Starch-Lipid Interactions and Formation of Resistant Starch in High-Amylose Barley

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ABSTRACT

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Starch was isolated and purified from a barley selection with 42.3% amylose in the starch. The white- and brown-layer starch fractions differed in chemical composition and endothermic properties; amylose was higher in the starch of the brown layer (49.3%) than in the starch of the white layer (43.5%). After being autoclaved for 1 hr at 121°C, the starches were reacted during cooling from 100°C to ambient temperature with sodium stearoyl lactylate, distilled monoglycerides, diacetyl tartaric acid esters of mono-diglycerides (DATEM), and ethoxylated monoglycerides. Formation of amylose-lipid complexes was indicated by peaks in differential scanning calorimetry thermograms at temperatures from 100 to 112°C. Endothermic transitions from 154 to 162°C (mean, 158°C) reflected the presence of crystallized amylose. Yields of enzyme-resistant starch (RS) from a single autoclaving-cooling cycle were 7.1 and 4.0% for the white- and brown-layer starches, respectively. Those yields decreased to 0.7–5.3 and 0.7–2.1%, respectively, when RS was prepared after the starches were complexed with the various emulsifiers. Complexes between distilled monoglycerides, DATEM, and ethoxylated monoglycerides and amylose from the brown starch layer attained relatively higher values of melting enthalpies than those from the white layer. It is postulated that amylose crystallization (as measured by enthalpies of the 158°C endotherm) that is involved in the formation of RS is competitively affected by the complexation of amylose with lipids. The effects of complexation on yields and enthalpy of RS from high-amylose maize and barley starches differed widely.

Starch is the major reserve storage polysaccharide of barley endosperm, where it occurs in the form of large lenticular A-granules and small spherical B-granules. The starch type can vary from waxy (traces of amylose), to regular (about 25% amylose), to high (35–50%) amylose (Pomeranz 1987). During the isolation of barley starch, small granules 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 large starch granules (McDonald and Stark 1988).

One characteristic of starch, especially its linear amylose fraction, is its ability to form inclusion complexes with a variety of inorganic and organic ligands. The ligands enter the helical cavities of the amylose molecules and form molecular inclusion complexes. In the presence of ligand molecules, amylose undergoes rapid conformational ordering (from coil to helix), which promotes aggregation of helices into partially crystalline V structures. The complexes are heat-stable and insoluble in aqueous media at pH 7. Among the starch complexes known are those with iodine (Banks et al 1971), flavor components in foods (Osman-Ismail and Solms 1973), alcohols (Kuge and Takeo 1968), free fatty acids (Raphaelides and Karkalas 1988), emulsifiers (Krog 1971), and many surfactants (Kim and Robinson 1979).

Lipids or surfactants act as texture modifiers when added to starch-containing foods. For example, saturated monoglycerides and sodium stearoyl lactylate are added to baked goods because of their ability to retard firming and retrogradation of starch (Krog et al 1989). The complex-forming ability of amylose with monoglycerides and related surface-active monoacyl lipids has been exploited in breadmaking to retard staling (Krog and Jensen 1970), in the manufacture of instant mashed potato granules to prevent stickiness (Hoover and Hadziyev 1981), and in extruded starch-containing products to control texture (Launay and Lisch 1983). It was stated also that formation of complexes prevents leaching of amylose during gelatinization, inhibits the swelling of starch granules heated in water, and reduces the water-binding capacity of the starch (Eliasson 1985).

Differential scanning calorimetry (DSC) of amylose-lipid complexes shows a reversible transition at 95–130°C, well above the melting endotherm of starch crystallites at 65–72°C. The high-temperature transition was assigned to the melting of the amylose-lipid complex (Biliaderis 1991). It has been assumed that amylopectin, because of its short outer chains, does not effectively complex with lipids. However, Gudmundsson and Eliasson (1990) recently provided evidence for such interaction by calorimetry, in which amylopectin-lipid complexes exhibited a transition at temperatures usually associated with the amylose-lipid complex.

Retrogradation of amylose-containing starches was shown to include the formation of an enzyme-resistant starch (RS) fraction that comprises short-chain linear α-glucans (Russell et al 1989). This form of starch, undigestible in vitro and in vivo, is found in food products processed by methods using relatively high moisture contents, such as cooking, baking, and autoclaving (Englyst et al 1983). RS is composed of noncovalently bonded crystallites of amylose. Crude RS preparations contain native starch lipids and protein originating from added enzymes used in the isolation procedure (Russell et al 1989). Interchain amylose association in the RS fraction was indicated by an endothermic transition at 155–158°C in DSC thermograms (Sievert and Pomeranz 1989, Szczodrak and Pomeranz 1991). There is considerable interest in the nutritional implications of RS in foods, since a relatively slow rate of starch hydrolysis in the gastrointestinal tract of humans is associated with low glycemic responses and may have some of the physiological effects of dietary fiber (Englyst and MacFarlane 1986). The observation that amylose content and yield of RS are positively correlated (Sievert and Pomeranz 1989) focused interest on high-amylose varieties of cereals, like amylomaize and barley, as potential sources for RS production.

