Starch and Enzyme-Resistant Starch from High-Amylose Barley

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ABSTRACT

A Glacier variety selection with a 43% amylose content was used for isolation and purification of barley starch. The starch was separated into two fractions that varied in granule size, and the two fractions were assayed using chemical, microscopic (scanning electron microscopy), and thermoanalytical methods. Large and small barley starch granules were different in both chemical composition and endothermic properties; the small starch granules were higher in amylose than the large granules. Heat-moisture treatment (autoclaving at 121°C) with subsequent cooling was used to produce amylase-resistant starch (RS) from purified high-amylose starch samples. The formation of RS in barley starch was strongly affected by the number of autoclaving-cooling cycles; increasing the number of cycles from one to 20 raised the RS yield from 6 to 26%. Differential scanning calorimetry thermograms showed that all isolated RS preparations exhibited an endothermic transition over a similar temperature range (116–177°C), with a mean peak temperature at 158°C, which could apparently be attributed to the melting of RS amylose crystallites. The maximum melting enthalpy for RS from barley, 37 J/g, was achieved by 12 repeated autoclaving-cooling cycles. The thermodynamic data indicated that changes in the quality of RS occurred during autoclaving-cooling cycles.

Barley is the fourth most important cereal (after wheat, rice, and maize), consisting of about 12% of the world's total cereal production (Mac Key 1981). It is mainly used as a feed grain but also finds substantial application in the brewing and distilling industries, especially in the manufacture of malt, and in the food industry. Owing to its nutritional and chemical properties, in particular a high dietary fiber (DF) content and a high proportion of soluble viscous DF components, barley is considered the most suitable grain in the human diet (Munck 1981, Björck et al 1990). Recent clinical studies have also demonstrated the hypoglycemic and hypocholesterolemic function of barley β-glucans in human feeding trials (Anderson 1980, Newman et al 1989).

Starch, the major reserve storage polysaccharide of barley, occurs in large lenticular and small spherical granules. The starch type can vary in certain genotypes from regular (75% amylopectin, 25% amylose), to high-amylose (about 50% amylose), to waxy (up to 100% amylopectin) (Pomeranz 1985, 1987; Hoseney 1986). During the isolation of barley starch, the small granules have a tendency to associate with the protein fraction (brown layer), which appears on top of centrifuged suspensions of crude starch. The lower, white layer obtained after centrifugation contains mostly the large starch granules. McDonald and Stark (1988) pointed out that discarding of the brown layer (widely applied in procedures for isolation of barley starch) results in severe loss of small granules and affects the ratio of small to large granules. The authors suggested that the brown layer should be purified separately and then added back to the white layer to give representative starch granule preparations.

Recently, much interest has been focused on a particular type of starch that is undigestible in vitro and in vivo, i.e., resistant starch (RS) (Englyst and Cummings 1987, Sievert and Pomeranz 1989, Siljeström et al 1989). This type of amylase-resistant starch is formed in food products processed by methods that use relatively high moisture contents, such as cooking, baking, and autoclaving (Englyst et al 1983, Siljeström and Asp 1985). The reduced bioavailability of RS in the human gastrointestinal tract has particular significance to diabetics because it lowers their insulin demand (Jenkins et al 1983). Moreover, malabsorbed starch reaching the colon can exert physiological effects similar to those of DF (Björck et al 1987, Jenkins et al 1987, Schneeman 1989). Examination of RS by enzymatic methods (Berry 1986), iodine-binding capacity (Ring et al 1988), column permeation chromatography (Berry et al 1988, Siljeström et al 1989), differential scanning calorimetry (Sievert and Pomeranz 1989, 1990), and X-ray diffraction (Sievert et al 1991) indicates that retrograded amylose is mainly responsible for the generation of RS and that RS represents noncovalently bonded crystallites within the amylose component of starch. The observation that the RS yield of autoclaved starch suspensions increased with increasing amylose content (Sievert and Pomeranz 1989) also supports this hypothesis. The presence of interchain amylose associations in the RS fraction was demonstrated by an endothermic transition at about 155°C in differential scanning calorimetry thermograms (Sievert and Pomeranz 1989, 1990). In addition to amylose crystallites, crude RS preparations contain native starch lipids and protein originating from added enzymes used in the isolation procedure (Russell et al 1989). It also appears that lipids in the RS fraction may be in a noncomplexed form and may adhere to the undigested starch matrix (Sievert and Pomeranz 1990).

The hypothesis that the amylose-amylopectin ratio is an important determinant of RS formation focused interest on high-amylose varieties of cereals as potential sources for RS production. Most of the earlier studies (with the exception of the one by Björck et al 1990) concentrated on the formation of RS from wheat and amylomaize starches. The objectives of this study were to reevaluate various factors that contribute to formation of RS; to isolate and characterize small and large starch granules from high-amylose barley and produce RS from the isolated starches; and to characterize the RS by enzymatic, microscopic (scanning electron microscopy [SEM]), and thermodynamic methods.

MATERIALS AND METHODS

Chemicals

Amyloglucosidase, A-3042, from *Aspergillus niger*; protease, P-5147, type XIV, from *Streptomyces griseus*; protease P-5380, Type VIII, from *Bacillus licheniformis*; peroxidase, P-6782, type VI-A, from horseradish; glucose oxidase, G-6766, from *A. niger*, and *o*-dianisidine, D-3252, were purchased from Sigma Chemical Co., St. Louis, MO. Heat-stable α-amylase, Takalite L-340, from *B. licheniformis*, was obtained from Miles Laboratories, Inc., Elkhart, IN.

