest to chips and pellets. As the leader of cassava starch production (Figure 1), Thailand is also the only country where modified starches from cassava are produced in large

[1]*Department of Biotechnology, Faculty of Agro- Industry, Kasetsart University, Bangkok, Thailand;* [2]*Kasetsart Agricultural and Agro-Industrial Product Improvement Institute, Kasetsart University, Bangkok, Thailand;* [3]*National Center for Genetic Engineering and Biotechnology, Bangkok, Thailand;* [4]*Agro Food Resources (Thailand) Co., Ltd., Bangkok, Thailand*

scale. Around 50% of the starch (native and modified) are employed locally in the food and non-food industries, the remainder is exported. This commodity generates significant revenue for the country (Table 2) and the future is promising. Growth of the starch industry sector is, in part, a substantial driving force that has generated large-scale cassava planting for commercial purpose in Thailand. From the experience in Thailand, this paper describes the unique modification of cassava starch.

Table 1

World production of cassava roots in 2001.

Country	Volume (million tons)
Nigeria	33,854,000
Brazil	24,481,356
Thailand	18,283,000
Congo	15,959,000
Indonesia	15,800,000
Ghana	7,845,440
Tanzania	5,757,968
India	5,800,000
Mozambique	5,361,974
China	3,750,900
Other	38,723,751
Total	175,617,389

Source: [1].

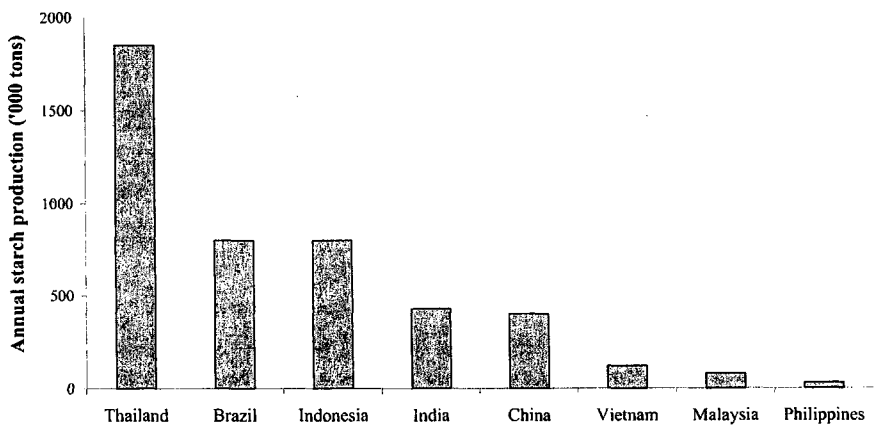

Fig. 1. Cassava starch production in various countries.
Source: [3, 6].

Table 2

Export volume and value of Thai cassava starch.

Cassava starch	Volume (tons)			Value (million Baht*)		
	1999	2000	2001	1999	2000	2001
Native starch	699,175	1,044,087	724,393	4,817.43	6,148.86	5,241.89
Modified starch	331,604	365,571	344,738	5,606.95	6,257.29	6,063.75
Sago pearl	15,508	15,470	14,455	164.64	150.14	150.49
Total	1,046,287	1,425,128	1,083,586	10,588.84	12,556.29	11,456.13

*1 USD = 45 Baht
Source: [6].

Modification

Native Starch

The term "native starch" is defined as the product extracted from cassava roots, which is called "starch" – not "flour" by the modern separation process [4]. The standard for starch content in native cassava starches is not less than 96% (dry basis). For modification purposes, native starch of the specification summarized in Table 3 is used.

Table 3

Standard Specification of native cassava starch for modification purpose

Property	Specification
Moisture content (% maximum)	13 %
Ash (% maximum)	0.2 %
Fiber (cm^3 per 50g wet starch, maximum)	0.2
pH	5.0 to 7.0
Whiteness (Kett scale, minimum)	90
Viscosity (Barbender Unit, minimum)	600
Sulfur dioxide content (ppm, maximum)	100
Residue (ppm, maximum)	300

Modification of cassava starch in Thailand

The starch modification sector is one of the most important industries in Thailand. This industry began as the production technology of cassava starch developed from small- to large-scale and starch quality improved. One of the main driving forces was the high market demand; both domestically and internationally, for the diversified

cassava-based products produced by the modification technology. The modified cassava starch and derivatives currently produced at the commercial scale can be categorized based on the technology approach as summarized in Figure 2.

Physical modification

This group of modified starches involves the treatment of cassava starch by physical means such as shear force, blending and thermal treatment. A combination of heat treatment and shear force has been used to produce many extruded products and snacks. The well-known products for cassava starch are alpha starch and heat-moisture treated starch obtained by a thermal process.

Alpha starch

Alpha starch or pregelatinized starch began to be a major industry in the late 1980's during the eel-farming boom when farms required a cold water soluble binder. Alpha starch from cassava gives specific properties such as high transparency, absence of foreign odors, good color carrier properties and high viscosity. The total production capacity for all alpha starch in Thailand is about 50,000 tons/year. The manufacturing process involves drying of 30–40% (dry solid) cassava starch slurry on a roller drum drier heated to 160–170°C by direct steam (Figure 3). Presently alpha starch is produced as food grade, and is used in many industries (Table 4).

Table 4

Specification of food-grade alpha starch produced from Thai cassava starch

Property	Specification
Moisture content (% maximum)	13
pH	4.5-7.0
Viscosity (Barbender Unit*, minimum)	800**
Ash (% maximum)	0.2
Pulp (cm^3, maximum)	0.2
Cyanide (ppm)	nil
Residues (ppm, maximum)	300
Whiteness (Kett scale, minimum)	90
Sulfur dioxide content (ppm, maximum)	30

*Using 6% starch (dry basis)

**Upon the customer's request and application

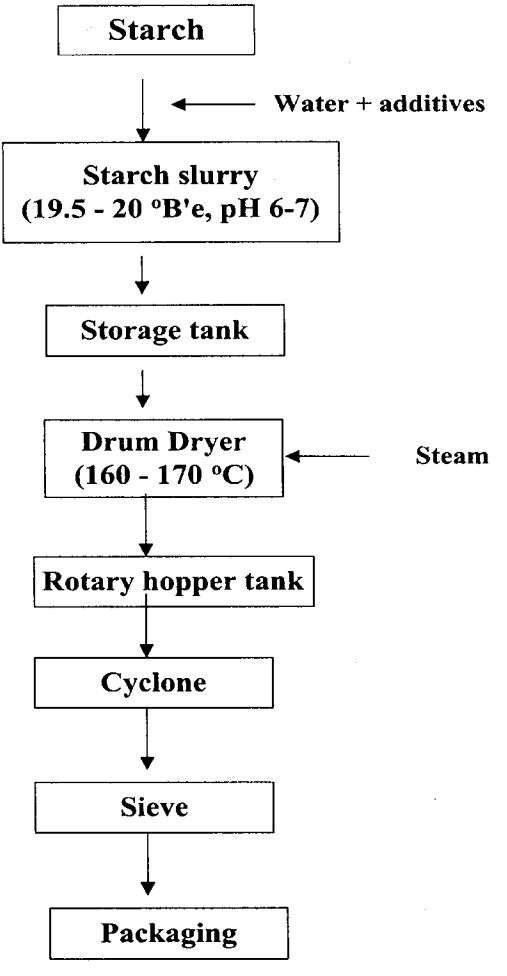

Fig. 3. Alpha starch process.

Heat-moisture treated starch

Heat-moisture treated starch is the oldest physically modified cassava starch. It had been manufactured in early time since the settling pond was used for separation of starch cake. The collected starch cake from the pond containing about 50% moisture content was used as the starting material for making heat-moisture treated starch. After being dried overnight on a hot floor (50 to 80°C), the dried starch was ground, sieved and packed. The product was accepted as flour with special name called *Tao starch*, not cassava starch. This starch is preferentially used as the product improver for many Thai desserts and food recipes replacing the traditional starch extracted from *Tacca*

pinnatifida (Tao Yai Mom) tubers, which is rare and more expensive than cassava starch. At present, all starch factories operate the modern separation technique instead of settling. The current manufacturing process, then, starts with soaking dried cassava starch overnight in ceramic or cement ponds. The moisture content of starch cake is about 50 % and wet starch is then dried on the hot floor. The produced starch has a remarkably different pasting profile from the native one (Figure 4).

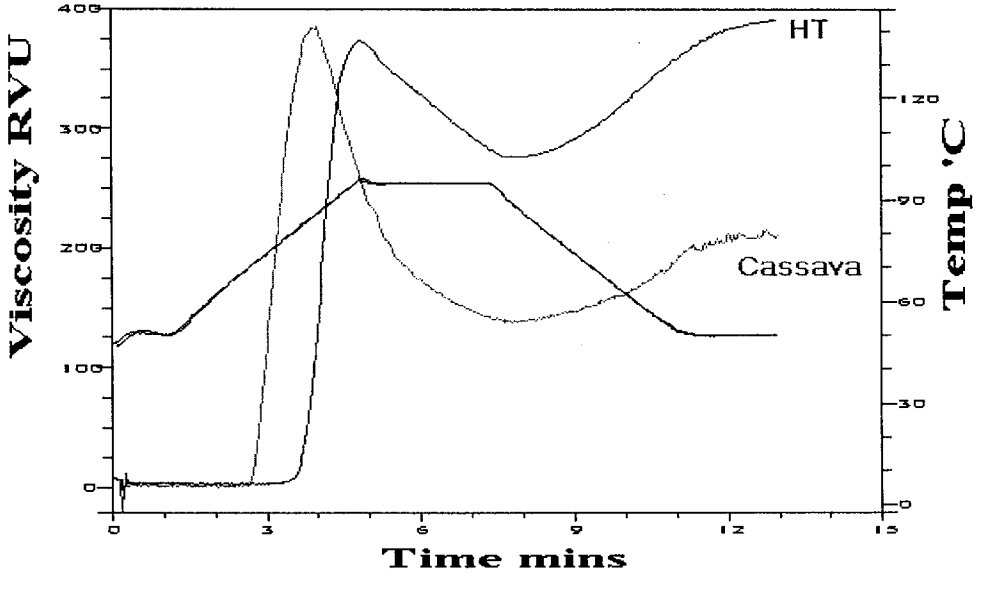

Fig. 4. Paste viscosity profiles as determined by a Rapid Visco Analyzer (using 3g starch of 14% moisture content in 25g of distilled water) of heat-moisture treated (HT) and native cassava starches.

Sago pearl or tapioca pearl

Sago pearl is one of the unique products produced from cassava starch in Thailand. Originally, this pearl was made from starch obtained from the stem of sago (*Metroxylon* spp.) palms which occur naturally only in the Southern part of Thailand. Due to the scarcity of sago starch, the technology of making sago pearls from cassava starch was developed and the product, called tapioca pearls, is used in some food products. The process for producing sago pearl involves heat-moisture treatment and a mechanical process (Figure 5). Similar to heat-moisture treatment, the starch is wetted overnight in ceramic or cement ponds to reach 50% moisture content. Wet starch is shaped to sphere-like by shaking continuously and the products subjected to dry heat process at 250–300°C. The pearls are cooled before being subjected to another drying

process at a lower temperature (50–80°C) on the hot floor. The pearls are then graded and packed. When cooked, the pearl has a very unique characteristic as the pearl's surface is soft and transparent but inside is hard and opaque. Nowadays, the amount of starch used in the sago industry is about 60,000 tons per year, accounting for 6% of total domestic cassava starch consumption.

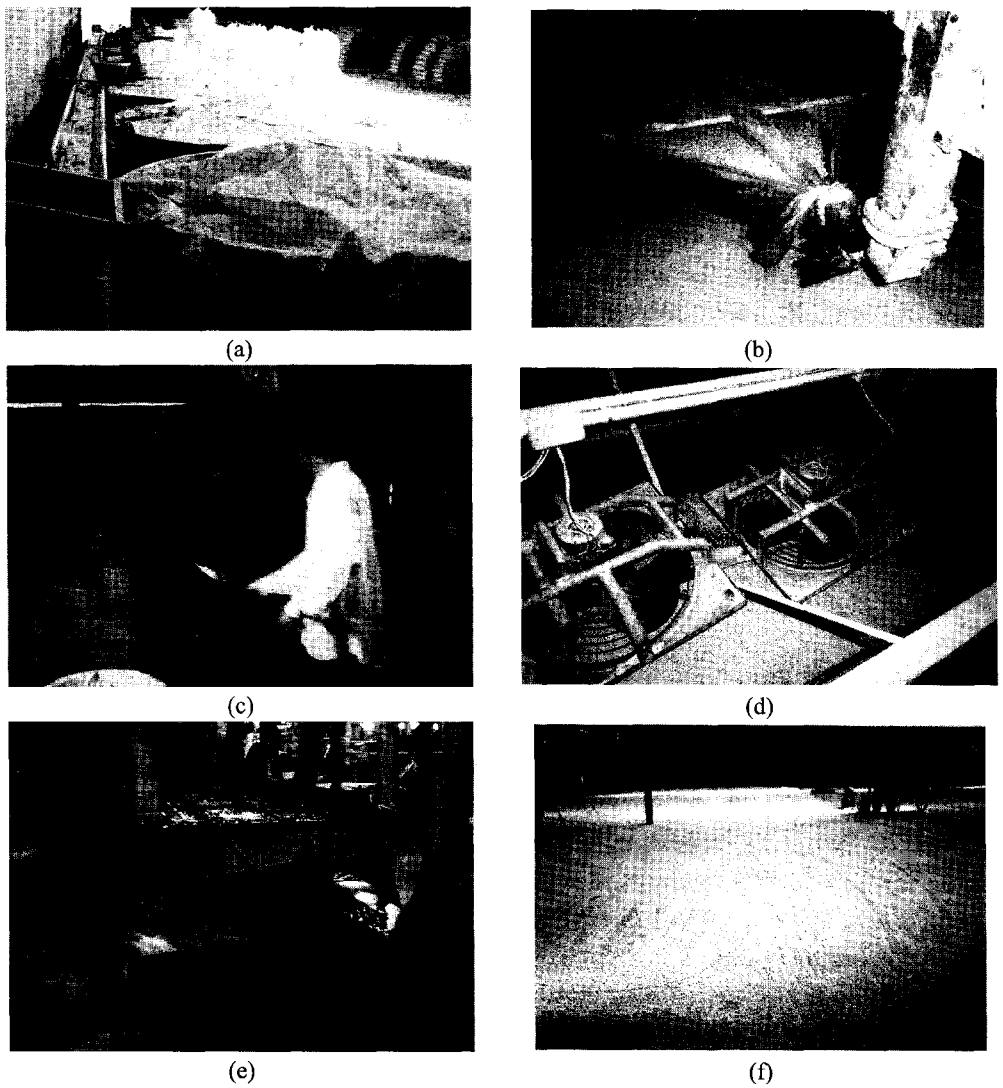

Fig. 5. Sago pearl process (a) wetting cassava starch (b) shaping the pearl (c) dry-heat process (d) cooling the pearl (e) sizing the pearls and (f) drying on the hot floor.

