Effects of Heat Treatment of Barley Starches on In Vitro Digestibility and Glucose Responses in Rats[1]

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Cereal Chem. 73(5):588–592

ABSTRACT

Starches were purified from barley flours milled from Waxbar, Glacier, high-amylose Glacier (HAG) and hull-less high-amylose Glacier (HHAG) cultivars. Wheat starch, maize amylopectin, maize amylose, and normal maize starch were used for comparative controls. Starches were either boiled or moisture-autoclaved (3 or 12 times) with subsequent cooling overnight, after which enzyme-resistant starch (ERS) was measured. In vitro digestibility and hydrolysis rates over time were determined. Postprandial glucose responses in rats were investigated with starches from Waxbar, Glacier, HAG, and wheat. Production of ERS varied from 0.6% in waxy starches to 18.6% in the high-amylose barleys, compared to 44.2% in maize amylose starch. Boiling of starches produced only marginal effects on digestibility and hydrolysis rates, and no effects on blood glucose levels in rats. Autoclaving, however, produced significant differences in digestibility and blood glucose responses between starch types. Digestibility of waxy starches was not changed in vitro ($P > 0.05$), but blood glucose in rats was increased ($P < 0.05$) after ingestion of autoclaved Waxbar barley starch. In contrast, digestibilities of HAG and HHAG starches were reduced by 14 and 20% ($P < 0.001$) after 3 and 12 autoclaving-cooling cycles. Autoclaved HAG starch significantly lowered glucose peaks in rats compared to Waxbar and Glacier starches at 30 min ($P < 0.01$). The in vivo results corresponded to the in vitro study, which demonstrated that digestibilities of different cereal starches followed the pattern: waxy > normal > high-amylose starches after heat-moisture autoclaving, possibly due to the formation of ERS from the amylose component.

Both rate and extent of starch hydrolysis in vitro are regarded as predictors of metabolic responses to complex carbohydrate in vitro (O'Dea and Holm 1985). It has been well established that starch hydrolysis differences correlate with postprandial blood glucose changes in humans under experimental conditions (Brand et al 1985, Bornet et al 1989, Lund and Johnson 1991).

Amylomaize starch has been shown to be less susceptible to amylolysis than normal maize (Dreher et al 1984). High-amylose maize starch has been shown to have a positive lowering effect on blood glucose and insulin levels, as well as triglycerides and cholesterol in humans (Behall et al 1988, 1989; Amelsvoort and Weststrate 1992). Goddard et al (1984) observed similar responses from high-amylose rice. Behall et al (1989) concluded that long-term intake of a high-amylose starch may benefit individuals with elevated glucose and insulin levels and apparent insulin resistance, as in early adult-onset diabetes and for hyperlipidemic subjects.

High-amylose barley starch purified from the high-amylose mutant of Glacier (HAG) was also found to be less susceptible to α-amylase than was normal barley starch (Pomeranz et al 1972). Calvert et al (1976) reported that rats consumed less of a purified diet prepared with HAG starch than of a similar diet containing starch prepared from normal Glacier (NG) and, consequently, gained at a slower rate. In another study, Calvert et al (1981) fed diets prepared with HAG and NG to growing swine and found that barley starch type did not significantly affect gain or feed consumption. Rubin et al (1974) demonstrated HAG to be nutritionally superior to five other barleys, including Glacier, as measured by growth of weanling rats. Newman et al (1978) confirmed these findings and suggested that the superiority of the HAG was due to a higher lysine content of HAG protein as compared to NG protein.

[1]Contribution J-3080, Montana Agricultural Experiment Station. Partial funding for this research was supplied by the Montana Wheat and Barley Committee, Great Falls.

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Publication no. C-1996-0805-07R.

Xue et al (1991) showed that blood glucose levels in broiler chicks fed two uncooked (raw) milling fractions (flour and red dog) from HAG and HHAG barleys were not significantly flattened when compared with that of NG barley milling fractions and were higher than that of the maize control. Plasma cholesterol, however, was significantly reduced in chicks fed the high-amylose flour and red dog fractions. Björck et al (1990) reported that the amylose-to-amylopectin ratio in different barley genotypes (waxy, normal, and high-amylose starch) produced no differences in enzymatic hydrolysis when the barley flour was boiled. Autoclaving the flour, however, produced a concomitant decrease for in vitro digestibility with increased amylose content. This was explained by an increased amount of ERS (3%) and the formation of amylose-lipid complexes.