Barley

A covered barley selection of cv. Glacier, high in amylose, from the 1988/89 crop was obtained from S. E. Ullrich, Agronomy Department, Washington State University. Crude flour from the barley contained 54.5% starch (dry matter [dm] basis), of which 43.1% was amylose; 15.7% crude protein (N × 6.25); 3.0% ash; and 3.4% lipids.

Isolation of Starch

Starch was isolated from the grains by a modification of the method of McDonald and Stark (1988). Barley kernels were cracked lightly by passing them through a sample mill (Tecator,

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Cemotec 1090) and dehulled in an air-aspirated grain cleaner (Kice-DT4, Kice Metal Products Co., Inc., Wichita, KS). After steeping in 0.02*M* HCl and neutralizing with 0.2*M* NaOH, the pearled material was rubbed gently in a mortar with water and the resulting slurry was successively sieved through 130- and 73-μm polypropylene screens. The residue was homogenized in a Waring Blendor with water; the mixture was screened; and the process was repeated three to four times. The lower, white layer obtained on centrifugation (1,700 $\times$ *g* for 20 min) of barley starch suspensions was purified six times by the toluene shaking procedure (McDonald and Stark 1988). The pigmented fractions (proteinaceous brown layer, tailings) on top of the starch were pooled and purified three times by protease XIV (5 mg/g of starch in 30 ml of incubation mixture) and six times by the toluene shaking procedure. The separated starch layers, crude flour, crude brown layer, and residue after starch isolation were used for further characterization.

Enzyme-Resistant Starch

The formation, isolation, and determination of RS were performed according to the methods developed by Sievert and Pomeranz (1989). The following modifications were introduced: the starch-water ratio used for RS formation was 1:5. The suspensions were autoclaved for 1 hr at 121°C and cooled overnight in a refrigerator (4°C). The treated samples were vacuum-dried. RS was estimated by an enzymatic-gravimetric assay using a heat-stable bacterial α-amylase and fungal amyloglucosidase. In another series of experiments, a procedure was added between the digestion of sample with amylase and amyloglucosidase. Protease VIII (0.2 ml, 10 mg per sample) was added, and the sample was