Chemical Modification

This group of products is prepared by chemical reaction. The most popular are oxidized starch and acid-modified starch for paper industry. The production of hydroxy – ethylated starch, cationic starch and amphoteric starch from cassava for paper industry is prepared only in a small scale. Starch acetate and phosphate are the most produced products for food industry.

Oxidized starch

Oxidized or chlorinated starch is one of the biggest-volume products produced from cassava starch. The preparation of oxidized starch is normally accomplished by the reaction of starch with sodium hypochlorite (NaOCl) under alkaline conditions. Oxidized starch is used at the size press as a surface sizing on wide range of uncoated free sheets to strengthen the paper surface. Traditionally, it is applied at 40 to 45 tons per ton of paper. Based on estimated paper consumption in Asian countries, an additional demand of about 240,000 tons of oxidized starch is expected each year [7].

Cassava starch, as a dominant source of starch in Asian countries, possesses a strong film, clear paste, good water holding properties and stable viscosity and should be the most suitable material for paper industry in this region.

Characteristics of oxidized cassava starch are influenced by oxidation conditions. Compared to the strong oxidized starches, the mild oxidized starch (prepared by 1,000 ppm active chlorine at pH 10.5) produces a stable high paste viscosity, which is called – Stabilized high viscosity starch [2].

Acid modified starch

Acid modified starch is also a well-known product in many Thai cassava starch factories. This product is normally prepared during the production of native starch. The preparation involves the addition of acid (usually hydrochloric acid) to the starch slurry (≈ 20°Be) at the temperature below the gelatinization temperature. After the reaction is finished and neutralized with soda ash, the starch slurry is concentrated, dewatered and dried. The acid modified starch should give the viscosity less than 30 cPs and pH about 5.0–6.0.

A main characteristic of acid modified cassava starch is the low tendency of the starch to retrograde compared to other starches. The handling of acid modified cassava starch under 70–85° C does not create any film-forming problem in storage tanks.

Starch acetate

Starch acetate is a representative of modified cassava starches for the food industry. The high volume of consumption is in food seasoning and sauce industry. The

normal preparation process of starch acetate is the reaction of vinyl acetate monomer (max 7.5% of starch dry weight) to cassava starch under an alkaline aqueous suspension. The standard allowance of acetyl groups in modified starch for food application is 2.5% as the maximum level.

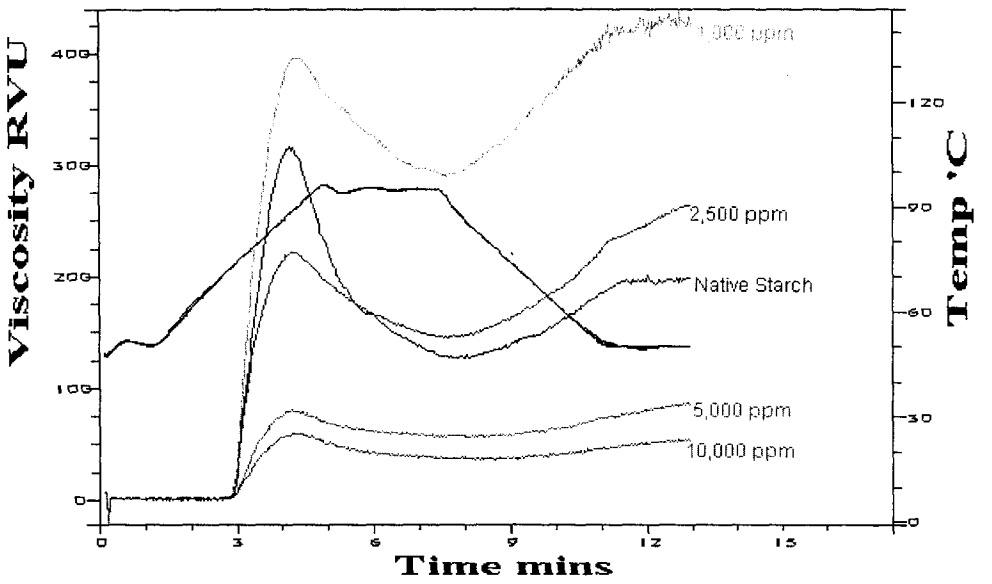

Fig. 6. Paste viscosity as determined by a Rapid Visco Analyzer (using 3g starch of 14% moisture content in 25g of distilled water) of oxidized cassava starches prepared by using different levels of sodium hypochlorite (0 to 10,000 ppm).

Other modified cassava starches for food products and their regulations

- Monostarch phosphate
  - Preparation: Orthophosphoric acid, Sodium orthophosphate, Sodium tripolyphosphate
  - Product regulation: Phosphate (calculated as phosphorus) not more than 0.4% (0.5% for potato and wheat starch)
- Distarch phosphate
  - Preparation: Sodium trimetaphosphate, Phosphorus oxychloride
  - Product regulation: Phosphate (calculated as phosphorus) not more than 0.04% (0.14% for potato and wheat starch)
- Starch succinate
  - Preparation: Succinic oxide, Octenylsuccinic anhydride
  - Product regulation: Octenyl succinic group not more than 0.3%

- Hydroxyl - propyl starch
  Preparation: Propylene oxide (max 10%)
  Product regulation: Propylene chlorohydrin not more than 1 mg/kg and hydroxy - propyl group not more than 7.0%
- Hydroxy - propyl - distrach phosphate
  Preparation: Sodium trimetaphoaphate, Phosphorus oxychloride and propylene oxide (not more than 10%)
  Product regulation: Propylene chlorohydrin not more than 1 mg/ kg and Hydroxy - propyl group more than 4.0%
- Acetylated distrach phosphate
  Preparation: Phosphorus oxychloride and vinyl acetate not more than 7.5% (in case of acetic anhydride not more than 10%)
  Product regulation: Acetyl group not more than 2.5%
  Phosphate (calculated as phosphorus) not more than 0.04% (0.14% for potato and wheat starch)

Starch hydrolysate and derivatives

This industry sector consumes the biggest volume of cassava starch produced in Thailand. The major product of this group is as a sweetener; important are glucose and fructose syrup. Glucose syrup is further used as the starting material for other industries; the biggest one is monosodium glutamate/ lysine (Table 5).

Table 5

Expected annual demand for cassava starch for the production of sweeteners and MSG/lysine in Thailand.

Products	Quantity of starch used (tons/year)	Product yield (kg/kg of starch)
High fructose (42% dry solid)	54,000	1.00
Glucose syrup	60,000	0.90-0.95
Dextrose monohydrate	20,000	1.75
Dextrose anhydrous	500	0.50
Sorbitol	30,000	1.20
MSG/Lysine	233,000	0.42

Source: [5, 6]

Sweeteners (glucose/fructose/sorbitol)

In Thailand, there are 14 factories manufacturing glucose syrup (two also produce sorbitol) and two large international sorbitol producers (Ueno Co., Ltd., Japan and Lucky Chemical Co., Ltd., Korea). There are two factories producing high fructose

syrup (about 54,000 tons per year). All factories prefer to apply the enzyme process for hydrolyzing starch and isomerizing glucose to produce glucose and fructose syrup.

Monosodium glutamate (MSG) and lysine

Highest consumption of native cassava starch in Thailand is by the MSG (four factories) and lysine (one factory) industries. Starch consumption for production of these products is in the proportion of 80:20 by the MSG and lysine industries, respectively. Production of commercial MSG in Thailand utilizes only two carbohydrate sources for inoculation including molasses and cassava starch. To produce one ton of MSG, factories need either about 2.4 tons of cassava starch or 7.0 tons of molasses.

Other starch hydrolysate and derivatives

- Citric acid

There are only two factories manufacturing citric acid in Thailand. One uses cassava pulp from starch factories as the raw material (about 5-6 tons/day) for its solid state (surface) fermentation. The other, recently established, uses cassava chips as the raw material for its submerged fermentation process. About 40 tons of chips are needed to produce 6 tons of citric acid per day.

- Maltodextrin

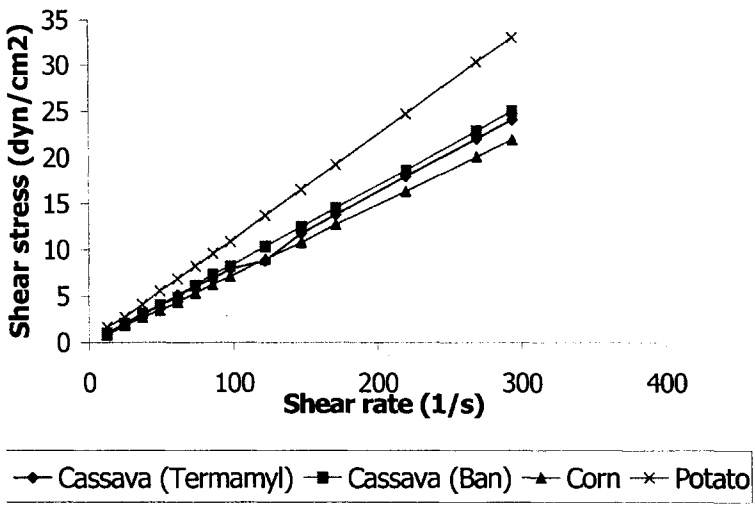

Fig. 7. Shear rate and shear stress of maltodextrin DE 5 (20% solution at 25°C) obtained from cassava starch prepared by two types of enzymes (I and II), corn starch and potato starch.

Maltodextrin produced from cassava starch in Thailand usually has the dextrose equivalent value greater than 10 (DE = 10, 14 and 17). The production of maltodextrin

with DE < 10 is still limited to the low yield due to the filtration problem caused by the retrogradation of starch hydrolysate prior to spray drying. The process involves the hydrolysis of cooked starch with microbial enzymes and, when the reaction is terminated, the hydrolysate is filtered and spray-dried. Significant properties such as solubility, viscosity and water adsorption capacity, of cassava-based maltodextrin are much more similar to corn-based than potato-based maltodextrin (Figure 7).

Conclusion

Cassava can be more than a subsistence crop that contributes to the sustainability of millions of farmers. With technology development, the high-starch containing roots of this crop can be converted to starch, an important material for other upstream industries of many value-added products by modification technology.

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MODYFIKACJA SKROBI TAPIOKOWEJ

Streszczenie

Tapioka (*Manihot esculenta*, Crantz) jest ważną rośliną uprawianą w wielu krajach Afryki, Ameryki Południowej i Azji. W Tajlandii roślina ta zajmuje poczesne miejsce. Jest ona ważna jako surowiec dla olbrzymiego kompleksu przemysłowego liczącego się w kształtowaniu ekonomii kraju. Korzenie tej rośliny odznaczają się wysoką zawartością skrobi toteż połowa zbiorów (20 milionów ton rocznie) jest przerabiana przez przemysł skrobiowy. Skrobia tapiokowa odznacza się szeregiem użytecznych właściwości takich, jak lepkość kleików, przejrzystość tych kleików i wysoka stabilność na zamrażanie i rozmrażanie, mające znaczenie w wielu zastosowaniach. Szczególnie skrobia natywna o wysokiej czystości nadaje się do modyfikacji fizycznej, chemicznej i enzymatycznej, prowadzących do polepszenia jej właściwości funkcjonalnych i zachęcających do poszerzenia zastosowań przemysłowych. W pracy opisano sposób modyfikacji skrobi tapiokowej wdrożony w Tajlandii na skalę przemysłową.

TETSUYA YAMADA, TAKUO ADACHI

MOLECULAR CHANGE OF STARCH GRANULES WITH DRY/OIL HEAT TREATMENT AND ITS APPLICATION IN FOOD

Summary

Molecular change of starch granules and its reaction in the simple system with heat in the cases of 11 starch species (normal-, amylo-, waxy-maize, normal-, waxy-wheat, normal-, waxy-rice, potato, sweet potato, cassava, sago) were studied . Dry heat treatment of starch samples was carried out at 200°C for 0.5 h, 1 h and 2 h. Oil treatment involved heating of starch in soy bean oil, methyl laurate and methyl oleate and kept at 190°C. After heating, SEM showed no changes of the granule images but they became instantly water soluble after 2 h heat treatment. GPC studies revealed that every 2 h starch sample was largely disrupted and became smaller than amylose but fairly larger than oligosaccharide. Hence, one assumed that starches are disrupted to cluster unit. Decomposition ratio of amylose was different among plant origin. In general, tuber starch was far more resistant to heat than cereal starch. Oligosaccharides formed in this processing are all anhydro type. Heat treatment in oil gave almost same effect on starch and the decomposition took place more rapid than in air. However, treatment time did not bring increase of oil incorporation into starch granule and there was little difference among oil species.

Introduction

Many baked foods including bread, cookies and biscuit are very common all over the world. However, there are only limited studies on molecular change of their starches in the food on such processing. Because, foods are complex systems of several materials and processing, hence, situation of starch in a food is dependent on other residing with it components. As the result, molecular changes of all components are closely related to each other. Especially, behaviour of lipids is related to starch modification. At first, it is essential to recognize that starch in baked food ordinary exists in the granular state. Starch granule has the fine structure, multi-dimensional structure with radial direction. Also granule has a canal leading from surface to hilum. Naturally, these structures are related to gelatinization and formation of complex with other

Dr. Tetsuya Yamada; Professor, Faculty of Agriculture, Meijo University, Tenpaku, Nagoya, 468-8502, Japan, Dr. Takuo Adachi; Associate professor, the same address

components in processing. For example, we reported that there are more saturated fatty acids incorporated into starch granule as inclusion complex than unsaturated fatty acids when starch is treated with fatty acid mixture under ultra high pressure (700 MPa) [1]. Probably this observation might be related to amylose situation in granule with relation to that of amylopectin. This fact might affect physical property of food. Hence, it is difficult to study properties and behaviour of starch in food.