The dietary fiber content of barley, largely in the form of (1→3),(1→4) mixed linked β-D-glucans (β-glucans), may contribute to the glycemic effect. Dietary fiber is known to flatten glucose curves in humans (Jenkins and Jenkins 1985). Oat β-glucan has been reported to lower blood glucose and insulin in rats (Vachon et al 1988) and humans (Wood et al (1994). Barley β-glucan has been shown to lower cholesterol in hypercholesterolemic individuals (Newman et al 1989). In addition to the high-amylose content, high-amylose Glacier barley is also relatively high in β-glucan (7%). Therefore, effects of DF and other components in the barley may confound starch effects on glucose responses when whole barley meal is fed.

The objectives of these studies were to: 1) estimate the formation of ERS during the autoclaving process with different ratios of amylose and amylopectin in barley and maize starches; 2) determine in vitro digestibility and hydrolysis rate of barley and maize starches with different amylose and amylopectin content and heat treatments (autoclaved vs. nonautoclaved); and 3) evaluate the glucose responses of autoclaved barley starch compared to wheat starch in rats.

MATERIALS AND METHODS

Barley Flour

Waxbar, a waxy hull-less barley, Glacier, a covered normal starch barley, and two high-amylose mutants of Glacier, covered and hull-less, were grown in Arizona in 1990. The barleys were

milled through a MIAG MULTOMAT eight-roll dry mill at the Western Wheat Quality Laboratory (Washington State University, Pullman, WA). Flour was made up of 2nd break (B), 3rd B, 1st middling (M), 2nd M, 3rd M, and 4th M streams (2B–4M, 2nd break flour to 4th middling) from each cultivar.

Starch Isolation

Starches were isolated from barley flours (2B–4M) by a modification of the method of Morrison and Laignelet (1983) and Szczodrak and Pomeranz (1991). After steeping briefly in 0.02*M* HCl and neutralizing with 0.02*M* NaOH to pH 7.6, the starch suspensions were incubated for 24 hr at 37°C with protease (5 mg/g, Type XIV, Sigma) and 0.01% thimerosal (T512, Sigma) to free the starch. The suspension was filtered through 88- and 61-μm mesh sieves to remove the fiber. The residue was homogenized (homogenizor, Brinkmann Instruments, Co. Westbury, NY) with water and screened again. The filtered suspension was centrifuged at 2,000 rpm for 15 min, and the supernatant was discarded. The brown and white layers were combined and purified with an 8:1 mixture of water and toluene by a shaking procedure (McDonald and Stark 1988). As a result of this treatment, the denatured protein and dissolved fat were concentrated in the supernatant toluene layer, while the purified starch granules precipitated in the aqueous layer. The toluene layer was separated from the aqueous layer, and the procedure was repeated until a completely clear toluene layer was obtained. The wet starches were air-dried.

Wheat starch (unmodified), maize amylopectin, maize starch, and maize amylose were purchased from Sigma Chemical Co., St. Louis MO. Analyses of purified starches were made for protein, ether extract, ash (AOAC 1984) and starch (Åman and Hesselman 1984). Amylose percentage was determined with gel filtration chromatography (Torneport et al 1990).

Starch Treatment (Formation of ERS)

The formation and determination of ERS were according to the methods described by Szczodrak and Pomeranz (1991). The starch and water ratio used for ERS formation was 1:5. The suspension was autoclaved for 1 hr at 121°C using a thermostatically controlled autoclave and cooled overnight at 4°C. Starches were subjected to 3 and 12 autoclaving-cooling cycles. The treated samples were vacuum-dried from –40°C to room temperature (freeze dryer, FTS Systems Co., Stone Ridge NY). All the materials were ground on a Wiley mill (A. H. Thomas Co., Philadelphia, PA) to pass a screen with 0.5-mm diameter openings.

ERS Determination

ERS was estimated by an enzymatic-gravimetric assay as described by Sievert and Pomeranz (1989). It was considered as the residue remaining after incubation of the sample with a heat-stable α-amylase (A-3306, Sigma) and amyloglucosidase (from *Aspergillus niger*, A-9913). This assay was a modification of the AOAC method for the determination of insoluble dietary fiber (AOAC 1984).

