

CHANGES IN MOLECULAR MASS AND CRYSTALLINE STRUCTURE OF STARCH ISOLATED FROM IMMATURE CEREALS

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In the study starch samples isolated from wheat, rye and barley grains harvested at various stages of maturity were compared in respect to their structure and molecular weight. During maturation of wheat, rye and barley grain, *i.e.* in early-waxy, late-waxy and full maturity stages, a change was observed in molecular weight of amylose and amylopectin, that depended on the variety of cereal and starch granularity. Intrinsic viscosity of pastes prepared from starch isolated in various stages of grain development depended on molecular weight of amylopectin. Irrespective of maturation stage, all studied starch granules displayed properties characteristic for mass and surface fractals. Fractal dimension of structures present in developing starch granules increased with molecular weight of amylopectin, while the diameter of the observed mass and surface structures remained almost constant in the whole period.

INTRODUCTION

The synthesis of large and small starch granules in cereals is not uniform. The synthesis of large granules (A type, with diameter 10 μm) occurs mainly in the peripheral part of endosperm, starts immediately after fertilization and continues during the whole period of cereal development. In the inner cells of embryo the size of those granules is therefore above 10 μm . But those of the granules which are produced in the later period of grain development could not reach this size before the end of starch synthesis. Nevertheless, they should not be regarded as typical small granules (B type), because the majority of small granules is synthesized in the inner part of endosperm in the later period, *i.e.* 30–40 days after blooming, and their average size does not change during cereal ripening [Rahman *et al.*, 2000].

In a fully matured cereal grain (wheat, rye and barley), the number of small granules is above 80%, while that of large granules is only 10% [Evers *et al.*, 1999; Peng *et al.*, 1999; Take-da *et al.*, 1999; Verwimp *et al.*, 2004; Farmakis *et al.*, 2008].

The most intensive accumulation of starch takes place at milky stage of development [D'Egidio *et al.*, 1996]. During this 20-day long period, endosperm tissues gather more than 90% of starch. At the end of the milky stage the intensity of starch production significantly lowers and at the next waxy stage the process is completely stopped. During grain development the rate of synthesis of starch glucans is observed to alter. At first starch granules consist of 80–90% of amylopectin, then the percentage of amylose rises [Kang *et al.*, 1985; D'Egidio *et al.*, 1996]. Starch accumulation is accompanied not only

by the increase in the number of starch glucans but also by a change in the molecular weight of amylose and amylopectin. The long, linear amylose chains are continuously elongated and slightly branched [Banks & Muir, 1980].

Due to the polydispersion of starch, there is a need to use average molecular weight values. The number average molecular weight is connected with the properties that depend on the number of molecules in sample, such as osmotic pressure. The weight average molecular weight is more important for properties that depend more on the size of molecules, *e.g.* light scattering and viscosity. By means of gel permeation chromatography it is possible to obtain both weight and number average molecular weights. Additional information about molecular weight may be obtained from intrinsic viscosity of starch in good solvents [Pfanemüller, 1992; Praznik, 1992].

It is common knowledge that the maximum absorbance of an amylose iodine complex is between 640 and 660 nm, while amylopectin forms iodine complexes with the maximum absorbance ranging from 520 to 540 nm [Burchard, 1985]. Praznik *et al.* [1983, 1986] state that a high ratio of E640/E525 is a sufficient proof of the presence of amylose. Low values of this ratio, corresponding to high absorbance at 525 nm, suggest the prevalence of short-branched glucans, *i.e.* amylopectin. These values, measured for each fraction obtained by GPC allow to obtain molecular weight distributions of both major types of starch glucans.

Starch isolated from mature grains contains lamellar regions formed by a sequence of amorphous and crystalline areas [Imberty *et al.*, 1991; Gallant *et al.*, 1997; Yuryev *et al.*,

2004; Vermeylen *et al.*, 2005; Sanderson *et al.*, 2006]. Due to the differences in electron density between those areas, they may be detected by small angle X-ray scattering. Many authors [Cameron & Donald, 1992; Jenkins *et al.*, 1993; Pikus *et al.*, 2000; Yuryev *et al.*, 2004; Pikus, 2005; Vermeylen *et al.*, 2005; Sanderson *et al.*, 2006] regard those patterns as the representation of starch fractal structure. It is then possible to classify them as mass, pore or surface fractal types [Berrett & Peleg, 1995].

