Isolation and Characterisation of Starch from Different Barley and Oat Varieties

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Abstract

Šubarić D., Babić J., Lalić A., Ačkar Đ., Kopjar M. (2011): **Isolation and characterisation of starch from different barley and oat varieties**. Czech J. Food Sci., **29**: 354–360.

Starches were isolated from three oat (Dzoker, Zvolen and Sampionka) and three barley (Barun, Zlatko and Vanessa) cultivars. The gelatinisation and retrogradation characteristics, pasting properties, swelling power, and solubility of the isolated starches were analysed. The gelatinisation onset temperatures varied from 59.4°C to 61.4°C for the oat starches (OS), and from 58.4°C to 62.2°C for the barley starches (BS). BS displayed a higher retrogradation enthalpy (ΔH_r) than OS after 7 and 14 days storage at 4°C. OS-Sampionka had the lowest one while BS-Vanessa had the highest ΔH_r after 7 and 14 days of storage. Significant differences in pasting properties were observed between the OS and BS. OS showed higher values of maximum viscosity than BS and followed the order: OS-Dzoker > OS-Sampionka > OS-Zvolen > BS-Vanessa > BS-Zlatko > BS-Barun. The breakdown viscosities of BS were considerably lower than those of OS. OS had higher swelling power and solubility values than BS. The higher swelling and solubility values of the oat starches in conjunction with lower retrogradation suggest different applications of these starches. The results showed that, while the barley starches are suitable for such applications where high stability is needed during heating and shearing (low breakdown values), the oat starches have a great potential for the applications where high stability during storage is needed (low ΔH_r values). In addition, the barley starches are suitable for those applications where high water binding is undesirable, while the oat starches are applicable where low amounts of starch need to bind high proportions of water.

Keywords: oat; barley; isolation; starch properties

Oat and barley are ones of the major sources of cereal starch. In normal covered oat and barley grain, starch is the major constituent accounting for about 60% of dry matter, followed by total dietary fibre and protein with about 10%, respectively (Asp *et al.* 1992; Song & Jane 2000; Wang *et al.* 2007; Vigier *et al.* 2009). Fat, ash, and low molecular weight sugars are minor components constituting about 0.20% of dry matter, respectively (Andersson *et al.* 1999). Oats have recently attracted research and commercial attention mainly due to their high contents of β-glucan. The health effects of β-glucan are related to the cholesterol reduction, improved gastrointestinal function, and glucose metabolism (Pins & Kaur 2006). Barley grain is mainly used in the brewing and malting industries and for animal feeds.

The increasing demand for high-quality oat and barley as feed, food, and industrial raw material has led to the development of many different cultivars of oat and barley. Understandig the properties of their components is essential in order to achieve the desired properties of the finished product. It

Supported by the Ministry of Science, Education and Sports of the Republic of Croatia, Project No. 113-1130473-0571.

has been reported that oat and barley starches from different genotypes vary in chemical compositions and properties (Hoover & Vasanthan 1992; Oscarsson *et al.* 1997; Tester 1997). Starch is widely used in food industry as a thickening agent or stabiliser in order to provide some properties such as favourable texture and appearance. The functional properties of starches are important for their use in food products and industrial applications, especially the pasting properties and gelatinisation and retrogradation characteristics. Gelatinisation of starch is the phase transition of starch granules from an ordered to a disordered state during heating with excess water. It induces a number of changes in starch granules, such as swelling, exudation of amylose and amylopectin, granule disruption, loss of birefringence, and increased viscosity (Li *et al.* 2004; Šubarić *et al.* 2007; Babić *et al.* 2009b). The texture and acceptability of starch-containing foods correlates with the starch retrogradation which has been mainly described as the recrystallisation process of gelatinised starch. Retrogradation rate is affected by the ratio of amylose and amylopectin, molecular size, temperature, hydrocolloids, suga, and botanical sources (Aee *et al.* 1998; Babić *et al.* 2009b). The gelatinisation and retrogradation of starch have been intensively investigated with differential scanning calorimetry (DSC) (Sopade *et al.* 2004; Koo *et al.* 2005; Zhong & Sun 2005).