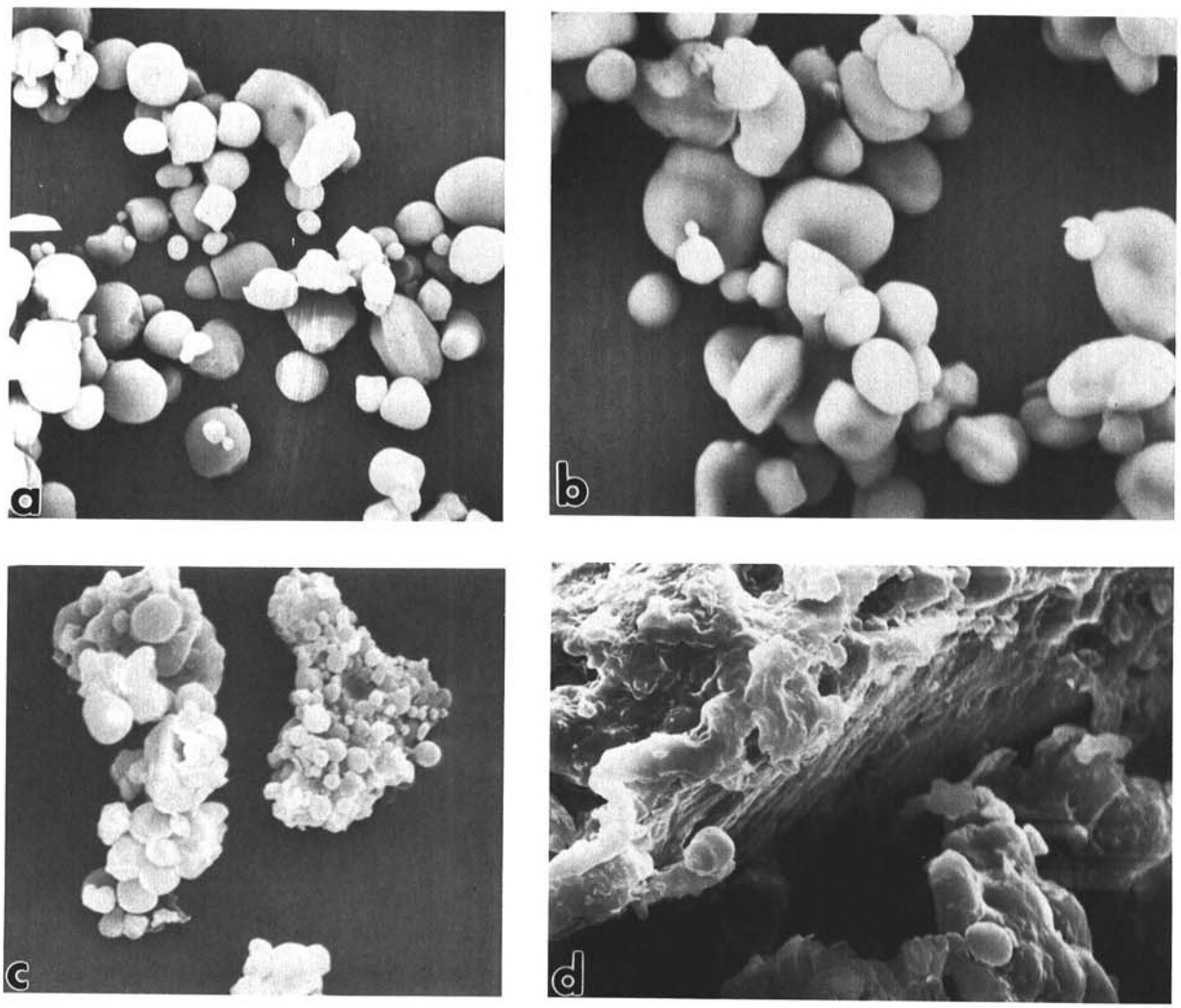

Fig. 1. Scanning electron micrographs of the purified brown barley starch layer (**a**), the purified white barley starch layer (**b**), the crude brown barley layer (**c**), and the residue after starch isolation (**d**).

TABLE I
Yield and Chemical Composition (% dry matter) of Starchy Materials Isolated from Covered Glacier High-Amylose Barley

Material	Yield	Crude Protein	Starch	Amylose (% of starch)	Ash	Free Lipids
Crude flour	100.0	15.7	54.5	43.1	3.0	3.4
Purified white layer[a]	18.0	0.9	97.8	44.5	0.2	0.5
Crude brown layer	20.7	16.8	78.0	46.4	0.5	2.0
Purified brown layer[b]	9.4	0.8	97.0	47.9	0.3	0.6
Residue after starch isolation	49.8	17.8	32.1	41.0	1.7	4.9

[a] Purified six times using toluene extraction.
[b] Purified three times using protease and six times using toluene extraction.

incubated at 60°C for 30 min (pH 7.5). After hydrolysis, the enzymatic extracts were centrifuged (2,200 × *g* for 10 min) and the supernatant was discarded. The insoluble residue (crude RS) was then washed four times using hot water (60°C) and autoclaved for 20 min at 121°C to inactivate the residual enzymes. Thereafter, a series of five washings using hot water (90°C) were performed to remove digested nonresistant starch and other by-products of the enzyme digestion process. The resulting purified RS was vacuum-dried, weighed, finely ground, and used for further characterization.

TABLE II
Differential Scanning Calorimetry of Starchy Materials Isolated from Covered Glacier High-Amylose Barley[a]

	Transition Temperatures[b] (T, °C)[c] and Transition Enthalpies (ΔH, J/g dry matter)[d]							
	Starch Gelatinization Transition				Amylose-Lipid Complex Transition			
Material	T_i	T_p	T_c	ΔH	T_i	T_p	T_c	ΔH
Crude flour	62.1	72.8	82.9	2.3	86.8	97.1	107.3	1.6
Purified white layer	54.5	66.6	82.3	11.0	90.2	103.8	112.4	3.1
Crude brown layer	56.5	70.3	82.1	5.4	90.4	102.5	112.4	2.7
Purified brown layer	56.2	66.6	81.7	10.2	87.2	103.2	111.4	4.2
Residue after starch isolation	63.6	75.9	86.8	0.9	90.2	99.6	110.8	0.7

[a] Values are examples of three determinations.
[b] T_i, T_p, and T_c = initial, peak, and completion transition temperatures, respectively.
[c] SD < 1.0°C, n = 3.
[d] SD < 10% of the mean, n = 3.

TABLE III
Effects of Autoclaving-Cooling Cycles on Enzymatic (Resistant Starch Yields) and Thermal Characteristics of a Purified White Starch Layer Isolated from Covered Glacier High-Amylose Barley[a]

Number of Autoclaving-Cooling Cycles	Resistant Starch Yield (%)	Transition Temperatures (T, °C) and Transition Enthalpies (ΔH, J/g dry matter)							
		First Transition				Second Transition (Resistant Starch)			
		T_i	T_p	T_c	ΔH	T_i	T_p	T_c	ΔH
0[b]	0.0	54.5	66.6	82.3	11.0	nd[f]	nd	nd	nd
0[c]	4.1	34.8	56.0	64.8	6.8	128.9	146.6	167.4	17.9
0[d]	2.4	39.1	54.5	63.3	4.3	130.3	143.7	169.7	13.3
0[e]	0.9	30.0	44.5	62.8	3.0	106.8	138.2	167.0	9.6
1	7.8	43.2	58.3	65.7	2.4	122.3	153.6	174.0	22.8
2	10.0	45.3	55.6	62.7	1.1	123.0	158.4	173.1	26.2
4	14.2	48.2	56.5	65.2	0.7	127.4	160.8	174.6	32.6
12	23.2	43.3	54.3	61.3	0.6	126.6	159.1	176.8	37.4
20	25.9	43.5	52.6	59.3	0.6	120.4	158.9	175.4	37.8

[a] Thermal characteristics determined by differential scanning calorimetry. Enthalpy values refer to vacuum-dried resistant starch. T_i, T_p, and T_c = initial, peak, and completion transition temperatures, respectively.
[b] Native pure starch.
[c] Pure starch dried under vacuum and treated with amylases.
[d] Pure starch treated with amylases (without preliminary vacuum drying).
[e] Pure starch treated with amylases and protease (without preliminary vacuum drying).
[f] None detected. See also Table II.

