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In Vitro Digestion Rate and Estimated Glycemic Index of Oat Flours from Typical and High β -Glucan Oat Lines

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ABSTRACT: The in vitro starch digestion rate and estimated glycemic index (GI) of oat flours and oat starches from typical and high β -glucan oat lines were evaluated along with the impact of heating on starch digestion. Flour from oat lines ('Jim', 'Paul', IA95, and N979 containing 4.0, 5.3, 7.4, and 7.7% β -glucan, respectively) was digested by pepsin and porcine pancreatin. To determine the impact of heating on starch digestion, oat slurries were prepared by mixing oat flour and water (1:8 ratio) and heating for 10 min prior to digestion. Viscosity, as measured on a Rapid Visco Analyzer, increased with increases in concentration and molecular weight of β -glucan. The in vitro starch digestion of oat flours and a control, white bread made from wheat flour, increased as the digestion time increased. Starch digestion of oat flour was slower than that of the control ($p < 0.05$). Heat treatment of oat-flour slurries increased the starch digestion from a range of 31–39% to a range of 52–64% measured after 180 min of in vitro digestion. There were no differences in starch digestibility among oat starches extracted from the different oat lines. The GI, estimated by starch hydrolysis of oat flours, ranged from 61 to 67, which increased to a range of 77–86 after heating. Oat-flour slurries prepared from IA95 and N979 lines with high β -glucan concentrations had lower GI values than did slurries made from Jim and Paul lines. Starch digestion was negatively correlated with β -glucan concentrations in heated oat-flour slurries ($R^2 = 0.92$). These results illustrate that the oat soluble fiber, β -glucan, slowed the rate of starch digestion. This finding will help to develop new food products with low GI by using oat β -glucan.

KEYWORDS: oat β -glucan, starch digestion, estimated glycemic index

■ INTRODUCTION

β -Glucan, a soluble fiber in oats, has gained much attention resulting from its health benefits to help decrease glucose uptake and insulin responses, lower cholesterol in the blood, and induce and prolong satiety.^{1,2} It has been suggested that the physiological activity of β -glucan is attributed to its effect in increasing the viscosity in the upper digestive tract,³ but the exact mechanism is not well understood.

The glycemic index (GI) characterizes the carbohydrates consumed in different types of foods on the basis of the postprandial level of blood glucose.^{4,5} The long-term intake of low GI foods has been reported to improve glucose control in people with diabetes and reduce insulin sensitivity of glucose intolerance.^{6,7} The rate of blood glucose rise is determined by the rate and extent of starch digestion.⁸ Several studies showed a high correlation between the rate of starch digestion and the glycemic response by various in vitro digestion methods that mimic the in vivo situation.^{5,9,10} The starch digestion rate is dependent on botanical origin, which determines the structural type and shape of starch granules and the ratio of amylose and amylopectin,¹¹ and thermal processing, which determines the extent of starch gelatinization.¹² Also, the presence of dietary fiber in foods influences the starch digestibility by changing the microstructure of foods, which decreases the susceptibility of starch to amylolytic attack,^{13,14} and by limiting water availability, which restricts starch gelatinization.^{15,16}

Oat β -glucan, as a soluble dietary fiber (SDF), may influence the starch digestibility and help to lower GI in foods. In an attempt to better understand this characteristic of β -glucan, four different oat types with typical and high β -glucan

concentrations were evaluated for their in vitro starch digestion rate and estimated GI, along with the impact of heating on starch digestion.

■ MATERIALS AND METHODS

Oat Flour and Oat Slurry Preparation. Two publicly available oat lines, 'Jim' and 'Paul', with a typical concentration of β -glucan, and two experimental oat lines developed at Iowa State University, IA95111 (IA95) and N979-5-4 (N979), with a high level of β -glucan, were selected for this study. All oat lines were grown at the Agronomy and Agricultural Engineering Field Research Center in Ames, Iowa, and harvested in 2010. The harvested oat kernels were dried and dehulled with an air-pressure dehuller (Codema, Eden Prairie, MN). The resulting oat groats were ground in an ultracentrifugal mill (ZM-1, Retch GmbH & Co., Hann, Germany) with a 0.5 mm sieve. Oat flours were then stored at 4 °C until used. To determine the impact of heating on in vitro starch digestion, oat-flour slurries were prepared by mixing oat flour and water (1:8 ratio) and heating for 10 min prior to digestion, which simulated preparation of oat porridge.¹⁷