In this study, we isolated starches from three oat (Dzoker, Zvolen and Sampionka) and three barley (Barun, Zlatko and Vanessa) cultivars. The gelatinisation and retrogradation characteristics, pasting properties, swelling power, and solubility of the isolated starches were analysed.

MATERIAL AND METHODS

Three cultivars of oat – Dzoker, Zvolen and Sampionka and three cultivars of barley – Barun, Zlatko and Vanessa were provided by the Agricultural Institute Osijek.

Whole oat and barley kernels were milled with a blender (type Ike Werke M20), for 1.5 min at full speed and suspended in distilled water at a 1:2 (w/v) ratio. The pH was adjusted to 11 with 1M NaOH and the dispersion was stirred for 20 h with a propeller. After the incubation, the slurry was filtered through a cheese cloth with additional distilled water. The starch suspension was allowed to rest for 30 min and then the supernatant was decanted and centrifuged at 3000 rpm for 10 minutes. The upper greyish layer was removed with a spatula, the residual starch was suspended in distilled water and passed through a flour sieve to remove the coarse bran. The starch milk was centrifuged at 3000 min^{-1} for 10 min and the top greyish layer was scraped off. This step was repeated until the starch was visually clean. The residual starch was suspended in distilled water, neutralised with 1.0M HCl and centrifuged again. The resulting starch was air-dried overnight and than at 40–45°C for 7 hours.

The gelatinisation and retrogradation properties of the isolated starches were analysed using differential scanning calorimeter DSC 822e (Mettler Toledo, GmbH Im, Langacher, Switzerland) equipped with STARe software. The starch samples were weighed into standard aluminium pans (40 µl) and distilled water was added with the help of a Hamilton microsyringe to achieve a starch:water suspension containing 65% water. The samples were hermetically sealed and equilibrated for 24 h at room temperature before the heat treatment in the DSC apparatus. The heating was performed at a rate of 10°C/min from 25°C to 95°C. After the heat treatment, the samples were cooled to 25°C and removed from the DSC. The starch gels were aged at 4°C and monitored for retrogradation after 7 days and 14 days. The retrogradation experiments were conducted at a heating rate of 10°C/min from 25°C to 95°C. The changes in enthalpy (ΔH in J/g of dry starch), onset temperature (T_o), peak temperature (T_p) and conclusion temperature (T_c) for gelatinisation and retrogradation were obtained from the exotherm DSC curves. The experiments were run in triplicates.

The pasting properties of the starch samples were determined using a Rapid Visco-Analyser (Model 803202, Brabender Gmbh & Co KG, Duisburg, Germany). Starch was suspended in water in 7% concentration (dry basis). 100 g of starch suspension was equilibrated at 50°C for 1 min, heated at 7.5°C/min to 92°C, held at 92°C for 15 min, cooled at 7.5°C/min to 50°C, and held at 50°C for 10 minutes. The experiments were run in triplicates.

Swelling power (SP) and solubility (SOL) were determined in triplicates, following the method of Babic *et al.* 2009. Starch dispersions (1%) were prepared by mixing starch with distilled water. The dispersions were heated at 65°C, 75°C, 85°C, and 95°C for 30 min in a temperature controlled

shaking water bath with constant shaking. After the heating, the samples were centrifuged at 4000 rpm for 30 minutes. The precipitated paste was separated from the supernatant and weighed (W_p). The supernatant was dried at 120°C for 4 h and weighed (W_s). The SOL is the percentage of dry mass of the solubles in the supernatant to the dry mass of the whole starch sample (W_0).

$$SOL = (W_s/W_0) \times 100 \quad (\%)$$

SP is the ratio of the weight of the swollen starch granules after centrifugation (g) to their dry mass (g).

$$SP = W_p/(W_0 - W_s) \quad (g/g)$$

The experimental data were analysed by analysis of variance (ANOVA) and Fisher's least significant difference (LSD) with significance defined at $P < 0.05$. All statistical analyses were carried out using software program STATISTICA 7 (StatSoft, Inc., Tulsa, USA).

