

## In Vitro Bile Acid Binding Activity within Flour Fractions from Oat Lines with Typical and High $\beta$ -Glucan Amounts

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Whole flours from four oat lines with different amounts of  $\beta$ -glucan (4.8–8.1%) were examined for their antioxidant activity and total phenolic and lignin concentrations. These data, along with the  $\beta$ -glucan percentages, were compared with bile acid (BA) binding. Only the lignin concentrations of the flours significantly ( $P < 0.01$ ) correlated with the BA binding values. The oat flours also were fractionated into bran, protein concentrate, starch, layer above starch, and soluble  $\beta$ -glucan (SBG)-free flour, and their BA binding capacities were evaluated. The bran fractions were the only fractions that bound greater BA than did the whole oat flours on dry matter basis. Extraction of the soluble  $\beta$ -glucan to create the SBG-free flour significantly ( $P < 0.01$ ) decreased the BA binding of the remaining flour. These data suggest that BA binding of the oat flours involves the synergistic interactions of the oat components, with  $\beta$ -glucan and lignin (insoluble fiber) having a great impact.

**KEYWORDS:**  $\beta$ -Glucan; oat; bile acid; fraction; antioxidant; phenolics; lignin

### INTRODUCTION

Oats (*Avena sativa* L.) are accepted as a multifunctional and nutritious food with an annual production of around 26 million tons in the world (1). Although consumed in considerably lower quantities worldwide than wheat and rice, oat has a potential advantage in that it is normally consumed as a whole-grain cereal, including all the nutritionally valuable portions. It is mainly consumed as a breakfast food or snack product. Oats are an excellent source of dietary fiber, such as mixed-linked (1 $\rightarrow$ 3, 1 $\rightarrow$ 4)- $\beta$ -D-glucan ( $\beta$ -glucan) and arabinoxylans and also contain a considerable amount of protein, vitamins, and minerals along with phenolic compounds and other bioactive phytochemicals that provide specific health benefits (2). Clinical studies have demonstrated that whole-grain oats can reduce total blood cholesterol and low-density lipoprotein cholesterol (LDL-C) levels (3, 4). The cholesterol-lowering activity generally is attributed to the soluble fiber fraction of oats, the  $\beta$ -glucan component.

The U.S. Food and Drug Administration (FDA) has approved a health claim for oat  $\beta$ -glucan, stating that the intake of whole oats, whole oat products, and soluble fiber from these sources, eaten as part of a diet low in saturated fat and cholesterol, may reduce the risk of coronary heart disease (5). However, the mechanism by which  $\beta$ -glucans lower the cholesterol level is not well-established. There are a wide variety of mechanisms by which whole grains may causally influence health. Nevertheless, the basis for protection is not known, and it remains likely

that no single compound in isolation confers protection, that the whole grain components may work synergistically to reduce risk for chronic disease (6–8). Components in whole grains that may be protective are diverse and include compounds that affect the gut environment (i.e., dietary fiber, resistant starch, and other indigestible compounds in whole grains), compounds that function as antioxidants such as trace minerals and phenolic compounds, and compounds that are phytoestrogens with potential hormonal effects. Rieckhoff et al. (9) examined the effects of various cereal fibers and various amounts of  $\beta$ -glucan on cholesterol and BA metabolism in vivo. Fecal BA excretion results of Rieckhoff's study showed that components other than  $\beta$ -glucan and soluble fibers also seem to be involved in the hypocholesterolemic effects of the fibers (6–8).

A useful way to test the cholesterol lowering effects of any dietary component, other than via in vivo studies, is by testing their in vitro bile acid (BA) binding capacity, which is less costly and more practical. The physical elimination of BA from the enterohepatic circulation necessitates increased synthesis of BA, which in turn consumes cholesterol. This elimination can be accomplished by preventing BA re-absorption in the ileum, thus increasing BA excretion in the feces (10, 11). Simply stated, biliary excretion increases the synthesis of BA, and it is the major mechanism by which the body disposes of excess cholesterol (10).

Several dietary components, oat flour, oat meal, oat bran extrudates, barley fiber, rice bran, wheat bran, corn bran, potato peels, locust bean gum, and raisins have been studied for their BA binding properties (2, 12–19). Eastwood and Hamilton (18), Story et al. (19), and Kay et al. (20) reported that lignin, a nonpolysaccharide component of fiber, has the greatest BA absorption capacity among fiber fractions. BA binding correlated