Proximate Composition. The moisture concentration of oat flours was analyzed by AACC Method 44-15A.¹⁸ The starch concentration was determined by following AACC Method 76-13 by using a Total Starch Kit (Megazyme International Ireland Ltd., Bray, Ireland). Proteins were analyzed by using an automatic nitrogen analyzer (Elementar Analzen System GmbH, Germany) with a nitrogen conversion factor of 5.7. Lipids were analyzed by following the gravimetric method after extraction with the mixture of petroleum

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Table 1. Composition of Oat Flours and MW of β -Glucan from Different Oat Lines

oat lines	composition ^a (% , dry weight basis)							β -glucan MW ^a ($\times 10^5$ g/mol)	
	starch	protein	lipid	β -glucan	SDF	IDF	ash	number-average MW	peak MW
Jim	60.4 \pm 0.2 a	12.3 \pm 0.1 c	7.0 \pm 0.2	4.0 \pm 0.4 c	4.1 \pm 0.3 b	3.7 \pm 0.1 b	2.7 \pm 0.6	6.1 \pm 0.5	8.9 \pm 0.4 b
Paul	56.8 \pm 0.6 ab	13.8 \pm 0.2 b	7.6 \pm 0.6	5.3 \pm 0.1 b	5.4 \pm 0.6 b	6.2 \pm 0.4 a	2.2 \pm 0.3	6.6 \pm 0.1	9.8 \pm 0.1 ab
IA95	54.4 \pm 1.3 ab	14.8 \pm 0.1 a	6.9 \pm 0.1	7.4 \pm 0.4 a	8.1 \pm 0.8 a	6.2 \pm 0.2 a	2.2 \pm 0.1	7.2 \pm 0.6	11.5 \pm 0.7 a
N979	51.9 \pm 2.8 b	13.6 \pm 0.1 b	7.5 \pm 0.4	7.7 \pm 0.1 a	9.7 \pm 0.9 a	6.3 \pm 0.1 a	2.2 \pm 0.1	5.7 \pm 0.8	7.7 \pm 0.4 c

^aValues are means \pm standard deviations. Values followed by different letters within a column are significantly different ($P < 0.05$).

ether and 2-propanol (3:2) in a Goldfish system (AACC Method 30-25). The concentrations of β -glucan in oat flours were enzymatically measured by AACC Method 32-23, with the application of a mixed β -glucan linkage kit (Megazyme). SDF and insoluble dietary fiber (IDF) were determined by using a total dietary fiber kit (Megazyme; AACC Method 32-07). The ash content in oat flour was determined by AACC Method 08-01. All analyses were run in triplicate, and the averages were reported on a dry-weight basis.

β -Glucan Molecular Weight (MW) Determination. For the determination of β -glucan MW, β -glucan was extracted from oat flours by using the procedure of Yao et al.¹⁹ The relative MW distribution of extracted β -glucan was determined by using size-exclusion high-performance liquid chromatography (SE-HPLC) according to the method of Sayar et al.²⁰ The SE-HPLC consisted of a solvent delivery module (model 210, ProStar, Varian Inc., Reodyne, CA), a 100 μ L loop injection valve, a guard column (Ohpak SB-G, Shodex Showa Denko K. K., Tokyo, Japan), three serially connected columns (Ohpak SB-806 HQ, Ohpak SB-805 HQ, and Ohpak SB-804 HQ; Shodex Showa Denko K. K.), and a refractive index detector (model 350, ProStar, Varian Inc.). The column temperature was 40 $^{\circ}$ C, and the flow rate of the mobile phase, Milli-Q water (Milipore, Bedford, MA) containing 0.02% sodium azide, was 0.5 mL/min. An aliquot was filtered through a 0.45 μ m filter (25 mm i.d., GD/X 25 nylon Syringe Filter, Whatman Inc., Piscataway, NY) before injection. β -Glucan MW standards (catalog no. P-MWBG, Megazyme) with MW values of 3.59×10^5 , 2.45×10^5 , 1.83×10^5 , 1.23×10^5 , and 0.40×10^5 g/mol were used to estimate the actual MW ranges of the extracted β -glucan. The number-average MW and peak MW were obtained by a line of log MW versus retention time of the HPLC chromatogram.¹⁹

