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ELECTROPHORETIC AND DSC STUDIES ON DIFFERENT WHEAT STARCHES WITH VARIOUS AMYLOSE CONTENTS

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Granule-bound starch synthase and the structural and thermodynamic properties of the isolated starches were compared using amylopectin (2.1% amylose content), normal (20.5–25.1% amylose content) and high amylose (≥39.5% amylose) wheat cultivars of Japanese, Italian and Russian selections. Amylopectin (2.1% amylose content), normal (20.5–25.1% amylose content) and high amylose (amylose rich containing ≥39.5% amylose) wheat cultivars were investigated using an electrophoretic technique to examine starch-granule bound synthase. High-sensitivity differential scanning microcalorimetry was used to examine the structural and thermodynamic properties of extracted wheat starches. *Wx-B1* protein was not present in normal and even in high amylose cultivars except for the normal amylose cultivar *Bilancia*. An increase in amylose content in starches is generally accompanied by a decrease both in the melting enthalpy and the melting temperatures of crystalline lamella, whilst the polymorphous structure of starches remains invariable (A-type). The melting cooperative unit, the thickness and the surface free energy, enthalpy and entropy of crystalline lamella were also determined. Classification of wheat starches with different amylose contents is offered.

INTRODUCTION

Starch is a complex biopolymer made up of two different glucan chains, amylose, an essentially linear polymer of glucose residues linked via α-1,4 linkages, and amylopectin, a branched α-1,4:α-1,6 D-glucan polymer. The relative amounts of amylose and amylopectin give starches their unique physical and chemical properties.

There are three main origins for the production of native starches and starch products in Europe, namely: maize, wheat and potato [Swinkels, 1985; Rope, 2000]. Starches extracted from various cultivars of maize are widely used in different branches of industry [Rope, 2000] due to diversity in their functional properties, depending, particularly, on the amylose/amylopectin ratio in starch granules [Swinkels, 1985; Rope, 2000; Friedman *et al.*, 1993; Zobel, 1988]. However, new cultivars of winter wheat containing either amylopectin or high amylose starches have been recently selected [Bocharnikova *et al.*, 2003; Chao *et al.*, 1989; Demeke *et al.*, 1999; Graybosch, 1998; Hayakawa *et al.*, 1997; Kiseleva *et al.*, 2004]. Intensive investigations into granule-bound starch synthases (GBSS I), structure, thermodynamic and functional properties of wheat starches have so far led to important results [Bocharnikova *et al.*, 2003; Chao *et al.*, 1989; Demeke *et al.*, 1999; Graybosch, 1998; Hayakawa *et al.*, 1997; Kiseleva *et al.*, 2004; Mangalika *et al.*, 2003; Miura & Tanii, 1994; Miura & Sugawara, 1996; Nakamura *et al.*, 1993, 1995; Wasserman *et al.*, 2004; Yasui *et al.*, 1996, 1997; Yoo & Jane, 2002a, b;Yuryev *et al.*, 2004].

Granule-bound starch synthase (GBSS I) also known as "*waxy*" protein, is the key enzyme for amylose synthesis [Graybosch, 1998]. In bread wheat GBSS is encoded by three *waxy* loci -, *Wx-A1*, *Wx-D1*, and *Wx-B1* – located on the short arms of chromosomes 7A and 7D and on the long arm of chromosome 4A, respectively [Chao *et al.*, 1989]. By the purification and the electrophoretic separation of wheat GBSS, the identification of the protein products of the three *Wx* loci was possible [Nakamura *et al.*, 1993], thus showing the existence of GBSS I allelism among wheat accessions and allowing the identification of *waxy* mutants. Wheat lines carrying one or two GBSS null alleles are lacking one or two GBSS isoforms, respectively, produce starch with reduced amylose content and are designated "partial-*waxy*", whereas the loss of all three GBSS isoforms gives rise to amylose-free starch, referred to as *waxy*. Wheat lines carrying one or two GBSS null alleles are lacking one or two GBSS I isoforms, respectively, produce starch with reduced amylose content and are designated "partial-*waxy*", whereas the loss of all three GBSS I isoforms gives rise to amy-