It has been postulated that the presence of complexing lipids affects the reassociation behavior of amylose upon retrogradation of starch and thus affects formation of RS. Some investigators suggested that a competitive mechanism exists between amylose retrogradation and formation of amylose-lipid complexes (Sarko and Wu 1978). Also, Slade and Levine (1987) reported that the crystallization of amylose-lipid complexes is favored over amylose retrogradation.

We recently isolated and characterized starch and RS from high-amylose barley (Szczodrak and Pomeranz 1991). We know of no work on starch-lipid interaction and its effect on formation of RS in barley. The aim of the present study was to investigate by enzymatic-gravimetric and thermoanalytical methods how complexing lipids (including those that are added as breadmaking improvers and those added as antistaling agents) affect the formation of RS in barley starch.

MATERIALS AND METHODS

Reagents

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*;

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o-dianisidine, D-3252; amylose, A-0512, from potato; and amylopectin, A-8515, from potato were purchased from Sigma Chemical Co., St. Louis, MO. Heat-stable α-amylase, Takalite L-340, from *B. licheniformis* was obtained from Miles Lab., Inc., Elkhart, IN. Sodium stearoyl lactylate (SSL), distilled saturated monoglycerides (monoglycerides), and diacetyl tartaric acid esters of mono- and diglycerides (DATEM) were from Grindsted Products, Inc., Industrial Airport, KS. Ethoxylated mono- and diglycerides (EMG) were from American Ingredients Co., Kansas City, MO.

Monoglycerides are emulsifying, starch complexing, and aerating agents used (among others) as crumb softeners for bread. The distilled monoglycerides we used were made from refined, hydrogenated lard (tallow). DATEM were from edible, refined vegetable fat. They are used primarily as a highly effective dough conditioner to improve processing tolerance of dough and to increase the volume of yeast-leavened baked goods. EMG, a versatile dough conditioner, is manufactured by glycerolysis of edible fats primarily composed of stearic, palmitic, and myristic acids, which are reacted with ethylene oxide. SSL is a multifunctional emulsifier, dough conditioner, and starch-complexing agent.

The amylose-complexing indexes of the emulsifiers are 72 for SSL, 92 for monoglycerides, 49 for DATEM, and very low for EMG (manufacturer's data). The emulsifiers are water dispersible above 50°C.

Barley

A hull-less, high-amylose barley selection of the cultivar Glacier, from the 1989 crop, was provided by S. E. Ullrich, Agronomy Department, Washington State University.

Isolation of Starch

Starch was isolated from barley by a modification of the method of McDonald and Stark (1988). Barley kernels were cracked lightly by passing through a sample mill (Tecator Cemotec 1090, Herndon, VA) at its widest aperture. After being steeped in 0.02*M* HCl for 17 hr at 4°C and being neutralized 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 × *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.

Determinations

Barley samples used for analyses were milled in a grinder (Udy Corp., Ft. Collins, CO) fitted with a 0.5-mm sieve. Protein was determined by Kjeldahl nitrogen (N × 6.25), method 46-11A; ash by dry combustion, method 08-01; moisture by oven drying for 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 analyzed essentially as described for dietary fiber (Prosky et al 1988). The starch was converted to glucose by successive treatments with bacterial α-amylase followed by protease and fungal amyloglucosidase. Liberated glucose was quantified with a glucose oxidase-peroxidase reagent (Lloyd and Whelan 1969), and starch content was expressed on a polysaccharide basis (glucose × 0.9). The content of amylose (as percent of starch) was estimated according to the method developed by Hovenkamp-Hermelink et al (1988). The analysis combines extraction of starch in perchloric acid with determination of absorbance at two wavelengths (618 and 550 nm) after staining in an iodine-potassium iodide (Lugol) solution. All samples were assayed at least in duplicate; average results of all analyses are given on a dry matter basis.

Preparation of Starch, Starch-Lipid Complexes, and Resistant Starch

Thermal processing with subsequent cooling of the starch sample was used for RS formation (Sievert and Pomeranz 1989). To study the effects of lipids on RS formation, SSL, monoglycerides, DATEM, or EMG were added during cooling of autoclaved starch according to the following procedure. Ten grams (dry starch basis) of barley starches (white or brown layer) were weighed into a 1,000-ml beaker and suspended in 100 ml of distilled water. The suspensions were then autoclaved for 1 hr at 121°C. When the temperature of the autoclave reached about 100°C during the cooling period, the starch samples were taken out from the autoclave and 25 ml of an aqueous 4% lipid dispersion (preheated at 60–90°C), or no emulsifiers (control sample), were added immediately to the hot (about 90°C) starch suspensions. The mixtures were cooled to ambient temperature under vigorous stirring, refrigerated at 4°C overnight, and vacuum-dried. The dried material was milled in a Udy grinder fitted with a 0.5-mm sieve, and the excess of the uncomplexed lipids in the starch sample was extracted (three times, 30 min each) with petroleum ether at a temperature less than 60°C. The petroleum ether was allowed to evaporate under a hood at room temperature.