Viscosity Measurement. The Rapid Visco Analyzer (RVA, Newport Scientific, Warriewood, Australia) was used to measure the viscosity of oat-flour slurries as a function of temperature, time, and stirring speed by using the pasting program.²¹ The test profile of the RVA included a stirring speed of 960 rpm for 10 s and 115 rpm for the remainder of the test and a temperature program increasing from 40 to 90 $^{\circ}$ C over 3 min, holding at 90 $^{\circ}$ C for 6.5 min, decreasing to 40 $^{\circ}$ C over 4.5 min, and holding at 40 $^{\circ}$ C for 5 min. To measure the viscosity, oat flour (13% w/v) was dispersed in 16.7 mM silver nitrate solution to inhibit natural β -glucan-degrading enzymes.²¹ All pasting curves were collected in triplicate, and the results were averaged. The peak and final viscosities were measured.

Extraction of Oat Starch. To compare in vitro starch digestibility of intact oat starch, the starch was extracted from oat flour according to the method given by Sayar et al.²² Oat flour (25 g) was mixed with 200 mL of 0.02 M sodium hydroxide solution at room temperature for 30 min. The mixture passed through a 106 μ m pore diameter sieve (no. 140), and the filtrate was centrifuged at 3200g for 20 min. The bottom solid layer of the pellet was transferred to a 50 mL centrifuge tube and mixed with distilled water. After the neutralization with 1 M hydrochloric acid, the mixture was centrifuged at 3200g for 20 min. The supernatant was discarded, and the pellet was dried at 40 $^{\circ}$ C overnight. The starch content was determined after drying.

In Vitro Starch Digestibility. The in vitro starch digestibility was determined by the method of Englyst et al.²³ and Regand et al.²⁴ with modifications. The enzyme solution for digestion was prepared as follows: 0.9 g of porcine pancreatin (EC 232.468.9, from porcine pancreas, activity $8 \times$ USP/g, Sigma-Aldrich, St. Louis, MO) was dispersed in 4 mL of distilled water and centrifuged at 1500g for 10

min. The supernatant (5.4 mL) was mixed with 0.8 mL of diluted amyloglucosidase [0.64 mL of amyloglucosidase (EC 3.2.1.3., 3300 U/mL, Megazyme) diluted to 0.8 mL of distilled water], and 0.2 mL of distilled water was added. This enzyme solution was freshly prepared for each digestion.

Oat flours, heated oat-flour slurries, oat starches, heated oat-starch slurries, corn starch (Sigma-Aldrich), and a control (white bread made from wheat flour) were weighed to 100 mg into 50 mL tubes, in triplicate, with 10 glass beads (5 mm diameter) added to each tube. Two milliliters of 0.05 M hydrochloric acid and 10 mg of pepsin were added to the tubes and incubated at 37 $^{\circ}$ C in a shaking water bath for 30 min. Four milliliters of sodium acetate buffer (0.5 M, pH 5.2) was added to each tube; the freshly prepared enzyme solution (1 mL) was added after 1 min intervals. The mixtures were then incubated at 37 $^{\circ}$ C in a shaking water bath. Aliquots (100 μ L) were taken at 0, 10, 20, 30, 60, 90, 120, and 180 min intervals and mixed with 50% ethanol (1 mL). These solutions were centrifuged at 800g for 10 min, and the hydrolyzed glucose content of the supernatant was measured by using the glucose oxidase-peroxidase assay (Megazyme). Total starch hydrolysis (%) in the oat flour or oat starch slurry was calculated as follows: total starch hydrolysis (%) = $\{[\text{released glucose weight} \times 160/182]/(\text{total starch weight in oat flour or oat starch slurry})\} \times 100$.

Estimation of GI. The kinetics of in vitro starch digestion was followed by a nonlinear model established by Goni et al.²⁵ The first order equation is $C = C_{\infty} (1 - e^{-kt})$, where C is the percentage of starch hydrolyzed at time t (min), C_{∞} is the equilibrium percentage of starch hydrolyzed after 180 min, and k is the kinetic constant. The parameters, C_{∞} and k , were estimated for each treatment based on the data obtained from the in vitro starch digestion. The area under the hydrolysis curve (AUC) was calculated by the following equation: $\text{AUC} = C_{\infty}(t_f - t_0) - (C_{\infty}/k)[1 - \exp\{-k(t_f - t_0)\}]$, where C_{∞} is the equilibrium percentage of starch hydrolyzed after 180 min, t_f is the final time (180 min), t_0 is the initial time (0 min), and k is the kinetic constant.