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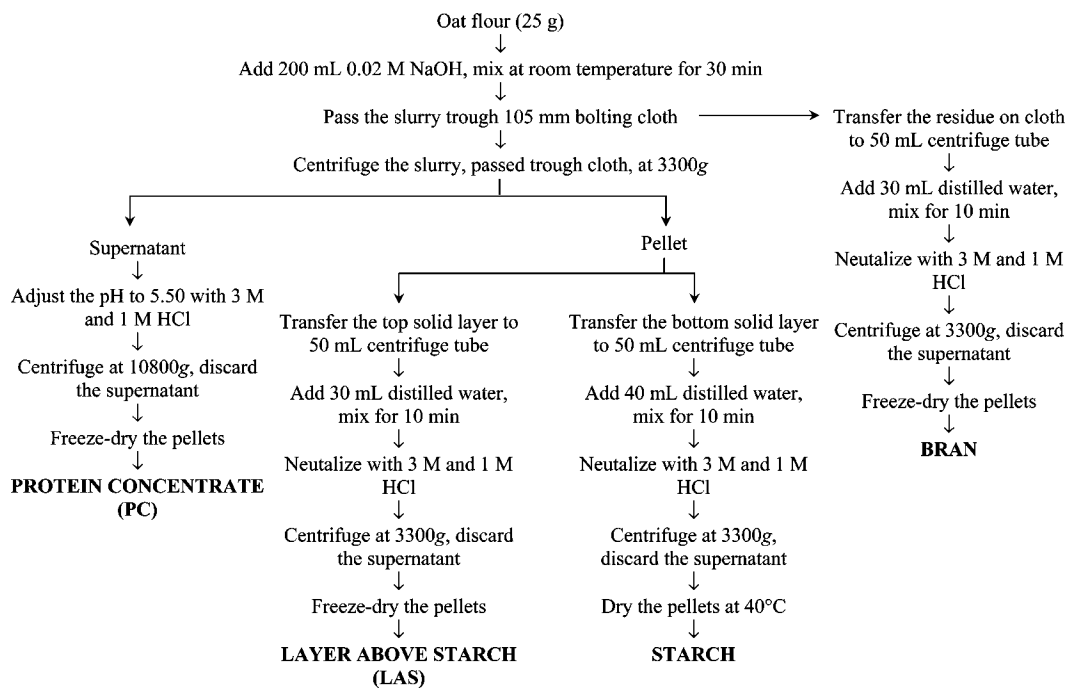


Figure 1. Fractionation diagram of oat flour.

well with the insoluble dietary fiber (2, 12, 15, 21) and total dietary fiber (2). Iron content also was correlated with the BA binding capacity of dietary fibers (15, 16). However, in our previous study (12) no significant correlations were found between the content and the molecular weight distribution of  $\beta$ -glucan in the oat flours and BA binding. BA binding also was not proportional to the soluble fiber content of cereal brans tested by Kahlon and Chow (14).

Accordingly, this study was undertaken to identify specific components of oat flours that have great impact on BA binding. The first objective was to determine the antioxidant activity, total phenolic content, and lignin content of the oat flours and to test the possible impact of these compounds on BA binding capacity. The second objective was to separate the oat flours, which have different  $\beta$ -glucan contents, into bran, protein concentrate, starch, and the layer above starch (LAS) fractions and to analyze the *in vitro* BA binding abilities of these fractions. Also, soluble  $\beta$ -glucans (SBG) were extracted from the oat flours, and the BA binding of both SBG-free flours and SBG-extracts were determined in order to evaluate the effects of soluble  $\beta$ -glucan.

## MATERIALS AND METHODS

**Oat Grains.** Two experimental oat (*Avena sativa*) lines, IA95111 and N979-5-2-4 (N979), and two publicly available cultivars, Jim and Paul, were used in this study. The oat lines chosen for this study displayed a broad range of  $\beta$ -glucan concentrations with significant differences among oat lines. The Jim and Paul lines are traditional varieties with normal  $\beta$ -glucan levels (4.8 and 5.3%). The experimental lines, IA95111 and N979, contained 7.64 and 8.05%  $\beta$ -glucan, respectively, which is greater than typical values reported for domestic *A. sativa* cultivars in the literature (3.7–5.0%) (22). Detailed information about the oat lines was given in Sayar et al. (12). The samples, except the naked variety, Paul, were dehulled with an air-pressure dehuller (Codema, Eden Prairie, MN), and the kernels, including Paul, were ground in an ultra centrifugal mill (ZM-1, Retch GmbH&Co, Haan, Germany) fitted with a 0.5 mm sieve.

**Chemicals.** DPPH (2,2-diphenyl-1-picrylhydrazyl), trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), Folin-Ciocalteu reagent, gallic acid, human salivary  $\alpha$ -amylase (EC 3.2.1.1), porcine

pepsin (EC 3.4.23.1), and pancreatin (from porcine pancreas, activity at least equivalent to 8 $\times$  USP specifications), sodium cholate, sodium deoxycholate, sodium glycocholate, and sodium taurocholate were purchased from Sigma-Aldrich (St. Louis, MO). All other chemicals used in the experiments were reagent or HPLC grade and were purchased from Fisher Scientific (Pittsburgh, PA).

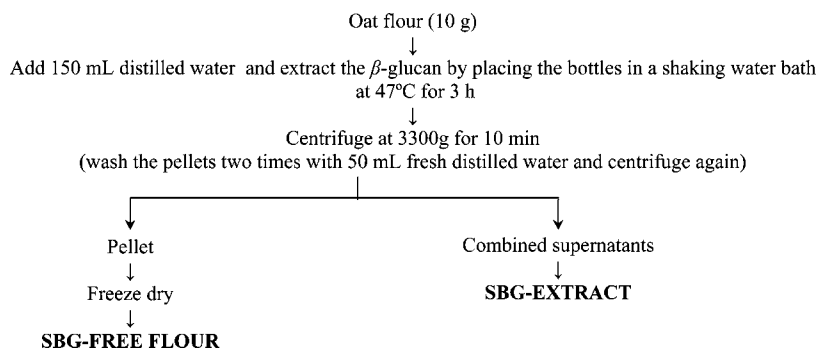
### Determination of Antioxidant Activity and Total Phenolics.