For the isolation of RS, 0.5 g of the autoclaved starch samples was suspended in 50 ml of phosphate buffer solution (pH 6.0, 0.05*M*) and incubated with 0.2 ml of heat-stable α-amylase (340,000 modified Wohlgemuth units per milliliter) at 100°C for 30 min. 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. No changes in DSC parameters were found under those conditions. Thereafter, a series of five washings using hot water (90°C) was performed to remove digested nonresistant starch, and other by-products of the enzyme digestion process. The purified enzyme-resistant starch was vacuum-dried, weighed, ground to pass a sieve with 0.5-mm openings, and used for further characterization.

DSC Measurements

The DSC thermograms were conducted with a DSC-4 instrument (Perkin-Elmer Corp., Norwalk, CT) fitted with a 3600 thermal analysis data station and a graphics plotter 2. An indium standard was used for temperature and enthalpy calibration. Samples (10 mg, dry starch basis) 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 room 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, temperatures of transition onset, peak (T_p), and completion were determined by data processing software (TADS, DSC-4, standard Program, Rev. C) developed by the Perkin-Elmer Corp. The transition enthalpy (ΔH) was calculated by the software from the peak area and expressed as joules per gram of dry matter. The values given are the means of at least three to four independent measurements.

TABLE I
Yield and Chemical Composition (%, dmb) of Starchy Materials Isolated from Hull-less Glacier High-Amylose Barley

Material	Yield	Crude Protein	Starch	Amylose (% of starch)	Ash	Free Lipids
Crude flour	100.00	15.4	59.7	42.3	2.7	3.7
Purified white layer[a]	27.8	0.4	98.5	43.5	0.2	0.3
Purified brown layer[b]	6.1	0.5	97.8	49.3	0.3	0.4

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

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

Material	Transition Temperatures (T, °C)[b,c] and Transition Enthalpies (ΔH, J/g dry matter)[d]							
	Starch Gelatinization Transition				Amylose-Lipid Complex Transition			
	T_o	T_p	T_c	ΔH	T_o	T_p	T_c	ΔH
Crude flour	61.5	71.7	84.8	3.9	89.6	99.9	110.2	1.6
Purified white layer	58.3	65.4	80.7	10.2	96.4	103.4	108.8	2.5
Purified brown layer	60.1	68.3	82.3	8.6	93.2	101.9	110.8	3.9

[a]Values are averages of three determinations.
[b]Transition temperatures of onset (T_o), peak (T_p), and completion (T_c).
[c]SD < 1.0°C, $n = 3$.
[d]SD < 10% of the mean, $n = 3$.

TABLE III
Effects of Temperature and Hydration on Transition Temperatures[a] (T, °C) and Transition Enthalpies[b] (ΔH, J/g dry matter) of Emulsifiers, Determined by Differential Scanning Calorimetry (DSC)

Emulsifiers and Thermal Treatment[c]	Weight of Preparation and Its Hydration							
	5 mg (without water)				5 mg + 10 µl of water			
	T_o	T_p	T_c	ΔH	T_o	T_p	T_c	ΔH
SSL								
Original (20–180°C)	44.4	49.1	61.2	80.5	...[d]	...	...	...
Reheated (20–180°C)	45.4	49.2	56.7	73.7	...	...	...	...
Original (20–100°C)	44.9	48.9	61.1	81.0	48.7	51.6	60.8	69.6
Reheated (20–100°C)	44.9	49.0	57.3	74.8	47.6	50.6	59.6	62.9
Reheated after wait[e] (20–100°C)	45.0	48.8	56.8	72.3	...	...	...	...
Monoglycerides								
Original (20–180°C)	66.6	71.4	79.2	178.8[f]	...	...	...	...
Reheated (20–180°C)	62.7	67.4	74.1	97.3[g]	...	...	...	...
Original (20–100°C)	66.4	71.6	79.8	172.6[f]	54.0	61.0	73.9	160.9[h]
Reheated (20–100°C)								
1[i]	63.5	68.2	76.2	100.9[g]	54.2	58.4	67.0	82.9[j]
2	63.5	68.2	75.8	101.1[g]	54.0	58.0	67.0	84.1[j]
3	63.5	68.2	76.5	101.3[g]	53.8	58.0	67.2	84.8[j]
4	63.5	68.2	77.0	101.2[g]	53.8	57.9	66.4	83.6[j]
5	63.5	68.2	77.1	101.5[g]	53.8	57.9	66.6	83.7[j]
Reheated after wait[e] (20–100°C)	63.7	68.3	76.0	99.4[g]	...	...	...	...
DATEM								
Original (20–100°C)	42.6	48.9	60.7	76.6	...	...	...	...
Reheated (20–100°C)	44.1	49.8	56.6	68.2	...	...	...	..
EMG								
Original (20–100°C)	23.3	32.8	38.9	71.4	...	...	...	...
Reheated (20–100°C)	22.5	32.5	39.7	57.4	...	...	...	...

[a]T_o, T_p, and T_c = onset, peak, and completion temperatures, respectively. SD < 1.0°C, $n = 3$.
[b]SD < 10% of the mean, $n = 3$.
[c]Original, no reheating was performed after the first DSC run; reheated, sample was reheated immediately after the first DSC run. Temperature ranges of the DSC runs are shown in parentheses. SSL = sodium stearoyl lactylate, monoglycerides = distilled monoglycerides, DATEM = diacetyl tartaric acid esters of mono- and diglycerides, EMG = ethoxylated monoglycerides.
[d]Not determined.
[e]After the first DSC run, the sample was kept at 100°C for 30 min, and then the second DSC run (at 20–100°C) was performed.
[f]β-crystal form.
[g]α-crystal form.
[h]β to lamellar mesophase.
[i]Number of reheatings.
[j]α to lamellar mesophase.