TABLE IV
Effects of Autoclaving-Cooling Cycles on Enzymatic (Resistant Starch Yields) and Thermal Characteristics of a Purified Brown Starch Layer Isolated from Covered Glacier High-Amylose Barley[a]

Number of Autoclaving-Cooling Cycles	Resistant Starch Yield (%)	Transition Temperatures[b] (T, °C) and Transition Enthalpies (ΔH, J/g dry matter)							
		First Transition				Second Transition (Resistant Starch)			
		T_i	T_p	T_c	ΔH	T_i	T_p	T_c	ΔH
0[c]	0.0	56.2	66.6	81.7	10.2	nd[g]	nd	nd	nd
0[d]	3.8	45.1	55.6	65.7	3.9	133.7	150.4	169.1	17.1
0[e]	2.2	39.1	51.7	62.8	4.1	129.4	153.4	164.4	8.9
0[f]	0.9	43.7	52.0	59.3	1.1	106.8	138.9	169.6	7.5
1	5.8	37.0	56.0	64.2	4.3	116.4	155.4	172.4	19.4
2	8.5	39.8	54.2	62.9	2.0	116.5	157.8	171.9	23.1
4	12.9	42.4	55.7	63.5	1.6	117.4	159.2	176.1	28.6
12	21.5	42.4	51.7	59.2	0.7	118.2	159.9	175.2	37.3
20	25.5	44.8	49.9	56.3	0.2	121.9	159.8	174.1	36.9

[a] Thermal characteristics determined by differential scanning calorimetry. Enthalpy values refer to vacuum-dried resistant starch.
[b] T_i, T_p, and T_c = initial, peak, and completion transition temperatures, respectively.
[c] Native pure starch.
[d] Pure starch dried under vacuum and treated with amylases.
[e] Pure starch treated with amylases (without preliminary vacuum drying).
[f] Pure starch treated with amylases and protease (without preliminary vacuum drying).
[g] None detected. See also Table II.

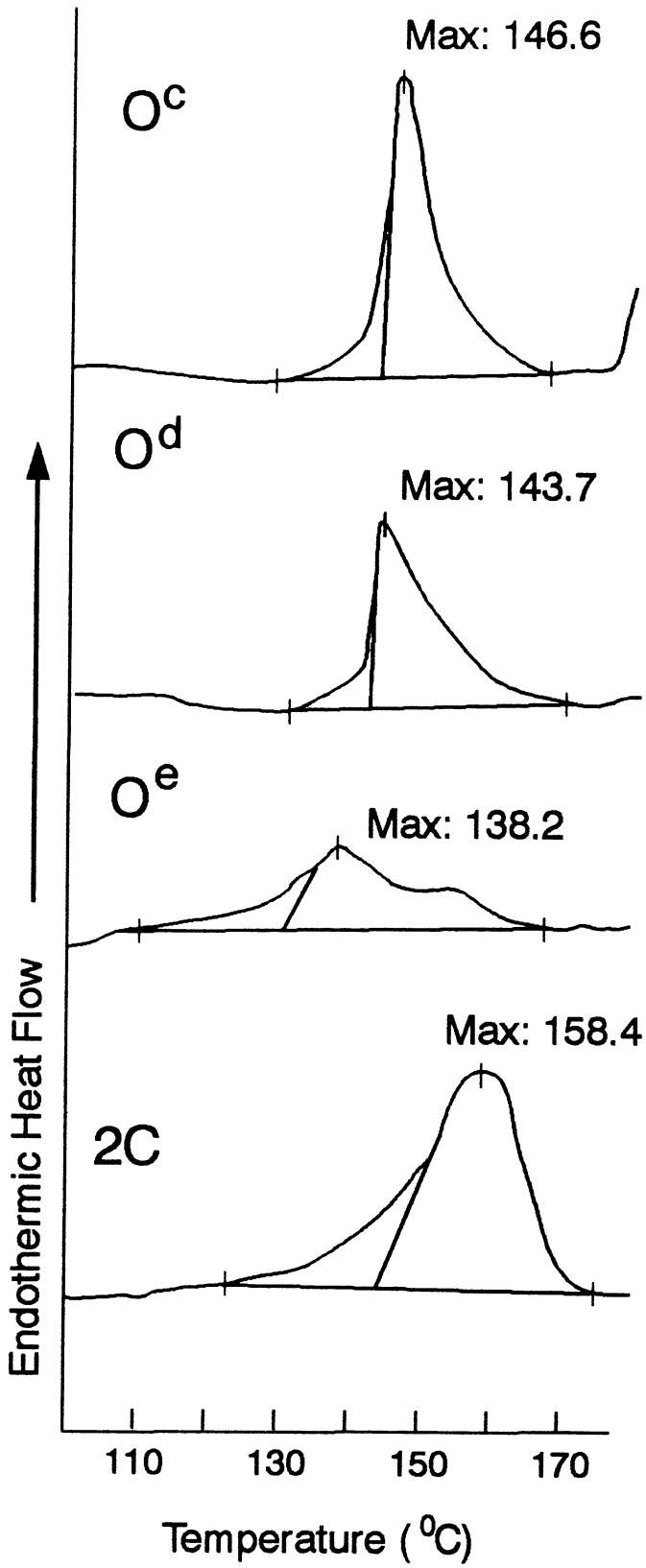

Fig. 2. Differential scanning calorimetric thermograms of the "apparent" and "true" resistant starch isolated from the purified white barley starch layer. O^c = residue obtained from starch dried under vacuum and heated with amylases, O^d = residue isolated from starch treated with amylases without preliminary vacuum drying, O^e = residue obtained from starch treated with amylases and protease without preliminary vacuum drying, 2C = resistant starch isolated after two autoclaving-cooling cycles. O = no autoclaving cooling cycle.