Mass fractal is an aggregate consisting of connected three-dimensional subunits. The fractal dimension (D_m) varies from 1 to 3 and equals to the values of scattering curve $- \alpha$. Bottom limit (1) denotes a lack of branching, while upper limit maximum branching. The increase in D_m is then equivalent to the rise of branching. Mass and pore fractals are classified as volume fractals, because the whole object is fractal. Surface fractals are self-similar only on their surface. Their fractal volume is between 2 and 3, which corresponds to between 3 and 4 ($\alpha=6-DS$) [Pikus *et al.*, 2000; Pikus, 2005].

The aim of the study was to determine changes in molecular weight, intrinsic viscosity and crystalline structure (as measured by SAXS) of starch isolated from immature cereals.

MATERIALS AND METHODS

MATERIALS

The study was performed on immature wheat, rye and barley grains harvested at various times and stored in a frozen state till the moment of starch separation.

Materials

Starch was isolated from wheat (variety *Almari*), rye (variety *Dankowskie Zlote*), and barley (non variety composition). Grain was harvested in the farm of the Experimental Station of the Agricultural University in Warsaw (Poland) in 1997 and 1998.

Harvesting

Harvest was carried out at different stages of grain maturity as given in Table 1. It was conducted on approximately 30th and 40th day after blooming. The days of harvesting were selected based on sensory analyses. The very first samples were collected in the early-waxy stage of maturity and the second samples were harvested 7-10 days later, in the late-waxy stage.

Grain processing

The heads included 20-40 cm of stem were gleaned manually with either reaping hook or knife and thresh into laboratory thresher. Then, the grain was crumbled with a meat cutter

and immediately refrigerated up to $-20(\pm 2)^\circ\text{C}$ to afford homogeneity of the mass. Immature grain was stored in cold and vacuum until starch was isolated [Gambuś *et al.*, 1994].

METHODS

Analyses of granular starch

Starch granularity

Granularity of starch was evaluated by Analysette 22 (Fritsch, Germany). The measurement was performed in water suspension, at ambient temperature. The results were expressed as percentage of starch granules of a given diameter in the sample.

Non-starch components

Total phosphorus, as an indicator of phospholipids, was measured according to Marsh [1959]. Crude protein content was measured with the Kjeldahl method [Richter *et al.*, 1969].

Semi-crystalline (lamellar) structure of starch

Semi-crystalline (lamellar) structure of starch was determined by means of Small Angle X-ray Scattering (SAXS). A cuvette (diameter 0.5 mm) with a dry sample was mounted in a Kratky camera (WAT Warsaw) with Ni filtered Cu radiation equipped with pulse-height analyser. The measurement was carried out under ambient conditions, in the range 2θ from 0.076 to 6.52 degrees with a changing step from 0.0076 to 0.038 degree and counting time of 100 s. All data presented in Table 4 were calculated using a modified Vonk's programme [Vonk, 1975].

Scanning electron microscopy

Microphotographs of starch were obtained in a Jeol ISM 5400 scanning electron microscope. Prior to the measurement, the samples were covered with carbon under vacuum.

Molecular parameters of starch

GPC analysis

Starch (50 mg) was dissolved in 2 mL of DMSO at 70°C , overnight, on a magnetic stirrer. Molecular weight distribution was estimated using gel-permeation chromatography equipped in the set of four columns of $\phi=16$ mm and the length of 350, 880, 880, and 860 mm, respectively. Columns were packed with Sephacryl/Pharmacia S-200, S-500 S-500 and S-1000 gels. Elution was carried out at room temperature with 0.003 mol/L Na_2CO_3 (flow rate 0.275 mL/min). The collected 130 fractions of 5 mL were determined for:

- i) sum of carbohydrates by means of the anthrone method (measurements were done at $\lambda=540$ nm) [Morris, 1948];
- ii) spectrophotometric measurements of iodine-starch complexes at $\lambda=525$ nm and $\lambda=640$ nm [Praznik *et al.*, 1983];
- iii) amylose and amylopectin content in each fraction: the blue value (BV) was used as an indicator (defined as the absorption of iodine in 100 mL of the solution containing 10 mg of dry matter of starch according to the equation: $W_n = E \cdot 10$ mg/d.m., where: E – absorbance at $\lambda=640$ nm,