Antioxidant activity and total phenolic content of the oat flours were determined both in the aqueous–organic extracts and in the nonextractable residue, according to the method given by Perez-Jimenez and Saura-Calixto (23). The radical scavenging capacity of the samples was measured according to the DPPH method (24), and the total phenolic was measured according to the Folin-Ciocalteu procedure (23). Antioxidant activity was expressed as micromoles of trolox equivalent (TE) per gram of flour on a dry basis (db), and the total phenolic was as milligrams of gallic acid equivalent (GA) per kilogram of flour (db).

**Determination of the Lignin Content.** Lignin was measured as the Klason lignin from 1.0 g of oat flour by the gravimetric method described by Theander and Westerlund (25). Klason lignin is the fiber material not soluble in 12 M sulfuric acid (26).

**Fractionation of the Oat Samples.** Fractionation processes were applied to the oat flours to obtain bran, protein concentrate, starch, and LAS fractions according to the method given by Wu et al. (27) with minor modifications (Figure 1). To extract SBG from the oat flours, a simple water extraction process was applied at 47 °C as shown in Figure 2. The solid part remaining after extraction of the SBG (SBG-free flour) was freeze-dried. The liquid part (SBG-extract) was used directly after measuring the total volume.

**Chemical Composition of the Fractions.** All analyses of oat flours and fractions were done in triplicate and reported on db. The moisture of oat flours was determined by AACC Method 44-15 A (28). The  $\beta$ -glucan concentration in flours and in fractions was determined enzymatically by AACC Method 32-23, (29), by using the mixed  $\beta$ -glucan linkage kit from Megazyme (Megazyme Ltd, Co. Wicklow, Ireland). Proteins were determined with the automatic nitrogen analyzer (elementar, Analysensysteme GmbH, Germany) by using a protein conversion factor of 6.25. Starch content in flours and fractions was analyzed by AACC Method 76-13 (29), by using a Total Starch Kit from Megazyme (Megazyme Ltd, Co., Wicklow, Ireland). Lipids were analyzed by the gravimetric method after extraction with petroleum ether on a Soxhlet system (Method 30-25) (29). 1-Propanol–water (3:1, v/v) was used to extract lipids from starch, LAS, and SBG-free flours. Ash was determined by using Method 08-01 (29).



**Figure 2.** Extraction of the soluble  $\beta$ -glucan (SBG) from whole oat flours.

**Table 1.** Total Antioxidant and Phenolic Content of the Oat Flours<sup>a</sup>

oat type	total antioxidants, (trolox equivalents) <sup>b</sup>	total phenolics, (gallic acid equivalents) <sup>c</sup>
Jim	15.8 a	4024.5 a
Paul	14.3 a	4454.3 b
IA95111	15.4 a	4066.3 a
N979	14.1 a	3807.6 a

<sup>a</sup> Values are means of three measurements. Values within a column followed by a common letter (a–d) are not significantly different ( $P > 0.05$ ). <sup>b</sup> mmol of trolox/g of sample (db). <sup>c</sup> mg of gallic acid/kg of sample (db).

**In Vitro Digestion and BA Binding.** An in vitro digestion process was applied according to the method given in the previous study (12). Briefly, oat flours and fractions were first cooked in boiling water and subjected to human salivary  $\alpha$ -amylase, porcine pepsin, and pancreatic enzymes, respectively. The BA mixture containing sodium cholate, sodium deoxycholate, sodium glycocholate, and sodium taurocholate was added before the pancreatic digestion step. The in vitro digested sample, solutions were centrifuged and separated into an insoluble part (pellets) and extract (supernatant). The supernatant was used for BA binding analyses, and the pellets were freeze-dried for other studies. Bulk digestibility (dry matter disappearance) of the samples was calculated from the following equation:

$$\text{bulk digestibility} = (M_S - M_P)/M_S \times 100 \quad (1)$$

where  $M_S$  is the dry weight of the sample used in the digestion, and  $M_P$  is the dry weight of the pellets (indigestible part).

The unbound BA was analyzed in the extract by using a Bile Acid Diagnostic Kit (Trinity Biotech plc, Bray Co., Wicklow, Ireland) as described (12). Aliquots from the extracts were diluted to fall within the BA concentration range of the test kit. The sample amounts used in the digestion process were 0.8 g for all the oat flours, brans, protein concentrates, starches, LAS, and SBG-free fractions. For SBG-extract, first the dry matter content of the extracts was determined, and then the volumes of extracts that contained 0.8 g of dry solids were taken directly to the in vitro digestion process.

**Statistical Analysis.** All analyses were conducted at a minimum in triplicate. Results were analyzed by using a statistical analysis software system (SPSS Version 12.0, SPSS Inc., IL). Differences among samples, fractions, and treatments were compared by using a least significant difference (LSD) test with a probability level ( $\alpha$ ) of 0.05. Bivariate correlations between variables were determined using Pearson's test at probability levels ( $P$ ) of 0.05 and 0.01.