Chemical Analyses

Protein was determined by the Kjeldahl nitrogen (N × 6.25), Method 46-11A; ash by dry combustion, Method 08-01; moisture by oven drying 1 hr at 130°C, Method 44-15A; and free lipids by exhaustive extraction with petroleum ether, followed by evaporation to constant weight under vacuum, Method 30-25 (AACC 1983). Starch was determined essentially as described for DF (Prosky et al 1988). The starch was converted to glucose by successive treatments with bacterial α-amylase followed by protease and fungal amyloglucosidase. The glucose released was measured with a glucose oxidase-peroxidase reagent (Lloyd and Whelan 1969), and results were expressed on a polysaccharide basis (glucose × 0.9). Quantification of amylose (as percent of starch) was done according to Hovenkamp-Hermelink et al (1988). All analyses were done at least in duplicate; average results of all analyses are given on a dm basis.

Differential Scanning Calorimetry

The thermograms from differential scanning calorimetry (DSC) were recorded with a Perkin-Elmer DSC-4 instrument fitted with a 3600 thermal analysis data station and a graphics plotter 2. An indium standard was used for temperature and energy calibration. Samples (10 mg, dm) were weighed into large-volume stainless steel capsules (Perkin-Elmer, No. 0319-0218). About 20 μl of distilled water was added, and the capsules were sealed by a quick press and allowed to equilibrate for 2 hr at ambient temperature. The samples were then heated from 20 to 180°C at a scanning rate of 10°C/min. A capsule with inert material (Al_2O_3) and water represented the reference sample. For each endotherm, initial (T_i), peak (T_p), and completion (T_c) transition temperatures were determined by a computerized system developed by the Perkin-Elmer Corp. The transition enthalpy (ΔH) was calculated from the peak area and expressed as joules per gram of dry matter. The values given are the means of three to four independent measurements.

Scanning Electron Microscopy

Samples of dried starches were evenly sprinkled on double-sided adhesive tape mounted on aluminum specimen holders. The samples were then coated with a thin layer of ionized gold by Technics Hummer V Sputtering and examined in a Hitachi S-570 scanning electron microscope at 20 kV.

RESULTS AND DISCUSSION

Isolated Starch

The yield and composition of purified starch layers obtained after centrifugation of starch suspensions from the covered high-amylose Glacier selection are presented in Table I. Values from the crude flour, nonpurified brown layer, and residue after starch isolation were included for comparison. The yields of starch in the purified white and crude brown layers were relatively low and averaged 19.4% of the dry kernel weight or 35.5% of the total starch in the grain. Recovery of 45% starch from the proteinaceous brown layer was achieved by enzymatic processing of tailings. Starch was the main component of both purified layers, in which its content reached 97–98%. The purified starch layers contained less than 1% protein and up to 0.3% ash and 0.6% free lipids. The percentage of amylose in the starch was 44.5 in the white layer and 47.9 in the brown layer; the difference was statistically significant at the 0.01 level. It should also be noted that relatively large amounts of starch (32%) remained in the residual material after starch isolation. Our results on amylose distribution in high-amylose barley starch granules differ from those of Stark and Yin (1986), who reported that small granules (brown layer) of regular (about 25% amylose) barley contained 4% less amylose than the large granules (white layer).

Characterization of Barley Starches by SEM and DSC

Figure 1 shows SEM micrographs of starch granules and other barley components. The purified brown starch layer was composed of a high percentage of small (2–5 μm in diameter) round granules

with only a few large (10–15 μm) lenticular granules visible (Fig. 1a); the white layer contained mainly large lenticular granules and a low proportion of small granules (Fig. 1b) The granule size, shape, and distribution appeared typical of normal barley starch, and the starch granules showed no apparent damage. The crude brown layer contained clusters of starch granules surrounded by a protein matrix (Fig. 1c). The residue after starch isolation represented a mixture of cell wall debris, protein fragments, and unreleased starch granules (Fig. 1d)

DSC data for isolated starchy materials are given in Table II. All preparations exhibited two prominent endothermic transitions over the temperature range of 50–120°C; the transitions corresponded to endotherms of starch gelatinization (54–87°C) and melting of the amylose-lipid complex (87–113°C). In both transitions, purified preparations attained much higher values of melting enthalpies than crude starch materials. Peak temperatures of starch gelatinization were much lower and melting enthalpies of both starch gelatinization and amylose-lipid complexes were higher in the purified starches than in the other materials (flour, crude brown layer, and residue). No significant differences in endothermic transition temperatures were observed between starches in the two purified starch layers. The mean peak temperatures and melting enthalpies were, respectively, 66.6°C and 10.6 J/g for starch gelatinization and 103.5°C and 3.7 J/g for amylose-lipid complexes. The values obtained are comparable to the data reported previously for barley starch by Bhatty and MacGregor (1988).

Resistant Starch from Barley

The purified high-amylose barley starches were then used for the formation, isolation, and characterization of RS. As stated previously, the isolated starches were examined by DSC over the range of 20–180°C.

In the temperature range of 116–177°C, no transition could be observed in native, ungelatinized starches (Table III). However, the same starch treated with amylases without an autoclaving-cooling cycle yielded 4.1% of material, which was assayed as RS. It exhibited an endothermic transition in the general RS region but with a lower peak temperature and melting enthalpy. The results suggested that some RS may be formed during the analytical procedure. According to some reports, substantial amounts of RS may be formed during freeze-drying of the product before analysis (Englyst et al 1982).