A hydrolysis index (HI) represents the rate of starch digestion, and estimated GI indicates the digestibility of the starch in oats in relation to the digestibility of starch in a reference material, white bread. The HI, a good predictor of glycemic response, was calculated by dividing the AUC of each treatment by the AUC of a reference (control, white bread). The GI was then estimated by using the following equation of Goni et al.:²⁵ $\text{GI} = 39.71 + 0.549\text{HI}$.

Statistical Analysis. Oat flours from each of the four lines were prepared in triplicate. All analyses were done in triplicate, and the values were averaged. Data on the replicate preparations were analyzed by using the analysis of variance (ANOVA), followed by least significant differences (LSD) for the comparison among treatments using the GLM procedure found in SAS version 9.1 (SAS Inst., Cary, NC) at $\alpha = 0.05$.

RESULTS AND DISCUSSION

Characterization of Oat Flours. The contents of starch, protein, lipid, β -glucan, and dietary fiber in the oat flours from different oat lines are shown (Table 1). The oat lines were selected for their differences in β -glucan concentrations in this study. The traditional oat lines, Jim and Paul, contained 4.0 and 5.3% β -glucan, respectively. The experimental lines, IA95 and N979, contained 7.4 and 7.7% β -glucan, which were greater

than in typical domestic cultivars. The starch content of oat lines ranged from 51.9 to 60.4%. The Jim and Paul oat flours, with low β -glucan concentrations, had greater concentrations of starch than did IA95 and N979 lines. The protein concentration of Jim oat line was lower than that of the other three oat lines ($p < 0.05$), but the value was within the range of common oats reported.^{19,26} The lipid concentrations of the four different oat lines were not different from each other. The concentrations of SDF (including β -glucan) and IDF of IA95 and N979 oat lines were greater than those of Jim and Paul lines. As expected, the SDF of oat flours was correlated with the β -glucan concentrations ($R^2 = 0.95$). Thus, the major component of SDF in oats was β -glucan. The Jim oat flour had the lowest IDF% among all oat flours.

The number-average MW values of β -glucan extracted from the four oat lines did not differ among lines ($p > 0.05$), ranging from 5.7×10^5 g/mol to 7.2×10^5 g/mol (Table 1). The peak MW of extracted β -glucan from the IA 95 oat (11.5×10^5 g/mol) was greater than from the Jim, Paul, and N979 oat lines. The peak MW of β -glucan extracted from the N979 oat line was the lowest, although this line had the greatest concentration of β -glucan. A positive correlation between β -glucan concentration and peak MW distribution was reported in a previous study.¹⁹ Heritable traits, such as genotype, impacted the β -glucan concentration and MW in these four oat lines.²⁷

Viscosity of Oat-Flour Slurries. The peak viscosity values of the oat-flour slurries were not different from each other; however, the final viscosity of the oat-flour slurry made from N979 oat line was lower than those of other three oat lines (Figure 1). Among three oat lines, Jim, Paul, and IA95, the

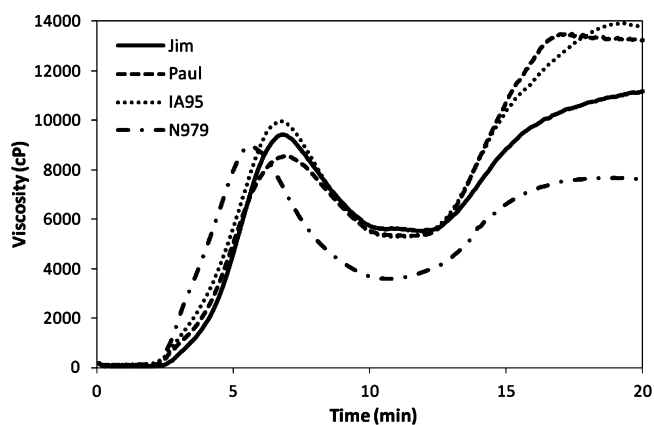


Figure 1. Viscosity profiles of different oat lines prepared as oat-flour slurries in silver nitrate solution. Values are the average of three replicates.

viscosity increased with increases in concentration and MW of β -glucan. However, the N979 line showed the lowest final viscosity despite containing the highest amount of β -glucan. In contrast, in a previous study, the N979 oat line grown in the years of 2002, 2003, and 2004 showed the greatest peak viscosity and final viscosity with the highest concentration and MW of β -glucan as compared to the Jim, Paul, and IA95 lines.¹⁹ The N979 oat line grown in the year of 2011 contained higher amounts of low MW β -glucan than in previous years, resulting in a reduction of viscosity (Table 1). The impact of β -glucan on viscosity was greater than that of starch in oat flour.²¹ Even

though the peak viscosity was high, this viscosity was more likely attributed to β -glucan than starch.