TABLE IV
Thermoanalytical Characteristics of Autoclaved Preparations of White Starch Layer Isolated From Hull-less High-Amylose Barley and Cooled in the Presence of Added Complexing Agents[a]

Emulsifier[b]	Transition Temperatures[c] (T, °C) and Transition Enthalpies[d] (ΔH, J/g dry matter)											
	First Transition				Second Transition (Amylose-Lipid Complex)				Third Transition (Resistant Starch)			
	T_o	T_p	T_c	ΔH	T_o	T_p	T_c	ΔH	T_o	T_p	T_c	ΔH
None	...[e]	...	...	...	101.1	107.4	115.6	1.6	146.6	154.4	165.2	4.8
SSL	...	...	...	...	109.4	111.8	128.5	21.1	...	...	...	...
Monoglycerides	56.0	60.7	71.4	11.9	96.9	106.5	117.4	6.7	143.3	155.0	164.1	2.0
DATEM	...	...	...	...	91.2	100.4	118.0	8.2	150.3	156.5	164.9	1.5
EMG	...	...	...	...	102.4	110.2	125.2	11.5	148.5	156.2	165.8	0.6

[a]Preparations represented a mixture of starch and resistant starch (crude resistant starch). Values are averages of three determinations.
[b]SSL = sodium stearoyl lactylate, monoglycerides = distilled monoglycerides, DATEM = diacetyl tartaric acid esters of mono- and diglycerides, EMG = ethoxylated monglycerides.
[c]T_o, T_p, T_c = onset, peak, and completion temperatures, respectively. SD < 1.0°C, $n = 3$.
[d]SD < 10% of the mean, $n = 3$.
[e]None detected.

RESULTS AND DISCUSSION

The hull-less cv. Glacier selection, high in amylose, was used for isolation and purification of barley starch. Table I shows the yield and chemical composition of purified starch layers obtained after centrifugation of starch suspensions. Crude barley flour was included as control. Yields of starch in the purified white and brown layers were only 27.8 and 6.1% of the dry kernel weight or 46.6 and 10.2% of the total starch in the grain, respectively. Starch averaged 98.1% of both purified layers. The purified starch contained less than 1% protein and up to 0.3% mineral components (ash) and 0.4% free lipids. The large (white layer) starch granules contained about 6 percentage points less amylose than the small (brown layer) granules. A higher amylose content in small starch granules was found in a previous study on starches isolated and purified from covered high-amylose Glacier barley (Szczodrak and Pomeranz 1991). Our results on amylose distribution in barley starch granules support those of McDonald et al (1991), who stated that in high-amylose barley, the amylose content of the small granules exceeded that of the large granules by about 8%. Previous studies in Glacier (high-amylose) barley (Evers et al 1974, Goering and DeHaas 1974, Morrison et al 1984) have reported the amylose contents of the small granules at maturity to be both higher (Evers et al 1974) and lower (Goering and DeHaas 1974) than that of the large granules.

DSC data for isolated starchy materials are summarized in Table II. All preparations exhibited two prominent transitions over a similar temperature range (55–111°C), corresponding to endotherms of starch gelatinization (55–85°C) and amylose-lipid complexation (89–111°C). In both transitions, purified barley starches attained higher melting enthalpies than the crude flour. No significant differences in endothermic transition temperatures were observed between starches in the two purified starch layers. The mean peak temperatures and melting enthalpies of the purified starches were 66.9°C and 9.4 J/g, respectively, for starch gelatinization and 102.7°C and 3.2 J/g, respectively, for amylose-lipid complexes. Those values agree with the values reported for barley starch by Bhatty and MacGregor (1988).

Effects of temperature and hydration on thermal characteristics of the emulsifiers are summarized in Table III for SSL, monoglycerides, DATEM and EMG. DSC data for original emulsifiers and for those heated and reheated to 100 and 180°C are presented. Included are runs in which some of the emulsifiers (SSL and monoglycerides) were reheated after they were kept for 30 min at 100°C. In the case of SSL, there was no large or consistent effect on transition temperatures; the enthalpy decreased as a result of heating by about 8 J/g, and enthalpies in the presence of water were lower than when the SSL alone was reheated. Similar results were obtained for DATEM and EMG. The largest changes occurred on reheating of monoglycerides. The drop in T_p was about 3°C, similar to that of the white starch layer autoclaved in the presence of monoglycerides (Table IV). Transition temperatures of monoglycerides heated in the presence of water (unlike those of SSL) dropped consistently and markedly (by about 10°C). In addition, the enthalpy values decreased to almost half as a result of reheating (and accompanying phase changes, Table III) and were consistently lower in monoglycerides reheated with water than without water. There was a large decrease after the first reheating of monoglycerides (presumably as a result of phase changes) and little change after additional reheating cycles. The purified high-amylose barley starches (white and brown layer) were then used in determining the effects of complexing lipids on formation of resistant starch. To evaluate the effect of lipids on crystallization of amylose, as assessed by enthalpies of the 158°C RS endotherm (i.e., the mean of endotherms from 154 to 162°C), we compared results from starch samples before and after amylolytic treatment.