Two transition ranges were recorded: around 55°C (probably uncomplexed lipid) and around 155°C (enzyme-resistant starch) (Tables III and IV). In light of the possibility of RS formation during the analytical procedure, a series of controls in addition to the native pure starch was included: starch dried under vacuum and heated with amylases, starch treated with amylases without preliminary vacuum drying, and starch treated with amylases and protease without preliminary vacuum drying.

Table III and Figure 2 indicate the possible formation of RS in isolated starch that did not undergo the autoclaving-cooling process. The yield of such "RS" was highest in starch dried under vacuum and treated with amylases. The yield decreased in samples treated with amylases without preliminary vacuum drying and decreased still further in samples treated with both amylase and protease without preliminary vacuum drying. Further examina-

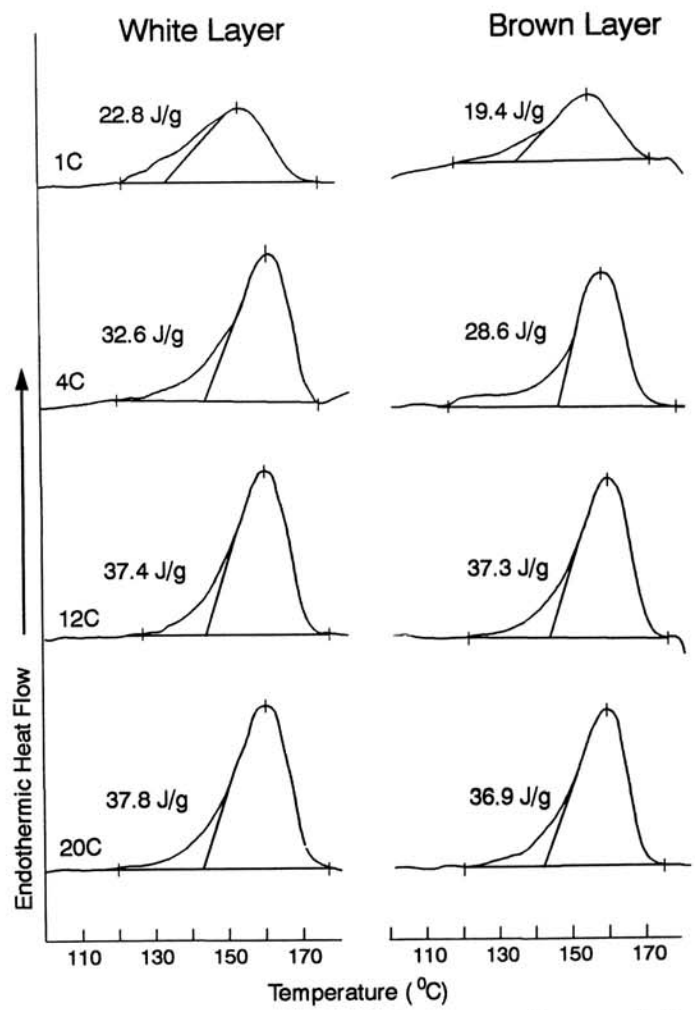

Fig. 4. Differential scanning calorimetric thermograms of vacuum-dried resistant starch isolated from purified white and brown barley starch layers after one, four, 12, and 20 autoclaving-cooling cycles (C). Starch-water ratio = 1:5, autoclaving temperature = 121°C.

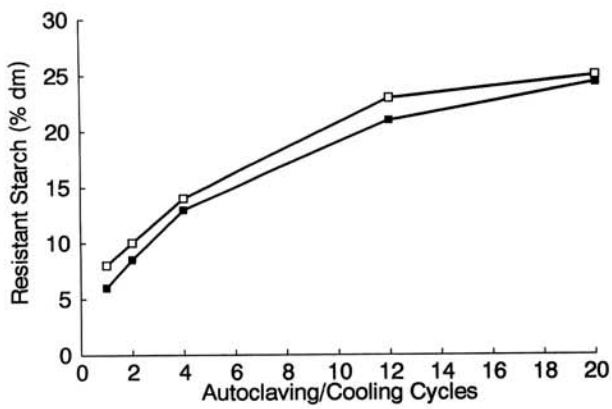

Fig. 3. Effect of repeated autoclaving-cooling cycles on yields of resistant starch from purified white (□) and brown (■) starch layers isolated from covered Glacier high-amylose barley.

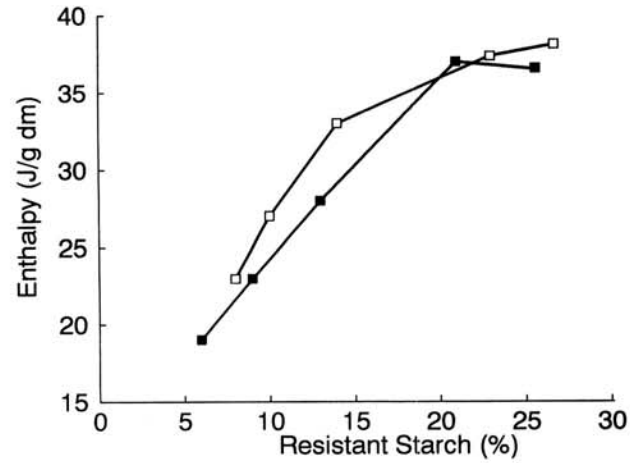

Fig. 5. Plot of enthalpy values of resistant starch versus yields of resistant starch from purified white (□) and brown (■) starch layers isolated from covered Glacier high-amylose barley.