TABLE 1. Harvesting dates of cereals used for starch isolation*.

| Stage of grain maturity | Cereal and date of harvest | | |
|-------------------------|----------------------------|---------------------------|---------------------------|
| | wheat | rye | barley |
| Early-waxy | 10.07.1997, 29.06.1998 | 03.07.1997, 29.06.1998 | 10.07.1997, 06.07.1998 |
| Late-waxy | 17.07.1997, 06.07.1998 | 17.07.1997, 06.07.1998 | 17.07.1997, 13.07.1998 |

*For comparison the cereals were also harvested at full stage of maturity.

d.m. – dry matter in 100 mL of the solution. Dry matter was calculated as total carbohydrates measured by anthrone method with the appropriate correction for the solution volume;

iv) weight-average molecular weight (\bar{M}_w) was estimated using calibration with pullulan standards (Shodex, P-10, P-50, P-200, P-800, 12200–853000 Da). Logarithmic molecular weights of standards were plotted against fraction numbers, and the slope and intercept of the resulting curve were estimated by linear regression.

Intrinsic viscosity

Intrinsic viscosity of starch dissolved in KOH (1 mol/L) was calculated, based on the viscosity results acquired using Ubbelohde-Rafikow viscometer at 30°C for 0.5, 0.4, 0.3, 0.2 and 0.1 starch concentration. Standard deviation of the method was 0.23 [Richter *et al.*, 1969].

Statistical analysis

The results were compared statistically by means of the Duncan's test, at a significance level of 0.05, with the use of computer programme Stat 1, Skierniewice 1998. All the measurement were done at least in duplicate.

RESULTS AND DISCUSSION

The change in molecular weight of amylose and amylopectin during maturation of wheat grains may be derived from size exclusion chromatography data. The molecular weight distribution of starch from 1998 is represented in Figures 1-3. Broad amylopectin peaks, which are observed in early waxy stage of starch maturity correspond to extremely high polydispersity of branched starch components. Their narrowing in later stages of maturity implies stepwise rise in weight of amylopectin during ripening. Although the largest branched molecules are larger than exclusion limit of the applied columns even at early stages of maturity, the last fraction containing high molecular-weight amylopectin is shifted from 0.35 MDa in an early-waxy phase of maturity to 1.4 MDa in a late-waxy and 1.8 MDa in a fully ripe phases. Smaller short-chain branched starch molecules are however present

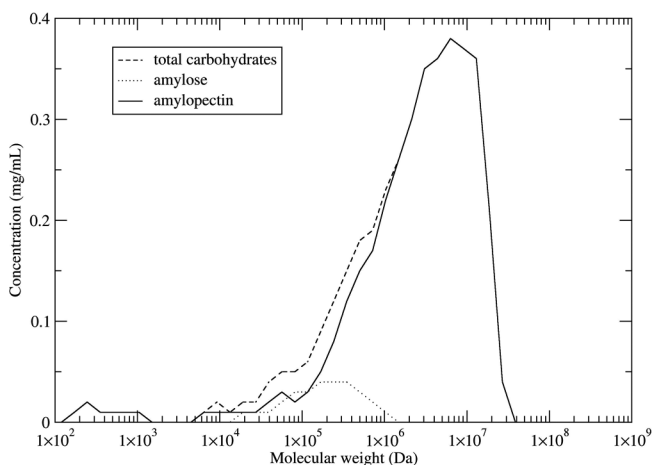


FIGURE 1. Molecular weight distribution of wheat starch amylose and amylopectin isolated at early-waxy stage of maturity in vegetative season of 1998.

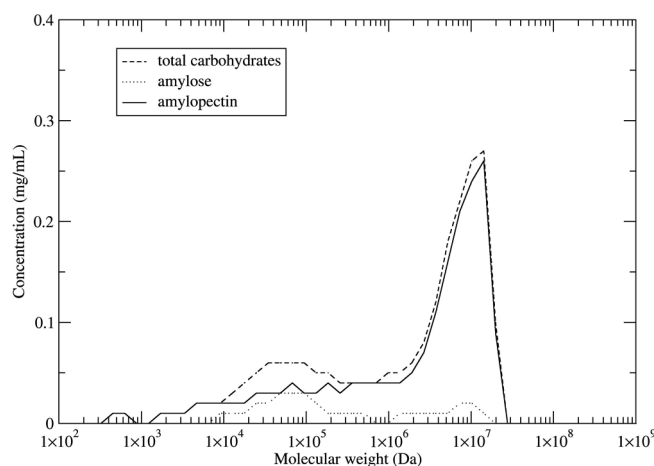


FIGURE 2. Molecular weight distribution of wheat starch amylose and amylopectin isolated at late-waxy stage of maturity in vegetative season of 1998.

