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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-

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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.

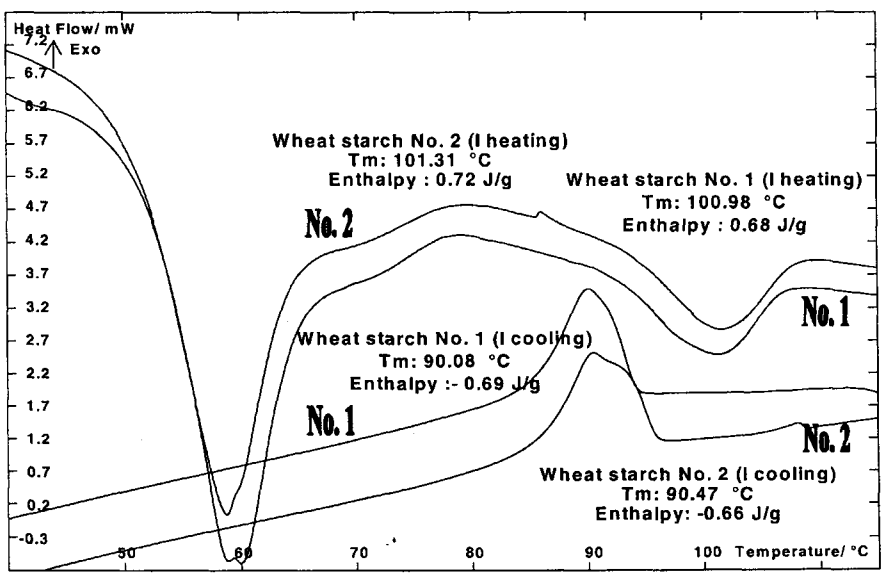

Fig. 1. DSC thermograms of native wheat starch preparation no 1 and no 2.

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}).

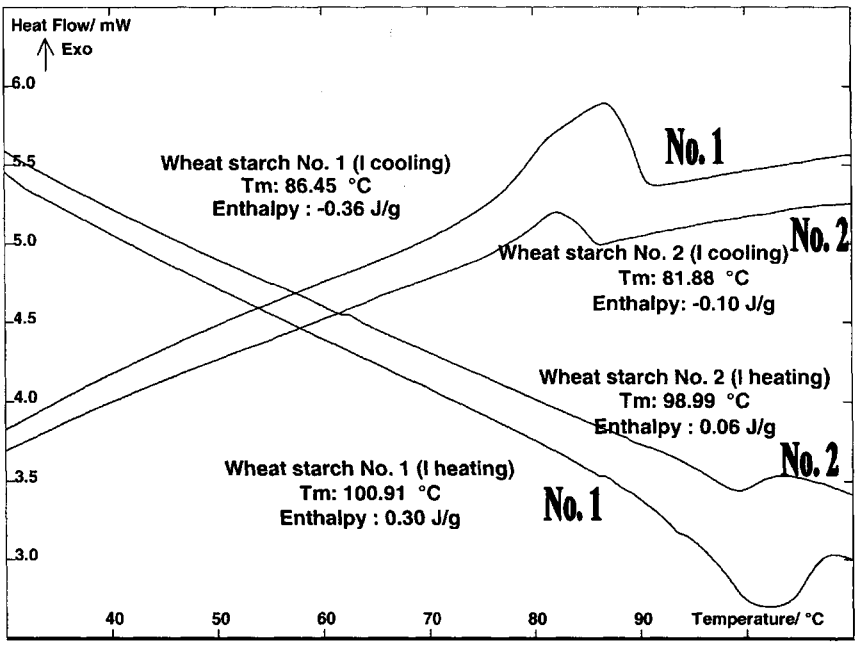

Fig. 2. DSC thermograms of wheat starches suspensions no 1 and no 2 digested with Termamyl S α–amylase.

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.

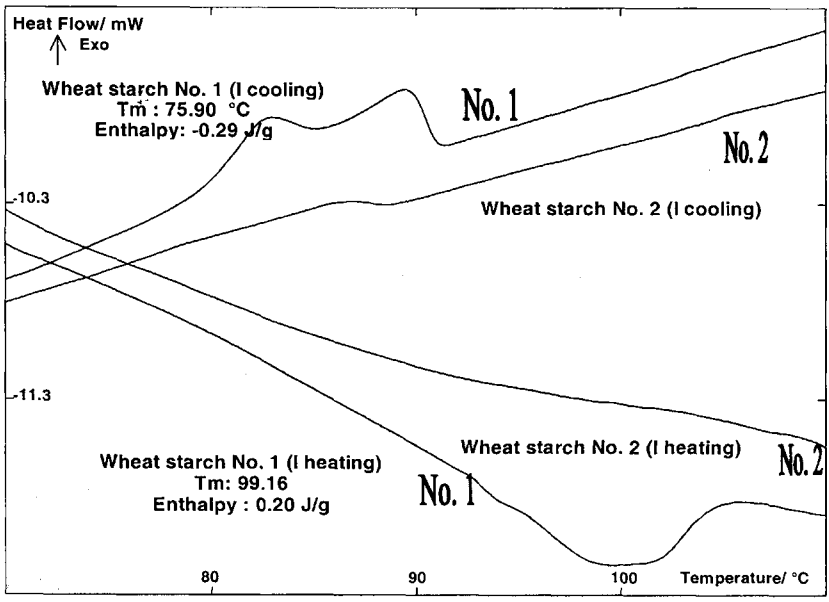

Fig. 3. DSC thermograms of wheat starches suspensions no 1 and no 2 treated for 48 hrs with AMG E glucoamylase and G – zyme lysophospholipase.

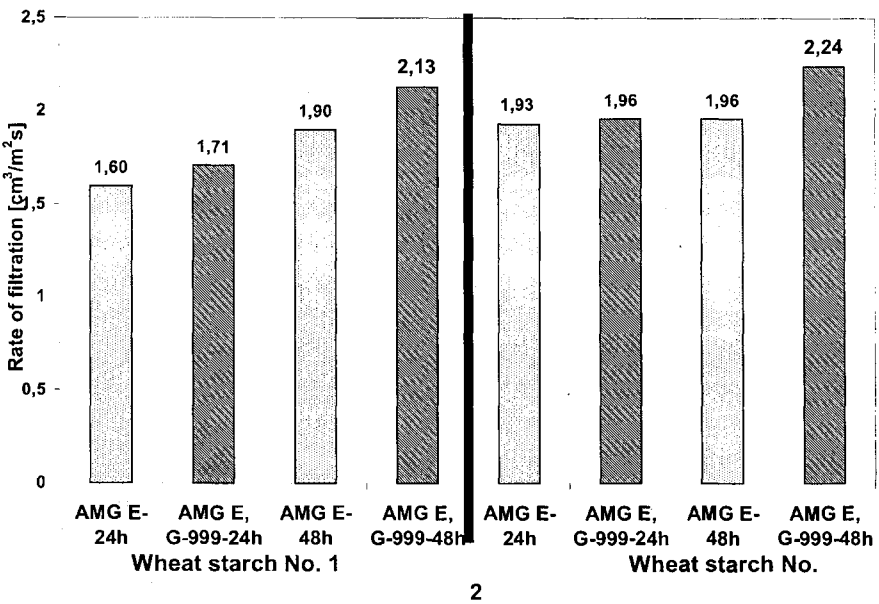

Fig. 4. Rate of filtration of glucose hydrolysate obtained from wheat starches no 1 and 2 treated for 48 hrs with AMG E glucoamylase or AMG E glucoamylase and G – zyme lysophospholipase.

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.

Acknowledgement

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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.