We have begun studies of starch and lipid in food processing from simple system and results of this study are presented in this report.

Materials and methods

Materials

Ten species of starches were adopted, and they were, A) normal maize, B) amylomaize, C) waxy maize, D) wheat, E) normal rice, F) waxy rice, G) potato, H) sweet potato, I) cassava, J) sago.

Methods

Dry heat treatment: each starch sample (5g) was placed in Petri dish and covered with aluminum foil followed by maintaining samples at 200°C for 0.5 h, 1 h and 2 h.

Subsequently, samples in solution of 40% perchloric acid [2] were subjected to GPC analysis on Toyopearl HW-65 and HW-50 columns. The concentration of eluted saccharides in each fractionated tube was measured by means of the phenol-sulfuric acid method.

Iodine color reaction spectra patterns of the samples were also measured using the same solution.

Oil heat treatment was carried out as follows: Starch (300 mg) was suspended in a lipid (1 ml) (rape seed oil, methyl laurate and methyl oleate) in a small vials and ultrasonicated then maintained at 190°C in the oven. After such treatment, samples were centrifuged. Upper lipid layer was removed by decantation overnight, then, the incorporated amount of lipid was calculated by subtraction of starch weight from total weight. Lipid amount of inclusion complex in the sample was measured with gas-chromatography. After removing the incorporated lipid with diethyl ether, the inclusion complex of lipid in amylose helices was extracted with chloroform-ethanol mixture (1:1), followed by gas-chromatographic determination.

Glucoamylase sensitivity of the sample was measured as following: The sample (100 mg) was suspended in water (20 ml), then it was kept at 40°C with gently shaking and glucoamylase solution (1 ml) (10 unit) was added stepwise. Amount of liberated glucose in the suspension was measured by the Sumner method.

Microscopic observation was carried under birefringent light on samples suspended in water, and electron scanning microscopic (SEM) pictures were taken after platinum coating.

Matrix Assisted Desorption Ionization-Time of Flight Type-Mass Spectroscopy (MALDI-TOF-MS) was measured by means of Voyager DE PRO System (Biosystems) after dispersion of samplse in 2,5-dihydroxy benzoic acid and sodium iodide solution.

Results and Discussion

SEM images of all species suggested that starches did not change after dry/oil heat treatment except random, small cracks on surface of some granules.

Microscopic observation of dry heated starch samples are diversified. Relatively many species have dark cross until 1 h treatment except waxy type, but after heating for 2 h, all specimens but these of cassava lost this cross. In contrast samples treated for 2 h in oil retained this cross.

Fig. 1 shows the result of glucoamylase sensitivity of samples. All species but waxy type starches showed increasing with heating time sensitivity to such treatment whereas waxy type starches showed opposite behaviour.

It is known that glucoamylase acts from non-reducing end of glucan chain. Hence, if the end moiety of its chain changes to non-glucose, the chain can not be hydrolysed and remains in intact. Reduced ability to amylolysis, remarkable in waxy type starches resulted probably from its sensitivity to thermolysis. Because, amylopectin has a cluster structure forming crystalline regions, amorphous regions in amylopectin bound two or more clusters. When heat is applied to starch, the water (including bound water) in granule is removed. As the result, stress should occur in structure-forming material. The stress might especially occur in the part between crystalline region, namely in amorphous region, because, the crystalline region is well organized and rigid. Disruption of starch in food processing is due to hydrolysis with water, but this disruption by dry/oil heating is rather a pyrolysis, hence, it induces anhydro-type or double bond formation at the scission part.

Fig. 2 shows MALDI-TOF-MS of fragments formed in the sample on the treatment. This figure suggests that they are dehyroxy substances, but it is not certain which type of anhydro or double bond it presents. This result strongly supports former explanation of the reason of heat-induces decrease of waxy starches to amylolysis.

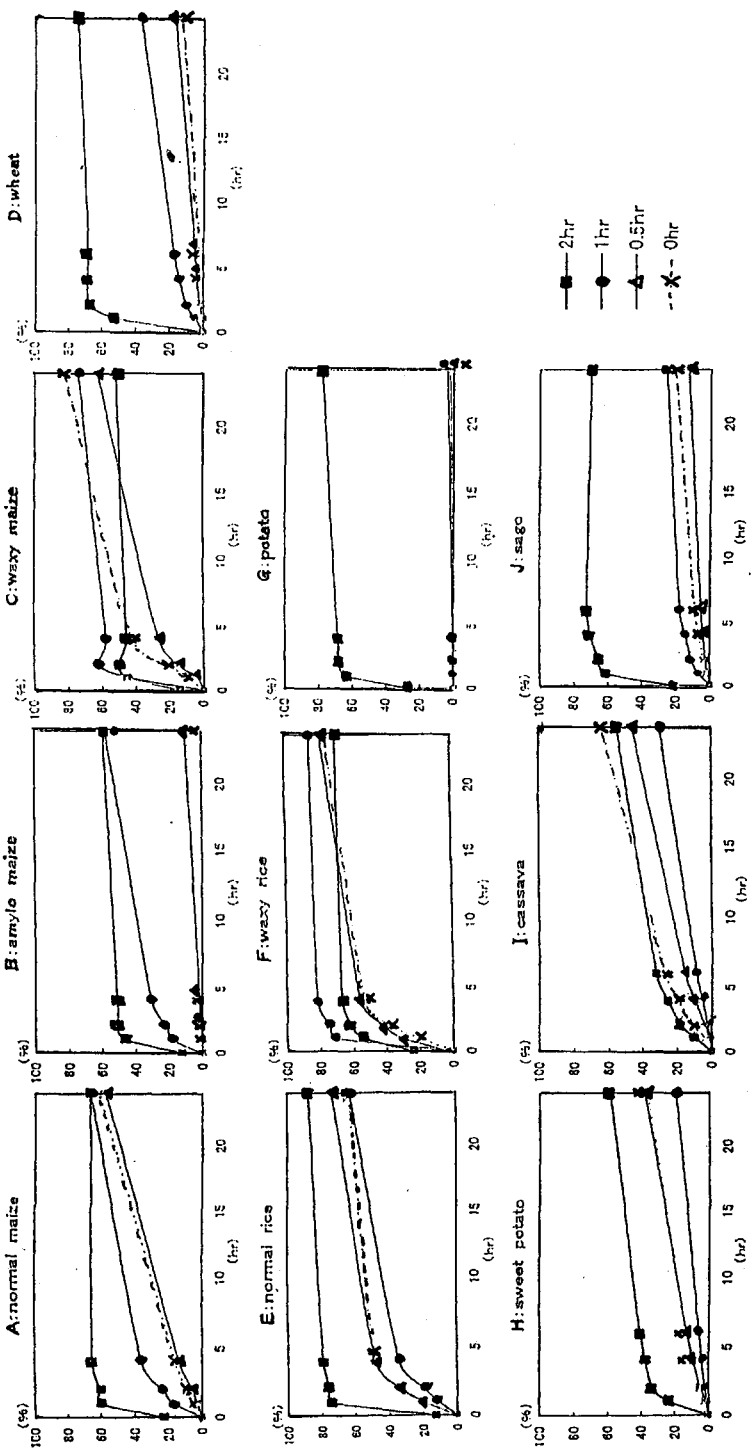
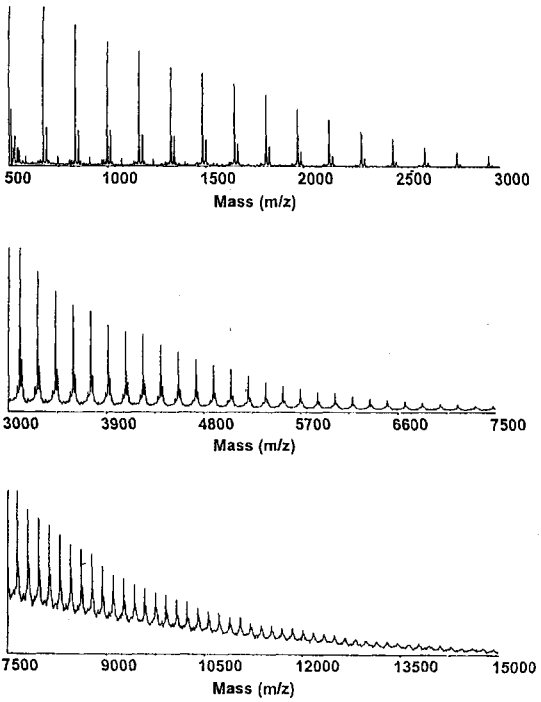


Fig. 1. Glucoamylase sensitivity of dry heated starch.



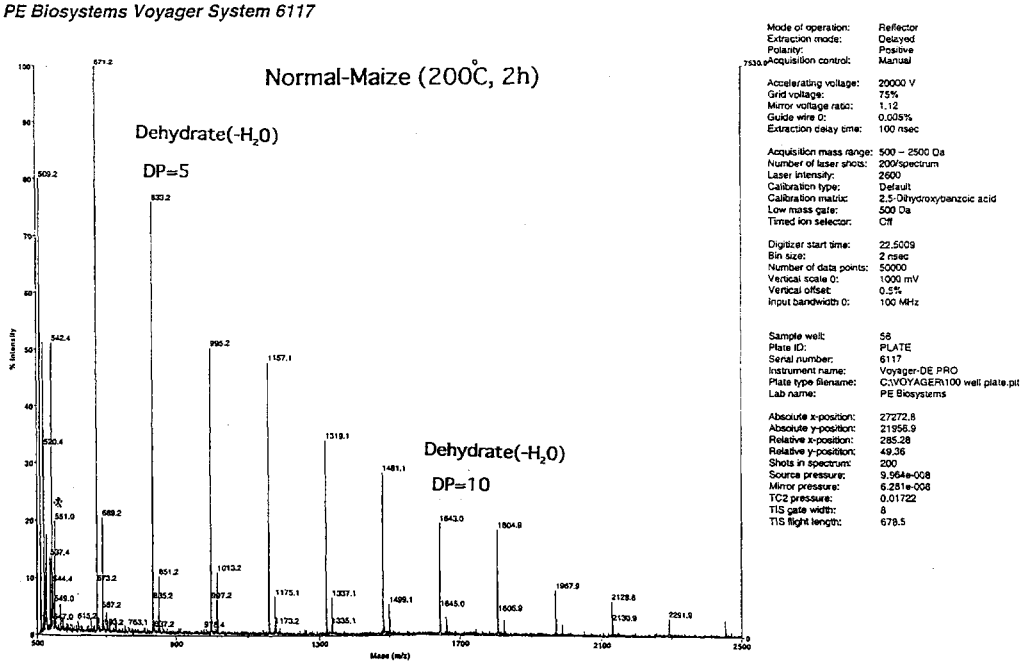


Fig. 2. MALDI-TOF-MS Spectra of dry heated starch (normal maize):
A: Wide range spectrum, B: Detail spectrum of small size fragment.

Table 1

Decomposition rate of amylose in starch granule with dry heat treatment

normal maize		ε680	Ratio(%)	λmax	ελmax	Ratio(%)
A	0hr	0.284	100	595.0~605.0	0.344	100
	1hr	0.078	27(73)	590	0.105	30(70)
	2hr	0.062	22(78)	475	0.024	7(93)

amylo maize		ε680	Ratio (%)	λmax	ελmax	Ratio(%)
B	0hr	0.495	100	605	0.588	100
	1hr	0.08	16(84)	575	0.127	21(79)
	2hr	0.047	9(91)	565	0.091	15(85)

waxy maize		ε680	Ratio(%)	λmax	ελmax	Ratio(%)
C	0hr	0.056	100	450	0.174	100
	1hr	0.015	28(72)	535.0~540.0	0.042	24(76)
	2hr	0.01	18(82)	530.0~535.0	0.033	19(81)

wheat		ε680	Ratio(%)	λmax	ελmax	Ratio(%)
D	0hr	0.309	100	610.0~615.0	0.345	100
	1hr	0.091	29(71)	595	0.118	34(66)
	2hr	0.073	23(77)	585	0.105	30(70)

normal rice		ε680	Ratio(%)	λmax	ελmax	Ratio(%)
E	0hr	0.172	100	590.0~605.0	0.211	100
	1hr	0.048	28(72)	570	0.077	36(76)
	2hr	0.032	18(82)	565.0~570.0	0.058	27(73)

waxy rice		ε680	Ratio(%)	λmax	ελmax	Ratio(%)
F	0hr	0.059	100	450	0.144	100
	1hr	0.015	25(75)	540.0~545.0	0.035	24(76)
	2hr	0.009	15(85)	520	0.34	23(77)

potato		ε680	Ratio(%)	λmax	ελmax	Ratio(%)
G	0hr	0.295	100	600	0.351	100
	1hr	0.128	25(75)	595	0.159	45(55)
	2hr	0.069	15(85)	580	0.107	30(70)

sweet potato		ε680	Ratio(%)	λmax	ελmax	Ratio(%)
H	0hr	0.234	100	605	0.275	100
	1hr	0.106	45(55)	595.0~600.0	0.132	48(52)
	2hr	0.06	25(75)	580	0.089	32(68)

cassava		ε680	Ratio(%)	λmax	ελmax	Ratio(%)
I	0hr	0.221	100	600.0~605.0	0.257	100
	1hr	0.083	37(63)	590.0~595.0	0.107	41(59)
	2hr	0.071	32(68)	590	0.095	39(64)

sago		ε680	Ratio(%)	λmax	ελmax	Ratio(%)
J	0hr	0.295	100	605.0~610.0	0.323	100
	1hr	0.075	25(75)	585	0.099	30(70)
	2hr	0.047	16(84)	580	0.072	22(78)