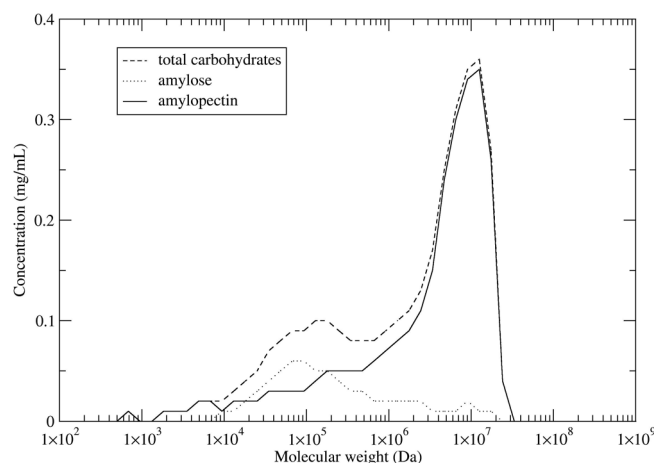


FIGURE 3. Molecular weight distribution of wheat starch amylose and amylopectin isolated at full stage of maturity in vegetative season of 1998.

in the samples, and still could be detected in the same range of molecular weights as amylose. Similar changes were observed in 1997.

The weight average values calculated from molecular weight distributions are represented in Table 2. A successive increase of molecular weights could be observed in wheat starch amylopectin from 1997. It is in accordance with the results of other authors [Banks & Muir, 1980; Praznik *et al.*, 1987].

According to Praznik *et al.* [1987], there is a small drop of weight average molecular weight of amylose fraction isolated from corn starch (1-10 weeks after blooming) in the last phases of grain development. The authors explained this by slight depolymerisation of linear glucans in this period.

The results represented in Table 2 confirm the observation mentioned above. The amylose in the late-waxy stage of maturity revealed slightly lower weight average molecular weight (\bar{M}_w) in comparison to amylose from early-waxy period. It seems that this lowering may be attributed to the rapid synthesis of small starch granules (type B) [Kang *et al.*, 1985; Bechtel *et al.*, 1990; Rahman *et al.*, 2000], that contain shorter

TABLE 2. Number and weight average molecular weights and amylose/amylopectin ratio of starches derived at different stages of grain maturity.