In Vitro Starch Digestibility. In vitro starch digestibilities of raw oat flours and heated oat-flour slurries from the Jim, Paul, IA95 and N979 lines are shown in Figure 2a,b. The starch

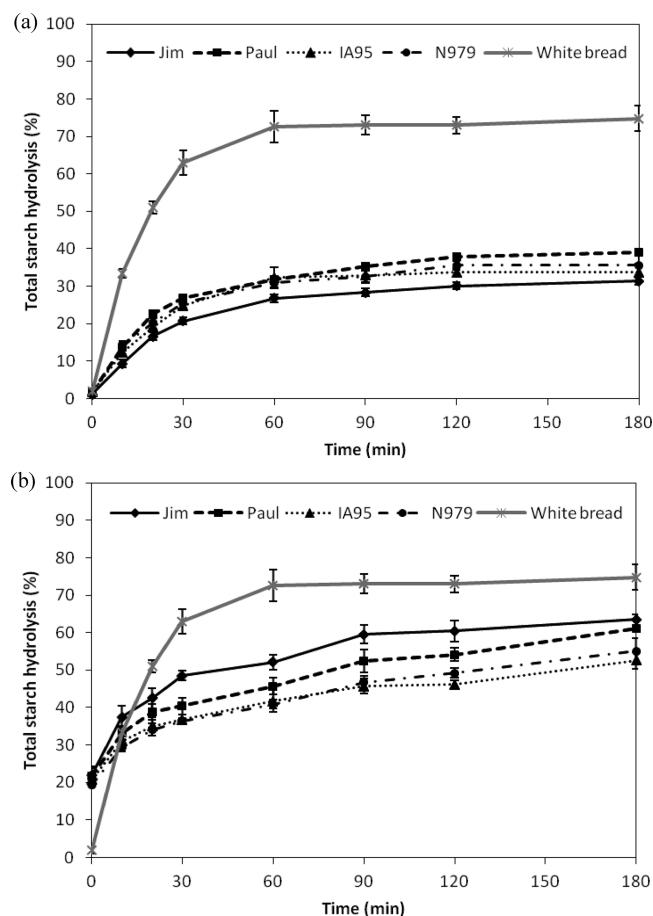


Figure 2. In vitro starch hydrolysis of raw oat flours (a) and heated oat-flour slurries (b) from different oat lines. Values are the average of three replicates.

hydrolysis of raw oat flours and the control increased as digestion time increased. The control showed a digestion value of $\sim 70\%$ after 180 min, which agreed with the value reported in the original protocol by Goni et al.²⁵ The starch digestion rate of raw oat flour was much slower than that of the control ($p < 0.05$). There were no differences in starch digestion of raw oat flours from Jim, Paul, IA95, and N979 oat lines.

Heat treatment of oat-flour slurries increased starch digestion from a range of 31–39% to a range of 52–64% measured after 180 min of in vitro digestion (Figure 2b). The starch digestibility of heated oat-flour slurries was lower than that of the control. The starch of the heated oat slurry from the Jim line had a higher digestibility than did those from the IA95 and N979 oat lines during in vitro digestion. The Jim oat contained less SDF, including β -glucan, and IDF than did the IA95 and N979 oats (Table 1). Heating changes the structure of starch in foods.¹² When starch granules in oat flours were gelatinized by heating, the structure was disrupted, thus increasing the susceptibility of enzymatic digestion. The presence of dietary fiber in oat flours possibly retarded the starch digestion by formation of a transient fiber network, thus decreasing the

susceptibility of enzyme attack.¹⁵ The SDF possibly limited the water availability as a consequence of soluble nonstarch polysaccharide hydration, which restricted starch gelatinization in oats.^{15,16}

The *in vitro* starch digestibility of oat starches, which were extracted from the four different oat lines to contain ~95% starch, showed that raw oat starches and corn starch were digested to 30% of total starch after 180 min of digestion (Figure 3a). The starch digestibility of raw oat starch was lower