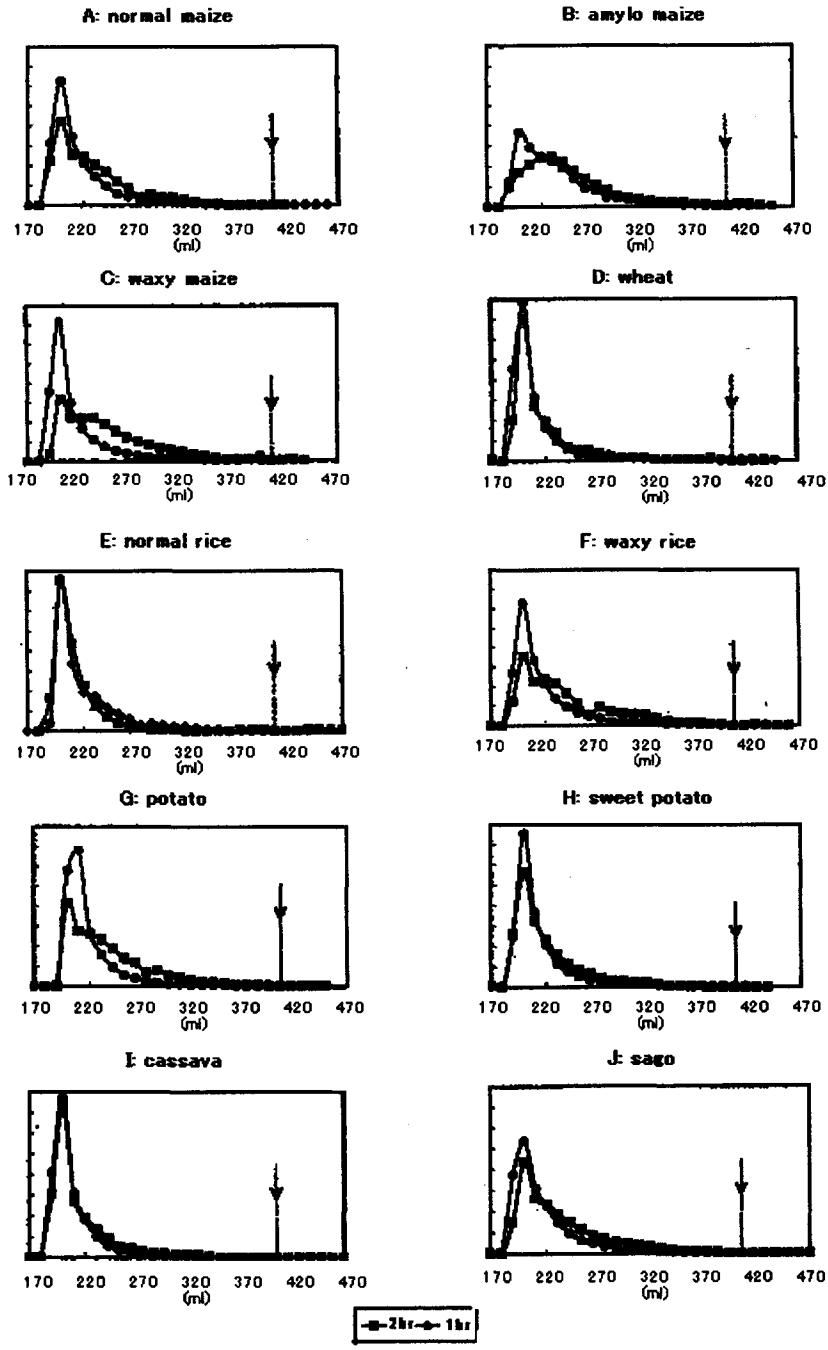


Fig. 3. GPC Profiles of dry heated starches on Toyopearl HW-65.

Table 1 shows amylose decomposition rate estimated with iodine color reaction. Amylose in root starches is more resistant to heat than that in cereal starch, and amylo maize starch is the most sensitive to the heat treatment. It is well known that there is more lipids included in amylose of cereal starch than in amylose of root starches and lipids readily form peroxide with heat in open air. Hence, it is suggested that amylose in cereal starches decompose more readily from amylose of root starches.

Fig. 3 shows GPC profiles of dry heated starches on Toyo-pearl HW-65. Decomposition of starch progressed with the heating time, and peaks finally converged to one peak at the end of elution pattern indicating that its molecular size corresponds to a species of lower molecular weight than amylose.

Table 2-A

Incorporation amount of lipid to starch granule suspended in lipid with heat treatment (soybean oil)

heating time(min)		0	20	40	60	120
Normal maize	weight(mg)	144	126	109	85	97
heating time(min)		0	20	40	60	120
Amylo maize	weight(mg)	178	110	90	95	106
heating time(min)		0	20	40	60	120
Waxy maize	weight(mg)	203	141	122	110	94
heating time(min)		0	20	40	60	120
Wheat	weight(mg)	125	84	74	71	79
heating time(min)		0	20	40	60	120
Normal rice	weight(mg)	276	281	123	198	131
heating time(min)		0	20	40	60	120
Waxy rice	weight(mg)	305	209	204	207	228
heating time(min)		0	20	40	60	120
Potato	weight(mg)	54	13	-8	1	17
heating time(min)		0	20	40	60	120
Sweet potato	weight(mg)	158	127	91	79	100
heating time(min)		0	20	40	60	120
Cassava	weight(mg)	107	142	108	107	114
heating time(min)		0	20	40	60	120
Sago	weight(mg)	159	44	43	41	66

starch: 300 mg

Table 2-B

Incorporation amount of lipid to starch granule suspended in lipid with heat treatment (methyl oleate)

heating time(min)		0	20	40	60	120
Normal maize	weight(mg)	193	136	134	128	104
heating time(min)		0	20	40	60	120
Amylo maize	weight(mg)	183	128	112	121	116
heating time(min)		0	20	40	60	120
Waxy maize	weight(mg)	202	141	145	122	98
heating time(min)		0	20	40	60	120
Wheat	weight(mg)	159	121	112	112	107
heating time(min)		0	20	40	60	120
Normal rice	weight(mg)	260	203	217	237	198
heating time(min)		0	20	40	60	120
Waxy rice	weight(mg)	256	238	236	210	192
heating time(min)		0	20	40	60	120
Potato	weight(mg)	90	2	28	-22	41
heating time(min)		0	20	40	60	120
Sweet potato	weight(mg)	176	133	120	96	96
heating time(min)		0	20	40	60	120
Cassava	weight(mg)	144	134	89	109	89
heating time(min)		0	20	40	60	120
Sago	weight(mg)	88	59	66	35	25

starch: 300 mg

However, GPC profiles of samples on HW-50 shown in Fig. 4 show that some fragments of the samples eluted at top position of the pattern. This fact indicates that these fragments are not so small. Results observed in Fig. 4 and Fig. 3 imply the same conclusion that major fragmentation might occur at amorphous region in amylopectin. Localisation of amylose in starch granule is still uncertain. Therefore, the problem whether amylose might form crystalline regions can not be solved. If amylose might form such regions, decomposition should be affected by amylopectin decomposition. Incorporation amount of lipid into the oil heated starch sample is shown in Table 2. Initial water content in starch should change with heat and approaches zero. Hence, the starch weight at each stage should be corrected but it is impossible.

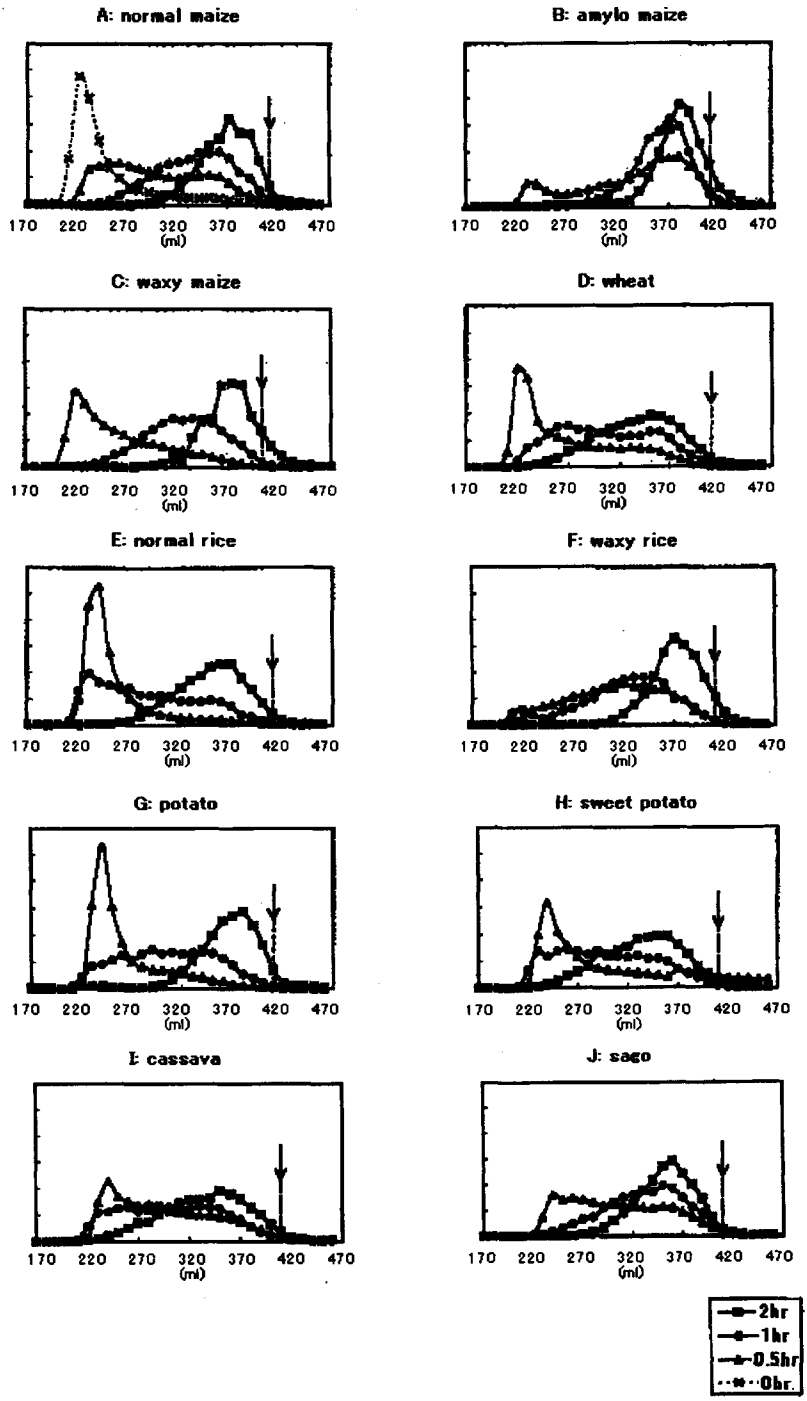


Fig. 4. GPC Profiles of dry heated starches on Toyopearl HW-50.

Ordinarily, the water content of starch is about 15%, hence it is equivalent to 45 mg in this case. This value does not contribute in any essential way to the total weight of the treated sample. However, one can say that lipid amount decrease with heat treatment time. This result is out of our expectation that vacant space in granule might increase by removing water, and lipid can easily penetrate into the space through fine cracks which might be formed with heat, and as the result, the incorporation amount of lipid might increase with the treatment.

In conclusion, baked food has fairly much water at initial stage of processing, hence on heating starch should be gelatinized to some extent. Amount of oil incorporated amount should be associated with this gelatinisation which contributes to expansion of starch granule and it adds a space for incorporation of lipids. Because of low water content in starch processed in our experiments, no increase in the oil amount in starch could be observed.

References

[1] Yamada T., T. Kato, S. Tamaki, K. Teranishi and M. Hisamatsu : Starch/Starke, 1998, **50**, 484-486.
[2] Tamaki S., M. Hisamatsu, K. Teranishi, T. Adachi and T. Yamada: Starch/Starke, 1998, **50**, 342-348.

ZMIANY NA POZIOMIE MOLEKULARNYM W GAŁECZKACH SKROBIOWYCH W CZASIE OGRZEWANIA ICH NA SUCHO ORAZ W OLEJU I ZASTOSOWANIE ZAOBSERWOWANYCH ZJAWISK W TECHNOLOGII ŻYWNOŚCI

Streszczenie

Na rynkach światowych powszechnie spotyka się szereg wypieków jak np. ciasteczka i biszkopty. Niewiele jednak pojawiło się w literaturze prac poświęconych zmianom na poziomie molekularnym w znajdującej się w tych produktach skrobi. Badania takie są utrudnione przez obecność lipidów, białek i innych składników.

Próbowano ustalić jakie zmiany zachodzą w gałeczkach skrobiowych 11 botanicznych odmian skrobi (kukurydziana zwykła, wysokoamylozowa i woskowa, pszenna zwykła i woskowa, ryżowa zwykła i woskowa, ziemniaczana, ze słodkich ziemniaków, tapioki i sago). Skrobie przetrzymywano w stanie suchym w 200°C przez 30 minut, jedną i dwie godziny. Ogrzewanie w oleju sojowym oraz estrach metylowych kwasu laurowego i olejowego prowadzono w 190°C w tych samych przedziałach czasowych. Ogrzewanie nie zmieniało obserwowanego pod skanningowym mikroskopem elektronowym wyglądu. Zmieniała się jednak rozpuszczalność gałeczek w wodzie. Po dwugodzinnym ogrzewaniu gałeczki niemal natychmiast się rozpuszczały.

Podatność na trawienie glukoamylazą zmieniała się w sposób zależny przede wszystkim od botanicznego pochodzenia gałeczek. Pod tym względem wpływ ogrzewania na skrobie woskowe był nieznaczny. Badania chromatograficzne (chromatografia żelowa) wskazują, że prawdopodobnie w wyniku ogrzewania doszło do zniszczenia klasterów, a nie samych cząsteczek polisacharydów. Skrobie bulw były odporniejsze na termolizę od skrobi zbożowych.

R. ZIOBRO, A. NOWOTNA, A. GOLACHOWSKI, H. GAMBUŚ,
M. HERNIK, R. SABAT

THE INFLUENCE OF EXTRUDER'S TEMPERATURE PROFILE ON THE CHARACTERISTICS OF PROCESSED STARCH

Summary

The study concerned the influence of extruder's temperature profile on physico-chemical characteristics of processed cereal starches. The products obtained at higher temperature were more expanded and less dense, however there were differences in response of various starches on the change of processing parameters. Solubility and water binding capacity of extruded corn starch strongly depended on thermal conditions in extruder while in case of rye and triticale no significant relation was observed.

Introduction

During extrusion cooking starch is being transformed by mechanical and thermal energy [1, 2]. The extent of physico-chemical changes occuring under extrusion depends on process parameters i.e. temperature, screw configuration and speed [1, 3, 12]. It also depends on moisture content in a raw material [1, 2, 3, 12].

Optimal values of these parameters, especially temperature and moisture content, depend on starch chemical characteristics such as amylose content [3, 4], that influence physical (and mainly rheological) parameters of starch melt inside extruder.