| Source of starch | Stage of grain maturity | Amylose (%) | Amylopectin (%) | AM- \bar{M}_n (g/mol) | AM- \bar{M}_w (g/mol) | AP- \bar{M}_n (g/mol) | AP- \bar{M}_w (g/mol) |
|------------------|-------------------------|-------------|-----------------|-------------------------|-------------------------|-------------------------|-------------------------|
| 1997 | | | | | | | |
| Wheat | early-waxy | 24 | 76 | 9.6x10 ³ | 1.3x10 ^{6a*} | 5.2x10 ³ | 4.0x10 ^{6a} |
| | late-waxy | 26 | 74 | 2.4x10 ⁴ | 1.1x10 ^{6a} | 4.7x10 ³ | 4.8x10 ^{6b} |
| | full | 27 | 73 | 1.5x10 ⁴ | 1.7x10 ^{6a} | 1.5x10 ⁴ | 6.6x10 ^{6c} |
| Rye | early-waxy | 17 | 83 | 1.9x10 ⁴ | 4.6x10 ^{5b} | 1.9x10 ⁴ | 6.6x10 ^{6c} |
| | late-waxy | 16 | 84 | 1.7x10 ⁴ | 2.8x10 ^{5a} | 7.8x10 ³ | 6.3x10 ^{6b} |
| | full | 16 | 84 | 1.0x10 ⁴ | 4.3x10 ^{5b} | 3.0x10 ⁴ | 5.1x10 ^{6a} |
| Barley | early-waxy | 17 | 83 | 8.8x10 ⁴ | 4.4x10 ^{6a} | 4.9x10 ⁴ | 1.2x10 ^{7a} |
| | late-waxy | 19 | 81 | 1.1x10 ⁴ | 3.7x10 ^{6a} | 1.6x10 ⁴ | 9.6x10 ^{6a} |
| | full | 20 | 80 | 5.9x10 ⁴ | 3.9x10 ^{6a} | 7.5x10 ³ | 1.2x10 ^{7a} |
| 1998 | | | | | | | |
| Wheat | early-waxy | 8 | 92 | 3.2x10 ⁴ | 2.6x10 ^{5a} | 1.6x10 ⁴ | 5.4x10 ^{6a} |
| | late-waxy | 15 | 85 | 4.5x10 ⁴ | 2.0x10 ^{6c} | 3.2x10 ⁴ | 6.2x10 ^{6b} |
| | full | 20 | 80 | 6.5x10 ⁴ | 1.4x10 ^{6b} | 6.2x10 ⁴ | 6.7x10 ^{6c} |
| Rye | early-waxy | 16 | 84 | 7.6x10 ⁴ | 4.1x10 ^{5a} | 2.0x10 ⁵ | 8.2x10 ^{6a} |
| | late-waxy | 18 | 82 | 1.0x10 ⁵ | 1.0x10 ^{6c} | 4.3x10 ⁵ | 8.2x10 ^{6a} |
| | full | 18 | 82 | 8.0x10 ⁴ | 7.6x10 ^{5b} | 1.1x10 ⁵ | 8.3x10 ^{6a} |
| Barley | early-waxy | 18 | 82 | 9.7x10 ⁴ | 2.4x10 ^{6a} | 6.0x10 ⁴ | 1.0x10 ^{7a} |
| | late-waxy | 18 | 82 | 9.1x10 ⁴ | 2.0x10 ^{6a} | 3.8x10 ⁵ | 9.7x10 ^{6a} |
| | full | 20 | 80 | 9.1x10 ⁴ | 2.2x10 ^{6a} | 1.9x10 ⁵ | 1.0x10 ^{7a} |

AM- \bar{M}_n – the number average molecular weight of amylose; AM- \bar{M}_w – the weight average molecular weight of amylose. AP- \bar{M}_n – the number average molecular weight of amylopectin, AP- \bar{M}_w – the weight average molecular weight of amylopectin. *The values denoted by different letters in columns for each vegetative season of every cereal differ significantly at a significance level of 0.05.

amylose chains. The increase in their content may in turn cause the change in average molecular weight of amylose. In the case of amylopectin, a small but significant increase of weight average molecular weight was observed.

Starch isolated from mature wheat contains amylose with slightly higher \bar{M}_w , as the relative content of A granules increases, due to their constant growth [Bechtel *et al.*, 1990; Shi *et al.*, 1994]. Amylose chains present in those granules are longer, which is reflected in the average values.

In the vegetative season of 1998 a similar tendency as in 1997 was observed in weight average molecular weight,

but in the case of wheat starch the increase of \bar{M}_w of amylose was observed in late-waxy stage and the decrease after maturation (Table 2). This is probably caused by the different granularity of wheat starch in those vegetative seasons. The number of small granules present in mature wheat starch in 1998 was much higher than that recorded in 1997 (Table 5 and Figures 4, 5).

Weight average molecular weight of amylopectin from rye harvested in 1997 regularly decreased during grain maturation (Table 2). It could be caused by excessive enzymatic hydrolysis occurring in this period as a result of unsuitable

