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PROPERTIES OF AMYLOSE-LIPID COMPLEXES FROM DIFFERENT WHEAT VARIETIES HARVESTED IN DIFFERENT YEARS

OSOBINE AMILAZNO LIPIDNOG KOMPLEKSA RAZLIČITIH SORTI PŠENICE UBIRANE U RAZLIČITIM GODINAMA

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ABSTRACT

Samples of starch derived from three wheat varieties harvested in 2001, 2002 and 2003 were analyzed. Thermodynamic properties of amylose-lipid complexes (AML) formed in these starches and their dependence on weather conditions during wheat cultivation were determined. Dissociation and reassociation of AML and properties of their polymorphic forms were characterized by differential scanning calorimetry (DSC). It was found that starch samples derived from the same wheat variety but harvested in different years had different composition. Also enthalpies of dissociation and reassembling of their AML and shapes of peaks observed during cooling of starch slurries were different and depended on the year of wheat culture. We concluded that environmental conditions during wheat growth have a significant effect on starch properties.

***Key words**: amylose-lipid complex polymorph, wheat starch, differential scanning calorimetry.*

REZIME

U radu su analizirani uzorci skroba koji su dobijeni iz tri sorte pšenice ubirane 2001, 2002 i 2003 godine. Određene su termodinamičke osobine amilazno-lipidnog kompleksa (AML) formiranog u analiziranom skrobu u zavisnosti od vlažnosti zemljišta tokom vegetaije. Disocijacija i resocijacija AML i osobine njhovih poliformnih oblika su određene kalorimetrijskim diferencijalnim skeniranjem. Utvrđeno je da uzorci skroba dobijeni iz iste sorte ali ubirani u različitim godinama imaju različiti sastav. Takođe, otkriveno je da entalpija disocijcije i reasocijacije AML i oblici pikova na dijagramima tokom hlađenja skroba su različiti i zavise od godine ubiranja pšenice. Zaključeno je da uticaji okoline tokom rasta pšenice imaju signifikantni efekat na osobine skroba.

***Ključne reči**: amiloza – lipid kompleks, pšenični skrob, diferencijalno kalorimetrijsko skeniranje.*

INTRODUCTION

Wheat is one of the most important sources of starch *(Ružić, 2001; Biliaderis and Lazaridou, 2009)*. Starch is the principal reserve polysaccharide of higher plants. It is a mixture of linear amylose and branched amylopectin, which are both polymers of glucose. Amylose is known to form inclusion complexes with iodine, alcohols and certain lipids (*Putseys et.al, 2010; Morrison et al., 1993; Sarko and Zugenmaier, 1980)*. Some authors reported existence of amylopectin-lipid complex (*Eliasson, 1994)*. Complexing with iodine is used for determination of amylose content in starch.

Formation of amylose-lipid complexes with fatty acids and phospholipids, which are included in starch granules, occurs both during biosynthesis of starch and during its gelatinization. These complexes are also formed with emulsifiers (*Eliasson, 1994; Morrison et al., 1993)*.

According to Biliaderis and Galloway, formation of AML comprises two steps such as an entrapment of lipid molecules within the interior of amylose chains followed by crystallization of the inclusion complex. Two polymorphic forms of AML are formed during these processes: amorphous (I) and semicrystalline (II). The first of them is generated at lower temperature (*Putseys et.al, 2010; Biliaderis and Seneviratne, 1990)*.

The crystalline structure of AML, which is usually referred to as type V structure, was characterized in detail by X-ray crystallography (*Godet et al., 1995; Biliaderis and Seneviratne, 1990)*. Biliaderis and Galloway demonstrated that form I of AML had the spectrum typical of amorphous structure while the form II had the spectrum of type V.

Differential scanning calorimetry is widely applied for determination of properties of AML. Both the melting temperature of AML and the energy required for its dissociation in aqueous medium are estimated by this method. Melting temperature of AML depends on many factors including the temperature of its generation, the length of amylose chains and the structure of lipid. Amorphous AML melt at 96-104°C while the crystalline ones at approximately 120°C (*Biliaderis and Seneviratne, 1990)*.