## RESULTS AND DISCUSSION

**Antioxidant Activity, Total Phenolic Content, and Lignin Content of Oat Flours in Relation to BA Binding.** The antioxidant activity and total phenolic and lignin contents of the oat samples were determined, and the possible effects of these compounds on BA binding were investigated. **Table 1**

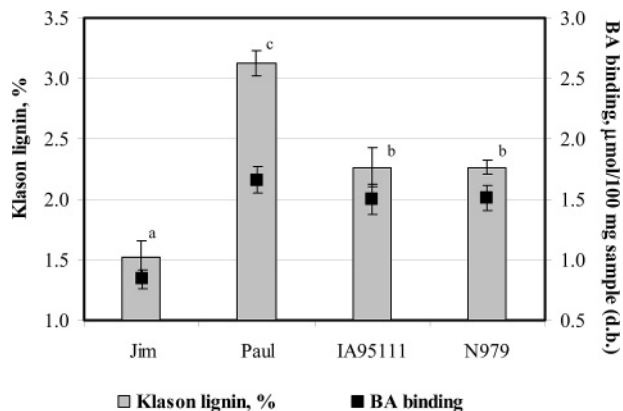
summarizes the antioxidant capacity and total phenolic contents of the oat flours. The values given in **Table 1** are the sum of the extract and the residue values for antioxidant activity and total phenolic content. The aqueous–organic extracts gave antioxidant activities of 1.7–2.9  $\mu\text{mol TE/g}$  and total phenolic contents of 1531.6–1823.0 mg GA/kg. The residue of the aqueous–organic extracts gave antioxidant activities of 11.9–12.9  $\mu\text{mol TE/g}$  and total phenolics of 2276.0–2631.3 mg GA/kg. Perez-Jimenez and Saura-Calixto (23) reported antioxidant activity as 1.63  $\mu\text{mol TE/g}$  in the extract and 8.92  $\mu\text{mol TE/g}$  in the residue for wheat flour. For oat bran in the same study, the values were reported as 3.68  $\mu\text{mol TE/g}$  in the extract and 30.59  $\mu\text{mol TE/g}$  in the residue (23). Perez-Jimenez and Saura-Calixto (23) also determined the total phenolic content for wheat flour as 3.440 and 2.510 mg GA/kg and for oat bran as 1.950 and 9.710 mg GA/kg in the extract and the residue, respectively.

In our study, only low correlations occurred between antioxidant capacity and BA binding ( $R^2 = 0.31$ ,  $P > 0.05$ ) and between total phenolic content and BA binding ( $R^2 = 0.07$ ,  $P > 0.05$ ). Additionally, there were no significant correlations ( $P > 0.05$ ) between antioxidant capacity, total phenolic, and/or  $\beta$ -glucan concentration of the samples.

Although, the health benefits of the natural antioxidants have been studied extensively, there is a lack of information in the literature about their impacts on BA binding. The general interest in the health benefits of antioxidants is their ability to react with free radicals, which are believed to trigger the initiation phase of several diseases (30), rather than their hypocholesteremic effects. The results from this study showed that antioxidant activity and phenolic content of the oat flour are not directly involved in BA binding.

The Klason lignin is a noncarbohydrate part of dietary fiber and can consist of lignin, modified lignin, unavailable cell wall protein, polymers originating from Maillard reactions, and tannin–protein complexes (26). Significant differences were found between the Klason lignin content of the oat flours, with the greatest content for Paul (**Figure 3**). The Klason lignin content of the flours and fractions was not significantly correlated ( $P > 0.05$ ) with the  $\beta$ -glucan content. Values for the Klason lignin were similar to those reported by Manthey et al. (26) for some oat flours. Positive correlations were found between lignin content and BA binding capacity of the oat flours ( $R^2 = 0.72$ ,  $P < 0.01$ ).

Eastwood and Hamilton (18) reported that the lignin fraction of dry-grain preparations was the main component responsible for bile salt binding by these substances. Lignin also was the most effective component in the case of BA binding by alfalfa and wheat bran (19). Isolated lignin also has been shown to be active in binding BA (20, 31). It was speculated that the main



**Figure 3.** Klason lignin content and bile acid (BA) binding capacity of the flours. Different letters on top of the bars indicate significant differences ( $P < 0.05$ ) in Klason lignin content.

**Table 2.** Yields of the Extracted Fractions from Oat Flours (%)<sup>a</sup>

oat type	SBG <sup>b</sup> -free flour	flour fractions				total <sup>e</sup>
		bran	PC <sup>c</sup>	starch	LAS <sup>d</sup>	
Jim	85.4 c	4.8 a	18.6 ab	54.7 d	9.4 a	87.5 c
Paul	82.6 b	7.0 c	20.0 b	46.0 c	12.2 b	85.2 bc
IA95111	80.6 ab	5.5 ab	20.5 b	41.9 b	12.2 b	80.1 ab
N979	78.2 a	6.1 b	16.6 a	37.7 a	13.9 b	74.3 a

<sup>a</sup> Values are means of three measurements. Values within a column followed by a common letter (a–d) are not significantly different ( $P > 0.05$ ). <sup>b</sup> Soluble  $\beta$ -glucan. <sup>c</sup> Protein concentrate. <sup>d</sup> Layer above starch. <sup>e</sup> Sum of the yields of the flour fractions (bran + PC + starch + LAS).

mechanism by which lignin bound bile salts was by hydrophobic binding (18, 20).