RESULTS AND DISCUSSION

Differential scanning calorimetry (DSC) thermal properties of oat (OS) and barley (BS) starch suspensions (35%) are shown in Table 1. The gelatinisation onset temperatures varied from 59.4°C to 61.4°C for OS, and from 58.4°C to 62.2°C for BS. The gelatinisation peak temperatures of the oat starches were higher than those of the barley starches. Among the OS, cv. Sampionka had the highest value of gelatinisation enthalpy (ΔH_g = 10.15 J/g) followed by cv. Zvolen (9.35 J/g), while it was the lowest for cv. Djoker (7.88 J/g). For BS, ΔH_g were in range 8.25–8.66 J/g and followed the order: cv. Zlatko (8.25 J/g) < cv. Vanessa (8.45 J/g) < cv. Barun (8.66 J/g). These differences could be explained by the differences in the amylose contents present in the starches, total molecular dimensions, and internal mobilities as well as by the proportion of long chains in the amylopectin structure (BeMiller & Whistler 2009).

It has been reported that retrogradation consists of two separable processes. The first stage is governed by the gelation of amylose solubilised during gelatinisation, and the second stage is induced by the recrystallisation of amylopectin within the gelatinised granules. The enthalpy of recrystallised starch melting is lower than that of gelatinisation, in agreement with the fact that the melting of recrystallised starch during storage is always easier than the melting of native starch granules (Duran *et al.* 2001). The retrogradation ratio increased with time and at lower temperature (Babic *et al.* 2006). The results for the retrogradation parameters of oat (OS) and barley (BS) starch gels are shown in Table 2. Barley starches displayed a higher retrogradation enthalpy (ΔH_r) than oat starches after 7 days and 14 days storage at 4°C. The retrogradation enthalpies of BS varied from 3.36 J/g to 3.82 J/g for the storage period of 7 days, and from 4.24 J/g to 4.54 J/g for the storage period of 14 days. OS-Sampionka had the lowest ΔH_r (2.3 J/g after 7 days and 3.76 J/g after 14 days), while BS-Vanessa had the highest ΔH_r (3.83 J/g after 7 days and 4.54 J/g after 14 days) after 7 and 14 days of storage. The retrogradation enthalpy values of the OS samples after 7 days and 14 days of storage followed the order: Sampionka < Dzoker < Zvolen. These differences might be related to the sometimes unusually high lipid and protein contents in the starch, which may form complexes with these components, causing changes in the

Table 1. DSC gelatinisation properties of oat and barley starch (35%, w/w, db)

	T_o (°C)	T_p (°C)	T_e (°C)	ΔH_g (J/g)
OS-Dzoker	60.3 ± 0.26^b	64.1 ± 0.19^a	68.7 ± 0.05^a	7.88 ± 0.21^a
OS-Zvolen	61.4 ± 0.18^c	64.9 ± 0.06^b	69.9 ± 0.13^b	9.35 ± 0.17^b
OS-Sampionka	59.4 ± 0.33^a	64.3 ± 0.13^a	70.3 ± 0.34^b	10.15 ± 0.14^c
BS-Barun	61.5 ± 0.24^b	63.6 ± 0.38^b	66.7 ± 0.09^b	8.66 ± 0.14^b
BS-Zlatko	58.4 ± 0.19^a	61.1 ± 0.07^a	64.6 ± 0.23^a	8.25 ± 0.16^a
BS-Vanessa	62.2 ± 0.06^c	64.6 ± 0.16^c	67.8 ± 0.09^c	8.45 ± 0.03^{ab}

OS – oat starch; BS – barley starch; T_o –onset temperature; T_p – peak temperature; T_c – conclusion temperature; ΔH_g – gelatinisation enthalpy. Values in the same column with different superscripts (a–c) are significantly different ($P < 0.05$)

Table 2. Retrogradation properties of oat and barley starch starch gels after 7 days and 14 days at 4°C