Batches of starch derived from the same wheat variety but cultivated in different years have different chemical composition and physicochemical properties, which results from the impact of climatic conditions (*Tester and Karkalas, 2001)*. Thus also the properties of AML generated in starches from different wheat varieties grown in different years are likely to be diverse. They can have slightly different structure and thermodynamic features. Their edifice can be more or less ordered what can be related to different climatic conditions during starch synthesis each year. Hence, their susceptibility to dissociation can also be distinguishable. Studies on potato starch showed that one of the factors which determined their internal structure was temperature during the growth of these plants *(Tester* et al., *1999)*.

Presented studies aimed at determination of thermodynamic properties of AML formed in batches of starch from different wheat varieties and harvested in 2001, 2002 and 2003, as well as their resistance to dissociation and the structure of the adopted polymorph.

MATERIAL AND METHOD

Starch was isolated from three wheat varieties. Winter wheat varieties Jawa, Sakwa and Symfonia were obtained from the Institute of Plant Breeding in Strzelce (Poland). The crops were harvested in 2001-2003. The examined wheat varieties rank among different quality groups (according to the classification of

wheat varieties Central Institute of Crops Researches). The following starches were chosen for further investigation as they represented samples that should show differences in the chemical composition and physicochemical properties.

Starch was isolated from wheat grains in the laboratory according to the traditional technology of Martin *(Kerr, 1950*) with some modifications. Starch was washed with water from the dense dough and then the contaminating substances were removed from its suspension. Additional steps of sedimentation and centrifugation were introduced to obtain starch of 96-98 % purity. Starch was purified by sieving through sieves (250, 200 and 63 μm), sedimentation and centrifugation. Then, it was dried on the air.

Dry matter of starch was determined by gravimetric method *(PN-84/A-74706*). Starches were dried for 3h at 130˚C. They were analyzed for the contents of protein (Kjeldahl method) (*Krełowska-Kułas, 1993)*. Total and starch lipids were determined by Weibull–Soxhlet method (*PN-84/A-74706*). Starch was hydrolyzed with 25% hydrochloric acid for 30 min, in order to release all lipids fraction. Lipids were separated from hydrolysates (washed with hot water) and dried on the filter. Extraction was conducted in the automatic Soxteh apparatus during 45 min with petroleum benzine (at 135˚C).

Amylose content in starch samples was assayed according to Gibson et al., using a commercial kit Megazyme Amylose/Amylopectin AM/AMP 01/96 (Wicklow, Ireland). This method is based on binding of nonreducing amylopectin ends by Concanavalin A (Con A), which leads to precipitation of this starch fraction.

Preparation of samples

Each sample of starch (60 mg) was dispersed in 1 ml of DMSO while being mixed at a vortex mixer. The tube was then placed in a boiling water bath. After 1 min, the tube was removed and vortexed at high speed, then returned to the water bath for 15 min with intermittent stirring. During the initial sample dispersion, care was taken to avoid sample being splashed up to the internal walls of the tube.

To remove lipids, 4 ml of 95% (v/v) ethanol was added to precipitate the sample. A further portion (2 ml) of ethanol was added, the tube capped and the suspension was mixed by inversion and left overnight at 4ºC. The precipitate was collected by centrifugation (3500 *g* 5 min). The supernatant was discarded and the tube was drained for 10 min onto tissue paper. The pellet was suspended in DMSO (1 ml) by vortex mixing and completely dispersed by heating in a boiling water bath for 15 min with mixing. Dilute Con A solvent (2 ml) was mixed with the sample solution, which was then transferred quantitatively by repeated washing with dilute Con A solvent to a 25 ml volumetric flask. The sample was then diluted to volume with dilute Con A solvent. Subsequent amylose analysis commenced within 60 min to circumvent the possibility of amylose retrogradation. This solution was termed Solution 1. A reagent blank of DMSO (1 ml) diluted to 25 ml with Con A solvent in 25 ml volumetric flask was prepared and analyzed concurrently.

