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STRUCTURE AND FUNCTIONALITY OF BARLEY STARCHES

Abstract

Amylose contents of prime starches from non-waxy (regular) and high-amylose barley determined by colorimetric method were 24.6% and 48.7% respectively, while the waxy starch showed only a trace (0.04%) of amylose. The isoamylase debranched amylopectin showed little difference between non-waxy and high-amylose barleys, while amylopectin from waxy barley had significantly higher percentage of fraction with degree of polymerization (DP) < 15 (45%).

The x-ray diffraction pattern of waxy starch differed from non-waxy and high-amylose. Waxy starch had sharper peaks at 0.58 nm, 0.51 nm, 0.49 nm and 0.38 nm than non-waxy and high-amylose starches. The *d*-spacing at 0.44 nm characterizing the amylose-lipids complex was the most evident for high-amylose starch but was not observed in waxy starch.

DSC thermograms of prime starch of non-waxy and high-amylose barleys exhibited two prominent transition peaks: one above 60°C corresponded to starch gelatinization, and the second above 100°C corresponded to the amylose-lipid complex. The starch from waxy barley had only one endothermic peak of gelatinization of amylopectin with an enthalpy value of 16.0 J/g.

The retrogradation of gelatinized starch of three types of barley stored at 4°C showed that amylopectin recrystallization rates of non-waxy and high-amylose barley were comparable when recrystallization enthalpy was calculated based on the percentage of amylopectin. No recrystallization peak of amylopectin was observed in waxy barley.

Storage time showed a strong influence on the recrystallization of amylopectin. The enthalpy value for non-waxy barley increased from 1.93 J/g after 24 hr of storage to 3.74 J/g after 120 hr. When gel was rescanned every 24 hr a significant decrease in enthalpy was recorded.

A highly statistically significant correlation (r = 0.991) between DSC values of retrograded starch of non-waxy barley and gel hardness was obtained. The correlation between starch enthalpy value and gel hardness of starch concentrate indicates that texture of gel was mainly due to its starch structure and functionality. The relationship between properties of starch and starch concentrate might favor the application of barley starch concentrate without the necessity of using the wet fractionation process.

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Introduction

Starch in barley is the largest single component, representing up to 65% of kernel dry weight and providing a valuable source of energy. Despite such availability of barley starch, relatively little research has been carried out on its functional properties, when compared to wheat or corn starches. Part of the reason for such neglect is the fact that a high proportion of barley grain is used in animal feed without any processing. Another reason could be the difficulty of isolating the starch from barley as a pure product by the wet process, which is complex, lengthy and requires a large amount of water. The high water-holding capacity of barley meal is primarily due to the presence of β-glucans, which absorb a lot of water and make isolation of starch by the wet process difficult.

Previous studies on barley starch have been carried out primarily on non-waxy types of barley cultivars in which starch contains about 25% amylose, while starches of two other types of barley, waxy and high-amylose, have attracted interest only recently (Czuchajowska et al 1992). During any food preparation, starch undergoes partial or complete gelatinization, as well as interacting with other food components (Czuchajowska and Smolinski 1997). Therefore, in order to promote greater utilization of barley in human consumption, research on thermal behavior of isolated starches and on flours of different types of barley must be conducted.

Well-documented results of this research will be of great value to the food industry in selecting the right type of barley for a specific product or process.

Therefore, the objectives of this study were:

- to evaluate the thermal behavior of starches isolated from non-waxy, waxy and high-amylose barleys;
- to examine the thermal behavior of flours differing in composition as the result of abrading; and,
- to study the retrogradation rate of amylopectin and the gel strength of three types of starch as compared to the strength of gel from starch concentrate.

Materials and methods

Samples

Three types of hull-less barley, non-waxy (cv. Glacier), high-amylose (cv. Glacier) and waxy (cv. Wanubet) were provided by Dr. C.W. Newman from Montana State University and Dr. S. Ullrich from Washington State University. The barley starch was isolated from three types of barley by wet process according to the methodology by Szczodrak and Pomeranz (1991).

Abrasion of barley samples

Barley was abraded using a Tangential Abrasive Dehulling Device (TADD, Venables Machine Works, Ltd., Saskatoon, Canada) to 10%, 20% and 40% of kernel weight.