TABLE I
Yield and Chemical Composition of Starches

Sample	Yield	Starch[a]	Amylose[b]	Protein	Lipid	Ash
Waxbar barley	51.3	94.1	7.3	0.05	0.13	0.09
Glacier barley	56.0	92.6	29.4	0.16	0.07	0.21
HAG barley[c]	50.8	92.3	40.3	0.25	0.07	0.25
HHAG barley[d]	43.3	92.6	40.5	0.19	0.04	0.22
Wheat (S5127)[e]	···	90.4	25.0	0.03	···	0.18
Maize amylopectin (A7780)[e]	···	93.6	···	0.05	0.03	0.10
Maize normal (S4126)[e]	···	94.2	25.0	0.09	0.05	0.06
Maize amylose (A7043)[e]	···	74.3	70.0	0.69	0.01	0.09

[a] Dry matter (%).
[b] Starch (%) values provided by Sigma Chemical Co. (St. Louis, MO).
[c] High-amylose Glacier.
[d] Hull-less high-amylose Glacier.
[e] Sigma product number in parenthesis.

TABLE II
Yield of Enzyme Resistant Starch from Autoclaving-Cooling Cycles[a]

	Cycles		
Sample	0	3	12
Maize amylopectin[b]	0.2 (0.0)[c]	0.3	0.5
Waxbar barley starch	0.2 (7.3)	0.2	0.3
Maize starch[b]	0.2 (25.0)	8.1	14.1
Wheat starch[b]	0.5 (25.0)	8.8	14.0
Glacier barley starch	0.7 (29.4)	7.4	12.1
HAG barley starch[d]	1.2 (40.3)	12.4	18.6
HHAG barley[e]	1.8 (40.5)	11.3	16.7
Maize amylose[b]	23.1 (70.0)	33.1	44.2

[a] Dry matter (%).
[b] Starch from Sigma Chemical Co. (St. Louis, MO).
[c] Values in parentheses are percentage of amylose (Table I).
[d] High-amylose Glacier.
[e] Hull-less high-amylose Glacier.

TABLE III
Relative Digestibility of Starches In Vitro with Different Heat Treatments[a,b]

Sample	Raw[c]	Boiled[d]	3 Cycles[e]	12 Cycles[f]	*P* Value[g]
Maize amylopectin	19.7 ± 0.4 b[h]	76.6 ± 0.3 a	77.5 ± 0.9 a	76.9 ± 0.6 a	>0.05
Waxbar barley	24.3 ± 0.3 a	74.9 ± 0.8 ab	75.8 ± 0.8 a	73.3 ± 0.6 b	>0.05
Maize	14.1 ± 0.2 d	75.5 ± 0.7 a	70.8 ± 0.7 b	64.2 ± 1.0 c	<0.001
Wheat	18.8 ± 0.4 b	76.8 ± 0.6 a	69.0 ± 0.5 b	64.4 ± 0.2 c	<0.001
Glacier barley	15.7 ± 0.2 c	73.4 ± 0.8 bc	70.4 ± 1.3 b	64.3 ± 1.5 c	<0.01
HAG barley[i]	13.4 ± 0.2 d	72.1 ± 0.7 c	61.4 ± 0.5 c	57.2 ± 1.1 d	<0.001
HHAG barley[j]	11.5 ± 0.2 e	72.0 ± 0.6 c	62.4 ± 0.3 c	57.8 ± 0.4 d	<0.001
High-amylose Maize	5.3 ± 0.4 f	44.8 ± 0.7 d	49.1 ± 0.8 d	38.6 ± 0.9 e	<0.001

[a] Measured as starch degree of hydrolysis (%) with α-amylase after 60 min of incubation at 37°C.
[b] Data are means of 4–14 observations.
[c] Native starch without heat treatment.
[d] Boiled before incubation.
[e] Autoclaved 3 times, dried, and reboiled before incubation.
[f] Autoclaved 12 times, dried, and reboiled before incubation.
[g] Significance levels of autoclaving effects compared to boiled effects.
[h] Values with different superscript letters are differ significantly ($P < 0.05$ to $P < 0.001$).
[i] Covered high-amylose Glacier.
[j] Hull-less high-amylose Glacier.