Determination of amylose

An aliquot (0.75 ml) of Solution 1 was transferred to a 2 ml Eppendorf microfuge tube. Con A solution (0.75 ml) was added, the tube capped and the contents mixed by repeated inversion. The tube was allowed to stay for 60 min at the room temperature and centrifuged (10 min 2000 *g*) in a microfuge. An aliquot (1 ml) of the supernatant was transferred to a 10mL centrifuge tube and mixed with 0.2 M acetate buffer, pH 4.5 (1 ml) to reduce pH to ~5. The tube was stoppered with a glass marble, heated in a boiling water bath for 5 min to denature the Con A and equilibrated to 40ºC by incubation in a water bath for 5 min. *Alpha*-amylase/amyloglucosidase reagent (1 ml) was added with mixing and incubation was continued at 40ºC for 30 min. The tube was then centrifuged at 2000 *g* for 5 min at room temperature. Duplicate aliquots (1 ml) of the supernatant were mixed with GOPOD reagent

(4 ml) and incubated at 40ºC for 20 min. The absorbance was determined against blank at 510 nm. The absorbance at 510 nm of 100 μg/ml glucose standard (0.15 ml plus 0.85 ml H_2O) was determined concurrently.

Determination of total starch

An aliquot (0.25 ml) of Solution 1 was mixed with 0.2 M acetate buffer, pH 5.0 (2 ml) and *alfa*-amylase/amyloglucosidase reagent (1 ml) in a centrifuge tube. The tube was incubated for 30 min at 40ºC and cooled to room temperature before an aliquot (1 ml) was mixed with GOPOD reagent (4 ml), incubated at 40 ºC and the absorbance at 510 nm determined as described above.

Calculation of amylose

The amylose content is measured as the ratio of the glucose derived from the supernatant after treatment with Con A and the glucose derived from total starch solution, expressed as a percentage.

$$\%Amylose = \frac{A_{510}Supernatant\,ConAx6x100}{A_{510}Starchx13} = \frac{A_{510}Supernatant\,ConAx46.2}{A_{510}Starch} \quad (1)$$

where is: 6 and 13 are the dilution factors for the Con A and total starch extracts respectively.

The content of amylose-lipid complexes, formed in the examined wheat starch samples was calculated on the basis of the difference between the amounts of true and apparent amylase *(Morrison, 1995).*

DSC studies

The differential scanning calorimetry (DSC) was used to study the dissociation and reassembling of amylose-lipid inclusion complexes. Characteristic temperatures and enthalpies of AML dissociation/reassociation were determined using a Setaram micro-DSC 3 differential microcalorimeter (Caluire, France). Starch samples were directly weighed into stainless steel pans and left for 12 h prior to analysis to achieve the same humidity in all the examined starch samples. Solid substance content in starch samples was maintained at 30%. The reference pan contained water. Each sample was heated from 20ºC to 120ºC and cooled to 20ºC at a rate of 1ºC/ min. This temperature range (20ºC ÷ 120ºC) included known temperatures of AML dissociation (80ºC ÷ 120ºC).

X-ray diffraction

The powder samples were stored in a sealed vacuum box over saturated $CaCl_2$ solution (relative humidity 75% at 20-25°C) for 48 h in order to standardize the water content of the samples at about 12%. Crystallinity was measured by wide angle X-ray scattering using Bruker AXS D5005 X-ray diffractometer (AXS GmbH, Karlsruhe, Germany). The X-ray generator equipped with a copper tube operating at 40 kV and 50 mA produced radiation of approximately 154 nm wavelength. Data were recorded over an angular rate of 4º to 38º (2θ). The diffractograms covered the range from 4º 2θ to 38º 2θ.

Statistical evaluation

The data were elaborated statistically using STATISTICA 7.1 software package (Stat Soft Inc. Tulsa, USA). The data reported are average of triplicate observations. The results were reported as means ± standard deviations of replicates. The differences between means were determined by the T-test at p=0.05.

RESULTS AND DISCUSSION

Properties of starch depend first of all on the genotype of the plant, but also on conditions experienced during the growth. Apart from genetic determinants (*Vanstellandt and Delcour, 1999; Wooton et al., 1998; Zeng et al, 1997)*, temperature and availability of water (*Tester and Karkalas, 2001)* also determine the chemical composition and properties of starch synthesized by various wheat varieties. Average temperatures and monthly sum of rainfalls during growth of examined wheat varieties are presented in *Table 1*.