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lose-free starch, referred to as *waxy*. The effect of each locus on amylose production is different, the highest potency being shown by *Wx-B1*, followed by *Wx-D1* and *Wx-A1 A1* [Miura & Tanii, 1994; Miura & Sugawara, 1996]. At present, intensive investigations into granule-bound starch synthases (GBSS I), structure, thermodynamic and functional properties of wheat starches are in progress worldwide [Bocharnikova *et al.*, 2003; Chao *et al.*, 1989; Demeke *et al.*, 1999; Graybosch, 1998; Hayakawa *et al.*, 1997; Kiseleva *et al.*, 2004; Mangalika *et al.*, 2003; Miura & Tanii, 1994; Miura & Sugawara, 1996; Nakamura *et al.*, 1995; Wasserman *et al.*, 2004; Yasui *et al.*, 1996, 1997; Yoo & Jane, 2002a, b; Yuryev *et al.*, 2004], but Russian wheat cultivars with a 40%–45% amylose content [Bocharnikova *et al.*, 2003; Yuryev *et al.*, 2003] have not yet been examined with electrophoretic investigations.

It is known that an increase in amylose content in wheat starches is accompanied by changes of structural and thermodynamic properties. For example, as amylose content in wheat starches is increased from 1.5% to 39.5%, the following effects have been observed: (i) an increase in the average thickness of semi-crystalline growth rings, (ii) a decrease in the melting temperature of crystalline lamella, and (iii) the accumulation of defects in the granular structure [Bocharnikova *et al.*, 2003; Mangalika *et al.*, 2003; Yasui *et al.*, 1996]. However, these conclusions were tentatively drawn from investigations carried out with limited amount of wheat starches and therefore the may not be of general validity for every kind of wheat cultivars. A further aspect to investigate is the potential relationship between genetic characteristics of wheat cultivars with different amylose contents and structural and thermodynamic properties of the relevant starches.

The present paper concerns these subjects and reports the results of electrophoretic investigations of GBSS proteins from wheat cultivars with different amylose contents, and data on the structural and thermodynamic properties of the corresponding starches.

MATERIALS AND METHODS

MATERIALS

Plant materials

Wheat cultivars of Russian selection. "*Bulava*" cultivar was selected by means of chemical mutagenesis, using ethylene-imine as a mutagen, whilst wheat of "*Moskovskaja nizkostebel'naja*" was selected by traditional breeding. These wheats were grown in Central Russia (Moscow's regions) in the 2000 season. Both "*Bulava*" and "*Moskovskaja nizkostebel'naja*" cultivars are winter wheat.

Wheat cultivars of Italian selection. "*Nobel*", "*Serio*", "*Veronese*", "*Valledoro*", "*Marzuolo*" and "*Bilancia*" derived from different breeding programmes by public and private companies. With the exception of *Serio* and *Bilancia*, the other cultivars were selected more than twenty years ago, while *Marzuolo* is a local variety and is the only spring wheat.

Wheat cultivar of Japanese selection. Near-isogenic perfect *waxy* type of the spring wheat "*Chinese Spring*" developed through a double-haploid method [Mangalika *et al.*, 2003] was used in the study. The amylopectin wheat was grown at the National Agricultural Research Center for Hokkaido Region, Hokkaido, the northernmost island of Japan, in the 2001 season.

Starch preparation and determination of amylose content. Native starches from wheat samples except for amylopectin wheat were extracted according to Richter *et al.* [1968]. The extraction method of native starch from amylopectin wheat has recently been described in the work of Mangalika *et al.* [2003].

Amylose content of starch samples of "*Bulava*" and "*Moskovskaja nizkostebel'naja*" was determined using a spectrophotometric method described previously [Yuryev *et al.*, 2003]. Amylose content of starches from "*Nobel*", "*Serio*", "*Veronese*", "*Valledoro*", "*Marzuolo*", "*Bilancia*" and amylopectin wheat was determined by using the enzymatic kit supplied by Megazyme International Ireland Ltd. (Bray Business Park, Co Wicklow, Ireland). The Megazyme method was adapted for the determination of amylose content in the case of amylopectin wheat.

METHODS

Electrophoresis. GBSS I was extracted from distal halves of single grains according to Zhao & Sharp [1996] (with minor modifications) and examined with SDS-PAGE electrophoresis on 15% polyacrylamide gel.

High-sensitivity differential scanning microcalorimetry. Calorimetric investigations of starch dispersions in water (0.3% dry matter, sample volume 0.5 cm^3 in sealed cells) were performed using a high sensitivity differential scanning microcalorimeter DASM-4 (Puschino, Russia) from 10–120°C with a heating rate of 2 K/min and excess pressure of 2.5 bar. Distilled water was used as a reference material. The heat capacity scale was calibrated using the Joule-Lenz effect for each run. Corrections for dynamic lag and residence of the samples in calorimetric cell were not necessary under conditions used [Danilenko *et al.*, 1994; Andreev *et al.*, 1999].