Starch Hydrolysis In Vitro with α-Amylase

The amount of maltose released by treatment with pancreatin was determined spectrophotometrically with 3,5-dinitrosalicylic acid (1% in 0.4*N* NaOH containing 30% sodium potassium tartrate) according to the method of Björck et al (1987) and Wootton and Chandhry (1979), with modifications. About 500 mg of dry starch was added to 50 ml of 0.05*M* K-Na phosphate buffer (pH 6.9), containing 0.04% (w/v) NaCl. The solution was boiled in a water bath for 10 min and cooled to 37°C. A volume of 0.2 ml of α-amylase solution containing 504 U/ml was added to the starch suspension. The α-amylase solution was prepared from porcine pancreatic α-amylase (A-6255, Sigma, containing 27 mg of protein/ml and 1,260 U/mg [1 U liberates 1 mg of maltose from starch in 3 min at pH 6.9 and 20°C]). The starch suspension was then incubated at 37°C for up to 1 hr. Samples (0.2 ml) were withdrawn at timed intervals and analyzed for reducing sugar with dinitrosalicylic acid reagent. Maltose was used as a standard. Percent hydrolysis was calculated as milligrams of maltose from standard curve per milligram of starch × 100. The digestibility of the starches was calculated on the basis of percent hydrolysis measured after 1 hr of incubation, at which point an equilibrium or end point of hydrolysis was reached.

Glucose Tolerance Test In Rats

Diets. Starches prepared from Waxbar, Glacier, and HAG barleys and wheat were treated with different heat-moisture treatments (nonautoclaved, autoclaved for 3 and 12 cycles), boiled for 15 min in deionized water and 7.5% starch solutions with stirring, and cooled overnight at room temperature (≈21°C). Glucose was included as a reference.

Animals, treatments and analysis. Male rats (104) from Sprague-Dawley, eight weeks old, weighing an average 240 g were individually housed in a light-controlled (12 hr dark and 12 hr light), approved laboratory animal facility for a five-day adaptation period. During this time they were fed Purina Laboratory Chow free-choice. The rats were fasted for 24 hr and then gavaged via a stomach tube with 10 ml of the starch solutions (three treatments per starch) or glucose (eight rats per diet). The starch solutions provided ≈300 mg of dry matter per 100 g of body weight. Blood samples (1 ml) were taken from the tail artery of each rat at 0, 30, 60, 120, and 240 min after gavaging. Blood serum glucose levels were measured with a Kodak Ektachem DT 60 Analyzer (Eastman Kodak Co., Rochester, NY), using a glucose oxidase reaction and colorimetric determination. The rats were then fed Purina Laboratory Rat Chow for two weeks, randomized, and the test repeated as described. All procedures were approved by Montana State University's Animal Care Committee, according to the National Research Council guidelines (NRC 1985).

Analyses of purified starches were made for protein, ether extract, ash (AOAC 1984) and starch (Åman and Hesselman 1984). Amylose percentage was determined with gel-filtration chromatography (Torneport et al 1990).

Statistical Analysis

Main effects were analyzed using SAS general linear models procedure for repeated measurement; differences between means were analyzed by least squares means. Correlation coefficients (r) were determined by Pearson's CORR procedure (SAS 1988).

RESULTS AND DISCUSSION

Starch Isolation

The yield and chemical composition of purified barley starch are shown in Table I. Starch yield from barley flours ranged from 43.3 to 56%, which is similar to that reported by Szczodrak and Pomeranz (1991). The dry barley starches were determined to be ≈92.5% pure using soluble starch (S-9765, Sigma) as the standard. The total of crude protein, ether extract, and ash content of each barley starch was <0.3%, which was similar to that in the starches from Sigma. The lower starch value (74.3%) of maize amylose starch was possibly due to the high level of ERS (23%) resulting from the extraction process (Table II). The amylose content of barley starches varied from 7.3% in Waxbar to 40% in HAG and HHAG starches. In contrast, maize starches varied from 0% amylose in maize amylopectin to 70% amylose in maize amylose starch.