**Extraction and Proximate Composition of the Oat Flour Fractions.** An alkaline extraction process was used to fractionate the oat flours as shown in **Figure 1**. The high amount of  $\beta$ -glucan in IA95111 and N979 flours resulted in a high-viscosity slurry that was difficult to screen from the bolt cloth during bran removal. This difficulty was overcome by diluting the slurry with more sodium hydroxide solution (0.02 M) during screening. The residual amount of viscous SBG settled with the bran on the bolt cloth and was removed by centrifugation, after washing and neutralization. Bran fraction yields were between 4.8 and 7.0% db, with the highest value for Paul flour (**Table 2**). Proteins were precipitated from the supernatant of the centrifuged extracts, which passed through the bolt cloth at pH 5.50. High centrifuge speed was critical for the separation of the precipitated proteins. The yields of the protein concentrate (**Table 2**) were very close to the values reported by Cluskey et al. (32). Starch yields were negatively correlated with the  $\beta$ -glucan content, which was not unexpected because the starch content also was negatively correlated with the  $\beta$ -glucan contents (12). The LAS fraction was removed manually from the top of the white starch fraction by viewing the color differences. All the extracted and dried fractions were kept at  $-20^{\circ}\text{C}$  until used to prevent decomposition.

Bran contained considerable amounts (9.2–11.6%) of  $\beta$ -glucan (**Table 3**), which may correspond to the fraction that is insoluble in the extraction solution used. Protein, starch, lipid, and ash content of the bran fraction were in the range of the values reported by Cluskey et al. (32) and Wu et al. (27) for oat bran from Wyndmere and Garland varieties. Protein concentrates represented 16.6–20.5% of the starting material

**Table 3.** Proximate Composition of the Oat Flour Type, Soluble  $\beta$ -Glucan-Free Flour, and Flour Fractions, % (db)<sup>a</sup>

component	oat type	flour	SBG <sup>b</sup> -free flour	flour fractions			
				bran	PC <sup>c</sup>	starch	LAS <sup>d</sup>
$\beta$ -glucan <sup>e</sup>	Jim	4.8 a	0.8 a	9.2 a	0.5 a	<0.1	3.6 c
	Paul	5.3 b	1.0 b	9.7 a	0.5 a	<0.1	2.7 a
	IA95111	7.6 c	1.4 c	11.6 b	1.5 b	<0.1	3.4 b
	N979	8.1 d	1.3 c	11.3 b	3.4 c	<0.1	3.3 b
protein	Jim	15.5 a	15.5 a	12.7 b	71.5 b	0.5 a	1.8 a
	Paul	17.8 b	18.5 b	12.2 b	71.3 b	0.6 a	1.5 a
	IA95111	19.1 c	20.9 c	12.4 b	69.8 ab	0.6 a	1.5 a
	N979	18.6 bc	18.8 b	9.6 a	68.0 a	0.5 a	1.7 a
starch <sup>e</sup>	Jim	63.3 c	72.4 c	31.7 a	0.6 a	96.6 a	84.8 a
	Paul	57.6 b	68.3 bc	26.3 a	0.4 a	98.2 a	86.1 a
	IA95111	56.8 b	65.9 ab	29.0 a	1.0 a	99.1 a	86.4 a
	N979	54.4 a	61.1 a	30.5 a	2.1 b	99.6 a	86.8 a
lipid <sup>e</sup>	Jim	6.8 a	4.2 a	1.9 a	25.1 b	0.8 a	1.3 a
	Paul	8.0 b	6.8 b	2.3 a	23.7 b	0.9 a	1.4 a
	IA95111	6.8 a	6.6 b	1.7 a	20.0 a	0.8 a	1.7 a
	N979	7.2 a	6.7 b	1.7 a	20.4 a	0.9 a	2.3 a
ash	Jim	2.2 a	0.9 a	5.2 b	1.7 a	0.8 a	3.5 a
	Paul	2.5 a	1.0 a	5.0 b	1.7 a	0.8 a	3.0 a
	IA95111	2.5 a	0.9 a	5.2 b	2.2 b	0.9 a	3.4 a
	N979	2.5 a	0.9 a	4.4 a	2.9 c	0.8 a	2.5 a

<sup>a</sup> Values are means of three measurements. Values within a column and within single component followed by a common letter (a–d) are not significantly different ( $P > 0.05$ ). <sup>b</sup> Soluble  $\beta$ -glucan. <sup>c</sup> Protein concentrate. <sup>d</sup> Layer above starch. <sup>e</sup> Data from Sayar et al. (12).