Table 1. Average temperatures and rainfalls during wheat cultivation in April, May and June in 2001-2003

Name	2001			2002			2003		
	IV	V	VI	IV	V	VI	IV	V	VI
Temperatures [°C]	7	14	15	8	17	17	7	15	17
Rainfalls [mm]	86.5	41.4	67.2	40.5	73.5	28.2	16	40.8	21.4

Chemical composition

The contents of dry matter, protein, true and apparent amylose, total and starch lipids and AML were assayed in samples of starch derived from grains of four wheat varieties harvested in 2001, 2002 and 2003 *(Tab.2)*. Starch isolated from grains of the same wheat variety but harvested in different years had different chemical composition. Concentrations of true and apparent amylose in starch obtained from Sakwa and Symfonia varieties depended on a year of cultivation. True amylose content varied from 26 to 34% d.m. and from 30 to 34% d.m. for Sakwa and Symfonia varieties, respectively, while the apparent amylose content ranged from 21 to 26% d.m. and from 23 to 28% d.m. *(Tab. 2)*. Almost unchanged contents of true amylose (28% d.m.) and apparent amylose (20-21% d.m.) were observed in case of starch from Jawa variety *(Tab. 2)*.

The amount of apparent amylose in batches of starch from Sakwa wheat variety from 2001 and 2002 as well as 2001 and 2003 were significantly different according to T-test. There were no differences between batches from harvests in2002 and 2003.

However in starches from Jawa and Symfonia wheat varieties statistically important differences in apparent amylase content were observed for samples from 2001 and 2003 and also 2002 and 2003, whereas there were no differences between batches from harvests in 2001 and 2002.

In case of true amylose there were no significant differences observed for examined starches, both between varieties and year of harvest.

Chemical composition of the latter starch was the least affected by the year of cultivation.

Table 2. Chemical composition of starches isolated from different wheat varieties harvested in 2001-2003

Name	Sakwa			Symfonia			Jawa		
	2001	2002	2003	2001	2002	2003	2001	2002	2003
Dry matter [%]	89.48 ±2.03 ^{a}	86.86 ±1.89 ^{b}	92.51 ±1.94 ^{b}	90.04 ±1.88 ^{a}	88.34 ±1.76 ^{a}	92.52 ±2.01 ^{b}	89.69 ±1.25 ^{a}	89.85 ±1.83 ^{a}	93.98 ±1.18 ^{b}
Apparent amylase [% d.m.]	26.08 ±2.37^{a}	21.83 ±0.45^{a}	22.24 ±0.18^{a}	25.29 ±0.18^{a}	28.19 ±1.62^{a}	23.35 ±0.24^{a}	20.86 ±2.86^{a}	21.05 ±1.68^{a}	20.24 ±0.53^{a}
True amylase [% d.m.]	29.22 ±1.37^{a}	26.44 ±0.88^{a}	34.16 ±1.73^{a}	30.59 ±1.09^{a}	34.36 ±0.51^{a}	33.73 ±1.52^{a}	28.25 ±1.35^{a}	28.80 ±1.34^{a}	28.19 ±1.57^{a}
Lipids [% d.m]	0.61 ±0.04^{a}	0.47 ±0.01^{a}	0.55 ±0.06^{a}	0.51 ±0.06^{a}	0.50 ±0.02^{a}	0.86 ±0.04^{a}	0.50 ±0.04^{a}	0.53 ±0.02^{a}	0.61 ±0.02^{a}
Starch lipids [% d.m.]	0.50 ±0.06^{a}	0.38 ±0.04^{a}	0.43 ±0.03^{a}	0.43 ±0.05^{a}	0.41 ±0.08^{a}	0.47 ±0.06^{a}	0.41 ±0.05^{a}	0.43 ±0.07^{a}	0.51 ±0.04^{a}
Amylose-lipid complex [% d.m.]	3.14^{a}	4.34^{ab}	11.92^{b}	5.30^{a}	6.17^{ab}	10.38^{b}	7.39^{a}	7.75^{ab}	7.95^{b}

$^{a, ab. b}$ Values with similar letters in a row do not differ significantly ($p < 0.05$) according to T-test.