Formation of ERS

Yields of ERS from the different cereal starches with 0, 3, and 12 autoclaving-cooling cycles are shown in Table II. ERS formation varied with increasing percentage of amylose content and severity of the heat treatments. ERS formation >1% as measured in HAG and HHAG starches that did not undergo the autoclaving-cooling process was similar to values reported by Szczodrak and Pomeranz (1991). The extremely high level of ERS (23%) in the maize amylose before autoclaving is not readily explained, other than that the high level of amylose (70%) may make this starch more susceptible to ERS formation during the boiling process. Yield of ERS from waxy starches (maize amylopectin and Waxbar starch) was <0.6% even after 12 autoclaving-cooling cycles. In contrast, nonwaxy starches were strongly affected by the autoclaving heat treatment, with the affected high-amylose starches yielding the greatest amount of ERS. The 16.7–18.6% ERS produced in the HHAG and HAG barleys, respectively, are similar to that (21%) reported by Szczodrak and Pomeranz (1991). Although ERS in the native maize amylose was exceptionally high, it was further increased from 23 to 44% after 12 autoclaving cycles.

A significant positive correlation between amylose content and ERS formation was found for native starch ($r = 0.79$, $P < 0.05$), for starch autoclaved 3 times ($r = 0.96$, $P < 0.01$), and for starch autoclaved 12 times ($r = 0.97$, $P < 0.0001$). Similar correlations between ERS formation and amylose percentage have been reported by Berry 1986, Björck et al 1990, Szczodrak and Pomeranz 1991. These data support the earlier hypothesis that retrograded amylose is mainly responsible for the generation of ERS (Berry 1986, Berry et al 1988, Ring et al 1988, Siljeström et al 1989, Sievert and Pomeranz 1989, Sievert et al 1991, Szczodrak and Pomeranz 1991). Because the lipid content was <0.07% in HAG and <0.01% in maize amylose, amylose-lipid complexing probably had no influence on ERS formation .

In Vitro Digestibility and Hydrolysis Rates of Starches

The in vitro digestibilities of raw and heat-treated starches are shown in Table III. Raw starches had a much lower ($P < 0.01$) digestibility values (5.3–24.3%) than did boiled starches (44–76.8%). Raw waxy barley starch (Waxbar) showed the highest ($P < 0.05$) susceptibility to α-amylolysis (24.3%), followed by maize amylopectin, wheat, and the Glacier barleys. These results differ from those of Björck et al (1990), in which raw wheat starch had much lower digestibility (6.2%) than that of any other raw starches (>22%).

Hydrolysis rates of all starches subjected to 0 (boiled only), 3, and 12 autoclaving and cooling cycles are illustrated in Figures 1–3, respectively. The hydrolysis rates of boiled high-amylose starch suspensions were marginally lower than those of waxy and normal starches, with the exception of maize amylose, which was much lower than that of any of the other starches. The effect of starch type on hydrolysis rate and total digestibility was significantly magnified by autoclaving (Figs. 2 and 3). Hydrolysis rates and total digestibility of waxy starches were not affected ($P > 0.05$) by the autoclaving-cooling treatment (Figs. 1–3). In contrast, rates of hydrolysis and total digestibilities of nonwaxy starches were significantly lowered ($P < 0.001$) by autoclaving. Similar results from barley flour have been reported by Björck et al (1990). The total digestibilities of HAG and HHAG starches were reduced by 14 and 20%, after 3 and 12 autoclaving-cooling cycles, respectively. Therefore, with increasing amylose content, a concomitant decrease occurred for in vitro digestibility of autoclaved starch. Digestibility of

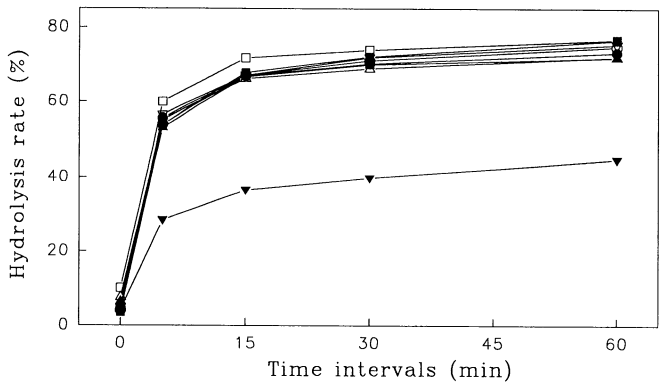

Fig. 1. Degree of starch hydrolysis (%) with boiled treatment. Samples: Waxbar barley (○); Glacier barley (●); high-amylose Glacier (Δ); hull-less high-amylose Glacier (▲); wheat (□); maize amylopectin (■); maize starch (▽); maize amylose (▼).