Chemical analyses

Protein contents (N x 6.25) of samples were determined by a Leco nitrogen analyzer (Leco Corporation, St. Joseph, MI) equipped with a thermal conductivity detector. Moisture content was determined by oven drying for 1 hr at 130°C (AACC Method 44-15A, 1995), ash content by dry combustion for 16 hr at 580°C (AACC Method 08-01, 1995) and free lipids content by petroleum ether extraction (AACC Method 30-25, 1995). Starch content was determined according to Prosky et al 1988. Beta-glucans content was measured enzymatically, as described by Ahluwalia and Ellis (1984).

Characteristics of starch

Amylose content of prime starch was determined by colorimetric method (Knutson and Grove 1994) and by high-performance size-exclusion chromatography (HPSEC). The starch solubilization procedures for HPSEC for estimation of amylose content was carried out according to Bradbury and Bello (1993). The amylose content of starch was determined by HPSEC as the ratio between total peak area and the peak area corresponding to amylose (Kobayashi et al 1985). Isoamylase-debranched amylopectin was prepared according to the method described by Yuan et al (1993) and modified by Lin and Czuchajowska (1997). DP of the linear fractions in debranched amylopectin was calculated as MW/162 (Bradbury and Bello 1993). Solubilized starch (100 μL) or isoamylase-debranched starch solution was injected into a two-column HPSEC system (Bio-Sil SEC 125, 300 x 7.8 mm, Bio-Rad, Richmond, CA) using 30% DMSO as the mobile phase at a flow rate of 0.5 mL/min. The two-column system was preceded by a guard column (80 x 7.8 mm) and a 2 μm precolumn filter (A315, Upchurch Scientific, Oak Harbor, WA). The HPSEC system consisted of an autosampler (model 1050, Hewlett Packard, Wilmington, DE), a solvent-delivering system (model 2350 HPLC pump and model 2360 gradient programmer, ISCO, Inc., Lincoln, NE), a differential refractometer (R401, Waters Associates, Inc., Milford, MA) and a computer equipped with HPLC 3D ChemStation software (Hewlett Packard). The columns and detector (sensitivity at 32Tx) were maintained at a constant temperature (35°C). The HPSEC system was calibrated using four pullulan standards (Polymer Laboratories, Amherst, MA) with MW 112,000, 22,800, 5,900 and 738, respectively. A linear relationship ($r^2 = 0.992$) between log molecular weight and retention time was obtained.

Wide-angle x-ray diffraction of barley starch

X-ray diffraction patterns of the starches were recorded on a Siemens D 500 diffractometer (Madison, WI) operating at 35 kV, 30 mA. Diffractograms were obtained from 4° 2θ to 30° 2θ with a step of 0.05° 2θ, counting 4 sec on each step. Integrated normalized intensities were calculated on the basis of the number of counts recorded by the scintillation counter (Sievert et al 1991, Czuchajowska et al 1991).

Differential scanning calorimetry (DSC)

Thermal behavior of three types of barley starches and flours was followed by DSC, as described by Czuchajowska and Pomeranz (1989), on a Perkin-Elmer DSC-2 instrument (Perkin-Elmer Corporation, Norwalk, CT).

Experiment 1

In the first experiment 30 sub-samples of dry starch (10 mg) isolated from non-waxy and waxy barley were weighed into large pans, water was added (20 μL), and then the pans were sealed and scanned from 20°C to 180°C at a heating rate of 10°C/min. Next, the 30 pans were divided into six sets of five pans each and stored at 4°C. Each set was rescanned once after 24 hr, 48 hr, 72 hr, 96 hr and 120 hr intervals.

Experiment 2

In the second experiment, contrary to the first, six replications of non-waxy and waxy prime starch were scanned at every 24 hr period up to 120 hr. After each scanning the pans were stored at 4°C to evaluate the effect of repeated heating on the intensity of retrogradation of non-waxy and waxy barley starches.

Pasting properties of abraded barley

The pasting properties of the material were measured by Brabender Visco-Amylograph according to Shuey and Tipples (1980).

Hardness of gel prepared from prime starch and starch concentrate

The texture of gel representing 60% of the inner part of the barley kernel (starch concentrate) was measured using a TX-XT2 Texture Analyzer (Stable Micro System, Haslemeres, England). The gel was prepared in a Brabender Visco-Amylograph. The slurry of 10% starch or 10% flour was heated to 90°C and held for 20 min at that temperature. The hot paste was poured into molds (30 mm in diameter and 35 mm in height), covered to avoid evaporation of water and stored at 4°C for up to 120 hr. Texture of the gel was measured by using two attachments: a plexiglass plunger (12 mm diameter) or a disc (60 mm diameter).