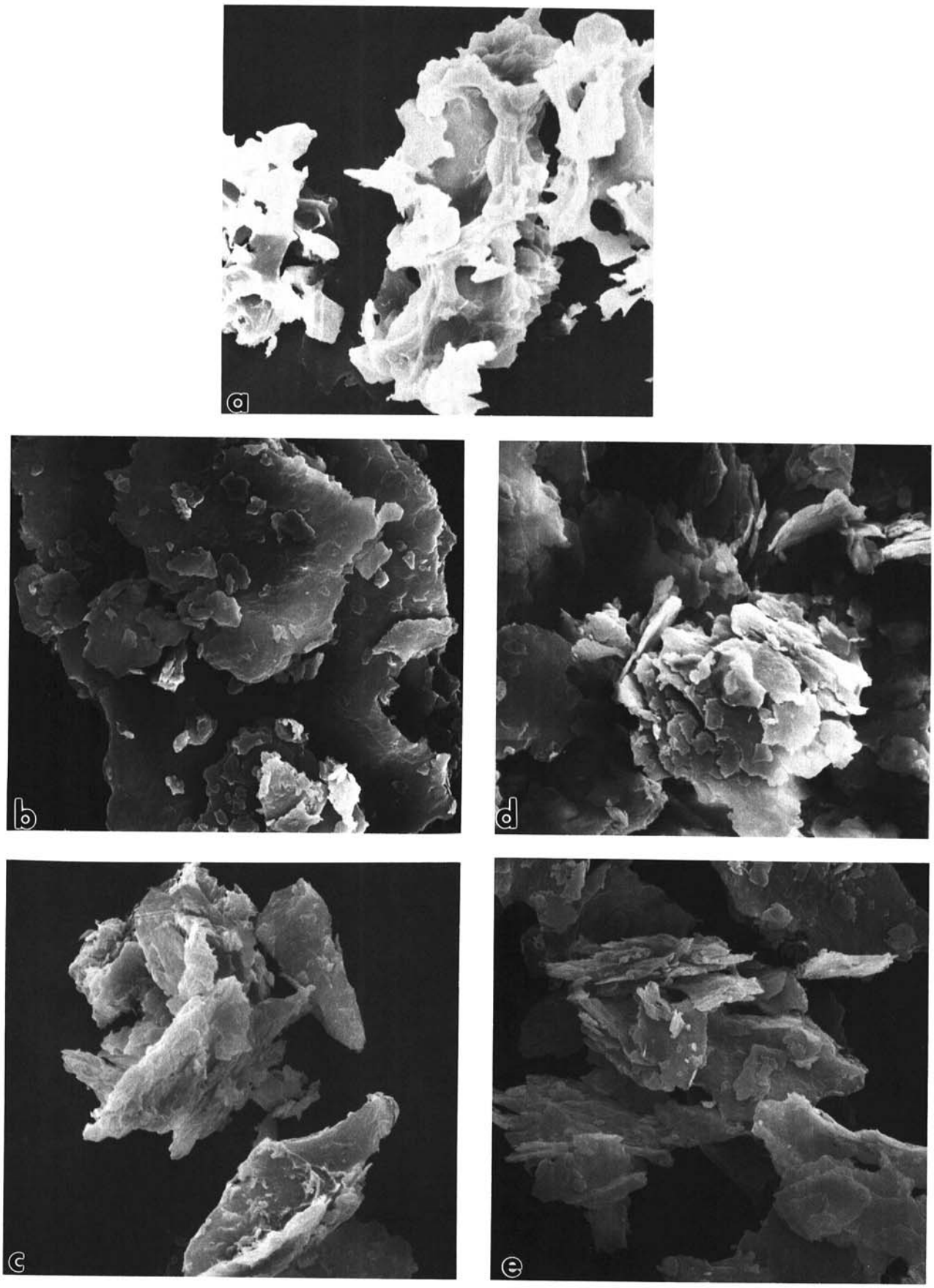

Fig. 6. Scanning electron micrographs of vacuum-dried resistant starch isolated from: amylomaize VII starch after one autoclaving-cooling cycle (C) (**a**), purified white barley starch layer after one C (**b**), purified white barley starch layer after four C (**c**), purified brown barley starch layer after one C (**d**), purified brown barley starch layer after four C (**e**).

tion of the results (second transition) shows, however, that the four control samples (those with no autoclaving-cooling cycles) showed no peak enthalpy around 155°C, the most reliable criterion of RS formation (Fig. 2). Thus, the presence of RS should be established by the enzymatic-gravimetric method and confirmed by DSC of the 155°C peak.