White Starch Layer

Thermal characteristics of autoclaved preparations of the white starch layer before enzymatic treatment are given in Table IV. The transitions, between 82 and 129°C, were assumed to be due to melting of amylose-lipid complex crystals and between 139 and 166°C were attributed to melting of crystallized amylose. In the control sample (without emulsifier added) the small endotherm (1.6 J/g) with T_p at ~107°C reflected melting of a complex between amylose and native starch lipids. Addition of commercial emulsifiers (SSL, monoglycerides, DATEM, or EMG) during cooling of the autoclaved white starch layer induced complexations of amylose with the added lipids. The highest melting enthalpy (21.1 J/g) was exhibited by the amylose-SSL complex and the lowest enthalpy (6.7 J/g) by the amylose-monoglyceride complex. The latter also exhibited a relatively high melting endotherm peak (11.9 J/g) around 61°C, which probably derived from melting of "free" (uncomplexed) lipid.

The high interactions with SSL and EMG are surprising and are not in agreement with their amylose-complexing capacities (see also Czuchajowska et al 1991). Reheating of the amylose-monoglyceride complex immediately after the first run reduced the enthalpy value by approximately 65% and shifted the endotherm peak from 60.7°C (Table IV) to 55.2°C. Since all lipids were added in excess, the lower melting enthalpy of the monoglyceride complex compared to the enthalpies of the other amylose-lipid complexes indicated a low complexing capacity. As can be seen from the data in Table IV, all starch samples gave smaller melting enthalpies for transition in the 155°C RS region than did the control preparation (4.8 J/g). Melting enthalpies for amylose-lipid complexes correlated negatively with those manifested in the resistant starch region. The higher the melting endotherm for the amylose-lipid complex, the lower the enthalpy for RS. In the case of the amylose-SSL complex no transition in the RS region was recorded, which suggests that almost all the amylose fraction was involved during gelatinization in formation of this complex.

After enzymatic treatment of the mixture of starch-lipid complexes and crystalline amylose with the thermostable α-amylase, most of the starch-lipid complexes were apparently removed. Yields of RS from complexed starch samples were lower than yields from the control sample, and reduction in yields depended on the complexing agent. The combined data of Tables IV and V indicate that the complexing ability of the added lipid, as demonstrated by melting enthalpies of the endotherms between 100 and 112°C, was negatively related to the yield of RS. Whereas SSL-complexed starch gave the highest melting enthalpy and lowest yield of RS, monoglyceride-complexed starch had the lowest enthalpy and highest yield. It can be concluded that good complexing agents such as SSL and DATEM effectively compete with amylose chains involved in the formation of RS during cooling of autoclaved barley starch. Since amylose-lipid complex crystals are liable to enzyme degradation (Holm et al 1983), the formation of complexed amylose in autoclaved-cooled starch samples apparently accounted to a large extent for the reduction in yields of RS. Removal of complexes of amylose with SSL, monoglycerides, DATEM, and EMG as a result of the amylolytic treatment was indicated by the disappearance of the corresponding melting transitions (100–112°C) in DSC thermograms of RS residues (Tables IV and V). These complexes may not be completely hydrolyzed. Indeed, some may have been transformed to RS. The only exception was the amylose-EMG complex, which still gave a small endotherm (1.5 J/g) after enzymatic treatment (Table V). The low solubility in water of the EMG complex may have contributed to its relative stability.

The small endotherms at about 50 and 55°C, observed in RS residues from starches complexed with SSL and monoglyceride (Table V), most likely derived from the melting of "free" SSL and monoglycerides that were released from amylose-SSL and amylose-monoglyceride complexes during the enzymatic treatment. This is in agreement with previous findings (Sievert and Pomeranz 1990), suggesting the presence of lipids that are released from the complex and adhere to nonhydrolyzed starch structures in some RS residues.

The 158°C endotherm of RS residues was considered to represent crystallized amylose fragments in RS material. Upon com-

parison of yield data of RS and enthalpy values of the 158°C endotherm of RS preparations (Table V), it became apparent that substantial decreases in yields of RS from complexed starch samples were accompanied by some increases in melting enthalpies of the 158°C endotherm for SSL and DATEM only. For monoglycerides and EMG the decrease in yield was accompanied by a decrease in enthalpy.

Brown Starch Layer

Similar results and relations concerning the effect of complexing lipids on the formation of RS were obtained for the purified brown starch layer (Tables VI and VII). The data in Table VI show that the melting enthalpies for amylose-monoglyceride, -DATEM, and -EMG complexes reached relatively higher values in the brown starch layer than in the white layer. This may be related to a higher (about 6%) amylose content in small starch granules. Also, in the case of monoglycerides and EMG, RS residues isolated after amylolysis gave higher melting enthalpies (27.0 and 15.3 J/g, respectively; Table VII) in comparison to those obtained from the white layer of barley starch (9.8 and 11.1 J/g, respectively; Table V).