The average values of the thermodynamic parameters were determined as described elsewhere [Andreev *et al.*, 1999; Bocharnikova *et al.*, 2003; Danilenko *et al.*, 1994; Matveev *et al.*, 2001], using five measurements at 95% significance level and converted to a dimension per mole anhydroglucose unit (162 g/mol).

Values for vant Hoff enthalpy (ΔH^{vH}) were calculated according to Andreev *et al.* [1999], Danilenko *et al.* [1994], and Matveev *et al.* [2001].

Values for the melting cooperative unit (ν) and the thickness of crystalline lamellae ($L_{crl.}$) for starches with symmetry DSC-trace were calculated according to Andreev *et al.* [1999], Bocharnikova *et al.* [2003], Danilenko *et al.* [1994], and Matveev *et al.* [2001], and are presented in equations 1 and 2 as follows:

$$\nu = (\Delta H^{vH})/(\Delta H_m) \quad (1)$$

where ΔH_m is the experimental melting enthalpy of crystalline lamellae;

$$L_{crl.}=0.35\nu \quad (2)$$

where, according to Gernat *et al.* [1993], there is pitch height of 0.35 nm per anhydroglucose residue in double helix.

To calculate the thermodynamic parameters characterising surface of face sides of crystalline lamellae of the starches, symmetrical DSC endotherms were used applying the Thomson-Gibbs' equation (3) [Bershtein & Egorov, 1994; Bocharnikova *et al.*, 2003]:

$$T_m=T^o_m[1-2\gamma_i/(\Delta H^o_m\rho_{crl}L_{crl})] \quad (3)$$

where T^o_m and ΔH^o_m are the melting temperature and the melting enthalpy respectively of a hypothetical crystal with unlimited size (a perfect crystal), γ_i is the free surface energy of face sides of crystalline lamellae, while ρ_{crl} and L_{crl} are respectively the density and the thickness of the crystal. Also, the parameter qi which is the surface enthalpy of crystalline lamellae, can be calculated from equations (4–5):

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

and

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

Since the values of the melting temperature (T^o_m) and the melting enthalpy (ΔH^o_m) for a perfect crystal are not available, calculations were performed assuming the values of T^o_m (366.5 K) and the ΔH^o_m (35.5 J/g) for A-type spherulytic crystals [Whittam *et al.*, 1991]. In addition, density, ρ_{crl}, of A-type structures (1.48 g/cm) [Whittam *et al.*, 1991] and L_{crl}, ΔH_m and T_m values of the investigated starches were used.

RESULTS AND DISCUSSION

Electrophoresis

Amylose content of all starches investigated is reported in Table 1, together with the allelic composition at the loci coding for the granule-bound starch synthase. At first approach the starches can be subdivided into three types, namely: amylopectin (2.1% amylose), normal (20.5%–25.1% amylose) and high amylose (≥39.5% amylose), although, as shown below, such classification is rather rough.

Functional GBSS I alleles at all the three loci (Wx-A1*a*, Wx-B1*a*, Wx-D1*a*) were present only in cultivar *Bilancia* (Figure 1). All the other cultivars, although having the null Wx-B1*b* allele (Figure 1, Table 1), show the same or even higher content of amylose. In particular, the very high amylose content of *Bulava* and *Moskovskaja nizkostebel'naja* could be explained by: (1) the presence of an unknown, till now, allele at one of the *Wx* loci. This allele controlling the amylose synthase with increased activity has been probably induced by *Agropyron glauca* present in the pedigree of both cultivars; (2) one of the *Wx* loci has been duplicated as a result of unequal crossing-over at crossing of *Agropyron glauca* with wheat or through some other mechanism followed by higher yield of amylose synthase.

Unfortunately, the enzymatic protein has been investigated only by SDS electrophoresis; therefore a definite answer about the exact mechanism of the process is not yet affordable, additional investigations being still necessary.