Although the relation between extrusion temperature and physico-chemical properties of extruded starch has been well documented, there are few reports comparing the behaviour of various starch sources under the same extrusion conditions. To check the differences corn, rye and triticale starch were examined.

Ziobro R., Nowotna A., Gambuś H., Sabat R., Katedra Technologii Węglowodanów, Akademia Rolnicza, al. 29 Listopada 46, 31-425 Kraków

Material and methods

Commercial corn starch was obtained from Diacel (corn). Triticale, and rye starch were isolated by laboratory method [14]. Prior to extrusions moisture content of starch samples was adjusted to 16%. Extrusion was performed by using single screw laboratory extruder Brabender 20DN working at 210 rpm. Compression ration was 4:1 and die diameter 3 mm. High temperature (HT) experiments were done by maintaining 140, 160 and 170°C in subsequent extruder's sections. Low temperature (LT) profile was 95, 120 and 150°C.

Expansion ratio and density were measured according to Ryu and Walker [16]. Each mean was an average of ten replications. After milling in a laboratory roller mill the extruded starch samples were subjected to following analyses. Total phosphorus content by Marsh [9] method (standard deviation: $s_x = 0.93$, coefficient of variation: $v_x = 2.23\%$) and amylose content by Morrison and Laingnelet method [11] ($s_x = 0.44$, $v_x = 1.7\%$). Water binding capacity (at 60°C $s_x = 0.14$, $v_x = 2.5\%$; at 90°C $s_x = 1.0$, $v_x = 5.4\%$) and solubility (at 60°C $s_x = 0.26$, $v_x = 5.0\%$; at 90°C $s_x = 0.29$, $v_x = 2.5\%$) were measured by Richter [14] method modified for extrudates [18]. Molecular characteristics of native and extruded starch was obtained by means of size exclusion chromatography [13, 18]. All measurements were done twice.

Results and discussion

The quality of products obtained by extrusion depends on processing temperature. Chinnaswamy and Hanna [4] report, that maximal expansion occurs for corn starch samples over 125°C, depending on amylose content. They have also observed that 50% of amylose in sample is optimal for the product quality.

Triticale and rye starch samples extruded at higher temperature exhibited higher expansion and lower density in comparison to LT samples (fig. 1). In this aspect cereal starches seem to be different from potato starch, in which case the density of extrudates was higher when barrel temperature was elevated [8].

In order to compare extruded starches with native ones, they were milled and examined in a similar way. To eliminate the influence of variability in natural starch characteristics, the quotients of values measured for extruded and native starch were used.

Fig. 2 represents the change in apparent amylose content upon extrusion. In our previous study [7] significantly enlarged amount of unbranched potato starch constituents has been observed after extrusion. This increase is related to mechanical disruption of glycosidic bonds in amylopectin, which results in its partial debranching and release of linear glucans [5, 6]. However, in the present study such pattern has been

observed only in case of corn starch. No substantial trends in this aspect has been found for HT and LT samples.

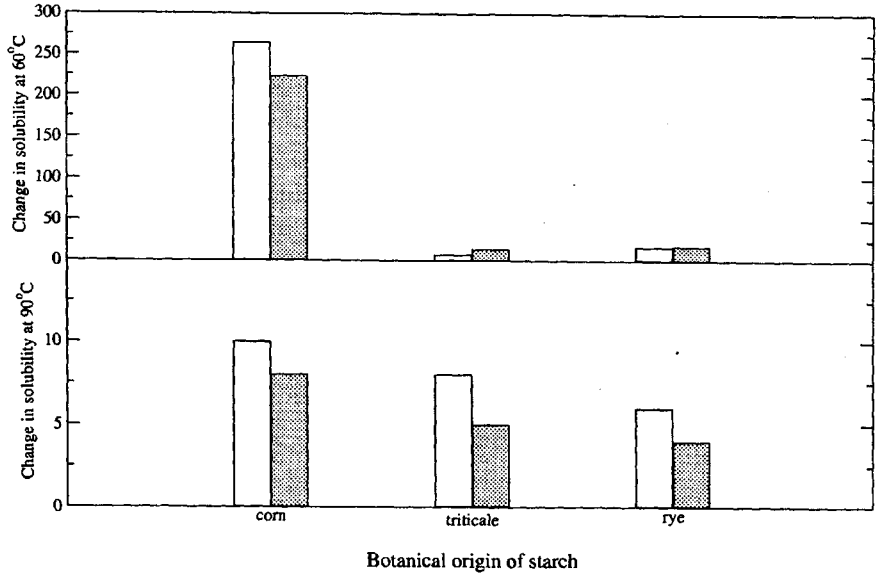


Fig. 1. Dependance of degree of expansion and density of starch extrudates on extrusion temperature (white - 140, 160, 170°C, gray - 95, 120, 150°C)

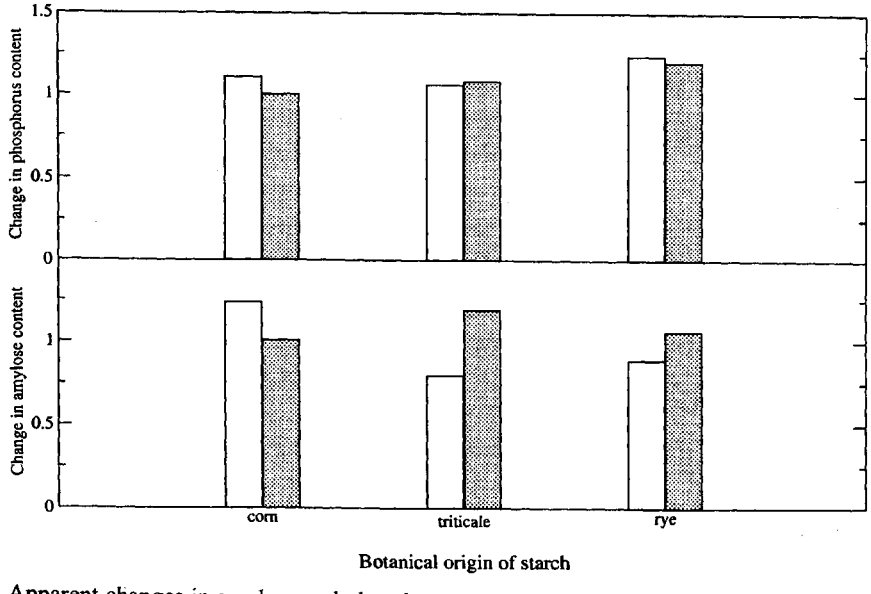


Fig. 2. Apparent changes in amylose and phosphorus content after extrusion at different thermal conditions (white - 140, 160, 170°C, gray - 95, 120, 150°C)

As it is well known, the phosphorus is present in cereal starches mainly in fat fraction consisting of lysophospholipids. As it was expected, the samples extruded in different conditions did not differ in this aspect (fig. 2). The slight aberrance from 1 observed in case of rye starch should not be considered as a real increase in phosphorus after extrusion, but is probably due to the different drying behaviour of native and extruded starch, which can result in varying dry mass of the samples.

Although under the applied process parameters only 1–2% of glycosidic bonds can be broken [15], and branching points are only a small part of them, the observed decrease in weight average molecular weight of starch and mainly its branched component is dramatic [18]. However it seems that such degradation is relatively independent on the applied temperature in the studied range (tab. 1).

Table 1

Weight average molecular weight of amylopectin present in starch samples extruded at different temperatures (HT - 140, 160, 170°C, LT - 95, 120, 150°C).

Starch origin	M_w of amylopectin [x10^6 g/mol]	
	LT	HT
Corn	2	1.7
Triticale	2	2.2
Rye	1.9	2

Mercier and Feillet [10] denote that corn, wheat and rice starches extruded at 180°C can absorbe maximum amounts of water. Our results (fig. 3) are in agreement with those findings. Water binding capacity at 60°C of HT starches was higher than of LT ones, although a pronounced effect was observed only in case of corn starch. At 90°C no such trend could be observed, probably due to excessive solubilisation of starch samples.

Solubility of extruded starch at 60°C was an order of magnitude higher than of native samples (fig. 4). This effect was especially visible in case of corn starch, which in native form is less soluble than other cereal starches [17]. Higher temperature of extrusion gave products more soluble in water at 90°C, which is in accordance with the results of Mercier and Feillet [10].

The results prove that extrusion temperature is an important parameter in starch processing and influences many important parameters of the obtained product. The extent of thermal effects depends however on starch origin.

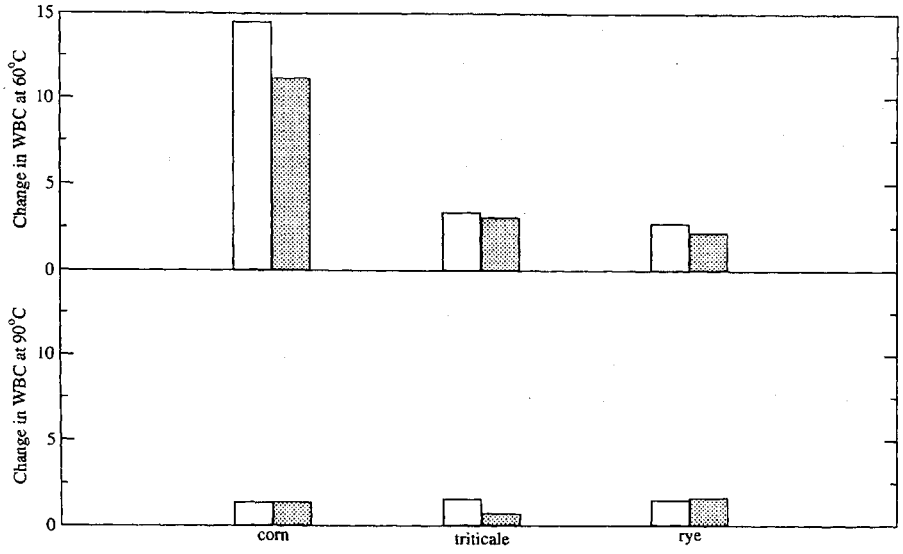


Fig. 3. Change in water binding capacity of starch samples after extrusion at different thermal conditions (white - 140, 160, 170°C, gray - 95, 120, 150°C)

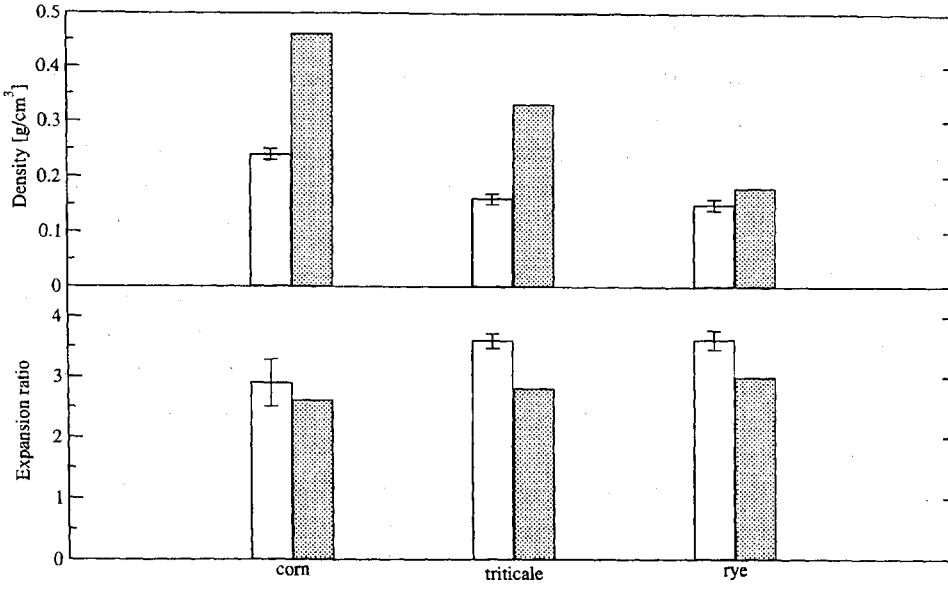


Fig. 4. Change in solubility of starch samples after extrusion at different thermal conditions (white - 140, 160, 170°C, gray - 95, 120, 150°C)

Conclusions

1. Products obtained at lower temperatures had lower expansion ratio and higher desity in comparison to those produced at high temperature.
2. The use of different temperature did not significantly influence total phosporus, irrespective of starch origin. The change in apparent amylose content was different for various samples.
3. The use of higher temperature resulted in higher water binding capacity and solubility at 60°C.
4. Weight average molecular mass of starch extrudates calculated from SEC profiles did not considerably change with different processing conditions.

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WPŁYW TEMPERATURY PROCESU NA WŁAŚCIWOŚCI EKSTRUDOWANYCH SKROBI

Streszczenie

W pacy przebadano fizycznochemiczne i molekularne właściwości skrobi kukurydzianej, pszenżytniej i żytniej poddanej procesowi ekstruzji w jednoślimakowym ekstruderze w temperaturze 140-160-170°C, jak również w (80-95)-120-150°C. Produkty otrzymane w niższej temperaturze charakteryzowały się mniejszą ekspansją i większą gęstością. Ponadto ekstrudowana skrobia kukurydziana, uzyskana w niższej temperaturze, charakteryzowała się mniejszą zdolnością wiązania wody i rozpuszczalnością w wodzie, w porównaniu do otrzymanej w wyższej temperaturze.

V.N. KISLENKO, N.I. LITOVCHENKO

KINETICS OF PARTICLE FORMATION IN THE GRAFT POLYMERIZATION OF ACRYLIC MONOMERS ONTO POLYSACCHARIDES

Summary

The kinetics of polymer particle formation and distribution of acrylic monomers between a solution and polymer particles at the graft polymerization of acrylic monomers onto polysaccharides have been investigated. The polyacrylic particle number increased at the first stage of polymerization of soluble monomers. At the second stage of polymerization, the number of particles was always constant. The concentration of adsorbed monomer decreased during the polymerization process. The monomer concentration in dispersed phase was higher than in solution.

The mathematical model taking into account the rate of monomer polymerization, aggregation of oligomer radicals and their conformation changes, adsorption of monomer in the polymer particles has been proposed. Based on the experimental results, some constants of the process were calculated.