Also total lipid and AML contents were different in starch samples isolated from Sakwa and Symfonia wheat varieties. Lipid contents were 0.47-0.61% d.m. in Sakwa starch and 0.50-0.86% d.m. in Symfonia starch whereas AML contents varied from 3 to 11% in Sakwa starch and from 5 to 10% in Symfonia starch *(Tab.2)*. These fluctuations were smaller in case of starch from Jawa wheat variety. Starch lipid content varied from 0.38 to 0.50% d.m. for Sakwa; from 0.41 to 0.47 for Symfonia and from 0.41 to 0.51% d.m for Jawa wheat starch variety. The highest contents of AML were observed in starch isolated from wheat grains harvested in 2003 irrespective of the variety.

No dissimilarities in total and starch lipids content were observed (*Tab.2*), however there were significant differences in AML content, between batches of starch from 2001 and 2003 harvest for all examined wheat varieties.

Our findings of chemical composition of wheat starches were similar to results of other authors (*BeMiller and Whistler, 2009; Vanstellandt and Delcour, 1999; Yun and Quail, 1999; Swinkels, 1985)*. Moisture content (13% d.m.) and total lipids content (0.8-2% d.m.) in wheat starch determined by other authors were similar to our findings (13% and 0.47-0.86 d.m. respectively). Amylose content in literature is about 28%, while our findings showed the amount of apparent amylose was 20-28% d.m. and true amylose 26-34% d.m.

Characteristic of amylose-lipid complex

DSC analysis included determination of temperatures of dissociation/reassembling (Td/Tr) of AML from the examined starch samples and the values of enthalpy ($\Delta H_d/\Delta H_r$) of these

processes. The results were consistent with that earlier reported for wheat starch (*Rakszegi* et *al., 2003; Tufvesson et al., 2001*).

Temperatures of dissociation (T_d) of AML generated in the examined starch samples varied from 98.73°C (Jawa) to 100.86°C (Symfonia) for wheat harvested in 2001 (*Tab.2)*, from 99.81°C (Jawa) to 100.50°C (Sakwa) for wheat harvested in 2002 *(Tab.2)* and from 98.41°C (Jawa) to 101.58°C (Symfonia) *(Tab.2)* for wheat harvested in 2003.

Table 3. Characteristic of AML transitions in starches from Sakwa, Symfonia and Jawa varieties harvested in 2001-2003

Name	Sakwa			Symfonia			Jawa		
	2001	2002	2003	2001	2002	2003	2001	2002	2003
T_d [ºC]	99.98 ±0.83[a]	100.50 ±0.08[a]	100.29 ±0.03[a]	100.86 ±0.46[a]	100.36 ±0.66[a]	101.58 ±0.41[a]	98.73 ±0.49[a]	99.81 ±0.39[a]	98.41 ±0.80[a]
ΔH_d [J/g d.m.]	1.60 ±0.01[a]	1.55 ±0.02[a]	2.11 ±0.18[a]	1.56 ±0.09[a]	1.45 ±0.05[a]	1.52 ±0.04[a]	1.36 ±0.09[a]	1.59 ±0.00[a]	1.73 ±0.00[a]
T_r [ºC]	89.22 ±0.22[a]	88.90 ±0.09[a]	88.72 ±0.31[a] 91.36 ±0.24[a]	89.67 ±1.04[a]	88.12 ±1.10[a] 90.90 ±0.90[a]	88.55 ±0.27[a] 90.46 ±0.55[a]	87.97 ±0.09[a]	89.68 ±0.31[a]	87.77 ±0.77[a]
ΔH_r [J/g d.m.]	-1.28 ±0.07[a]	-1.29 ±0.00[ab]	-1.82 ±0.02[b]	-1.14 ±0.05[a]	-1.33 ±0.00[ab]	-1.42 ±0.02[b]	-0.77 ±0.07[a]	-1.42 ±0.05[ab]	-1.73 ±0.05[b]

[a,ab,b] Values with similar letters in a row do not differ significantly ($p < 0,05$) according to T-test.

$\Delta H_d / \Delta H_r$ –enthalpy of dissociation/reassociation of amylose-lipid complex

T_d / T_r – temperature of dissociation/reassociation of amylose-lipid complex

The range of these temperatures (98-101°C) does not allow to unambiguously predict which of two possible AML forms (amorphous or semi-crystalline) occurs in the examined starch samples, although the relationships between the temperature of dissociation of amylose-lipid inclusion complexes and their crystallinity are known *(Iturriaga* et al., *2004; Wooton and Mahdar, 2003)*. Values of enthalpy of AML dissociation/reassociation ($\Delta H_d/\Delta H_r$) during successive steps of heating and cooling of starch slurries were determined on the basis of DSC thermograms *(Tab.3)*.