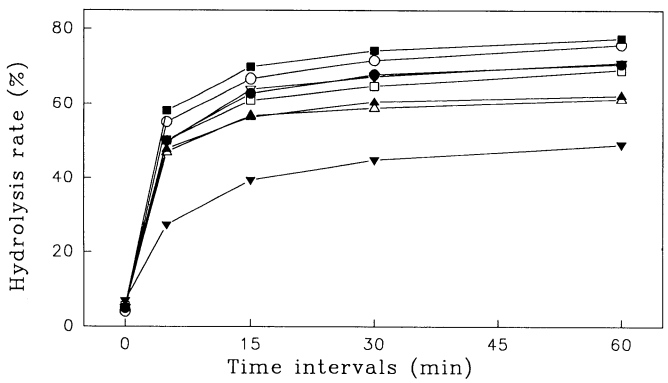

Fig. 2. Degree of starch hydrolysis (%) with 3-cycle autoclaved treatment. Samples: Waxbar barley (○); Glacier barley (●); high-amylose Glacier (Δ); hull-less high-amylose Glacier (▲); wheat (□); maize amylopectin (■); maize starch (▽); maize amylose (▼).

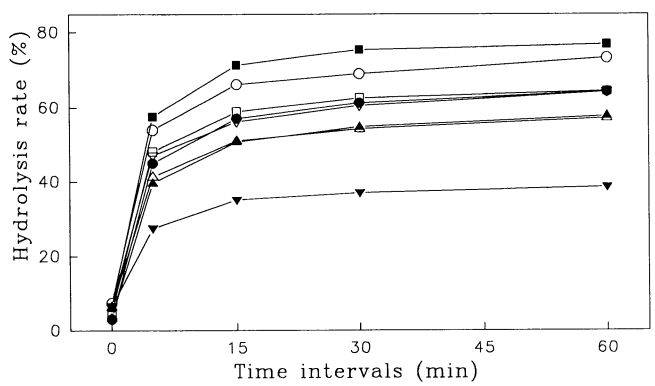

Fig. 3. Degree of starch hydrolysis (%) with 12-cycle autoclaved treatment. Samples: Waxbar barley (○); Glacier barley (●); high-amylose Glacier (Δ); hull-less high-amylose Glacier (▲); wheat (□); maize amylopectin (■); maize starch (▽); maize amylose (▼).

starch suspensions was significantly ($P < 0.001$) negatively correlated with amylose content ($r = -0.98$) and ERS formation ($r = -0.97$) in different heat treatments.

These data demonstrate that the hydrolysis rates of different cereal starches followed the pattern: waxy > normal > high-amylose starches after heat-moisture autoclaving, possibly due to the formation of ERS from the amylose component.

Glucose Responses in Rats

Postprandial glucose responses in rats after ingestion of 13 different diet solutions are shown in Figures 4–6. All time intervals showed no significant differences in postprandial glucose levels in

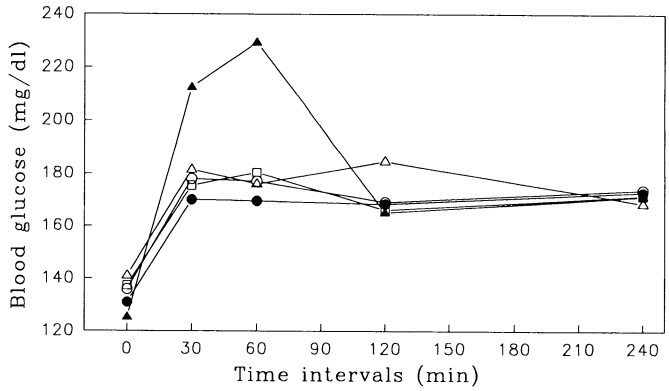

Fig. 4. Glucose responses in rats fed boiled starches. Samples: glucose (▲); Waxbar starch (○); Glacier starch (●); high-amylose Glacier starch (Δ); wheat starch (□).