Gel prepared from waxy barley was too weak to stand by itself. Therefore, to have comparable data, texture of gels prepared from non-waxy, high-amylose and waxy barleys was measured using the plexiglass plunger. The gels were penetrated once by the plunger to 30% of their height and the force of penetration recorded. Non-waxy and high-amylose barley gels were compressed by disc to 30% of the gel height. Hardness of gel was recorded. The texture of gels was measured during a 120 hr period in 24 hr intervals.

Statistical analysis

All tests were run at least in duplicate. Least significant difference (LSD), analysis of variance (ANOVA) and correlation analysis were performed using the Statistical Analysis System (SAS Institute, Cary, NC 1986).

Results and discussion

Effect of abrasion on composition of barley

The changes in barley composition due to abrasion at 10%, 20% and 40% are summarized in Table 1. Compared to whole kernel, protein content significantly decreased when the percentage of abrading was increased. A similar pattern was observed for all three types of barley. The largest change in protein content, a decrease of 4.2%,

Table 1

Composition of abraded barley

Type of Barley	Abrasion Level	Protein (%)	Ash (%)	Free Lipids (%)	Starch (%)	Total β-glucans
Regular	Whole kernel	13.6 a	2.11 a	2.60 a	67.6 d	6.2 ab
	10%	12.3 b	1.68 b	1.88 b	73.7 c	6.6 a
	20%	11.6 c	1.45 c	1.47 c	77.2 b	6.8 a
	40%	10.2 d	1.00 d	0.95 d	82.9 a	6.8 a
High-amylose	Whole kernel	12.5 a	2.05 a	2.16 a	65.2 d	5.6 c
	10%	10.3 b	1.62 b	1.62 b	71.0 c	6.4 b
	20%	9.9 c	1.36 c	1.18 c	77.8 b	6.5 b
	40%	8.3 d	0.92 d	0.80 d	81.0 a	7.1 a
Waxy	Whole kernel	15.5 a	2.61 a	2.65 a	65.6 d	6.60 cd
	10%	14.6 b	1.90 b	1.76 b	70.5 c	6.87 c
	20%	13.5 c	1.46 c	1.23 c	74.6 b	7.31 b
	40%	11.9 d	0.96 d	0.86 d	78.0 a	8.00 a

[a] Values with different letters in a column within each type of barley are significantly different at the 5% level.

took place in high-amylose barley at 40% abrading, compared to whole grain, while non-waxy and waxy barleys decreased in protein content by 3.4% and 3.6%, respectively, compared to whole grain. The ash and free lipids content decreased more than two times, due to the removal of 40% of the outer layer of the kernels. Contrary to protein, ash and free lipids, starch content increased by abrading. In the 40% abraded kernel starch content increased by 12.4% in waxy barley, and by more than 15% in non-waxy and high-amylose barleys. Similar changes in barley composition due to abrading were reported by Baik and Czuchajowska (1997). In all three types of barley β-glucans content was highest in the inner part when abraded at the 40% level.

Composition of isolated barley starches

The composition of purified prime starch is presented in Table 2. As indicated by average protein and ash contents of 0.5% and 0.2%, respectively, all three types of barley starches were of high purity. Beta-glucans were not detected in these starches. Independent of applied methodology, the highest amylose content was found in starch from high-amylose barley, followed by starches from non-waxy and waxy barleys. The amylose content determined by the colorimetric method in these three types of starches agreed with previous work by Czuchajowska et al (1992). The waxy starch had 0.04% amylose, while amylose content in high-amylose barley reached almost 50%. The prime starch from non-waxy barley had an amylose content of around 25%, which is close to most starch of wheat.

Table 2

Composition of prime starch from three types of barley

Type of Barley	Protein (%)	Ash (%)	Starch (%)	Amylose in Starch	
				HPSEC (%)	Iodometric (%)
Regular	0.56 b	0.21 b	97.4 b	32. 7 b	24.6 b
High-amylose	0.61 a	0.18 c	98.4 a	39.7 a	48.7 a
Waxy	0.35 c	0.23 a	97.8 b	7.4 c	0.04 c

[a] Values with different letters in a column are significantly different at the 5% level.