Introduction

At the graft polymerization of acrylic esters onto water soluble polysaccharides, the stable core-shell polymer dispersions are formed [1-3]. The stability of dispersions is associated with the amphyphility of the graft copolymers, where the nucleus of the polymer particle is formed by polyacrylic chains and its shell is formed by the soluble polysaccharide chains bound chemically to the nucleus. The degree of adsorption saturation of such particles, determined by adsorption titration with sodium oleate is about 70–90%. The grafting degree with respect to acrylic polymer increases from 30 to 100% when the initial concentration of monomer in the reaction mixture decreases.On the other hand, the part of polysaccharide chemically bound with polyacrylate decreases in this case.

V.N. Kislenko, N.I. Litovchenko, National Lviv Polytechnic University, 12 Bandera St., Lviv 79013, Ukraine

Investigation of the kinetics of polymerization of acrylic monomers onto water soluble polysaccharides showed [4, 5] that the first stage of the process is the reaction between the initiator radical with polysaccharide macromolecule and initiation of the graft polymerization. In this stage, the efficiency of the grafting reaches 100%. The homopolymer of acrylic monomers is formed in the second stage of polymerization, when the polymer particles have been formed. The efficiency of grafting decreases till to minimum.

Materials and methods

The acrylic monomers, methyl acrylate, methyl methacrylate and butyl acrylate were purified by rectification. Ammonium peroxydisulfate, used as initiator of the polymerization, was recrystallized twice from water. Carboxymethyl cellulose was purified by precipitation with hydrochloric acid from water solution. Industrial water soluble potato starch was used without purification.

The average particle radius was determined by the nephelometric method and calculated by the Shifrin-Slonim equation [6].

The monomer concentration was determined by the bromide-bromate method. The monomer concentration in solution was determined after centrifugation of dispersed phase. The concentration of monomer, adsorbed in the polymer particles was calculated according to equation:

$$c_a = c_t - c \qquad (1)$$

where c_t was the total concentration of monomer and c wass the concentration of monomer in solution after centrifugation.

The molecular mass of polyacrylate chains, extracted by toluene from the graft copolymer, was determined by the viscosimetric method [7].

Results and discussion

Investigation of the particle size during the monomer polymerization at the initial concentration of monomer 18–70 g/l showed that the radius of polymer particles was practically constant for methyl acrylate (Fig. 1) and butyl acrylate. An increase in the initial monomer concentration in the reaction mixture led to an increase in the radius of polymer particles at the constant concentration of polysaccharide. The particle radii increased slightly with decrease in the initial polysaccharide concentration.

The monomer concentration in solution was practically constant during polymerization for butyl acrylate graft polymerization onto starch. In this case, on polymerization monomer formed mostly monomer droplets or monomer - polymer particles. At the same time, methyl acrylate was practically soluble in water within the concentra-

tion region investigated. In course of polymerization the concentration of methyl acrylate in solution as well as the concentration of adsorbed monomer decreased (Fig. 2).

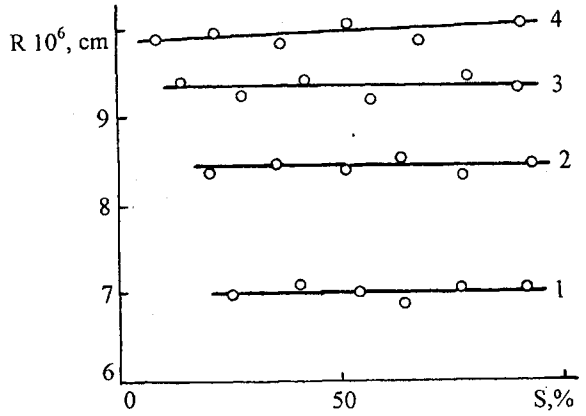

Fig. 1. Relationship between average radius of polymer particles and monomer conversion in the graft polymerization of methylacrylate onto starch at the initial monomer concentration of 18 (1), 37 (2), 54 (3) and 72 g/l (4).

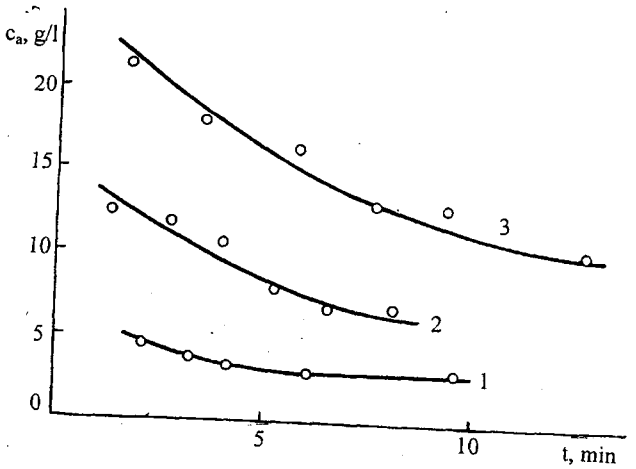

Fig. 2. Plot of the adsorbed monomer concentration vs time at the graft polymerization of methylacrylate onto carboxymethyl cellulose at the initial monomer concentration of 40 (1), 61 (2) and 82 g/l (3).

The change of the total concentration of monomer in the reaction mixture proceeded according to first order kinetics with respect to the monomer:

$$d[M]_t/dt = -k_e[M]_t \quad (2)$$

where k_e was the effective rate constant of polymerization.

For methyl acrylate, methyl methacrylate and butyl acrylate the molecular mass of polyacrylic chains of graft copolymer increased with the initial concentration of monomer in the reaction mixture. The linear relationship between the volume of polymer particles and molecular mass is showed in Fig. 3. Evidently, it was associated with an increase in the monomer concentration in the monomer - polymer particles. The monomer content in the unit volume of disperse phase rose with increase in the initial monomer concentration in the reaction mixture (Fig. 4). It was higher than the monomer concentration in the solution during the methyl acrylate polymerization. Evidently, the large oligomer radicals adsorbed the monomer from water solution. A high monomer concentration around the oligomer radical its solubility in water - monomer mixture increased and the flexibility of polyacrylic chains also increased.

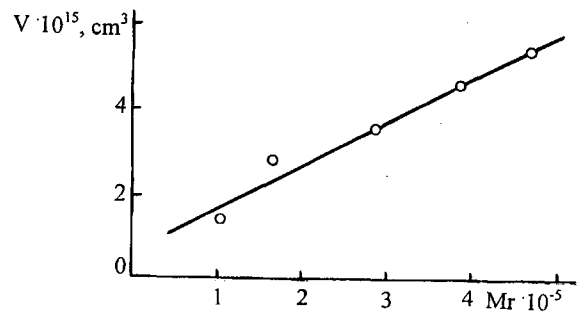

Fig. 3. Relationship between particle volume and molecular mass of polymethylacrylate at the graft polymerization of methylacrylate onto carboxymethyl cellulose.

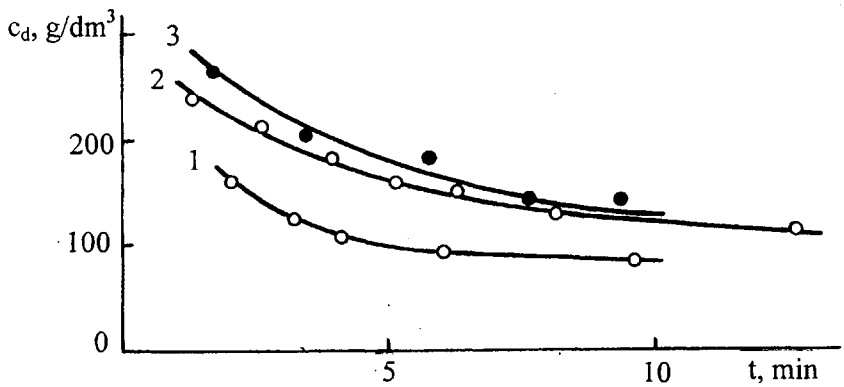

Fig. 4. Plot of the mass of monomer adsorbed in the unit volume of dispersed phase vs time for the graft polymerization of methylacrylate onto carboxymethyl cellulose at the initial monomer concentration of 40 (1), 61 (2) and 82 g/l (3).

The number of polymer particles in the unit volume of dispersion increased at the initial stage of methyl acrylate polymerization (Fig. 5), reaching maximum in 10 min. and then it remained practically constant. Therefore, the particle aggregation did not proceed until the high monomer conversion was achieved For all monomers the number of particles in the unit volume of the reaction mixture increased with the initial monomer concentration.

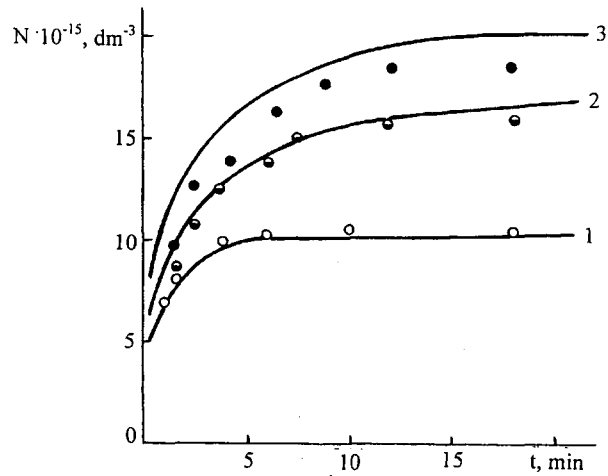

Fig. 5. Plot of the particle number in unit volume of dispersion vs time for the graft polymerization of methylacrylate onto starch at the initial monomer concentration of 40 (1), 61 (2) and 82 g/l (3). Curves are calculated according to Eq. (6).

Experimental data showed that the graft polymerization of monomers low soluble in water proceeded within monomer droplets. The initiation of polymerization passed through the stage of adsorption of polysaccharide radical on the surface of monomer droplets. The efficiency of polymerization initiation was very low. Initiation of polymerization of water soluble monomers proceeded in the aqueous solution. In this case, the dispersed primary particle consisted of polyacrylic chains, a monomer and water in the initial stage of polymerization. The monomer content in the dispersed phase was below 30%, and the content of polyacrylate in it did not exceed 20% at the initial stage of the process. Then the monomer diffused into the polymer particles from the reaction medium and water diffused from the particles. The polymerization process proceeded generally in particles in the second stage of polymerization.

The rate of a change of a polymer particle number in the unit volume of dispersion as a function of monomer and initiator concentration could be described by equation:

$$dN/dt = k_{ad}N_AN\{F_1/2 - [F_1^2/4 + 2k_dfk_i[S_2O_8^{2-}][M]/k_{ag}]^{1/2}\} + 2k_dfk_iN_A[M][S_2O_8^{2-}] \quad (3)$$

where k_d, f, k_i, and [M] were the rate constant of initiator decomposition, the initiation efficiency, the rate constant of initiation of polymerization, and [M] is the monomer concentration in water phase, respectively, k_{ad}, N, k_{ag} and k_f were the rate constant of oligomer radical adsorption onto polymer particles, the particle number in unit volume of dispersion the rate constant of oligomer radical aggregation leading to the particle formation, and the rate constant of a conformation changes of oligomer radical that leads to particle formation, respectively. $F_1 = k_f/k_{ag} + k_{ad}N/k_{ag}$

The rate of a change of the concentration of monomer, adsorbed in polymer particles, could be expressed by equation:

$$d[M_a]/dt = k_c V[M]\, dN/dt + k_{di} SN[M]([M_s]-[M_a]) - k_n[M_a] \quad (4)$$

where k_c, k_{di}, S, and $[M_s]$were the coefficient, the rate constant of diffusion, the surface area of one particle, and the monomer concentration when the particle is total saturated with monomer, respectively, $[M_a]$ and k_n were the concentration of the adsorbed monomer and the rate constant of polymerization, respectively.

Under condition of $c = [M]Mr$ and $F_3 = 2k_d f k_i N[M][S_2O_8^{2-}]/k_e >> dN/d[M]$, one can obtain Eq. (5) From Eqs.(2) and (3):

$$N\, dN/dc = -k_f F_3/(k_{ad}Mr) - k_{ag}k_e F_3^2/(Mr^2 k_{ad}^2 N_A)\, c/N \quad (5)$$

As it is shown in Fig. 6, experimental data on the kinetics of particle formation at the methyl acrylate graft polymerization follow a linearity according to Eq.(5). Correlation coefficient was 0.971. From the intercept on the axis of ordinate, $k_f F_3/(k_{ad}Mr) = (7\pm2)\cdot 10^{29}\ l^{-1}\ g^{-1}$, and from the tangent of the slope angle of straight line, $k_{ag}k_e F_3^2/(k_{ad}^2 N_A Mr^2) = (7.7\pm0.6)\cdot 10^{44}\ l^{-1}\ g^{-2}$.

Obtained ratios of rate constants provided the number of polymer particles in the unit volume of dispersion during polymerization according to the Eq. (6).

$$N = \{3 \int_0^\infty [k_{ag}k_e^2 F_3^2/(Mr^3 k_{ad}^2 N_A)\, c_o^2 \exp(-2k_e t) + k_f k_e F_3/(Mr^2 k_{ad})\, N c_o \exp(-k_e t)]dt\}^{1/3} \quad (6)$$

where $c = c_o \exp(-k_e t)$.

Fig. 6 shows that calculated curves properly describe the experimental data.

The rate of a change of the concentration of adsorbed monomer was described by Eq.(4). Eq.(4), if $k_n = k_e$, can be transformed into equation:

$$Y = k_d c_s/k_e + k_c X \quad (7)$$

where $Y = c_a/(c_S F_4)$, $X = VN/(SF_4)$, $F_4 = \int_{co}^{0} (N/c\, dc)$, $c_s = [M_s]Mr$.

F_4 was calculated by means of numerical integration taking into account the rate constant ratios found above and

$$S = 4\pi R^2 \text{ and } V = 4/3\ \pi R^3 \quad (8)$$

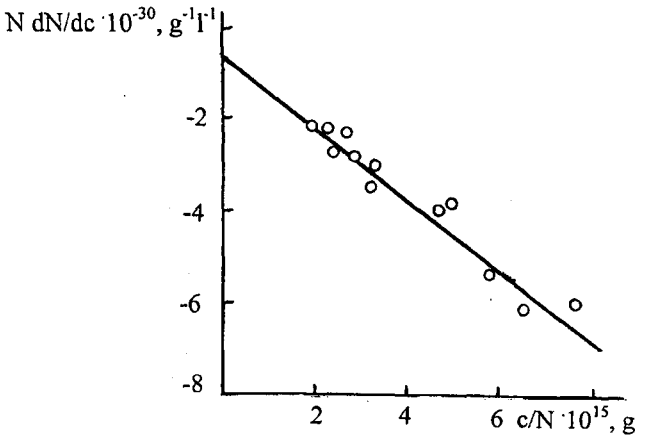

Fig. 6. Relationship between particle number in unit volume of dispersion and monomer concentration in solution when plotted according to Eq. (5) for the graft polymerization of methylacrylate.