High-sensitivity differential scanning microcalorimetry

Original DSC-curves related to the melting of 0.3% aqueous dispersions (Figure 2) show the typical endothermic transitions [Andreev *et al.*, 1999; Bocharnikova *et al.*, 2003; Kiseleva *et al.*, 2004; Yasui *et al.*, 1996; Yuryev *et al.*, 2002b; Zobel, 1988]. The low-temperature endotherm is attributed to the melting of the crystalline lamellae, while the high temperature peak is ascribed to the dissociation of the amylose-lipid complexes and/or the melting of single-helical V_h-type crystallites. Because of the low amylose content of amylopectin wheat, the second transition is (almost) absent for this type of starch. All the thermodynamic melting parameters related to both crystalline lamella and amylose-lipid complexes (Table 2), generally are in agreement with previously published data [Andreev *et al.*, 1999; Bocharnikova *et al.*, 2003; Yasui *et al.*, 1996; Yuryev *et al.*,

TABLE 1. Amylose content (%) and allelic composition at the GBSS I loci of the cultivars under investigation. (*a* is a functional allele, *b* is a null allele not functional).

Wheat cultivar	Amylose content (%)	Allelic composition		
		Wx-A1	*Wx-B1*	*Wx-D1*
Amylopectin	2.1	b	b	b
Marzuolo	20.5	a	b	a
Valledoro	21.8	a	b	a
Veronese	23.6	a	b	a
Bilancia	23.9	a	a	a
Nobel	25.0	a	b	a
Serio	25.0	a	b	a
Bulava	39.5	a	b	a
Moskovskaja nizkostebel'naja	45.0	a	b	a

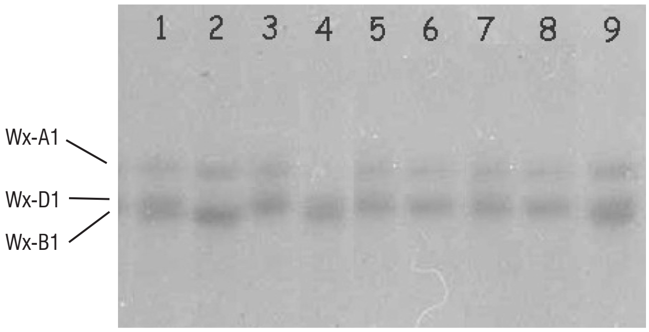

FIGURE 1. SDS-PAGE patterns of GBSS extracted from bread wheat samples. The loci coding for the isoforms are reported on the left. Lanes 1 to 4: bread wheat markers for *wild type* (Wx-A1*a*, Wx-B1*a*, Wx-D1*a*) and *partial waxy* at Wx-D (Wx-A1*a*, Wx-B1*a*, Wx-D1*b*), Wx- (Wx-A1*a*, Wx-B1*b*, Wx-D1*a*) and Wx-A (Wx-A1*b*, Wx-1*a*, Wx-D1*a*) loci, respectively. Lanes 5 to 9: cvs *Nobel*, *Veronese*, *Bulava*, *Moskovskaja nizkostebel'naja* and *Bilancia*.

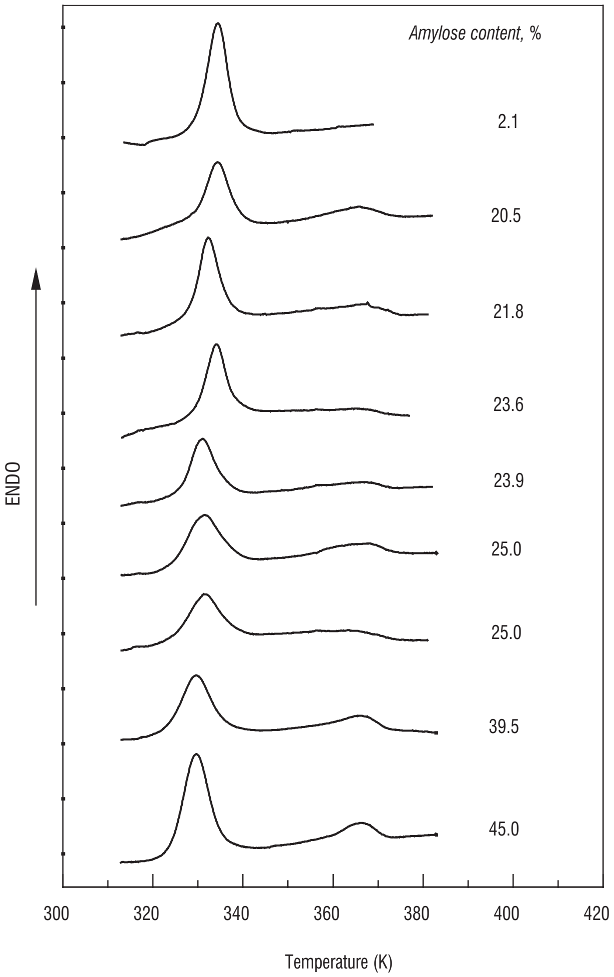

FIGURE 2. DSC-traces of wheat starches with different amylose contents.