As in the formation of RS from high-amylomaize starch (Sievert and Pomeranz 1989), an increase in the number of autoclaving-cooling cycles increased the yield of RS and its enthalpy. Similar results were obtained as a result of RS formation from the purified brown starch layer (Table IV). Increasing the number of cycles up to 20 raised the RS level from 6 to 26% (Fig. 3). Comparison of Tables III and IV shows that the RS preparations isolated from the two starch layers showed similar thermodynamic properties. They exhibited an endothermic transition in the temperature range of 116–177°C with a mean peak temperature at 158°C, probably due to dissociation of the crystalline structures of amylose. The melting temperature of recrystallized amylose was estimated at about 160°C (Biliaderis et al 1985, Ring et at 1987). Figure 4 illustrates the thermal profiles of RS isolated from white and brown starch layers after one to 20 autoclaving-cooling cycles. Differences in melting enthalpy values (about 3.4–4.0 J/g) were found between both layers after one and four cycles. The differences became smaller when the number of autoclaving-cooling cycles increased. The lower melting enthalpy values obtained for the brown starch layer could be attributed to the higher amylose content (over 3%) in small starch granules (brown layer) compared with that in the white starch layer. At 121°C, the partially ungelatinized brown starch layer yielded lower melting enthalpies than did the large granules in the white starch layer (Table II), particularly in RS isolated after one to four autoclaving-cooling cycles (Table IV). The thermograms in Figure 4 also show lower peak temperatures and broader peaks of melting enthalpy for RS residues isolated after one cycle than for those isolated after four to 20 cycles. The broader peaks probably reflect less organized crystalline structures, which require lower energies for the melting process. Our thermoanalytical results on retrograded barley starch are also in good agreement with those reported for other cereals such as amylomaize or wheat (Sievert and Pomeranz 1989, Siljeström et al 1989).

The data in Tables III and IV show that an increase in the number of autoclaving-cooling cycles was also associated with an increase in melting enthalpies. This increase was found only up to the 22% RS level, which was achieved by 12 repeated autoclaving-cooling cycles. Further cycles increased RS yield while the melting enthalpies remained constant (Fig. 5). The data suggest that changes in the quality of RS take place during autoclaving-cooling cycles. The plateau of the curves in Figure 5 probably reflects a limit of crystallization of RS structures formed under the conditions applied.

The vacuum-dried RS preparations exhibited an additional small thermal transition in the range from 37 to 66°C (Tables III and IV), which gradually disappeared as the number of autoclaving-cooling cycles increased. Similar observations were previously reported by Sievert and Pomeranz (1989) for vacuum-dried RS preparations obtained from amylomaize starch. It is not clear whether this thermal effect is derived from retrograded amylopectin, the association of which can be reversed by heating to about 70°C (Eliasson 1985, Zeleznak and Hoseney 1986), or whether other factors are involved in formation of this peak.

Amylose-lipid complexes that reach an endothermic transition at about 100°C (±20°C) (Kugimiya et al 1980, Biliaderis et al 1985) could not be detected in RS residues. This can be related to the use of the thermostable bacterial α-amylase for enzymatic isolation of RS. This enzyme has been shown to hydrolyze amylose-lipid complexes at high temperatures (Holm et at 1983).

Figure 6 shows SEM micrographs of RS preparations obtained from amylomaize VII starch and purified white and brown barley starch layers after one to four autoclaving-cooling cycles. The enzyme-resistant starch showed a completely different image than that of regular starch. The granular structure disappeared, and bigger, irregularly shaped particles were visible. Amylomaize VII RS residues obtained after one cycle formed an open, fluffy structure (Fig. 6a), while in RS preparations isolated from barley starch more compact forms predominated (Fig. 6b–e). In RS preparations obtained from white and especially brown starch layers after one cycle, small particles of residual starch granules were still evident in some parts of the material (Fig. 6b and d). After four autoclaving-cooling cycles, very compact and dense structures could be observed in RS derived from both starch layers (Fig. 6c and e). The higher melting enthalpy of barley RS after four cycles compared with that after one cycle might be related to the structural changes and stabilization.

TABLE V
Calculated Enthalpies[a]

Number of Cycles	White Layer	Brown Layer	Calculated Enthalpy in Brown/White Layer (%)
1	1.78	1.13	63.5
2	2.62	1.96	74.8
4	4.63	3.69	79.7
12	8.68	8.02	92.4
20	9.79	9.41	96.1

[a] Yield of resistant starch, percent dry matter multiplied by enthalpy (in J/g).

Czuchajowska et al (1991) introduced the term "calculated enthalpy" for the yield of RS (% dm) multiplied by the enthalpy (J/g) of the 155°C endotherm of RS. This theoretical value was used to take into account the effectiveness of different enzymes to isolate crystallized amylose from a starch sample and to deduce the amount of energy required to melt an isolated RS. The term can also be used to compare the yields and melting enthalpies from maize and barley or from large and small barley starch granules or the effects of numbers of autoclaving-cooling cycles. After 20 autoclaving-cooling cycles, the yields were 42 and 26% and the melting enthalpies 29 and 38 J/g, for RS in amylomaize VII and high-amylose barley, respectively; the corresponding calculated enthalpies were 12.2 and 9.9, respectively. Calculated enthalpies were consistently lower in RS for the brown layer than for the white layer (Table V). The difference in calculated enthalpies in RS for the two layers decreased, however, as the number of autoclaving-cooling cycles increased.

CONCLUSIONS

"Apparent" enzyme-resistant starch in high-amylose barley starch can be distinguished from "true" RS by its peak enthalpies, which are below ~155°C. Increasing the number of autoclaving-cooling cycles increased both the yield and the melting enthalpy of RS (the same as in high-amylose maize). The maximum yield was higher and the melting enthalpy at ~155°C was lower in RS from maize than in RS from barley. Yields, enthalpies, and "calculated enthalpy" (yield × enthalpy) were higher in RS from large starch granules (white layer) than in RS from small starch granules (purified brown layer). The differences decreased as the number of autoclaving-cooling cycles increased.

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