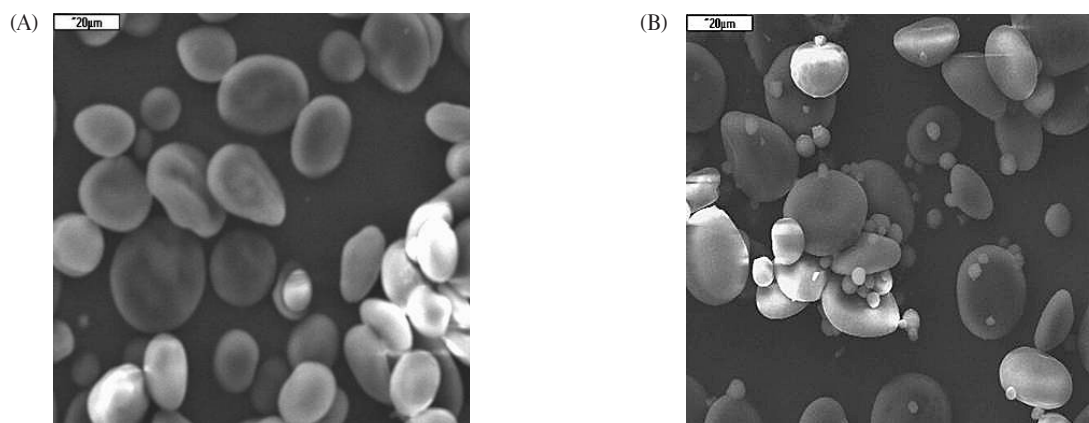


FIGURE 4. SEM image of wheat starch granules isolated at early-waxy stage of maturity (A) and full maturity (B) in 1997.

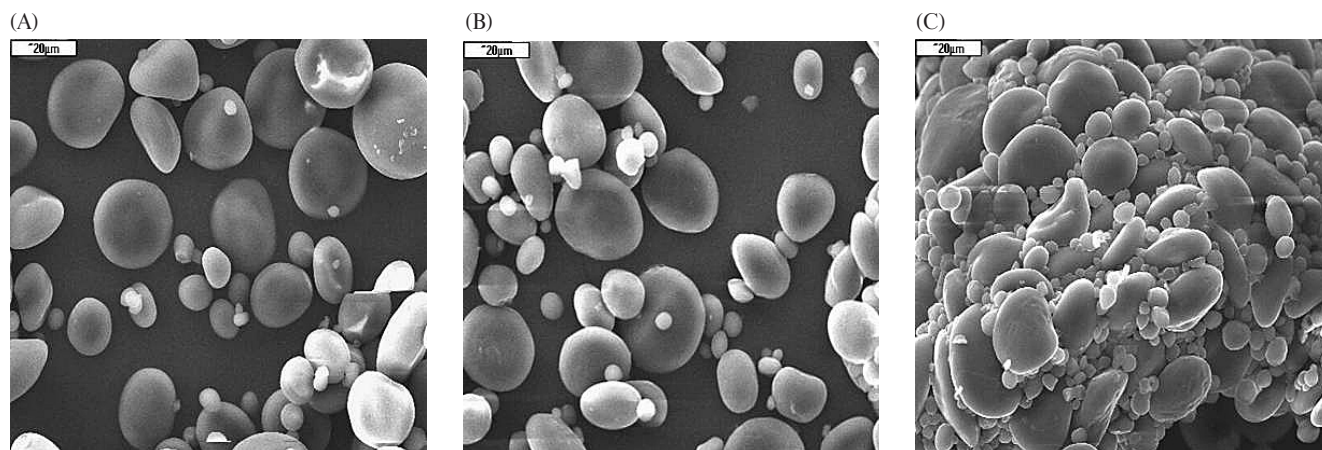


FIGURE 5. SEM image of wheat starch granules isolated at early-waxy stage of maturity (A), late-waxy stage of maturity (B) and full maturity (C) in 1998.

atmospheric conditions. Such a view is supported by very low viscosity of starch pastes [Gumul, 2002] obtained from mature rye, that sometimes behaved as newtonian fluids [Gambuś *et al.*, 2004]. Molecular weight of amylose after reaching the lowest values in the late-waxy stage of maturation significantly increased at full maturation. In the vegetative season of 1998 weight average molecular weight of amylopectin was constant from early-waxy till full maturation, while the highest \bar{M}_w of amylose was discovered at the late-waxy phase. Starch from mature rye contained amylose of medium size, its \bar{M}_w was significantly lower than in the late-waxy stage of maturation but significantly higher than at the early-waxy phase (Table 2).

Molecular weight of amylopectin in barley starch was constant in the whole studied period. It is in accordance with the earlier reports [Banks & Muir, 1980] that the molecular weight of barley amylopectin reaches the maximum 27 days after flowering (Table 2). The highest \bar{M}_w of amylose was found in the early-waxy stage of barley maturity, irrespective of the vegetative season. In the late-waxy stage of maturity it insignificantly decreased and then changed only a little (also insignificantly) at full maturation (Table 2).