2002b], although melting enthalpy of amylose-lipid complexes of starches from *Marzuolo*, *Nobel* and *Bilancia* cultivars slightly exceed the corresponding values for the other wheat starches [Andreev *et al.*, 1999; Bocharnikova *et al.*, 2003].

Except from *Marzuolo* and *Moskovskaya nizkostebel'naya* starches, the decreasing trend of the melting enthalpy of crystalline lamella observed with an increasing amylose content (Table 2) might be related to the decreased crystallinity [Bocharnikova *et al.*, 2003]. Similar trends, *i.e.* decreasing the melting thermodynamic parameters with an increasing amylose content, were reported for the crystalline lamellae of the other investigated wheat starches (Table 2). According to the current interpretation of the fusion of semi-crystalline synthetic polymers and starches [Bershtein & Egorov, 1994; Yuryev *et al.*, 2002a; Yuryev & Wasserman, 2003], a lower melting temperature can be related to changes of three parameters (equation 3), namely: polymorphous structure of crystal, thickness and free energy of the surface of face side of crystals.

According to X-ray data for wheat starches containing from 1.5% to 39.5% of amylose, the A-type polymorphous structure remains unchanged irrespective of the original wheat cultivar [Bocharnikova *et al.*, 2003; Graybosch, 1998]. Taking into account that passing from normal to high amylose content, A-type lattice structures are replaced by the C- [Wang *et al.*, 1998; Zobel, 1988], and the (B+B*)- [Yuryev *et al.*, 2002b] or C (A+B) [Gerard *et al.*, 1999] or B-types [Zobel, 1988], for maize and pea starches, respectively (one is observed at amylose content approximately 50% [Gerard *et al.*, 1999; Wang *et al.*, 1998; Yuryev *et al.*, 2002b; Zobel, 1988]), the changes could be expected also for wheat starch containing 45% of amylose.

It is well known that in the presence of 0.6 mol/L KCl the melting temperature of A-type starches increases by 10–12 K as compared to starch suspensions in pure water, whilst for B-type starches, the changes of the melting temperature are in the range of 2–4 K [Wang *et al.*, 1998]. Therefore an estimation of the polymorphous structure of wheat starch containing 45% of amylose can be attained by comparing the values of T_{crl} for starch suspensions in pure water and 0.6 mol/L aqueous solution of KCl (Figure 3). As it can be seen in Figure 3, the relevant differences of T_{crl} for 45% amylose wheat starch are about 9.8 K. This means that 45% amylose wheat starch should have an A-type structure similarly to the other wheat starches with different amylose contents [Zobel, 1988; Graybosch, 1998; Bocharnikova *et al.*, 2003; Eliasson & Larsson, 1993].

TABLE 2. Thermodynamic melting parameters of crystalline lamellae (melting temperature ($T_{m\ crl}$), melting enthalpy (ΔH_{crl}), the van't Hoff's enthalpy ($\Delta H^{V\,h}$), the melting cooperative unit (ν) and lamellear thickness (L_{crl}) of crystalline lamellae) and amylose-lipid complexes (melting temperature of amylose-lipid complexes (T_{aml}) and melting enthalpy (ΔH_{amc}) of amylose-lipid complexes) in wheat starches with different amylose contents.

Wheat varieties	Amylose content (%)	$T_{m\ crl}$ (K)	ΔH_{crl} (kJ/mol)[1]	$\Delta H^{V\,h}$ (kJ/mol)	T_{aml} (K)	ΔH_{amc} (kJ/mol)	ν anhydro-glucoses residue	L_{crl} (nm)
Amylopectin	2.1	334.5	3.1± 0.3	40.5	-	-	13.2	4.6
Marzuolo	20.5	332.0	3.3± 0.3	34.3	367.4	1.1	10.5	3.7
Valledoro	21.8	334.4	2.6± 0.2	29.3	367.2	0.8	11.4	4.0
Veronese	23.6	334.2	2.5± 0.2	30.3	367.0	0.4	12.0	4.1
Bilancia	23.9	330.9	2.3± 0.3	26.7	367.1	0.8	12.1	4.2
Nobel	25.0	331.4	2.4± 0.2	25.0	364.0	1.4	10.2	3.6
Serio	25.0	331.0	2.3± 0.2	23.9	362.4	0.7	10.2	3.6
Bulava	39.5	329.7	2.5 ± 0.3	28.6	366.8	0.4	12.1	4.2
Moskovskaya nizko-stebel'naya	45.0	329.5	2.8± 0.3	35.5	366.5	0.5	12.9	4.5
Mean							11.6±1.1	4.1±0.4