Fine structure of amylopectin

The distribution of average molecular weight of the branch chains of all three evaluated starches is summarized in Table 3. The average branch chain distribution of amylopectin showed little difference between non-waxy and high-amylose barleys. Waxy barley contained significantly more HMW and LMW, but less IMW fractions of debranched amylopectin than non-waxy and high-amylose barleys. Actual data were in agreement with those of MacGregor and Morgan (1984), in that HMW fraction of de-

branched amylopectin from waxy barley represented about 20% of the total relative peak area.

Table 3

Branch chain distribution of amylopectin debranched by isoamylase

Type of Barley	HMW[a] (%)	IMW (%)	LMW (%)
Regular	15.5 b[b]	47.5 a	37.0 b
High-amylose	16.3 b	46.4 a	37.4 b
Waxy	19.7 a	35.4 b	45.0 a

[a] HMW - Degree of Polymerization > 35: IMW - 35 < Degree of Polymerization < 15; LMW - Degree of Polymerization < 15.

[b] Values with different letters in a column are significantly different at the 5% level.

X-ray diffraction pattern of barley starches

All three barley starches exhibited the A-type x-ray diffraction pattern, as shown in Figure 1. A higher percent relative intensity (PRI) indicates a higher degree of crystallinity of starch. Major peaks of barley starches were observed around *d*-spacings 0.58 nm, 0.51 nm, 0.49 nm, 0.44 nm and 0.38 nm. Waxy barley starch differed from non-waxy and high-amylose barley by having sharper peaks at 0.58 nm, 0.51 nm, 0.49 nm and 0.38 nm. The *d*-spacing at 0.44 nm is characteristic of the amylose-lipid complex. No peak was observed in waxy starch at 0.44 nm, but high-amylose barley had the most evident peak of 0.44 nm of all three starches.

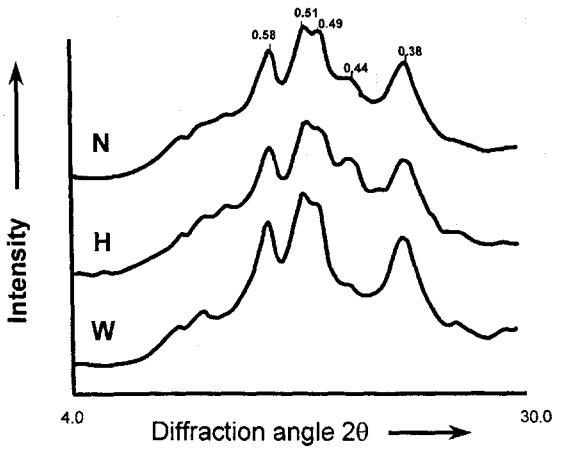

Fig. 1. X-ray diffraction patterns of barley starches. N, H and W indicate non-waxy, high-amylose and waxy starches. Numbers indicate the starch crystal *d*-spacings (2θ) of the major diffraction peaks.

Viscosity of abraded barley

The large changes in composition of barley kernels due to abrading (see Table 1) caused significant changes in thermal behavior of abraded barley. The increase in starch content due to abrading resulted in increased viscosity of all three types of barley. In non-waxy barley the viscosity increased from 680 BU of whole meal to 970 BU of flour from 40% abraded kernels (Table 4). This increase in viscosity was mainly due to an increase in starch content from 67.6% to 82.9%, since total β-glucans showed no significant changes due to abrading.

Table 4

Amylograph parameters of abraded barley

Type of Barley	Abrasion Level	Peak Temperature (°C)	Peak Viscosity (B.U.)
Regular	Whole kernels	85	680
	10%	85	860
	20%	85	930
	40%	85	970
High-amylose	Whole kernels	83	-
	10%	83	-
	20%	83	930
	40%	83	940
Waxy	Whole kernels	64	510
	10%	64	980
	20%	64	1380
	40%	65	1580

For high-amylose barley, viscosities were comparable to the non-waxy type. The largest changes in viscosity due to abrading took place in waxy barley. The viscosity of 40% abraded barley was three times higher than that of whole meal. It is interesting that this large increase in peak viscosity of all three types of barley was not accompanied by increases in peak temperature.