The changes of molecular weight during grain maturation are reflected in the values of intrinsic viscosity of starch pastes (Tables 2 and 3). The relationship between viscosity average molecular weight (\bar{M}_v) calculated from Mark-Houwink equation and weight average molecular weight (\bar{M}_w) obtained from GPC result is as follows [Richter *et al.*, 1969]: $\bar{M}_v \leq \bar{M}_w$.

Thus the intrinsic viscosity, which is directly connected to (\bar{M}_v) correlates to (\bar{M}_w).

As it could be seen from the results presented in Table 3, during wheat grain maturation in both vegetative season the values of intrinsic viscosity increased, similarly to the \bar{M}_w of amylopectin (Table 2). The lowest intrinsic viscosity as well as \bar{M}_w was found for rye starch obtained at full grain maturation in 1997, probably partly depolymerized in the grain. In 1998 both intrinsic viscosity and \bar{M}_w of amylopectin were constant, irrespective of the stage of grain maturation.

Similar trends in intrinsic viscosity and \bar{M}_w of amylopectin were found also for barley starch in both vegetative seasons.

According to Abou-Guendia & D'Appolonia [1973],

TABLE 3. Intrinsic viscosity of starch dissolved in 1 mol/L KOH.

| Source of starch | Stage of grain maturity | Intrinsic viscosity (g/cm ³) ⁻¹ |
|------------------|-------------------------|--|
| 1997 | | |
| Wheat | early-waxy | 118 ^{a*} |
| | late-waxy | 129 ^b |
| | full | 167 ^c |
| Rye | early-waxy | 155 ^c |
| | late-waxy | 146 ^b |
| | full | 137 ^a |
| Barley | early-waxy | 163 ^a |
| | late-waxy | 158 ^a |
| | full | 171 ^{ab} |
| 1998 | | |
| Wheat | early-waxy | 145 ^a |
| | late-waxy | 159 ^b |
| | full | 163 ^c |
| Rye | early-waxy | 158 ^a |
| | late-waxy | 159 ^a |
| | full | 151 ^a |
| Barley | early-waxy | 146 ^a |
| | late-waxy | not estimated |
| | full | 152 ^a |

*The values denoted by different letters in columns for each vegetative season of every cereal differ significantly at a significance level of 0.05.

the values of intrinsic viscosity depend on the cereal to a little extent but change significantly at various stages of maturation and in different environments.

Irrespective of maturation stage, all the analysed starches contained structures denoted as mass and surface fractals [Cameron & Donald, 1992; Jenkins *et al.*, 1993; Pikus *et al.*, 2000; Sanderson *et al.*, 2006]. The dimensions of those fractals changed from early-waxy stage of maturity to full maturation (Table 4). With grain maturation the fractal dimensions of both fractal types increased. The increase of mass fractal

TABLE 4. Crystalline structure (as measured by SAXS) of starch isolated from immature cereals.

| Source of starch | Stage of grain maturity | Value of scattering curve α | | Reliability factor R | | Range of scattering vector q [$1/\text{\AA}$] | | L_s [\AA] | L_m [\AA] |
|------------------|-------------------------|------------------------------------|-------------------|----------------------|--------|---|-----------|------------------------|------------------------|
| | | s $\alpha=6-D_s$ | m $\alpha=D_m$ | s | m | s | m | s | m |
| 1997 | | | | | | | | | |
| Wheat | early-waxy | 3.9493 | 2.5897 | 0.9988 | 0.9972 | 0.001÷0.03 | 0.03÷0.13 | ~3.3÷314 | ~7.7÷104 |
| | full | 3.9635 | 2.6735 | 0.9969 | 0.9936 | 0.098÷0.03 | 0.03÷0.15 | ~3.3÷320 | ~6.6÷104 |
| 1998 | | | | | | | | | |
| Wheat | early-waxy | 3.7151 | 2.6394 | 0.9988 | 0.9918 | 0.011÷0.03 | 0.03÷0.15 | ~3.3÷285 | ~6.6÷104 |
| | full | 3.9165 | 2.7517 | 0.9988 | 0.9906 | 0.010÷0.03 | 0.03÷0.14 | ~3.3÷314 | ~7.1÷104 |
| Rye | early-waxy | – | 2.3802 | – | 0.9800 | – | 0.03÷0.15 | – | ~6.6÷104 |
| | full | 3.8649 | 2.3941 | 0.9790 | 0.9733 | 0.011÷0.03 | 0.03÷0.14 | ~3.3÷295 | ~7.1÷104 |
| Barley | early-waxy | 3.7514 | 2.8112 | 0.9977 | 0.9912 | 0.010÷0.035 | 0.03÷0.15 | ~28.5÷314 | ~6.6÷104 |
| | full | 3.8074 | 2.8112 | 0.9974 | 0.9912 | 0.010÷0.03 | 0.03÷0.15 | ~3.3÷285 | ~6.6÷104 |