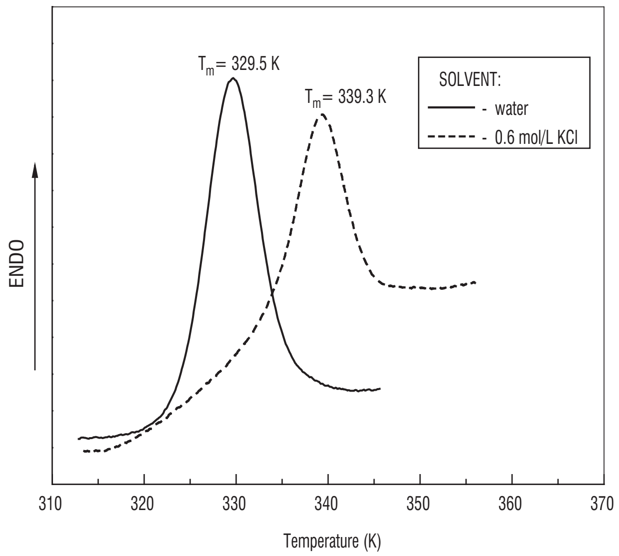

FIGURE 3. DSC-traces of wheat starch extracted from *Moskovskaya nizko-stebel'naya* cultivar. Endotherms correspond to the fusion of crystalline lamellae.

As it can be seen from Figure 2, an increase in amylose content up to 45% does not lead to changes in the symmetry of DSC peaks, which is in agreement with early published data concerning other wheat starches of different amylose contents [Bocharnikova *et al.*, 2003] This allows calculating the cooperative melting unit for the investigated starches and the thickness of their crystalline lamellae using the "two-state" melting model and equations 1,2. The calculation shows that the values of both the cooperative melting unit of starches and the thickness of crystalline lamella remain unchanged in spite of an increasing amylose content. Calculated average values of ν (11.6±1.1 anhydroglucose residues) and L_{crl} (4.1±0.4 nm) are closer to the corresponding ones of ν (13.3±1.6 anhydroglucose residues) and L_{crl} (4.7±0.5 nm) for wheat starches extracted from the other wheat cultivars [Bocharnikova *et al.*, 2003]. These (Table 3) and earlier published data [Bocharnikova *et al.*, 2003] enable the calculation of the average values of ν and L_{crl} for all wheat starches. These values are 12.1±0.3 anhydroglucose residues and 4.3±0.1 nm, correspondingly, irrespective of amylose content and type of cultivar.

Since on increasing amylose content, the polymorphous structure of wheat starches and thickness of crystalline lamella remain unchanged, according to the polymeric theory [Bershtein & Egorov, 1994] (equation 3) a major reason for the observed change of the melting temperature may be related to the change of the surface free of crystals (γ_i). The γ_i value is mainly governed by the surface entropy (s_i), which is in turn related to the concentration of structural defects (amylose tie-chains, molecular ordered chains, amylopectin B-chains) [Bershtein & Egorov, 1994; Wang *et al.*, 1998; Yuryev & Wasserman, 2003]. It is worth noting that a decrease in the s_i values has been observed [Bocharnikova *et al.*, 2003] for wheat starches with different amylose contents. The s_i values for the investigated wheat starches were calculated (Table 3) through equations 3–5 assuming the average L_{crl} value reported in Table 2. The overall trend of the experimental data indicates a decrease in s_i with an increasing amylose content of starch. However, the formation of defects in starches might be not proportional to the increase of amylose content sustained by biosynthesis since the investigations on the growth of crystal regions within starch granules of different potato cultivars undergoing ripening have shown that, in spite of the accumulation of amylose in the granules, the corresponding changes of the melting temperature are not correlated to the ripening time [Yuryev & Wasserman, 2003].

A better understanding of the overall correlation between the melting temperature of crystalline lamellae and amylose content of wheat starches comes from the data of this and previous investigations [Bocharnikova *et al.*, 2003], where the thermodynamic melting parameters of wheat starches were determined at low heating rate (2 K/min) and low starch concentration (0.3% dry matter), *i.e.* almost under quasi-equilibrium conditions [Yuryev *et al.*, 2002a]. At higher heating rates and concentrations the observed melting temperatures are higher because of the thermal lag of the instrument [Bershtein & Egorov, 1994; Whittam *et al.*, 1991] and seem to correlate with starch concentration.