	T_o (°C)	T_p (°C)	T_e (°C)	ΔH_r (J/g)
Retrogradation after 7 days at 4°C (n = 3)				
OS-Dzoker	41.3 ± 0.15^a	52.0 ± 0.30^b	60.3 ± 0.23^a	2.50 ± 0.09^c
OS-Zvolen	41.3 ± 0.24^a	51.0 ± 0.24^a	61.0 ± 0.27	4.09 ± 0.09^b
OS-Sampionka	41.4 ± 0.08^a	51.9 ± 0.35^b	60.2 ± 0.23^a	2.30 ± 0.07^a
BS-Barun	41.9 ± 0.13^a	51.3 ± 0.37^a	59.3 ± 0.10^a	3.36 ± 0.03^a
BS-Zlatko	42.3 ± 0.27^b	52.7 ± 0.35^c	60.8 ± 0.33^c	3.81 ± 0.07^b
BS-Vanessa	41.9 ± 0.18^a	52.0 ± 0.42^b	60.0 ± 0.29^b	3.83 ± 0.03^b
Retrogradation after 14 days at 4°C (n = 3)				
OS-Dzoker	41.0 ± 0.15^{ab}	50.6 ± 0.30^b	60.3 ± 0.23^a	4.39 ± 0.09^b
OS-Zvolen	40.8 ± 0.24^a	50.3 ± 0.23	60.0 ± 0.27^a	4.46 ± 0.08^b
OS-Sampionka	41.1 ± 0.08^b	51.6 ± 0.35^c	60.1 ± 0.22^a	3.76 ± 0.08^a
BS-Barun	41.2 ± 0.06^a	^{a}50.5 ± 0.37	60.2 ± 0.03^a	4.52 ± 0.01^b
BS-Zlatko	41.1 ± 0.01^a	^{a}50.5 ± 0.36	60.1 ± 0.23^a	4.24 ± 0.08^a
BS-Vanessa	41.2 ± 0.22^a	^{b}50.9 ± 0.26^b	60.2 ± 0.19^a	4.54 ± 0.14^b

OS – oat starch; BS – barley starch; T_o – onset temperature; T_p – peak temperature; T_c – conclusion temperature; ΔH_r – retrogradation enthalpy. Values in the same column with different superscripts (a–c) are significantly different ($P < 0.05$)

transition temperature and increments in the retrogradation enthalpy (Frederikson *et al.* 1998). The onset, peak, and conclusion temperatures of recrystallised starch melting were similar for all samples after 7 days and 14 days storage at 4°C. The onset temperatures were in range 41.0–42.3°C, peak temperatures 51.0–52.7°C, and conclusion temperatures 59.3–61.0°C. The retogradation temperatures for all samples were lower than the gelatinisation temperatures which is due to the formation of small or imperfect crystals during retrogradation (Paredes *et al.* 1994; Yuan *et al.* 1993).

The RVA pasting properties of the oat and barley starches are presented in Table 3. Significant differences were observed between the oat and barley starches and between different varieties in their behaviour during heating and cooling in excess water. The pasting properties of starch are affected by the starch granule size, amylose and lipid contents, and amylopectin structure. Amylopectin is primarily responsible for granule swelling, whereas amylose and lipid restrict the swelling (Tester & Morrison 1990).

Oat starches (OS) had higher values of maximum viscosity (V_{max}) than the barley starches (BS): OS-Dzoker (910 BU), OS-Sampionka (797 BU), OS-Zvolen (787 BU), BS-Vanessa (568 BU), BS-Zlatko (445 BU) and BS-Barun (432 BU). The characteristic difference between the oat and barley starch varieties can be attributed to their amylose and phospholipid contents. Phospholipids could form helical complexes with amylose and restrict granule swelling to a lower peak viscosity at a substantially higher pasting temperature (Song & Jane 2000). The barley starches showed an increase in viscosity during the holding period at 92°C (20 min) which means that there was a starch granule fraction that was still swelling in this period. All starch samples showed an increase in viscosity during cooling down to 50°C, with respect to the holding period at 92°C. This increase is indicative of the tendency of starch to retrogradation. Among OS, cv. Dzoker had the highest viscosity at 50°C (1750 BU) followed by cv. Sampionka (1549 BU), while it was the lowest for cv. Zvolen (1068 BU). Viscosity for BS at 50°C followed the order: Zlatko (668 BU) < Barun (820) < Vanessa (887).