Fig. 1 shows the representative DSC thermograms, which characterize AML formed in samples of starch isolated from the examined wheat varieties harvested in 2001-2003.

Enthalpy of dissociation of AML varied from 1.36 J/g d.m. (Jawa) to 1.60 J/g d.m. (Sakwa) for wheat cultivated in 2001 *(Tab.3)*, from 1.45 J/g d.m. (Symfonia) to 1.59 J/g d.m. (Jawa) for wheat cultivated in 2002 *(Tab.3)* and from 1.52 J/g d.m. (Symfonia) to 2.11 J/g d.m. (Sakwa) *(Tab.3)* for wheat cultivated in 2003. The observed differences in these values of enthalpy indicate that AML formed in the examined starch samples displayed different susceptibility to dissociation and had different structure.

The complexes generated in starch samples isolated from wheat harvested in 2003 had the highest values of enthalpy of dissociation. The widest range of values of this quantity was found for starch obtained from grains of Sakwa variety (1.60, 1.55 and 2.11 J/g d.m. in 2001, 2002 and 2003, respectively).

Comparison of chemical composition of the examined starch samples and properties of AML from these samples provides evidence of the correlation between the values of enthalpy of AML dissociation and AML contents in starch obtained from wheat grains harvested in 2001 and 2003.

The lowest values of enthalpy of AML dissociation were found for samples from 2001 (1.36 - 1.60 J/g d.m.), which contained the lowest amounts of AML (3.14-7.39%). In contrast, starch samples from 2003 were characterized by the highest values of enthalpy of AML dissociation (1.52-2.11 J/g d.m.) and the highest contents of these complexes (7.95-11.92 %) *(Tab.3)*. It was demonstrated that, according to T-Test, values of dissociation entalphy of amylose-lipid complex did not vary with statistical significance between wheat starch varieties.

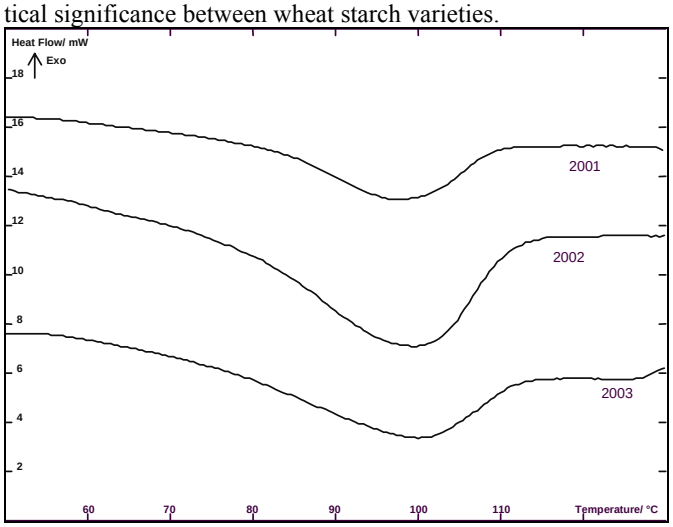

Fig. 1. Thermograms of dissociation of AML from starch of Symfonia variety harvested in 2001-2003

Different climatic conditions in 2001-2003 caused that batches of starch isolated from wheat grains of the same variety displayed diverse features. Enthalpy of AML dissociation depends on its amount and structure. More ordered crystalline structure is less susceptible to degradation and therefore crystalline (or semicrystalline) amylose-lipid inclusion complexes are decomposed at higher temperatures and this process requires more energy in comparison to dissociation of amorphous complexes. Studies on potato starch proved that cultivation at higher temperatures resulted in more ordered structure of starch (*Tester* et al., *1999)*. Our results revealed that the values of enthalpy of AML dissociation depended not only on wheat variety but also on climatic conditions during the culture.

All examined wheat varieties were cultivated under the same conditions of fertilizing and in the same sort of soil but the climatic conditions (temperature and intensity of raining) changed with a year. The highest temperatures during wheat cultivation were recorded in 2002 while in 2003 and 2001 they were slightly

lower (data recorded by the station of the Institute of Plant Breeding).