The influence of amylose content on the melting temperature of wheat starches extracted from different cultivars is shown in Figure 4. With the exception of starches from cvs *Verones* and *Valledoro*, the changes of the melting temperature with the amylose content can be represented with the function (1) (second order exponential decay; R^2=0.965). Taking into consideration that: (i) as a rule, an increase in amylose content leads to the accumulation of amylose tie--chains (defects) in starches located in both crystalline and amorphous lamella [Yuryev *et al.*, 2002b; Yuryev & Wasser-

TABLE 3. The values of surface free energy (γ_i) (calculated from equation 3), enthalpy (q_i) (calculated from equation 4) and entropy (s_i) (calculated from equation 5) of crystalline lamellae in wheat starches with different amylose contents.

Wheat varieties	Amylose content (%)	γ_i (J cm^{-2} 10^7)	q_i (J cm^{-2} 10^7)	s_i (J cm^{-2}K^{-1} 10^7)
Amylopectin	2.1	9.40	40.02	0.092
Marzuolo	20.5	10.14	36.72	0.080
Valledoro	21.8	9.43	47.21	0.120
Veronese	23.6	9.49	48.71	0.117
Bilancia	23.9	10.46	51.71	0.125
Nobel	25.0	10.32	50.21	0.120
Serio	25.0	10.43	50.21	0.120
Bulava	39.6	10.81	48.71	0.115
Moskovskay nizko-stebel'naya	45.0	10.87	44.21	0.101

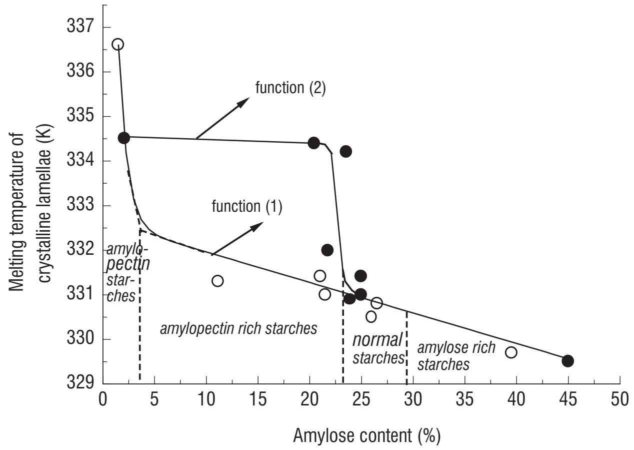

FIGURE 4. Melting temperature of crystalline lamellae for different wheat cultivars depending on amylose content. Dark points, present work; open points, from Bocharnikova *et al.* [2003]; functions (1) and (2) – see the text.

man, 2003], (ii) the fusion of the crystals begins from the locations of defects, and (iii) a larger content of defects leads to lower melting temperatures [Bershtein & Egorov, 1994], it can be disputed that, for the majority of wheat starches, an increase in amylose content may be really related to the accumulation of defects.

Since the melting temperature of starches is a function of environmental growing temperature, it can be supposed that the higher melting temperatures of wheat starches from cvs *Veronese* and *Valledoro* (as compared with the other starches containing more than 20% of amylose) can be due to a higher soil temperature during the maturation of *Veronese* and *Valledoro*. However, soil and temperature conditions for all the Italian selections were the same. Taking into consideration that the *waxy* starch of the Japanese selection and the *Veronese*, *Valledoro* starches have close melting temperatures, it can be disputed that some extra amylose is present, although not as defects in crystalline and amorphous lamellae. Accordingly, with the exception of 11.2%-amylose starch, function (2) can describe the correlation between melting temperature and amylose content (Figure 4).

The above considerations suggest that the formation of crystal structures within wheat starches with different amylose contents during starch biosynthesis (during maturation of plant) can follow various ways and, apparently being under environmental and genetic control.

Analyzing the functions (1) and (2) (Figure 4), the data relevant to 3.5% and 23.2% amylose deserve a special discussion. According to Swinkels [1985], starches with 24%–28% of amylose, *i.e.* comparable to starches from wild type plants, belong to the class of normal or regular starches, the differences between *waxy* and high-amylose starches being of minor relevance. High amylose wheat and barley starches containing up to 40%–42% of amylose and pea and maize starches containing up to 50%–80% of amylose belong to the same high-amylose class although differing from one another in many physical and physicochemical properties [Yuryev *et al.*, 2002b]. This view suggests a novel classification of starches. Starches containing less than 3.5% of amylose can be referred to as *waxy* starches. Starches with an amylose content ranging from 3.5 to 23.2% and from 23.2 to 28% can be referred to as amylopectin rich starches, and normal wheat starches, respectively.