DSC thermograms of starch

DSC thermograms of starch gelatinization and recrystallization of the three types of barley during storage are presented in Figure 2A and 2B. Both non-waxy and high-amylose barleys exhibited two prominent transitions over similar temperature ranges. The first transition temperature above 60°C corresponded to endotherms of starch gelatinization. The second transition above 100°C corresponded to the amylose-lipid complex. The retrogradation of gelatinized starch of three types of barley stored at 4°C for 2 weeks is graphically presented in Figure 2B. The enthalpy values of retrogradated

amylopectin of non-waxy and high-amylose barley were comparable when the recrystallization was calculated based on the percentage of amylopectin. No recrystallization peak of amylopectin was found in waxy barley. This result may be explained by the presence of a high percentage of low degree polymerization of branches of waxy amylopectin determined by HPSEC of isoamylase debranched amylopectin (Table 3).

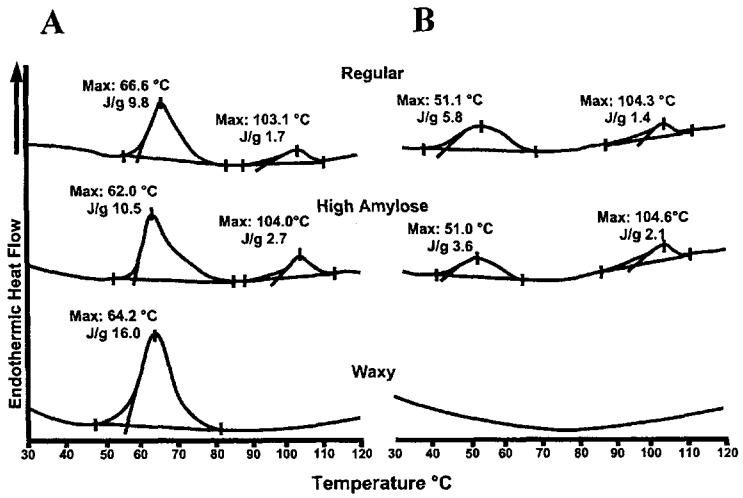

Fig. 2. DSC thermograms of prime starch from (non-waxy) regular, high-amylose and waxy barleys. A - first scan without storage; B - rescanned after two weeks of storage at 4°C.

Retrogradation of amylopectin – DSC study

The retrogradation rate of prime starch from non-waxy barley is graphically presented in Figure 3A. As indicated by the size of the enthalpy peak, it is clear that the retrogradation of amylopectin increases with storage time. After 120 hr, enthalpy of 3.74 J/g was recorded. It is interesting that the onset temperature of recrystallized amylopectin after 120 hr of storage was 38°C, almost 10°C lower than the onset of gelatinized starch. The lower temperature and lower enthalpy peak of recrystallized amylopectin indicates its less perfectly ordered structure. In waxy barley, again, the retrogradation of amylopectin did not occur during storage of the gel under the same conditions (Figure 3B). Since waxy starch did not show recrystallization enthalpy during storage in the first or second experiment, the changes in recrystallization due to rescanning of each gel 5 times are shown graphically only for starch of non-waxy barley (Figure 4A). Storage time showed a strong influence on the recrystallization of amylopectin (Figure 4A). The enthalpy value increased from 1.93 J/g after 24 hr to 3.74 J/g after 120 hr. However, a significant decrease in enthalpy was recorded when

gels were rescanned 5 times in 24 hr intervals (Figure 4B). These results indicate that not only is a certain amount of time needed to recrystallize amylopectin, but also that frequent melting can change the inner structure of amylopectin and delay retrogradation. This observation could be important to the food industry, because it may give processors an option to affect the texture of products by delaying recrystallization.

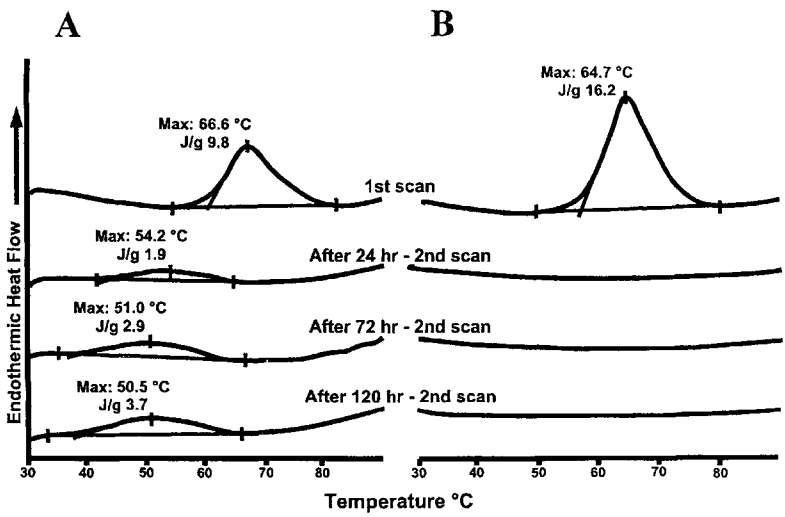

Fig. 3. DSC thermograms of starches from non-waxy (A) and waxy (B) barleys. Starches were scanned and rescanned a second time up to 120 hr at 4°C.