The breakdown viscosities of the BS were considerably lower than those of the OS: BS-Zlatko (3) BS-Vanessa (14), BS-Barun (18), OS-Zvolen (105), OS-Sampionka (344) and OS-Dzoker (570). The ability of starches to withstand heating at high temperature and shear stress is an important factor in many processes. High values of breakdown are associated with high peak viscosities, which in

Table 3. Pasting properties of oat and barley starches (7%, w/w, db)

	OS-Dzoker	OS-Zvolen	OS-Sampionka	BS-Barun	BS-Zlatko	BS-Vanessa
Pasting temperature (°C)	61.0 ± 0.3	62.3 ± 0.3	63.4 ± 0.4	71.9 ± 0.3	77.6 ± 0.4	73.2 ± 0.2
V_{max} (BU)	910 ± 9.0	787 ± 5.0	797 ± 1.5	432 ± 3.0	445 ± 3.5	568 ± 5.0
Viscosity at 92°C (BU)	444 ± 4.0	740 ± 6.0	742 ± 1.5	393 ± 2.5	346 ± 1.5	465 ± 3.5
Hold 20 min at 92°C (BU)	340 ± 3.0	681 ± 6.5	453 ± 2.0	414 ± 4.5	442 ± 5.0	554 ± 2.5
Viscosity at 50°C (BU)	1750 ± 9.5	1068 ± 7.0	1549 ± 5.5	820 ± 9.5	668 ± 8.00	887 ± 8.5
Hold 20 min at 50°C (BU)	1182 ± 9.5	941 ± 7.5	1374 ± 7.7	732 ± 3.0	645 ± 2.5	861 ± 9.5
Breakdown	570 ± 12.0	105 ± 11.0	344 ± 0.6	18 ± 0.6	3 ± 1.5	14 ± 0.6
Setback	1410 ± 12.5	386 ± 9.5	1089 ± 5.8	405 ± 11.1	225 ± 9.2	332 ± 8.1

OS – oat starch; BS – barley starch; V_{max} – maximum viscosity; Breakdown = V_{max} – viscosity at 92°C after 20 min; Setback = viscosity at 92°C after 20 min – viscosity at 50°C before holding. Values are means ± SD of triplicate

turn are related to the degree of the starch granules swelling during heating. The setback viscosity of OS was greater than that of BS. Hoover and Vasanthan (1992) explained that the setback values reflect the extent of water immobilisation around the charged centres of the starch components and those of free and helically complexed lipid molecules, rather than starch paste retrogradation. The association of water molecules with these charged centres would decrease the effective water concentration in the continuous phase, resulting in a rise in viscosity during the cooling cycle. The extent of water immobilisation (setback) may be greater in oat starches because of the presence of more charged centres (from the lipid) in the continuous phase (BeMiller & Whistler 2009).

Swelling power (SP) and solubility (SOL) of the oat (OS) and barley starches (BS) are presented in Table 4. When the temperature increased, all starches increased their SP and SOL values gradually, up to 95°C, as expected. The SP and SOL of the oat starches differed significantly at all temperatures measured. Among OS, cv. Dzoker had the highest values of SP and SOL at all temperatures measured, while these were the lowest for cv. Sampionka. For the Dzoker starch, the most important