Starches from *Bulava* and *Moskovskaja nizkostebel'naja*, which can accordingly be related to high amylose class, show a practically symmetric melting peak in the relevant DSC traces, just as high amylose barley starches that contain approximately the same amylose content [Yuryev *et al.*, 2002a, b], whilst the DSC traces of high amylose starches from maize and wrinkled pea with more than 50% of amylose show extraordinarily broad and asymmetric melting peaks [Friedman *et al.*, 1993; Gerard *et al.*, 1999; Matveev *et al.*, 2001; Yuryev *et al.*, 2002a; Yuryev *et al.*, 2002b; Zobel, 1988].

When passing from *waxy* (normal) to high amylose maize or pea starches, changes in the polymorphous structure and the thickness of crystalline lamellae [Gerard *et al.*, 1999; Jenkin & Donald, 1995; Matveev *et al.*, 2001; Yuryev *et al.*, 2002b; Zobel, 1988; Wang *et al.*, 1998] are observed; whereas wheat and barley starches have the same A-type polymorphous structure [Graybosch, 1998; Bocharnikova *et al.*, 2003; Vasanthan & Bhatty, 1996; Yuryev *et al.*, 2002a] and thickness of crystalline lamellae (Table 2) [Bocharnikova *et al.*, 2003]) in spite of their own amylose content. The data collected in the present work (Figure 2, Tables 2,3) complete together with those reported in previous papers on structural and thermodynamic properties of wheat, barley, maize and pea starches, and allow high amylose starches to be subdivided in two classes, namely:

(1) very high amylose starches (maize, pea with more than 50% of amylose) for which: (i) the melting peaks in the DSC-traces are asymmetric and very broad [Gerard *et al.*, 1999; Matveev *et al.*, 2001; Wang *et al.*, 1998; Yuryev *et al.*, 2002a, b; Zobel, 1988], (ii) the thickness of crystalline lamellae is in the range of 7.2–9.1 nm [Jenkin & Donald, 1995; Kozhevnikov *et al.*, 2001; Matveev *et al.*, 2001; Yuryev & Wasserman, 2003], (iii) the polymorphous structure of such starches is proposed to be of B- [Wang *et al.*, 1998; Zobel, 1988], or C (A+B)- [Gerard *et al.*, 1999], or (B+B*)–types [Kozhevnikov *et al.*, 2001; Matveev *et al.*, 2001; Yuryev *et al.*, 2002b], or B+V_h [Gernat *et al.*, 1993], *i.e.* a type polymorphous structure for such starches is under discusion till now;

(2) amylose rich starches (wheat, barley with an amylose content from 28–45%) for which: (i) the melting peaks in the DSC-traces are practically symmetrical, (ii) the thickness of crystalline lamellae is approximately 4.3 nm, and (iii) the same A-type polymorphous structure is found as in normal starches [Yuryev *et al.*, 2002a, b; Yuryev & Wasserman, 2003; Vasanthan & Bhatty, 1996].

CONCLUSION

Granule-bound starch synthase and the structural and thermodynamic properties of the isolated starches were compared using amylopectin (2.1% amylose content), normal (20.5–25.1% amylose content) and high amylose (≥39.5% amylose) wheat cultivars of Japanese, Italian and Russian selections. Amylopectin wheat lacked all the three loci (*Wx-A1*, *Wx-B1*, *Wx-D1*), while all the three loci were present in normal amylose cultivar *Bilancia*. All the other cultivars, including five normal amylose cultivars and two high amylose cultivars, lacked only *Wx-B1*. The high-sensitivity differential scanning microcalorimetry data demonstrated that the lower the melting enthalpy of crystalline lamella, the higher the amylose content in general. The results of T_{crl} in pure water and 0.6 mol/L KCl indicated that the polymorphous structure of high amylose wheat cultivar *Moskovskaja nizkostebel'naja* was A-type. There were no large differences in the melting cooperative unit and the thickness of crystalline lamella between amylopectin, normal amylose and high amylose starches. A general tendency for a decrease in the surface free entropy of crystalline lamella was not found with an increasing amylose content, suggesting that the formation of defects in starch is not proportional to the increase in amylose content.

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