(oat flour) and contained 68.0–71.5% protein ( $N \times 6.25$ ) (**Table 3**), which accounted for 61–86% of the proteins in the oat flours. Protein recovery values were lower in the case of the high  $\beta$ -glucan samples (IA95111 and N979). It is likely that the viscous solution decreased the separation efficiency of the centrifugation process. Highly pure starches (96.6–99.6%) were obtained by the fractionation method used. The small amount of protein (0.5–0.6%) probably resulted from the residual protein inside the starch granule. Lipid and ash contents of the starch fraction were in the range of other reported values (33). The ash content of the fractions might include some part of the sodium chloride resulting from neutralization of sodium hydroxide with hydrochloric acid solution.

To create flour without  $\beta$ -glucan, the SBG were extracted from the oat flours by means of a simple water extraction process at  $47^{\circ}\text{C}$ , below the starch gelatinization temperature (**Figure 2**). The yield values for the SBG-free flour (**Table 2**) show that, in addition to the  $\beta$ -glucans, some portions of the other flour components were removed by this process. The sum of the yields of the flour fractions were very similar to the yields of the SBG-free flours, suggesting that the discarded materials from both extraction processes (**Figures 1** and **2**) were similar. Also, the sum of the  $\beta$ -glucan content of the flour fractions (bran, protein concentrate, starch, and LAS) added to only 18.1–21.2% of the total  $\beta$ -glucan in the whole flours, demonstrating that most of the SBG were loosed during fractionation process. Proximate composition of the SBG-free flours (**Table 3**) indicated that 84–87% of the  $\beta$ -glucans were extracted, which is greater than the values we previously reported (12). The reason for the difference is that the inactivation step for the endogenous  $\beta$ -glucanases was not applied in the current study. Endogenous  $\beta$ -glucanases hydrolyze the  $\beta$ -glucan molecules to some extent, which facilitates the extraction of  $\beta$ -glucan. The ash content values also showed that a considerable amount of inorganic materials (corresponding to ash content) was removed during SBG extraction. Previous work showed that only a small amount of protein, some simple sugars, organic acids, water-

**Table 4.** Bulk Digestibility (dry matter disappearance) of the Oat Flours and Oat Flour Fractions, Calculated by Equation 1 (%)<sup>a</sup>

oat type	flour <sup>a</sup>	SBG <sup>b</sup> -free flour	flour fractions			
			bran	PC <sup>c</sup>	starch	LAS <sup>d</sup>
Jim	78.6 a	68.5 a	33.3 a	38.6 a	86.7 a	75.6 a
Paul	80.9 a	69.0 a	33.9 a	41.0 a	88.6 a	74.7 a
IA95111	76.9 a	68.5 a	33.4 a	39.9 a	85.9 a	73.3 a
N979	81.4 a	68.0 a	39.4 a	45.2 a	89.3 a	73.0 a

<sup>a</sup> Values are means of three measurements. Values within a column followed by a common letter (a–d) are not significantly different ( $P > 0.05$ ). <sup>b</sup> Soluble  $\beta$ -glucan. <sup>c</sup> Protein concentrate. <sup>d</sup> Layer above starch. <sup>e</sup> Data from Sayar et al. (12).

soluble vitamins, and glycosides are likely removed with the water extraction process (19).

#### BA Binding Capacities of Oat Flours and Their Fractions.

There were no significant differences ( $P > 0.05$ ) in bulk digestibility values among oat types for individual fractions (Table 4). High digestibility values were determined for the flours, SBG-free flours, starches, and LAS, but these values were low for bran and protein concentrate fractions. Overall digestibility of the bran used in this study was higher than values determined by Amerin et al. (34) for wheat aleurone (digestibility of 28–32%) but lower than for wheat bran (digestibility of 60%) (35). Possibly, the oat bran contains fewer digestible components than wheat bran. By using only pancreatin and amyloglucosidase for digestion, Drzikova et al. (2) reported 52% digestibility for oatmeal prepared from commercial oat kernels.