s – surface fractal, m – mass fractal, L_s – diameter of surface fractal, L_m – diameter of mass fractal, D_s – dimension of surface fractal, D_m – dimension of mass fractal

dimensions ($\alpha=D_m$, Table 4) may be attributed to the increase of molecular weight of amylopectin (Table 2), which involves the creation of new branches in this polymer [Pikus *et al.*, 2000; Pikus 2005]. The increase in surface fractal dimensions (D_s) may reflect the stepwise folding of starch granule surface corresponding to higher branching in the peripheral regions of starch granules [Jane, 1996; Pikus *et al.*, 2000; Pikus 2005].

The approximate fractal diameters may be calculated by Schmidt criterion [Schmidt, 1991]:

$$\frac{1}{q_{\max}} < Ls < Lm < \frac{1}{q_{\min}}$$

where: q_{\max} - upper value of scattering modulus, q_{\min} - lower value of scattering modulus, Ls - surface fractal diameter, and Lm - mass fractal diameter.

Because the range of fractal diameters is similar for “immature” and “mature” samples (Table 4) it seems, that the differences in X-ray scattering between these starch samples are caused not only by the size of molecules, but also by varying space arrangement of amylopectin and amylose. This in turn results from the changes in starch polymers and non-starch components of the granules, such as lipids and proteins, which could change the interactions between amylose and amylopectin and impact the formation of crystalline regions of starch granules. Protein content of starch granules was slightly changing over grain development, generally decreasing with time (Table 5). An increasing trend was observed for the content of phosphorus, of which 90% is bound with lipids (phospholipids) (Table 5) [Acker & Becker, 1971].

CONCLUSIONS

1. During maturation of wheat, rye and barley grain, *i.e.* in early-waxy, late-waxy and full maturity stages, there is a change in molecular weight (\bar{M}_w) of amylose and amylopectin that depends on species of cereal and starch granularity.

2. Intrinsic viscosity of pastes, prepared from starch iso-

TABLE 5. Starch granularity and non-starch components in starches derived at different stages of grain maturity.

| Source of starch | Stage of grain maturity | Granularity of starch <10 μm | Content of protein in starch (%d.m.) | Content of phospholipids in starch (%d.m.) |
|------------------|-------------------------|---|--------------------------------------|--|
| 1997 | | | | |
| Wheat | early-waxy | 10.28** | 0.20 ^a | 0.039 ^a |
| | full | 15.01 ^b | 0.19 ^a | 0.052 ^b |
| 1998 | | | | |
| Wheat | early-waxy | 13.52 ^a | 0.20 ^{ab} | 0.050 ^a |
| | full | 23.89 ^b | 0.17 ^a | 0.059 ^b |
| Rye | early-waxy | 6.90 ^a | 0.18 ^b | 0.025 ^a |
| | full | 14.00 ^b | 0.10 ^a | 0.028 ^{ab} |
| Barley | early-waxy | 14.88 ^a | 0.22 ^a | 0.059 ^a |
| | full | 14.93 ^a | 0.22 ^a | 0.075 ^b |

*The values denoted by different letters in columns for each vegetative season of every cereal differ significantly at a significance level of 0.05.

lated in various stages of grain development, depends on molecular weight of amylopectin, irrespective of cereal species.

3. Irrespective of maturation stage, all studied starch granules displayed properties characteristic for mass and surface fractals.

4. Fractal dimension of structures present in developing starch granules increased with molecular weight of amylopectin, while the diameter of the observed mass and surface structures remained almost constant in the whole period.

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