Table 4. Swelling power (SP) and solubility (SOL) of oat and barley starches

Sample	65°C	75°C	85°C	95°C
		SP (g/g)		
OS-Dzoker	9.9 ± 0.27^{c}	22.0 ± 0.28^{c}	31.1 ± 0.32	31.5 ± 0.25^{c}
OS-Zvolen	8.7 ± 0.10^{b}	13.2 ± 0.24^{b}	19.9 ± 0.06^{b}	30.7 ± 0.37^{b}
OS-Sampionka	6.6 ± 0.14^{a}	8.3 ± 0.20^{a}	12.8 ± 0.11^{a}	24.0 ± 0.45^{a}
BS-Barun	7.3 ± 0.26^{a}	9.3 ± 0.34^{c}	12.6 ± 0.26^{b}	20.8 ± 0.29^{a}
BS-Zlatko	7.9 ± 0.29^{b}	8.9 ± 0.26^{b}	11.8 ± 0.18^{a}	21.6 ± 0.27^{b}
BS-Vanessa	6.7 ± 0.23^{c}	8.2 ± 0.16^{a}	11.5 ± 0.21^{a}	21.8 ± 0.31^{b}
		SOL (%)		
OS-Dzoker	3.9 ± 0.07^{c}	17.7 ± 0.44	35.0 ± 0.40^{c}	35.5 ± 0.38^{c}
OS-Zvolen	0.9 ± 0.09^{b}	3.9 ± 0.17^{b}	14.4 ± 0.28^{b}	33.5 ± 0.41^{b}
OS-Sampionka	0.3 ± 0.03^{a}	0.8 ± 0.16^{a}	7.0 ± 0.27^{a}	15.6 ± 0.32^{a}
BS-Barun	0.9 ± 0.25^{a}	6.8 ± 0.15^{b}	10.7 ± 0.38^{b}	29.7 ± 0.20^{b}
BS-Zlatko	2.1 ± 0.24^{b}	5.4 ± 0.05	10.2 ± 0.28^{a}	28.4 ± 0.37^{a}
BS-Vanessa	0.9 ± 0.14^{a}	6.2 ± 0.20	9.8 ± 0.21^{a}	29.3 ± 0.24^{b}

Values are means ± SD of duplicate; values in the same column with different superscripts (a–c) are significantly different ($P < 0.05$)

increase in SP and SOL (22.0 g water/g starch; 17.7%) was recorded already at 75°C, and the maximum SP and SOL (31.5 g water/g starch; 35.5%) occurred at 95°C. Among barley starches, SP and SOL values were similar for all varieties at the same temperature. The BS begin to swell gradually at 75°C and 85°C, and reach maximal value of SP and SOL at 95°C (in range 20.8–21.8 g water/g starch; 28.4–29.7%). These maximum SP data of the investigated starches are low if compared to the cassava starch; 60.7 g water/g starch (Babić *et al.* 2007), but higher compared to some maize varieties (13.7–20.7 g water/g starch) (Sandhu & Singh 2007).

The difference found in SP and SOL between the oat and barley starches could be due to the structural differences, because amylose-amylopectin content, chain length, and chain length distribution are important factors in this pattern. The swelling power of starch has also been reported to depend on the water holding capacity of starch molecules by hydrogen bonding (Lee & Osman 1991). Hydrogen bonds stabilising the structure of the double helices in crystallites are broken during gelatinisation and are replaced by the hydrogen bonds with water, and swelling is regulated by the crystallinity of the starch (Tester & Morrison 1990). Further research is needed to define the molecular structure of isolated starches, but so far, the results suggest a close relationship between solubility and structure.

CONCLUSIONS

The initial gelatinisation temperatures for different varieties of oat starch (OS) were in the range of 59.4–61.4°C, and for barley starch (BS) of 58.4–62.2°C. The barley starches had higher retrogradation enthalpies (ΔH_r) than the oat starches after 7 days and 14 days storage at 4°C. OS-Sampionka had the lowest, while BS-Vanessa had the highest ΔH_r after 7 days and 14 days of storage. Significant differences in the pasting properties were observed between the OS and BS. The oat starches had higher values of maximum viscosity than the barley starches. The breakdown viscosities of BS were considerably lower than those of OS. OS had higher swelling power and solubility values than BS. The higher swelling and solubility values of the oat starches in conjunction with lower retrogradation suggest different applications of these starches.

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Received for publication August 9, 2010
Accepted after corrections September 16, 2010

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