CONCLUSIONS

SSL and EMG were the best complexing agents as indicated by melting enthalpies in the temperature range between 100 and

TABLE V
Effects of Added Complexing Agents on Enzymatic (Resistant Starch Yields) and Thermal Characteristics of Purified White Layer Isolated from Hull-less High-Amylose Barley[a]

Emulsifier[b]	Resistant Starch Yield (% dmb)	Transition Temperatures[c] (T, °C) and Transition Enthalpies[d] (ΔH, J/g dry matter)											
		First Transition				Second Transition (Amylose-Lipid Complex)				Third Transition (Resistant Starch)			
		T_o	T_p	T_c	ΔH	T_o	T_p	T_c	ΔH	T_o	T_p	T_c	ΔH
None	7.1	...[e]	...	...	...	...	...	...	...	116.1	157.7	172.8	22.4
SSL	0.7	44.1	50.0	57.3	0.9	...	...	...	...	141.1	159.1	173.1	29.8
Monoglycerides	5.3	49.9	54.9	62.2	2.6	...	...	...	...	155.4	162.3	171.5	9.8
DATEM	1.3	...	...	...	...	...	...	...	...	139.9	158.9	173.0	33.5
EMG	2.7	...	...	...	...	99.5	109.4	121.3	1.5	140.0	158.9	174.8	11.1

[a]Values are averages of three determinations.
[b]SSL = sodium stearoyl lactylate, monoglycerides = distilled monoglycerides, DATEM = diacetyl tartaric acid esters of mono- and diglycerides, EMG = ethoxylated monglycerides.
[c]T_o, T_p, T_c = onset, peak, and completion temperatures, respectively. SD < 1.0°C, $n = 3$.
[d]SD < 10% of the mean, $n = 3$.
[e]None detected.

TABLE VI
Thermoanalytical Characteristics of Autoclaved Preparations of Brown Starch Layer Isolated From Hull-less High-Amylose Barley and Cooled in the Presence of Added Complexing Agents[a]

Emulsifier[b]	Transition Temperatures[c] (T, °C) and Transition Enthalpies[d] (ΔH, J/g dry matter)											
	First Transition				Second Transition (Amylose-Lipid Complex)				Third Transition (Resistant Starch)			
	T_o	T_p	T_c	ΔH	T_o	T_p	T_c	ΔH	T_o	T_p	T_c	ΔH
None	...[e]	...	...	...	100.6	106.9	118.3	3.1	149.7	154.9	166.9	4.7
SSL	...	...	...	...	101.3	108.3	129.1	16.6	153.3	156.1	164.8	0.1
Monoglycerides	57.1	61.7	72.1	8.0	99.0	106.3	123.3	11.5	150.9	155.3	163.6	1.6
DATEM	...	...	...	...	91.8	100.5	116.8	10.1	151.8	160.2	167.0	2.4
EMG	...	...	...	...	105.4	110.7	129.6	14.8	153.0	156.6	163.9	0.2

[a]Preparations represented a mixture of starch and resistant starch (crude resistant starch). Values are averages of three determinations.
[b]SSL = sodium stearoyl lactylate, monoglycerides = distilled monoglycerides, DATEM = diacetyl tartaric acid esters of mono- and diglycerides, EMG = ethoxylated monglycerides.
[c]T_o, T_p, T_c = onset, peak, and completion temperatures, respectively. SD < 1.0°C, $n = 3$.
[d]SD < 10% of the mean, $n = 3$.
[e]None detected.

TABLE VII
Effects of Added Complexing Agents on Enzymatic (Resistant Starch Yields) and Thermal Characteristics of Purified Brown Layer Isolated from Hull-less High-Amylose Barley[a]

Emulsifier[b]	Resistant Starch Yield (% dmb)	Transition Temperatures[c] (T, °C) and Transition Enthalpies[d] (ΔH, J/g dry matter)											
		First Transition				Second Transition (Amylose-Lipid Complex)				Third Transition (Resistant Starch)			
		T_o	T_p	T_c	ΔH	T_o	T_p	T_c	ΔH	T_o	T_p	T_c	ΔH
None	4.0	...[e]	...	...	...	...	...	...	...	140.8	159.3	172.9	24.0
SSL	0.7	45.7	53.8	61.6	1.1	...	...	...	...	154.4	159.6	173.6	28.9
Monoglycerides	2.1	51.8	59.7	67.3	4.9	...	...	...	...	154.1	157.5	172.9	27.0
DATEM	1.1	...	...	...	...	...	...	...	...	150.4	160.4	175.2	29.2
EMG	1.9	...	...	...	...	96.4	106.1	121.3	0.4	150.6	159.4	172.5	15.3

[a]Values are averages of three determinations.
[b]SSL = sodium stearoyl lactylate, monoglycerides = distilled monoglycerides, DATEM = diacetyl tartaric acid esters of mono- and diglycerides, EMG = ethoxylated monglycerides.
[c]T_o, T_p, T_c = onset, peak, and completion temperatures, respectively. SD < 1.0°C, $n = 3$.
[d]SD < 10% of the mean, $n = 3$.
[e]None detected.