The average temperatures in April, May and June were 7, 14 and 15°C; 8, 17 and 17°C, and 7, 15 and 17°C, in 2001, 2002 and 2003, respectively *(Tab.1)*. That presumably altered the structure of wheat starch deposited each year and an edifice of amylose-lipid inclusion complexes. It was reflected by different shapes of peaks in DCS thermograms, which were related to re-assembling of AML during cooling of starch slurries and different values of enthalpy of dissociation of these complexes.

Intensities of raining during wheat cultivation (April, May and June) also changed with year in 2001-2003. They approached 86.5 , 41.4 and 67.2 mm; 40.5, 73.5 and 28.2 mm, and 16, 40.8 and 21.4 mm in 2001, 2002 and 2003, respectively (data recorded by the station of the Institute of Plant Breeding) (*Tab.1*). Water was much less available in 2003 as compared to 2001 and 2002 (from April to June), which also affected the growth of wheat. The highest values of enthalpy of AML dissociation were found for samples of starch isolated from wheat harvested in 2003 when the intensity of raining was the smallest.

For the starch samples obtained from wheat variety Sakwa harvested in 2003 and Symfonia harvested in 2002 and 2003 the double exothermic peaks related to AML reassembling were visible in DSC thermograms *(Fig. 2)*. This could result from the presence of two AML polymorphs. Different climatic conditions in the years 2001-2003 could contribute to alterations in starch structure. This assumption is confirmed by different contents of true and apparent amylose in starch isolated from Sakwa and Symfonia varieties.

Different shapes of peaks in DSC thermograms of starch samples derived from the same wheat variety but harvested in different years demonstrate that climatic conditions affected the structure of AML formed in these starches.

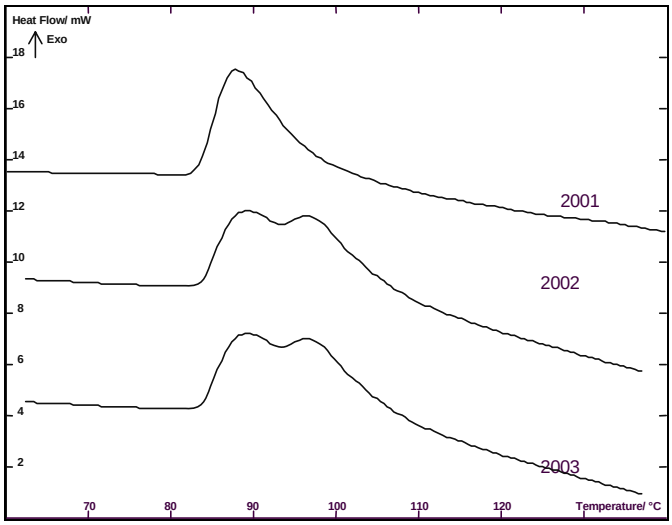

Fig. 2. Thermograms of reassociation of AML from starch of Symfonia variety harvested in 2001-2003

Fig. 2 shows representative DSC thermograms characterizing the step of cooling of starch gels with visible exothermic peaks, which are related to AML reassociation. The latter process occurred at temperatures ranging from 87.97°C (Jawa) to 89.67°C (Symfonia) for the batches of wheat harvested in 2001, and from 87.77°C (Jawa) to 89.45°C (Symfonia) for the batches of wheat harvested in 2003, while for samples from 2002 these temperatures ranged from 88.90°C (Sakwa) to 89.90°C (Symfonia) (*Tab.2*).

Values of enthalpy of AML reassembling (ΔHr) varied from –0.77 J/g s.s. (Jawa) to – 1.28 J/g s.s. (Sakwa) and from –1.29 J/g s.s. (Sakwa) to –1.42 J/g s.s. (Jawa) for the examined starches cultured in 2001 and 2002, respectively. For the starches grown in 2003 these values were slightly lower and ranged from –1.42 J/g s.s. (Symfonia) to –1.82 J/g s.s. (Sakwa) *(Tab.2)*. Statistically important differences in reassociation enthalpy of AML complex were observed for starches from all wheat varieties, between samples from 2001 and 3003. The rainfalls in 2001 were much higher than in 2003, which could affect starch composition and properties.