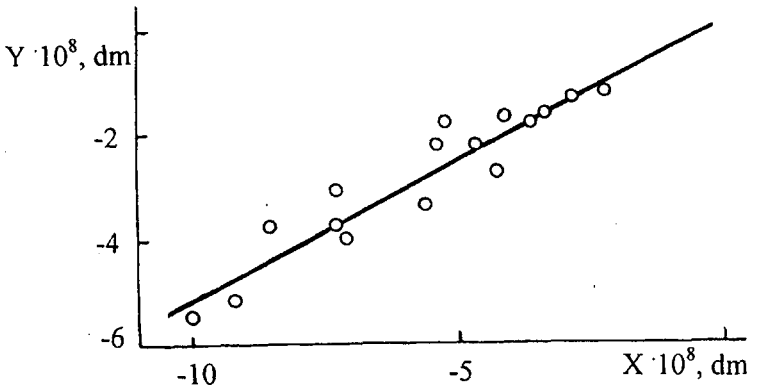

Fig. 7. Relationship between the concentration of adsorbed monomer and the monomer concentration in solution when plotted according to Eq. (7) for the graft polymerization of methylacrylate.

As showed in Fig. 7, experimental data of a change of adsorbed monomer concentration folowed the straight line according to Eq. (7) with the correlation coefficient of 0.937. The intercept on the axis of ordinate was close to zero. Thus, the value of $k_d c_s$ was below of experimental error. From the tangent of the slope of straight line, $k_c = (5.2 \pm 0.5) \cdot 10^{-3}$.

Therefore, monomer adsorption proceeded as the process of particle formation at low initial monomer concentration in the reaction mixture. Monomer concentration in particle was higher than in water phase for water soluble monomers.

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KINETYKA TWORZENIA CZĄSTECZEK W SZCZEPIONEJ POLIMERYZACJI MONOMERÓW AKRYLOWYCH Z POLISACHARYDAMI

Streszczenie

Badano kinetykę tworzenia polimerycznych cząsteczek i rozdział monomerów akrylowych pomiędzy roztwór i cząsteczki polimeru w trakcie szczepionej polimeryzacji monomerów akrylowych ze skrobią. Liczba cząsteczek poliakrylowych wzrastała w pierwszym etapie polimeryzacji rozpuszczanych monomerów. W drugim etapie polimeryzacji liczba tych cząsteczek utrzymywała się na stałym poziomie. Stężenie adsorbowanego monomeru malało w trakcie polimeryzacji. Stężenie monomeru w fazie zdyspergowanej było wyższe niż w roztworze.

Zaproponowano matematyczny model uwzględniający szybkość polimeryzacji agregację rodników oligomerowych i ich zmiany konformacyjne oraz adsorpcję monomeru w cząsteczkach polimerów. W oparciu o wyniki doświadczeń obliczono niektóre stałe procesu.

Introduction

Destylernia "Polmos" w Krakowie S.A. is leading producer of vodka and spirit professionally combining the century - old recipes and manufacturing secrets. The Company has been in existence for 70 years, modelling itself on the centuries-long tradition of distilling in the Cracow region. We produce many brands, which are well known and appreciated in Poland, and some are also marketed abroad.

Taking advantage of favourable circumstances in the first half of the 1990's, we undertook a significant investment program, becoming one of the most modern distilleries in Poland. Among other improvements, we constructed our own rectification division; this enhanced the company's image among consumers as a producer of the highest quality beverages.

We also invested in the future by creating a new brand, Cracovia Vodka. This requires a little explanation: there are many different alcoholic brands on the market in Poland, and Cracovia Vodka is undoubtedly one of these select few. This has been confirmed by the national and international awards and distinctions it has won already, but our greatest source of pride is the increasing consumer acceptance of Cracovia Vodka from month to month. We also produce baker's yeast, and for many years we had been co-operating with the Pepsi Cola Company.

In November 1998 our firm embarked on the road to privatisation by passing the first stage commercialisation. This means that the Cracow Distillery became a company owned by the Polish State Treasury as the sole shareholder. On 10th June 2000 The Ministry of State Treasury invited all interested investors for negotiations in purchasing the shares in not smaller amount than 51% of capital stock of Destylernia. As a result the Company entered the first step of privatisation.

Finding a strategic investor is now a major imperative.

If you represent a firm in the alcoholic beverage sector or have capital available to take advantage of this opportunity, consider the possibility of co-operating with the Cracow Distillery, a company with a rich tradition.

We welcome all inquiries and proposals.

History

The history of the Cracow Distillery begins shortly after World War I. After a century and a half of existence as a country divided up between its more powerful neighbours, the Polish authorities of the time, cognisant of the distilling industry's importance to the national economy, created the Polish Spirits Monopoly in 1925. It task was to form a coherent industry out of the scattered and disparate distillery businesses in the three formerly occupied sectors.

In 1926 the management of the State Spirits Monopoly, acting under an agreement with the Baczewski and Krakus distilleries, decided to locate a distillery in Dabie

near Cracow. The plant was commissioned on April 30, 1931. By contemporary European standards it was a very modern plant, with an efficient production line and well designed infrastructure for transport and for storage of raw materials and ready products. The distillery was completely independent of outside power sources, and immune to fluctuations in the market for raw materials; the volume of spirits storage was equal to a year's production. The plant's products, grounded in tradition and produced with the most modern technology of the times, quickly won the appreciation of consumers.

World War II spared the plant but was not merciful to its workers. Literally on the eve of liberation, the Nazis carried out mass executions in Cracow. Among the victims were 29 employees of the Cracow Distillery. A memorial plaque on the Company's administration building commemorates that tragic event and honours their memory.

After the war, production resumed quickly, still in the first half of 1945. In later years there were organisational changes in the spirits industry as the whole Polish economy was put under centralised control. As a result the plant was subordinated to a central board with its offices in Warsaw, having authority over more than twenty enterprises all over Poland. The spirits industry operated under this arrangement until the political changes of the late 1980's. The plant produced alcoholic beverages under shared trademarks for consumers in areas assigned by the state planning authorities. Often the label did not even say which plant had made the contents of the bottle.

In 1964 the Cracow Yeast Plant in Biezanow was merged with the Company. After six months of modernisation a new production line to turn out top quality yeast was commissioned. The end of the 1960's brought a large increase in demand for flavoured vodkas, which made it necessary for the Company to modernise and expand its facilities, so in the 1970's the plant gained a modern flavoured vodka division.

In January 1974 the Company took over the Pepsi Cola soft drinks plant in Nowa Huta from the Okocim Brewery. This division has been modernised and expanded many times since then, tripling its production capacity in the last twenty-odd years while continuing to meet Pepsi Cola's stringent quality standards. This co-operation provided us access to the world's latest technologies at a time when political realities kept Poland and other East Bloc countries isolated from the West, technologies which have been implemented in the other divisions.

The end of the 1970's saw a period of further restructuring of the unflavored vodka division. Worn out machinery from the 1960's was replaced by a high-capacity bottling line. Storage facilities were expanded. The 1980's did not bring major changes, though new equipment and technical ideas were continually introduced.

The political transformation in Poland in 1989 led in effect to the end of centralisation in the distilling industry. In 1991 the distilleries gained their independence. The enterprise in Cracow introduced several new brands of its own at that time. Among them, CYMES Vodka became a big hit. Its market and financial success powered the company's strong growth. The distillery was modernised through replacement of bottling and production lines. Buildings were renovated, and new office and warehouse buildings were constructed. Above all there was investment in the new product development, yielding Starka Krakowska (was produced and sold by The Cracow Distillery

till August 2000), Fiddler Vodka (was produced and sold by The Cracow Distillery till February 2000), and the most important one, Cracovia Vodka. In November 1998, the Cracow Polmos Distillery and a number of other distilleries were commercialised, becoming State Treasury-owned companies. This is the first step towards privatisation.

Since July 1999, as a result of the division of Polmos brands, Destylernia "Polmos" w Krakowie S.A. became the owner of Winiak Luksusowy 43%.

Since the beginning of the year 2000 the privatisation counsellor of Minister of Treasury of the State - EVIP International Sp. z o.o. has been operating in the firm. His task is to prepare the distillery for final privatisation.

A lot of changes in the company happen in order to achieve ISO 9001 Certificate of Quality. It is connected with the verification of assumed standards of work , the growth of the awareness of workers in the matter of personal responsibility for quality and gaining additional advantage over competition. It should be however remembered that the system of quality is the process of constant improving and achieving the certificate is not the final target of undertaken tasks.

As the result of organisational changes Yeast Production Plant in Kraków Biezanow achieved full independence on 1st, 2000.

Management Board

Management Board of Destylernia "Polmos" w Krakowie S.A.

Chairman
President
Msc Marian Cwiertniak
email: mcwiertniak@polmos.krakow.pl

Member of the Board
Director
Msc Andrzej Grela
email: agrela@polmos.krakow.pl

Contact

Destylernia "Polmos" w Krakowie S.A.
Poland, 31-553 Krakow, ul. Fabryczna 13

Main phone no. +48 12 411 89 00

Secretariat:
tel. +48 12 411 48 43
fax +48 12 412 14 33

Sales:
tel. +48 12 411 07 26
fax +48 12 411 87 86

Supplies:
tel. +48 12 411 21 70

Marketing:
tel. +48 12 411 66 88
email: market@polmos.krakow.pl

Yeast Plant
Poland, 30-860 Krakow, ul. Drozdzowa 5

Main phone no.
tel. +48 12 658 10 69
tel. +48 12 658 00 32

Sales and Marketing:
tel. +48 12 658 19 79

The History of Distilling in Cracow

Cracow's special role as the seat of royal dynasties for centuries and as a centre of science and culture have made it the scene of many firsts. Here, in the city whose heart is Wawel Hill and its castle, was the first Polish university, hospital, pharmacy, book

... and of course distillery, already in the late fifteenth century, as confirmed in a tome kept in the Jagiellonian University Library, written by Stefan Falimirz and published in 1534 (O ziolach i mocy yich, Herbs and their powers). Barrels and demijohns of aqua vitae, "water of life," called okowita in Poland and Lithuania, travelled on barges to Gdansk and in traders' wagons to Prague, Nuremberg, Vilnius and Kiev. These conveyances were a tasty morsel for bands of highwaymen. Income from distilling was an important item in the budget of the royal court, and honoured members of the Cracow distillers' guild enjoyed many privileges. According to a record from 1786, 35 distillers in Cracow were engaged in making spirits. The term wodka was coming into general use at that time.

The nineteenth century was a period of rapid growth in the spirits industry in Galicia, the Austrian-occupied sector of partitioned Poland. In 1835, 4,900 small distilleries were operating there. A tax was imposed on them the following year, and their numbers dropped to 1,900. They specialised in making flavoured liquors. A famous one of the time was Edward Urban's Distillery of Flavored Liquors, Rosoglios, Liqueurs and Rum, founded in 1877. It was located in the center of Cracow at 1 Wislna Street. M.A. Urbach entered the fray in 1899 by opening a brandy distillery. Piotr Porzycki and many others followed suit.

After the First World War there were eleven distilleries in Cracow, including the famous Krakus and Baczewski enterprises. In 1931 the Polmos Cracow Distillery was formed, resting on their tradition.

The city of Cracow

"The historical and architectural centre of Cracow which took shape during the course of almost a thousand years, is one of the most noteworthy artistic and cultural complexes in Europe" said UNESCO experts in their decision to enter the city on the UNESCO World Heritage List.

Cracow, along with Prague, Vienna and Budapest, shaped the political and cultural life of Central Europe through long centuries. One of the main centres of dynastic rule was the capital of the budding Polish state, and it filled that role up to the beginning of the seventeenth century. Around Wawel Castle - the residence of kings, the site of their coronations, and their place of eternal rest - a thriving town grew. The functionality of its thirteenth-century urban plan can be seen in the fact that it still works today: now as then, cultural, administrative and commercial life is focused around Cracow's 40,000-square-meter town square, the largest one in Europe.

Cracow's golden age was in the sixteenth century, during the reign of the last kings of the Jagiellonian dynasty. A community of illustrious scholars and scientists was grouped around the Jagiellonian University, founded in 1364 (Nicolaus Copernicus studied there). They formed a centre of humanist thought which exerted a significant impact on the Europe of their day. The castle on Wawel Hill took on a Renais-

sance look. Royal patronage of the arts was matched by the magnates and rich bourgeoisie who employed Italian masters to beautify their palaces and townhouses. Refugees from all over Europe, seeking protection from persecution, flowed into Cracow's Jewish quarter, Kazimierz. Poland was known for its tolerance.

At the end of the eighteenth century, when the Polish state disappeared from the map of Europe, Cracow became the spiritual capital of the Polish people, a symbol of its former glory and the site of patriotic demonstrations. It is no accident that on August 6, 1914, the first company of infantry under the leadership of Jozef Pilsudski began its march from Cracow, beginning the arduous task of reclaiming statehood.

By a fortunate accident of fate, the many catastrophes of history in this part of the world have spared Cracow's heritage buildings. About 600 of them present the story of the city's architecture beginning from the tenth century. There are more than half a million historical objects – art works, crafts and ethnographic exhibits – Besides the famous Jagiellonian University there are eleven other institutions of higher learning, a dozen or so theatres, dozens of art galleries, the Philharmonic...

Many people of world renown are connected with Cracow: Pope John Paul II, the Nobel laureates Czeslaw Milosz and Wislawa Szymborska, science fiction writer Stanislaw Lem and playwright Slawomir Mrozek, and composers Krzysztof Penderecki and Zbigniew Preisner to name a few. Cracow is the birthplace of the late Tadeusz Kantor's Cricot 2 Theatre. Here is the famous Stary Theatre, world-class events such as the Organ Music Days, Music in Old Cracow, International Short Film Festival, International Print Triennale, and the Architecture Biennale. The Gothic and Renaissance interiors of Cracow's heritage buildings provide a worthy setting for these events.