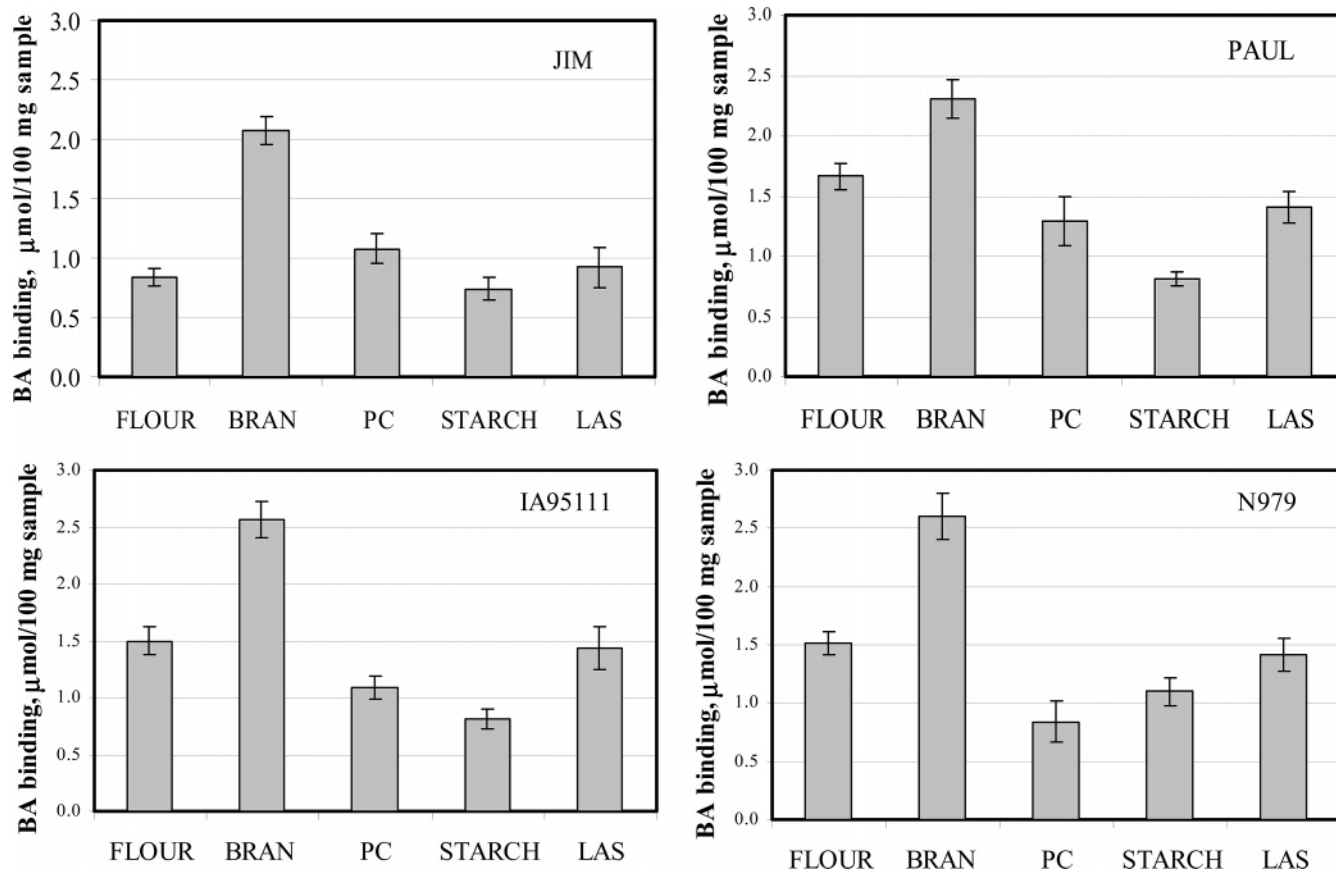
Starch digestibilities in the current study were greater than 80% (Table 4), which was expected, because all the samples were digested with human salivary  $\alpha$ -amylase. Amerin et al. (35) and Wood et al. (34) reported starch digestibility values

of greater than 90% for wheat aleurone and wheat bran. Protein digestibility values in the current study were lower than values reported for some cereal food samples (36, 37). Possibly, the heating step used in the *in vitro* digestion process in our study may not have been adequate to denature the protein, and/or the lower substrate:enzyme ratio used in this study as compared to the earlier study may have lowered protein digestibility values in the current study.

The *in vitro* BA binding values of the oat flours and flour fractions showed that bran had the greatest BA binding among all oat fractions, binding 1.5–2 times more BA than the flours (Figure 4). High BA binding of the bran fractions was expected because these fractions contain most of the insoluble dietary fiber, which was positively correlated with the BA binding of the same oat flours in our previous study (12). Drzikova et al. (2) also reported that the extrudates containing the greatest portion of the oat bran were the most effective in BA binding. Greater BA binding of bran fractions as compared to the oat flours, however, might arise from a concentration of binding components resulting from extraction of inactive materials, since a constant amount (0.8 g) of all materials was used to measure BA binding. Protein concentrate, starch, and LAS fractions also bound some BA but comparatively less than the flour samples (Figure 4). For each oat type, the starch fraction had the least BA binding among all its fractions, except for N979, where the PC had the least BA binding. The contribution of each fraction to the BA binding of each whole flour was calculated by:

$$\text{contribution of the fraction} = (\text{yield of the fraction} \times \text{BA binding of the fraction})/100 \quad (2)$$

where yield of the fraction was in percent (values given in Table 2) and BA binding of the fraction was in  $\mu\text{mol}$  of BA bound/

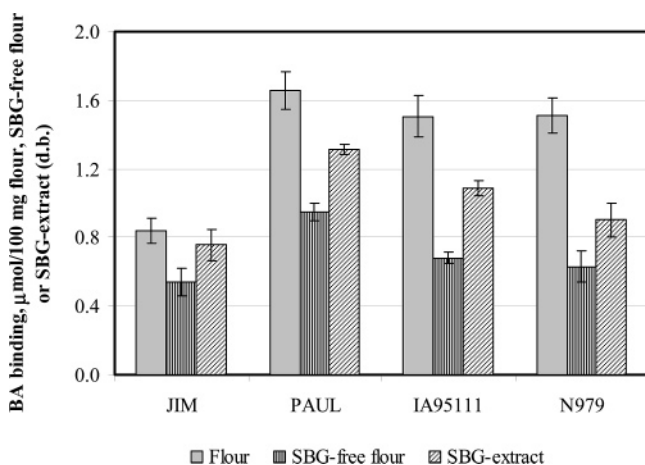


**Figure 4.** Bile acid (BA) binding capacity of the oat flour and their fractions. PC, protein concentrate; LAS, layer above starch.