Single exothermic peaks were observed in DCS thermograms of starch samples isolated from all the examined wheat varieties cultured in 2001. Double peaks were observed for starch from Symfonia variety grown in 2002 and for Sakwa and Symfonia varieties harvested in 2003 *(Tab.4)*. Two distinct exothermic transitions observed during cooling of these starch slurries *(Fig. 2*) imply that two polymorphs of AML were reassembled.

Single peaks were observed in DSC thermograms recorded during cooling of slurries containing starch isolated from wheat of Jawa variety irrespective of the year of harvesting. This suggests that weather conditions do not influence physicochemical properies of Jawa wheat variety as much as a genotype. In case of starches isolated from other wheat varieties, meteorogical factors had a higher effect on starch properties and AML polymorph form.

Character of exothermic peaks in DSC thermograms of starch samples isolated from the examined wheat varieties is shown in *Table 4*.

Table 4. The character of exothermic peaks in DSC thermograms providing evidence of AML reassembling during cooling of starch slurry

2001	2002	2003	Wheat variety
single	single	double	Sakwa
single	double	double	Symfonia
single	single	single	Jawa

To explain the observed re-association of AML inclusion complexes, documented by the appearance of a single or a double peak in DSC thermograms, starch derived from Symfonia wheat harvested in 2001, 2002 and 2003 was further analyzed. The gelatinized starch was dried and subjected to X-ray analysis, which revealed sharp peaks at a wavelength of 20. This result implies that the AML complex had a semicrystalline structure. The edifice of AML inclusion complex could be changed and reinforced during starch slurry heating and that gave rise to appearance of different AML polymorphs during cooling *(Fig.3)*.

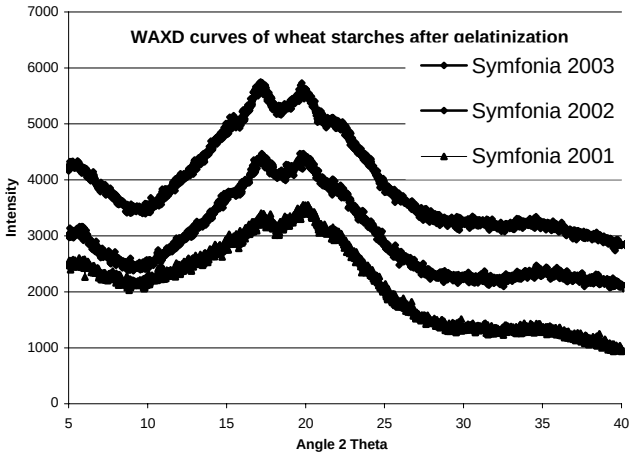

Fig. 3. X-ray scattering curves of starches from Symfonia wheat variety after gelatinization

All amylose-lipid inclusion complexes formed in the examined starches, both these with the single and double exothermal peaks, which provide evidence of AML reassembling, had the similar temperatures in maximum of the peaks. This implies that these complexes displayed the similar stability and susceptibility to dissociation.

CONCLUSION

Analyses of chemical composition of starch samples derived from three different wheat varieties harvested in years 2001-2003 showed that contents of amylose, lipids and amylose-lipid inclusion complexes were different in samples from the same wheat variety but grown in different years. Starch from wheat harvested in 2003 contained the highest amounts of AML, which were characterized by the highest enthalpy of dissociation, irrespective of the variety. The spring of 2003 was the driest among examined years. The differences in enthalpies of AML dissociation and the contents of the principal components of these starches could result from variations on the genetic level, the differences between the wheat varieties and fluctuations in climatic conditions during crop cultivation. Physicochemical properties of starch from Jawa wheat variety were stable (chemical composition, AML polymorh form, AML dissociation characteristics), which suggests that some wheat varieties are less susceptible to changing weather conditions and their properties are primarily determined by genotype.

AML formed in samples of starch isolated from the same wheat variety but harvested in different years were characterized by different shapes of peaks in DCS thermograms recorded during cooling of starch slurries which indicated that the reassociating complexes adopted different polymorphic forms. The latter phenomenon provides evidence that climatic conditions could affect properties of wheat starch and amylose-lipid inclusion complexes.

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