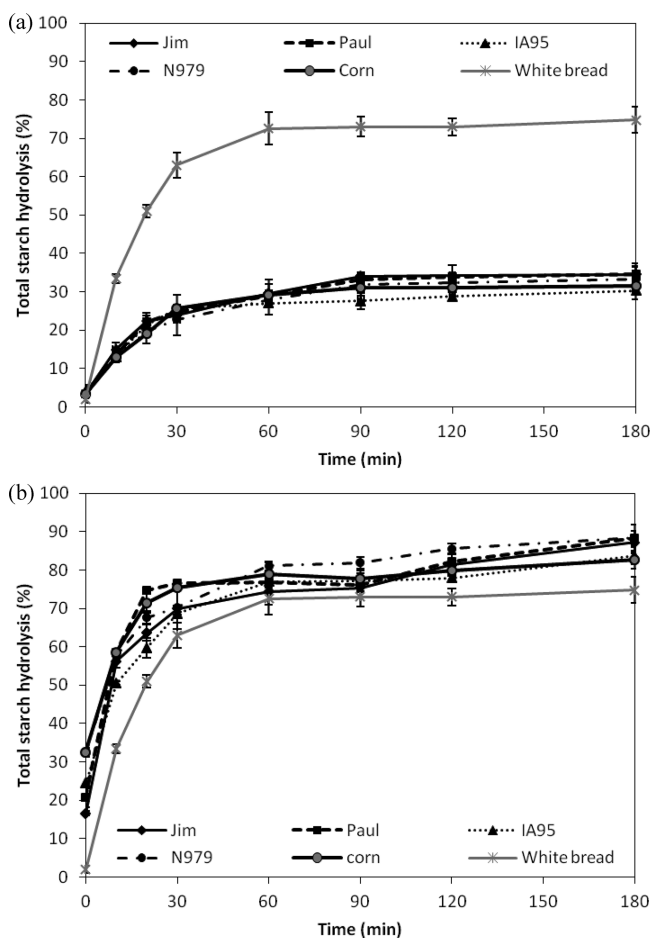


Figure 3. *In vitro* starch hydrolysis of raw oat starches (a) and heated oat-starch slurries (b) from different oat lines. Values are the average of three replicates.

than that of the control. Similar to the result shown for raw oat flour (Figure 2), heat treatment of oat starch slurries increased the digestibility of starch to a level greater than that of the control (Figure 3b). There were no differences in starch digestibility among heat-treated oat-starch slurries. The starch digestibility can be influenced by structures or conformation of starch, such as amylose and amylopectin ratio, granule size and type, and degree of crystallinity.²⁸ The relative proportions of amylose and amylopectin in the starch granules are known to impact enzymatic digestibility.¹² A high amylose content has been associated with reduced susceptibility to enzymatic hydrolysis of cooked rice and bean starches.^{29,30} The amylose content of oat starches from various oat cultivars was reported in the range of 25.2–29.4 and 19.6–24.5% of total starch.^{28,31} In the current study, the starch digestibility of oat starches from different oat lines was not different among lines, suggesting no differences in structure or conformation of starch extracted

from the Jim, Paul, IA95, and N979 oat lines. Further study is needed to fully investigate the impact of oat starch structural features attributed to different oat lines on the rate and extent of starch digestion.

HI and Estimated GI. The estimated GI of raw oat flours ranged from 61 to 67, which increased to a range of 77–86 after heating (Table 2). Heated oat-flour slurries prepared from

Table 2. Calculated HI and Estimated GI for Raw Oat Flours, Heated Oat-Flour Slurries, Raw Oat Starches, and Heated Oat-Starch Slurries from Different Oat Lines^a