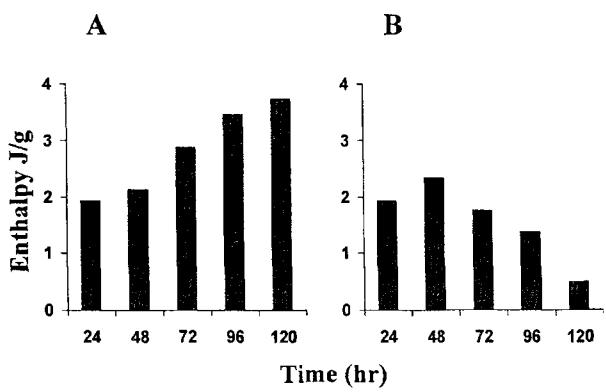

Fig. 4. Enthalpy values of retrograded starch from non-waxy barley. A - rescanned a second time after storage up to 120 hr at 4°C at 24 hr intervals. B - rescanned repeatedly at 24 hr intervals up to 120 hr.

Texture of gel

The effect of storage on texture of gels from prime starch and starch concentrate of three types of barley measured by plunger is graphically presented in Figure 5. The hardness of waxy starch gel was below 0.7 N and did not change during storage. Gels from non-waxy and high-amylose starch showed distinctly higher hardness than waxy starch, ranging from 5.8 N to 11.1 N (Figure 5A). The texture of gel from starch concentrate, also measured by plunger, showed a similar pattern, but slightly lower values (Figure 5B). Hardness of gel from waxy starch concentrate was below 0.6 N, while hardness of gels from non-waxy and high-amylose starch concentrate ranged from 5.2 N to 9.5 N. The hardness of gel from starch of non-waxy barley increased during storage from 4.1 N to 7.2 N, while hardness of gel from high-amylose starch increased from 6.6 N to 9.2 N. The higher relative value of gel hardness from high-amylose starch could be due to an almost 2 times higher amylose content in high-amylose starch than in non-waxy starch, as determined by colorimetric method. Hardness of gel prepared from prime starch and starch concentrate of non-waxy barley measured by disc are graphically presented in Figure 6. When the storage time was increased, the texture of gel increased significantly for both starch and starch concentrates. However, starch concentrate produced a softer gel due to the presence of other components. A statistically significant positive correlation was obtained between hardness storage time of gels prepared from starch and starch concentrate for non-waxy ($r = 0.997$) and for high-amylose barley ($r = 0.964$). The gradual increase in gel hardness (starch and starch concentrate) could be mainly due to retrogradation of amylopectin, especially since changes of gel hardness were greater for non-waxy than for high-amylose gel. Therefore, a relationship should exist between starch gel hardness and DSC enthalpy values during storage.

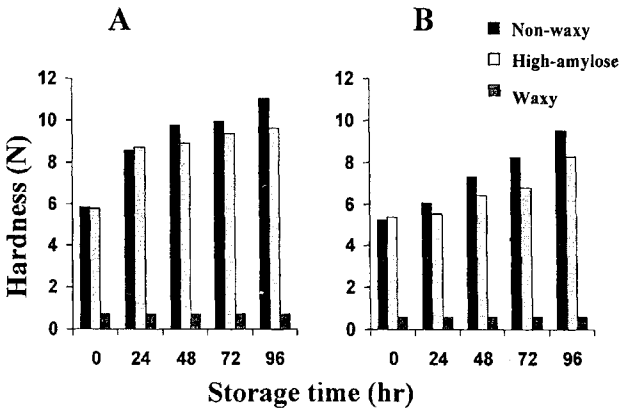

Fig. 5. Hardness of gels prepared from prime starch (A) and starch concentrate (B) measured using a TX-XT2 texture analyzer equipped with a plunger.