112°C (T_p) for both white and brown starch layers (Tables IV and Table VI). As stated before, with regard to the data for the white starch layer (Table IV), the melting enthalpies for amylose complexes with SSL and EMG are surprisingly high. After enzymatic hydrolysis, the RS residues obtained from both layers gave the highest temperature melting enthalpies (at around 158°C) for starch complexed with SSL and DATEM (compare Tables V and VII).

It appears that added lipids interacted with amylose chains that were involved in the formation of RS in the control samples in both maize (Czuchajowska et al 1991) and in barley (this study). The formation of crystallized amylose upon addition of lipids was thereby reduced. The data suggest that amylose-amylose association was hindered by the addition of lipids and that a competitive mechanism of amylose association and amylose-lipid complex formation would apply.

In the case of amylomaize VII starch (Czuchajowska et al 1991), the yield of RS decreased from 17.6 to 8.1% as a result of intermediate complexation with SSL; this was accompanied by more than doubling of the ΔH, from 15.5 to 32.0 J/g. In the case of barley white-layer amylose (this study), the corresponding yield decreased from 7.1 to 0.7% and was accompanied by a relatively small increase in ΔH from 22.4 to 29.8 J/g. The barley white-starch layer (43.5% amylose), the barley brown-starch layer (49.3%), and amylomaize starch (~70%) gave RS yields of 7.2, 4.0, and 17.6%, respectively. Thus the potential of the starches to form RS differs widely and is not necessarily related to their amylose contents. As mentioned before, the complexing abilities of the starches differ widely (see also Tables IV and VI).

A comparison between the effects of complexing with flour strengtheners (SSL and DATEM) and the softener (monoglycerides) is of interest. In the case of the white-layer amylose (Table V), ΔH of RS increased after intermediate complexing with the strengtheners and decreased after complexing with the monoglyceride preparation. As stated before, those effects are inversely related to the decrease in yields of RS. They may be related to the higher amylose-complexing capacity of SSL and DATEM than of EMG (Krog and Jensen 1970, Eliasson and Krog 1985), as well as to the nature of the RS formed from barley and maize amyloses.

The complexing capacities were in agreement with the decreases in enthalpies of the third transition around 155°C (Tables IV and VI). This points to a competitive mechanism. Also the decrease in yield of RS was in general agreement with complexing ability.

The relation between yield and enthalpy of RS is more difficult to interpret. The yield and enthalpy of RS cannot be equated. The third transition reflects only a part of RS. The contribution of RS to that transition and the magnitude of the enthalpy depend on several factors that include the properties of lipids and amylose-lipid complexes, and the three-dimensional organization of the complexes and of the crystalline and amorphous regions (Sievert et al 1991). Thus, lipids might interact with both moieties, albeit to different degrees, thereby leading to various proportions of crystalline regions in the residue. This, in turn, may affect the magnitude of the measured enthalpies. Finally, one cannot exclude the possible role of differences in chain length, molecular weight, and structure of amyloses in maize and barley in RS formation and complexation.

Those considerations notwithstanding, the peak measured by DSC around 155°C is probably the best single, simple physicochemical parameter of RS; its nature (shape, enthalpy, and temperature) characterizes the extent of retrogradation and/or crystallization of amylose in enzyme-resistant starch.

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LITERATURE CITED

AMERICAN ASSOCIATION OF CEREAL CHEMISTS. 1983. Approved Methods of the AACC, 8th ed. Method 08-01, approved April 1961, revised October 1976 and October 1981; Method 30-25, approved April 1961, revised October 1976 and October 1981; Method 44-15A, approved October 1975, revised October 1981; Method 46-11A, approved October 1976, revised October 1982. The Association: St. Paul, MN.

BANKS, W., GREENWOOD, C. T., and KHAN, K. H. 1971. The interaction of linear amylose oligomers with iodine. Carbohydr. Res. 17:25.

BHATTY, R. S., and MacGREGOR, A. W. 1988. Gamma irradiation of hulless barley: Effect on grain composition, beta-glucans and starch. Cereal Chem. 65:463.

BILIADERIS, C. G. 1991. The structure and interactions of starch with food constituents. Can. J. Physiol. Pharmacol. 69:60.

CZUCHAJOWSKA, Z., SIEVERT, D., and POMERANZ, Y. 1991. Enzyme-resistant starch. IV. Effects of complexing lipids. Cereal Chem. 68:537.

ELIASSON, A.-C. 1985. Starch gelatinization in the presence of emulsifier. A morphological study of wheat starch. Starch/Staerke 37:411.

ELIASSON, A.-C., and KROG, N. 1985. Physical properties of amylose-monoglyceride complexes. J. Cereal Sci. 3:239.

ENGLYST, H. N., and MacFARLANE, G. T. 1986. Breakdown of resistant starch and readily digestible starch by human gut bacteria. J. Sci. Food Agric. 37:699.

ENGLYST, H. N., ANDERSON, V., and CUMMINGS, J. H. 1983. Starch and non-starch polysaccharides in some cereal foods. J. Sci. Food Agric. 34:1434.

EVERS, A. D., GREENWOOD, C. T., MUIR, C. D. D., and VENABLES, C. 1974. Biosynthesis of starch granules. 8. Comparison of the properties of small and large granules in mature cereal starches. Staerke 26:42.