treatments		calculated HI	estimated GI
oat flour			
raw	Jim	39.0 ± 1.7 b	61.1 ± 0.9 b
	Paul	49.2 ± 2.9 a	66.7 ± 1.6 a
	IA95	44.5 ± 0.8 ab	64.2 ± 0.4 ab
	N979	46.2 ± 2.3 a	65.1 ± 1.3 a
heated	Jim	83.7 ± 1.3 a	85.7 ± 0.7 a
	Paul	78.3 ± 1.6 b	82.7 ± 0.9 b
	IA95	68.4 ± 0.9 c	77.2 ± 0.5 c
	N979	70.3 ± 2.4 c	78.3 ± 1.3 c
oat starch			
raw	Jim	45.3 ± 1.5 a	64.6 ± 0.8 a
	Paul	44.8 ± 3.0 a	64.3 ± 1.7 a
	IA95	39.8 ± 1.1 b	61.5 ± 0.6 b
	N979	42.6 ± 1.4 ab	63.1 ± 0.8 ab
	Corn	41.6 ± 1.6 ab	62.6 ± 0.9 ab
heated	Jim	115.3 ± 1.4 ab	103.0 ± 0.8 ab
	Paul	119.7 ± 1.8 a	105.4 ± 1.0 a
	IA95	111.7 ± 1.0 b	101.1 ± 0.6 b
	N979	119.1 ± 1.4 a	105.1 ± 0.8 a
	Corn	113.9 ± 2.4 ab	102.2 ± 1.5 ab

^aValues are means ± standard deviations. Values followed by different letters within a column are significantly different ($P < 0.05$).

IA95 and N979 lines with high β -glucan concentrations had lower GI values than did slurries made from Jim and Paul. The estimated GI was negatively correlated with β -glucan concentrations in heated oat-flour slurries ($R^2 = 0.92$). The estimated GI of oat starches extracted from oat flours of Jim, Paul, IA95, and N979 lines ranged from 62 to 64, which increased to a range of 101–105 after heating. Starch from the IA95 oat line tended to have the lowest HI and GI values, but there were no differences among oat types. The increased GI values of oat-flour slurries and oat-starch slurries after heating are mostly attributed to starch gelatinization. Ovando-Martinez et al.³⁰ observed that the surface of the starch granules in bean starch changed to be rough and flattened after cooking. This morphological change was caused by breaking and shattering of the cell during cooking. Previous work showed gelatinized corn starch to hydrolyze more readily than starch in the granular state.³² Starch gelatinized by heating was easily hydrolyzed by enzymes, thus releasing glucose and increasing the GI value.

Low GI values (low starch digestibility) of heated oat-flour slurries were attributed to the presence of dietary fiber in oats. The GI values of heated oat starch were not inherently different from that of corn starch (Table 2). This finding supported the view that low starch digestibility of heated oat flours was attributed to another component of oat flour, that of the major SDF, β -glucan. β -Glucan competed with starch for water and restricted the starch gelatinization. Because of the high water-binding capacity of oat β -glucan, it absorbs water more easily

than do starch granules and retains it during heating.³³ The limitation of starch gelatinization in high β -glucan oats, thus, reduced the rate of enzymatic hydrolysis of starch during in vitro digestion. Wood et al.³⁴ and Behall et al.³⁵ reported that the consumption of meals containing oat or barley β -glucan decreased the postprandial glucose rise as indicated by the GI.

The heating method of oat-based food products influenced the human glucose response. Regand et al.¹⁷ prepared four different types of oat-based food products including oat bread, porridge, granola, and pasta to study the effect of differently processed oat foods on human glycemic response. Among those foods, oat porridge, which was boiled at 100 °C and then simmered for 5 min, and oat granola, which was baked at 177 °C for 20 min, had the highest efficacy in attenuating the peak blood glucose response of human subjects. Both foods had a short preparation time resulting in less change in the physicochemical properties of β -glucan (viscosity, peak MW, and concentration) during processing. In addition, heating inactivated the native enzymes, which would break down β -glucan in oat flour, thus preventing changes in β -glucan properties.³⁶ β -Glucan in oats could help to produce a low GI value of food, which generates slow and moderate postprandial glucose and insulin response. This physiological effect of β -glucan will have a beneficial effect in the management of diabetes, obesity, and other diseases.

This study demonstrated the increased starch digestibility during in vitro digestion with heat treatment of oat flours. Heating of oat flours increased the starch gelatinization, thus increasing the susceptibility of enzymatic degradation of starch. The estimated GI values of heated oat-flour slurries from oat lines with high β -glucan concentrations were lower than those from oat lines with typical β -glucan concentrations. Starch digestion was negatively correlated with β -glucan concentrations ($R^2 = 0.92$). The estimated GI of the extracted oat starches from the different oat lines did not differ from each other, and all GI values increased after heating. These results illustrated that the oat SDF, β -glucan, retarded starch digestibility, resulting in a reduced GI value. Food products containing oat β -glucan can be developed to provide a low GI.

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Notes

The authors declare no competing financial interest.

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