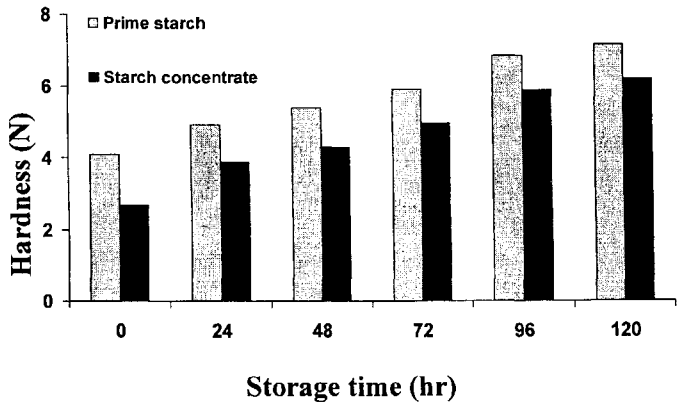

Fig. 6. Hardness of gels prepared from prime starch and starch concentrate of non-waxy barley measured using a TX-XT2 texture analyzer equipped with a disc.

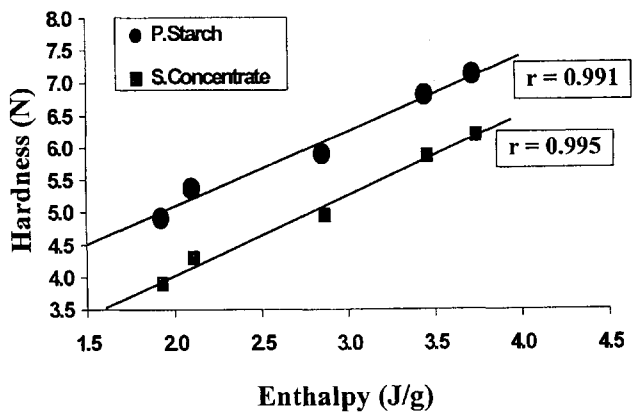

Fig. 7. Correlations between enthalpy value of prime starch and hardness of gels from prime starch and starch concentrate for non-waxy barley.

The correlations between DSC values (J/g) and gel hardness (N) for prime starch (r = 0.991) and starch concentrate (r = 0.995) of non-waxy barley are shown in Figure 7. The strong correlation between starch enthalpy values and hardness of starch concentrates indicates that the texture of starch gel concentrate is mainly due to its starch content, structure and functionality (Table I). The fact that this relationship appears for starch concentrate might be of particular importance for the food industry, because it would favor the application of barley starch concentrate without the necessity of using the wet fractionation process to isolate starch.

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SKŁAD, WŁAŚCIWOŚCI TERMICZNE I TEKSTURA ŻELU SKROBI SUPERIOR I SECUNDA Z FASOLI GARBANZO I GROCHU

Streszczenie

Otrzymano frakcje skrobi superior i secunda z fasoli garbanzo i grochu i określono ich właściwości fizyczne, termiczne oraz właściwości żelu z nich. W skrobi z fasoli garbanzo gałeczki o rozmiarach poniżej 35 μm stanowiły 85%, w skrobi grochowej gładkich odmian Latah 66,8% i SS Alaska tylko 18,4% ogólnej liczby gałeczek. W skrobi superior z garbanzo było 35,9% amylozy, w skrobiach grochowych powyżej wymienionych gładkich odmian amylozy było odpowiednio 44,5 i 48,8% a w skrobi z grochu pomarszczonej odmiany Scout amylozy było aż 86,0%. Skrobie secunda miały o co najmniej 8% więcej amylozy niż poprzednie. Endotermiczne entalpie dla skrobi z garbanzo i grochowych superior z odmian Latah i SS Alaska mieściły się w zakresie 12,1 do 14,2 J/g podczas gdy dla takiej frakcji ze skrobi Scout entalpia wynosiła zaledwie 1,1 J/g. Endotermiczna entalpia ze skanningowej kalorymetrii różnicowej i amylograficzne właściwości kleikujące skrobi superior wyraźnie zależały od zawartości amylozy ($P < 0,05$). Skrobie superior garbanzo i grochu odmian Latah i SS Alaska dawały bardzo zwarte, elastyczne żele. Skrobia superior z grochu odmiany Scout dawała mocne, lecz kruche żele. Ich twardość była wysoka wynosiła 21,8 N, a zwartość i sprężystość była niska (odpowiednio 0,29 i 0,82). Twardość żelu przechowywanego w 22 i 4°C wzrastała z zawartością amylozy w skrobi.