By itself all this would be enough to bring the annual throngs of tourists to this town. But beyond that – and this is not simply local pride talking – Cracow has a special atmosphere which is difficult to express but which the visitor will sense at every step and will long remember. It is the mingling of history and the here and now: the hourly bugle call from the tower of St. Mary's Church which halts in mid-note, recalling the sentry who trumpeted a warning of a Tartar onslaught and was slain by an enemy arrow; the June pageant of the Lajkonik, a folk character based on the story of a soldier who, it is said, got drunk and romped through town in the Turkish clothes of his fallen foe in the thirteenth century: the echo of old pagan rituals, also in June, when we float flower wreaths on the Vistula River; the annual competition for the silver cock, organised by the oldest municipal brotherhood of marksmen in Europe; the annual show of fanciful, glittering Christmas croches...

Brands

Clear vodkas
Cracovia Classic Polish Vodka
Cracovia Supreme Vodka
Swojska Vodka
Czysta Krakowska Vodka

Flavoured vodkas
Cracow Dry Gin
Przepalanka Krakowska - NEW
Akcent Krakowski - NEW

Brandy
Cracovia Very Old Vodka - NEW
Winiak Luksusowy
Winiak Jagiellonski 1410

Spirit
Spiritus Krakowski

The Cracow Distillery, a business with 70 -year tradition, is a recognised leader in the production of vodka and rectified spirits in Poland.

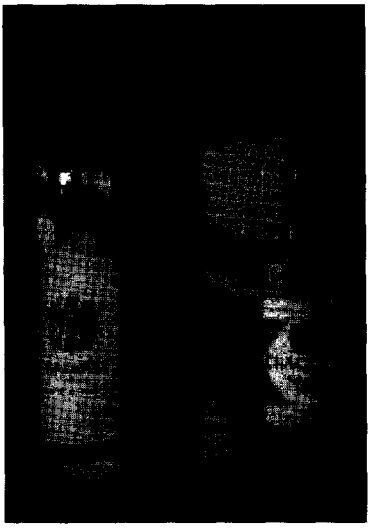
The Company skilfully combines the knowledge, formulations and techniques developed through many centuries with the latest technical achievements to produce an outstanding line of several dozen brands.

The Company's headquarters, Cracow, is a city with a splendid history and cultural heritage. Famous writers and musicians are not the only ones who are inspired by the city. The distillery, too, draws on Cracow's unique tradition in many ways, as can be seen from some of the brands on this list of our traditional, recently added and very latest products. among the most traditional products.

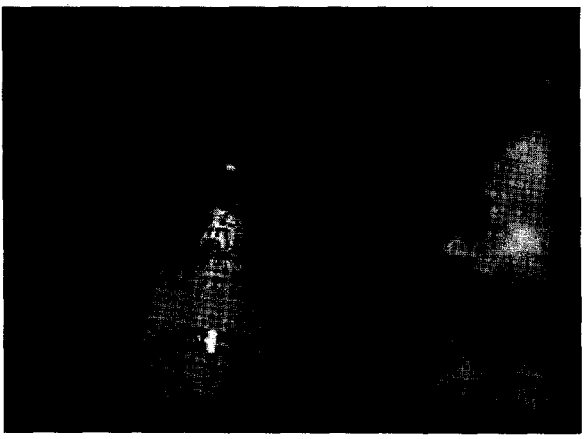
All the brands bottled by the Cracow Distillery are distinguished by their high quality. They bear informative, tasteful labels which reflect the unique character of these beverages. They are produced exclusively from natural ingredients. Both the Company and its products have earned recognition in Poland and abroad, reflected in numerous awards, medals and distinctions.

Cracovia Classic Polish Vodka 40%vol. - poj. 0,5l; 0,7l, Cracovia Supreme Vodka 42% vol- poj. 0,7l.

Cracovia Vodka is luxury unflavored vodka with a personality of its own, reflected in its name and its elegant presentation. It took first place in the Polish Agriculture and Food Ministry's competition for the most interesting alcoholic beverage of 1996, It was awarded the Export Opportunity Certificate at the 13th Food Products Trade Fair in 1997, and the Silver Mallow (1997) and Gold Mallow (1998) in Poland's National Ranking by Consumers of Goods and Services.



Cracovia Vodka is also an idea for winning another place for Polish vodka on foreign markets, this time for a brand with a beautiful and distinctive Polish image which can hold its own on store shelves and in bars, restaurants and hotels around the world.



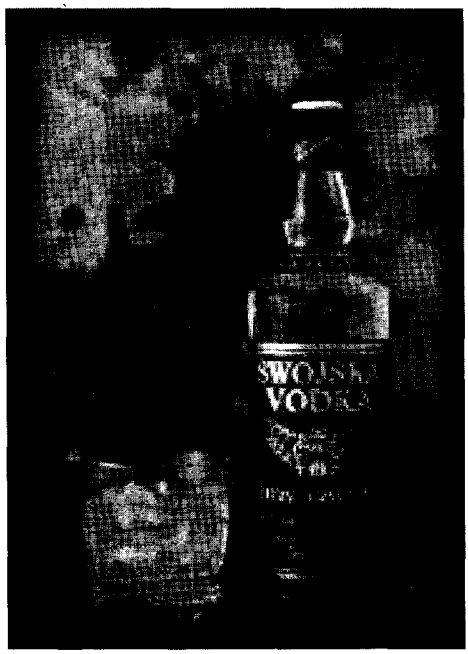
Cracovia Vodka genuine Polish vodka, has already made a splash abroad. It won the main award for the most interesting design in the annual competition organised by the prestigious London journal Wine and Spirit International in the white spirits category, and an honourable mention in the International Spirits Challenge 1998, an international tasting competition for alcoholic drinks, held in Great Britain.

Cracovia Vodka is above all a new way of thinking about the future. The Polish drinks market is flooded with products but not all of them can be thought of as brands. Beverages appear and quickly pass from the scene. Few win a firm place; most dissapear unnoticed by the majority of customers.

Cracovia Vodka is a brand, a permanent element of the market. It was introduced after several years of preparartion, including design work by David Taylor & Company Ltd., International Brand Design and Development studio based in London. At the distillery, a new rectification division was built to supply the best tasting spirits for CRACOVIA.

Swojska Vodka - 38% vol - poj. 0,1l; 0,25l; 0,5l.

Produced exclusively by the Cracow Distillery since 1993, Swojska Vodka is packaged and marketed with a deliberate nod to the pints and half pints that were a fixture of life in Poland in the 1950's and 1960's. But there the resemblance ends. This vodka is made from high quality potato spirits, so that Swojska is a relatively high quality vodka among the popular brands.

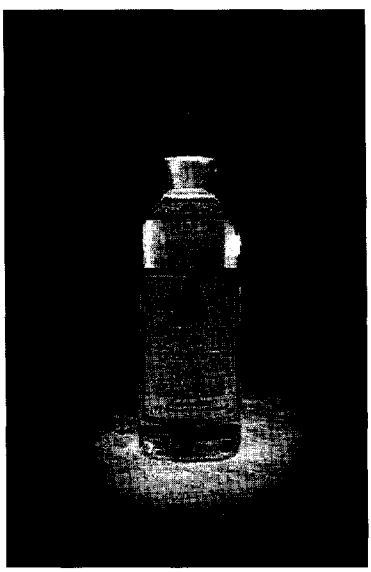


Serve well chilled, straight up or mixed like this:

Our Cocktail

1 part Swojska Vodka
1 part milk
1 part lemon juice
Sweeten and mix well.

Czysta Krakowska Vodka - 40% vol - poj. 0,1l; 0,25l; 05l.



Czysta Krakowska Vodka is the newcomer on Polish market within the clear vodkas category. Its recipe was developed in Destylernia "Polmos" w Krakowie S.A. in 2000 and it gains consistently its share of the market and popularity. Czysta Krakowska Vodka is crystal clear vodka, high quality of which is obtained thanks to the composition of best rectified spirit and cristal clear water.

Elegant and simple label deliver Cracow style and firm character to this vodka

Przepalanka Krakowska 40% vol.- poj. 0,1l; 0,25l; 0,5l

It is flavoured vodka strictly made according to very old Krak King's recipe used by people living in Cracow. Actually Destylernia "Polmos" w Krakowie S.A. on "consumer - gourmand's" request produces this vodka.

Akcent Krakowski - 30% vol. - poj. 0,11; 0,251; 0,51

It is the newest product of Destylernia "Polmos" w Krakowie S.A., which production started in April 2001. It is dry flavoured vodka made for clients appreciating excellent taste and quality.

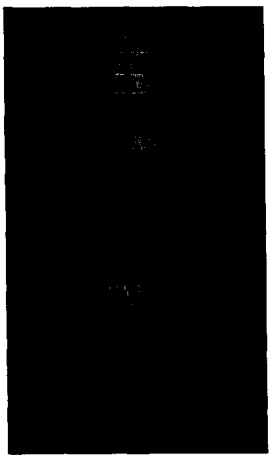
Cracovia Very Old Vodka - 40% vol.- poj. 0,50l;

Flavoured, dry, natural vodka is made according to old Cracow recipe. This vodka gets its noble and subtle taste during many years mellowing in storage in oak barrels. The brand is the result of the merger of classic product Cracovia with noble and traditional drink. Simple bottle referring to discreet elegance of Cracovia possesses neck label in old gold colour adding uniqueness and patina to the product.

Winiak Luksusowy - 43% vol. - poj. 0,5l

It is an original, high-quality brandy offered by Destylernia "Polmos" w Krakowie S.A. This liquor is produced on the base of few years wine distillate, mellowing in storage in oaken tanks. It has beautiful brown colour and excellent harmonised bouquet. The completion of totality is an original bottle.

Since July 15th, 1999 Destylernia "Polmos" w Krakowie S.A. is the owner of Winiak Luksusowy trademark.



Winiak Jagielloński 1410 - 40% vol. - poj. 0,1l; 0,7l

It is next high-quality brandy offered by Destylernia Kraków. This brandy is produced on the base of few years wine distillate, mellowing in storage in oaken tanks. The name of product - commemorates the period of glamour of Polish nation and Polish arms under the rule of Jagiellonian Dynasty strongly rooted in Polish people's consciousness. The year 1410 is the allusion to the Grunwald battle in which united forces of Polish Kingdom and Duchy of Lithuania crashed the army of Teutonic Order which had been plundering Polish country.

Spirytus Krakowski - 95% vol. - poj. 0,10l; 0,25l; 0,5l

Spirytus Krakowski is a novelty on Polish market, creating by Destylernia "Polmos" w Krakowie S.A. in 2000. From the beginning achieve its position on Polish market of spirits successfully. It is indebted to its quality valorous and elegant, classic label giving this product the Cracow style and emphatic character for success.

Prizes & Awards

1993

- Special Price of the Public - International Fair of Mountain Areas - Krynica '93 - Winiak Senator
- Medal KRAKOFOOD '93 - Galicyjska Vodka
- Award - Gold Medal - International Fair Tradexpo '93 - Sao Paulo - Starka Krakowska
- Gold Medal - II International Food Fair FOODTARG '93 - Katowice - The series of Cherry Liqueurs: Magnat, Queen Cherry, King Cherry Liqueur
- Award - in alcoholic beverages category - II International Food Fair Foodtarg '93 - Katowice - Cymes Vodka

1994

- The first Price in the Contest - III International Fair of Food Manufacture and Food Products POLFOOD '94 - Gdansk - Starka Krakowska
- Award - Food Fair of Malopolska '94 - Krakow - Specjal Vodka
- Silver AS'94 - for people and business company- Polish Promotion Corporation Ltd.
- Main Price - Partnerzy '94 - the price for the company

1995

The most interesting Product of the year 1995 - I price - The Ministry of Agriculture and Food Economy - Fiddler Vodka

- The price of the magazine "Polish Food"of The Ministry of Agriculture and Food Economy in the contest - "The most interesting promotion on the stand" at Polagra '95
- Medal for the best product - KRAKOFOOD 95 - Centus Vodka
- Gold Medal - International Poznañ Fair - POLAGRA '95 - Fiddler Vodka
- The price for the stand and the high quality of products - Nowosybirsk 1995

1996

- The award - Malwa '96 - Fiddler Vodka
- The award of X Warsaw Food Fair '96 - Cristal Cup - Cracovia Supreme
- The most Interesting Food Product of the Year 1996 - I price - Minister of Agriculture and Food Economy - Cracovia Vodka

1997

- Silver Malwa '97 - Cracovia Vodka
- Medal and Cup - The most Interesting Product of the Fair - IV Fair of Alcohols and condiments - Kielce '97 - Cracovia Supreme
- Diploma - Export Chance - XIII Warsaw Food Fair '97 - Cracovia Supreme
- Silver medal - Wine & Spirit International Design Awards 1997 - Cracovia Supreme Vodka

1998

- Gold Malwa '98 - Cracovia Supreme
- Seal of Approval - 1998 - International Spirits Challenge 1998 - Cracovia Supreme
- Gold Medal in category - Alcoholic Beverages of more than 25% of alcohol - Foodtarg '98 - Katowice - Cracovia Supreme
- Golden Grosz for the Best Company in Poland 1998 - the price of the weekly magazine Przekrój
- The Certificate of enrolment to the Book of Prizes and Awards for Economic Activity 1998 - Chamber of Commerce in Cracow

1999

- Medal for the Best Product of V Fair of Food, Drinks and Machinery for Food Industry in Cracow - KRAKOFOOD '99 - Cracovia Supreme
- Diploma of Prize Winner of IV Edition of Polish Promotional Contest Agro Polska - International Agriculture and Food Fair - AGROPOL Rzeszow '99 - Cracovia
- Medal - Product of High Quality for Eksportowa Vodka - V Cracow Meeting of Beverages Producers
- XII International Food Fair - FOODTARG '99 - Award in Alcoholic Beverages Category: 38% - Swojska Vodka 40% - Swojska vodka BIS
- Golden Grosz for the Best Company in Poland 1999 - the price of the weekly magazine Przekrój The Certificate of enrolment to the Book of Prizes and Awards for Economic Activity 1999 - Chamber of Commerce in Cracow

2000

- 14th edition of the contest Hat of Mercury 2000 main price - Cracovia Supreme
- Gold Medal - Wine & Spirit International - the International Spirits Challenge 2000 - Starka Krakowska
- Medal for the Best Product of 6th Fare of Food, Drinks and Machinery for Food Industry in Cracow Krakofood 2000 - Eksportowa Vodka 40% vol.
- Bronze Medal - Wine & Spirit International - The International Spirits Challenge 2000 Cracovia Supreme
- Gold Medal - Polagra Fair 2000 - Cracovia Supreme

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