GOERING, K. J., and DeHAAS, B. 1974. A comparison of the properties of large- and small-granule starch isolated from several isogenic lines of barley. Cereal Chem. 51:573.

GUDMUNDSSON, M., and ELIASSON, A.-C. 1990. Retrogradation of amylopectin and the effects of amylose and added surfactants/emulsifiers. Carbohydr. Polym. 13:295.

HOLM, J., BJÖRCK, I., OSTROWSKA, S., ELIASSON, A.-C., ASP, N.-G., LARSSON, K., and LUNDQUIST, I. 1983. Digestibility of amylose-lipid complexes in vitro and in vivo. Starch/Staerke 35:294.

HOOVER, R., and HADZIYEV, D. 1981. The effect of monoglycerides on amylose complexing during a potato granule process. Starch/Staerke 33:346.

HOVENKAMP-HERMELINK, J. H. M., DE VRIES, J. N., ADAMSE, P., JACOBSEN, E., WITHOLT, B., and FEENSTRA, W. J. 1988. Rapid estimation of the amylose/amylopectin ratio in small amounts of tuber and leaf tissue of the potato. Potato Res. 31:241.

KIM, Y. J., and ROBINSON, R. J. 1979. Effect of surfactants on starch in a model system. Starch/Staerke 31:293.

KROG, N. 1971. Amylose complexing effect of food grade emulsifiers. Staerke 23:206.

KROG, N., and JENSEN, B. N. 1970. Interactions of monoglycerides in different physical states with amylose and their anti-firming effects in bread. J. Food Technol. 5:77.

KROG, N., OLESEN, S. K., TOERNAES, H., and JOENSSON, T. 1989. Retrogradation of the starch fraction in wheat bread. Cereal Foods World 34:281.

KUGE, T., and TAKEO, K. 1968. Complexes of starchy materials with organic compounds. Part II. Complex formation in aqueous solution and fractionation of starch by l-menthone. Agric. Biol. Chem. 32:1232.

LAUNAY, B., and LISCH., J. M. 1983. Twin-screw extrusion cooking of starches: Flow behavior of starch pastes, expansion and mechanical properties of extrudates. J. Food Eng. 2:259.

LLOYD, J. B., and WHELAN, W. J. 1969. An improved method for enzymic determination of glucose in the presence of maltose. Anal. Biochem. 30:467.

McDONALD, A. M. L., and STARK, J. R. 1988. A critical examination of procedures for the isolation of barley starch. J. Inst. Brew. 94:125.

McDONALD, A. M. L., STARK, J. R., MORRISON, W. R., and ELLIS, R. P. 1991. The composition of starch granules form developing barley genotypes. J. Cereal Sci. 13:93.

MORRISON, W. R., MILLIGAN, T. P., and AZUDIN, M. N. 1984. A relationship between the amylose and lipid contents of starches from diploid cereals. J. Cereal Sci. 2:257.

OSMAN-ISMAIL, F., and SOLMS, J. 1973. The formation of inclusion

compounds of starches with flavor substances. Lebensm. Wiss. Technol. 6:147.
POMERANZ, Y. 1987. Barley. Pages 419-440 in: Modern Cereal Science and Technology. VCH Pub., New York.
PROSKY, L., ASP, N. G., SCHWEIZER, T. F., DE VRIES, J. W., and FURDA, I. 1988. Determination of insoluble, soluble, and total dietary fiber in foods and food products: International study. J. Assoc. Off. Anal. Chem. 71:1017.
RAPHAELIDES, S., and KARKALAS, J. 1988. Thermal dissociation of amylose-fatty acid complexes. Carbohydr. Res. 172:65.
RUSSELL, P. L., BERRY, C. S., and GREENWELL, P. 1989. Characterization of resistant starch from wheat and maize. J. Cereal Sci. 9:1.
SARKO, A., and WU, H.-C. H. 1978. The crystal structures of A-, B- and C-polymorphs of amylose and starch. Staerke 30:73.
SIEVERT, D., and POMERANZ, Y. 1989. Enzyme-resistant starch. I. Characterization and evaluation by enzymatic, thermoanalytical, and microscopic methods. Cereal Chem. 66:342.
SIEVERT, D., and POMERANZ, Y. 1990. Enzyme-resistant starch. II. Differential scanning calorimetry studies on heat-treated starches and enzyme-resistant starch residues. Cereal Chem. 67:217.
SIEVERT, D., CZUCHAJOWSKA, Z. and POMERANZ, Y. 1991. Enzyme-resistant starch. III. X-ray diffraction of autoclaved amylomaize VII starch and enzyme resistant starch residues. Cereal Chem. 68:86.
SLADE, L., and LEVINE, H. 1987. Recent advances in starch retrogradation. Pages 387-430 in: Industrial Polysaccharides. The Impact of Biotechnology and Advanced Methodologies. S. S. Stivala, V. Crescenzi, and I. C. M. Dea, eds. Gordon and Breach Science Publishers: New York.
SZCZODRAK, J., and POMERANZ, Y. 1991. Starch and enzyme-resistant starch from high-amylose barley. Cereal Chem. 68:589.

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