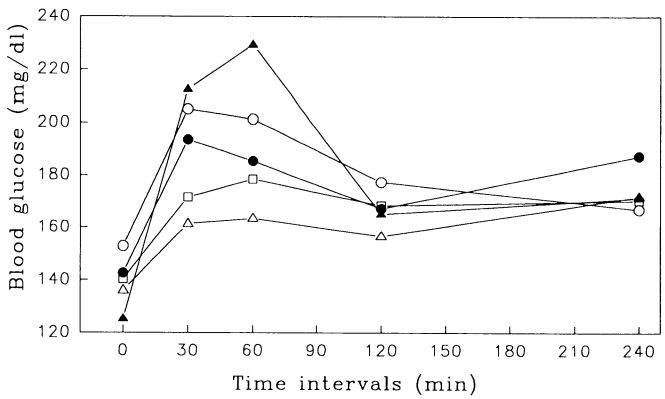

Fig. 5. Glucose responses in rats fed 3-cycle autoclaved starches. Samples: glucose (▲); Waxbar starch (○); Glacier starch (●); high-amylose Glacier starch (Δ); wheat starch (□).

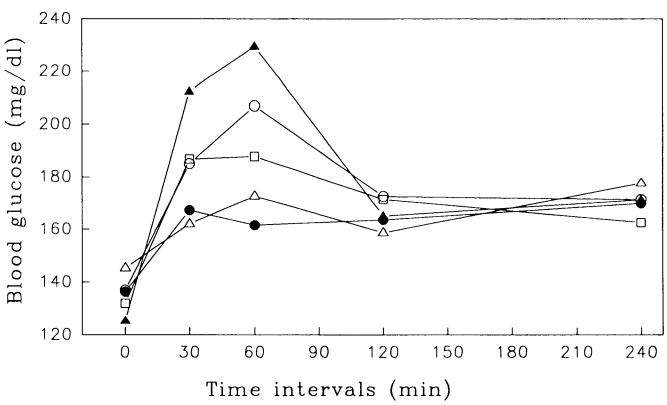

Fig. 6. Glucose responses in rats fed 12-cycle autoclaved starches. Samples: glucose (▲); Waxbar starch (○); Glacier starch (●); high-amylose Glacier starch (Δ); wheat starch (□).

rats fed any of the boiled starch solutions. After autoclaving, Waxbar barley starch produced an increased ($P < 0.05$) glucose peak at 30 min (3 cycles) and 60 min (12 cycles) when compared to the boiled Waxbar starch. This suggests that the waxy starch had increased susceptibility to enzymatic action, as observed by Goering and Eslick (1976). Autoclaved HAG barley starch produced lower glucose levels when compared to Waxbar at 30 min ($P < 0.01$) or at 60 min ($P < 0.05$) and when compared to Glacier starches at 30 min ($P < 0.01$) after 3 autoclaving cycles. There was a tendency for a delayed response to HAG starch at 240 min. Generally, starches autoclaved for 12 cycles produced the same pattern as that of 3 cycles. Differences were not significant ($P > 0.09$) among any treatments at 120 and 240 min. These in vivo results corresponded to the in vitro study, and demonstrated that glucose

absorption in rats from nonwaxy barley starches was reduced after heat-moisture autoclaving, possibly due to the formation of resistant starch from the amylose component. The glucose peaks in rats at 30 and 60 min after ingestion of autoclaved starches were closely correlated ($P < 0.05$) to in vitro digestibility ($r = 0.79$), amylose content ($r = -0.76$), and ERS ($r = -0.72$).

ERS formation in wheat, maize, and barley during heat-moisture processing may explain the lowered glucose responses to high-amylose starch, because ERS is believed to consist of retrograded amylose (Berry 1986, Ring et al 1988, Siljeström et al 1989, Szczodrak and Pomeranz 1991). Moreover, ERS is less bioavailable in the human gastrointestinal tract and exerts physiological effects similar to those of dietary fiber (Jenkins and Jenkins 1985, Björck et al 1987, Englyst and Cummings 1987, Schneeman 1989). Carbohydrate foods containing ERS have advantages to consumers because the starch is less available and tends to function more as dietary fiber.

These results imply that high-amylose grains have a great potential for food development for specialty markets, such as products for individuals with diabetes, where decreased availability or a slower rate of digestion is beneficial. High-amylose barley is a relative newcomer to cereal science investigation, and may have future industrial application.

ACKNOWLEDGMENTS

We wish to thank P. Hofer, K. Horsley, and R. Adair for technical assistance with chemical analysis, and L. Wang for assistance in manuscript preparation. Barley was grown by R. T. Ramage, USDA-ARS, Univ. Arizona at Tucson.

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[Received April, 3, 1996. Accepted June 27, 1996.]