**Table 5.** Contribution of the Individual Fractions on the Bile Acid Binding Capacity of the Flour Samples<sup>a</sup>

component	Jim	Paul	IA95111	N979
bran	0.10 ± 0.02	0.16 ± 0.01	0.14 ± 0.02	0.16 ± 0.01
PC <sup>b</sup>	0.20 ± 0.02	0.26 ± 0.05	0.22 ± 0.04	0.14 ± 0.04
starch	0.41 ± 0.03	0.37 ± 0.03	0.34 ± 0.05	0.41 ± 0.05
LAS <sup>c</sup>	0.09 ± 0.01	0.17 ± 0.01	0.18 ± 0.02	0.20 ± 0.04
sum <sup>d</sup>	0.79 ± 0.07	0.96 ± 0.04	0.88 ± 0.12	0.91 ± 0.12
flours <sup>e</sup>	0.84 ± 0.07	1.66 ± 0.11	1.50 ± 0.12	1.51 ± 0.10

<sup>a</sup> Values calculated by eq 2. Bile acid binding of the fraction is given as  $\mu\text{mol}$  of bile acid bound/total amount of fraction in 100 mg of flour. <sup>b</sup> Protein concentrate. <sup>c</sup> Layer above starch. <sup>d</sup> Sum of the BA binding values of bran, PC, starch, and LAS. <sup>e</sup> Bile acid binding of the oat flour samples,  $\mu\text{mol}$  of bile acid bound/100 mg of flour; data from Sayar et al. (12).

**Figure 5.** Bile acid (BA) binding capacity of the soluble  $\beta$ -glucan (SBG) free flours and SBG-extracts, comparison among flour types.

100 mg of sample. The contribution of each fraction is presented in **Table 5**. The sums of the BA binding contributions for all fractions for each oat type were lower than for the whole flour, suggesting possible removal of active components during fractionation or a synergistic impact of all flour fractions on BA binding that was lost when the parts were separated.

Removing the SBG to create the SBG-free oat flours decreased BA binding by almost 50% for all oat types, clearly demonstrating the involvement of  $\beta$ -glucan in binding (**Figure 5**). It is possible, however, considering that more than just the SBG were removed with the extraction process, that we might have eliminated some water-extractable components of the flours that also contributed to the binding. Neither the SBG-free flours nor the SBG extracts bound more BA than the flours. A simple calculation of the binding of both the SBG-free flours and SBG-extracts according to their partial ratio in the flour also did not add up to the binding that occurred in whole flour, thus demonstrating the synergistic impact of the oat fractions. Clinical studies (6, 8) also indicated a synergistic effect of the components in whole grains on cholesterol lowering. The dose-controlled clinical study by Davidson et al. (3) indicated the involvement of  $\beta$ -glucan in reducing serum cholesterol.

The findings from this study show that oat flours have considerable amounts of phenolic antioxidants and enhanced antioxidant activity, which seem to not be involved in BA binding. Interpretation of all data suggested that SBG has great impact in BA binding. Positive significant correlations also occurred between the lignin content of the oat flours and the BA binding. The BA binding by the oat flour fractions indicated that the hypocholesterolemic effect of oats involves the syner-

gistic impact of the oat components, with the soluble  $\beta$ -glucan fraction and lignin having considerable effects.

## ABBREVIATIONS USED

BA, bile acid; LAS, layer above starch; SBG, soluble  $\beta$ -glucan; DPPH, 2,2-diphenyl-1-picrylhydrazyl; trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; TE, trolox equivalent; GA, gallic acid equivalent.

## LITERATURE CITED

- Food and Agricultural Organization. Statistical Databases. FAOSTAT, Agricultural Data. 2005; <http://www.faostat.fao.org> (accessed July 14, 2005).
- Drzikova, B.; Dongowski, G.; Gebhardt, E.; Habel, A. The composition of dietary fibre-rich extrudates from oat affects bile acid binding and fermentation in vitro. *Food Chem.* **2005**, *90*, 181–192.
- Davidson, M. H.; Dugan, L. D.; Burns, J. H.; Bova, J.; Story, K.; Drennan, K. B. The hypocholesterolemic effects of  $\beta$ -glucan in oatmeal and oat bran: a dose-controlled study. *J. Am. Med. Assoc.* **1991**, *265*, 1833–1839.
- Karmally, W.; Montez, M. G.; Palmas, W.; Martinez, W.; Branstetter, A.; Ramakrishnan, R.; Holleran, S. F.; Haffner, S. M.; Ginsberg, H. N. Cholesterol-lowering benefits of oat-containing cereal in Hispanic Americans. *J. Am. Diet. Assoc.* **2005**, *105*, 967–970.
- FDA. Food labeling: Health claims; oat and coronary heart disease. Final rule. *Fed. Regist.* **1997**, *62*, 3584–3681.
- Jacobs, D. R.; Slavin, J.; Marquart, L. Whole grain intake and cancer: a review of the literature. *Nutr. Cancer* **1995**, *24*, 221–229.
- Slavin, J.; Jacobs, D. R.; Marquart, L. Whole-grain consumption and chronic disease: protective mechanisms. *Nutr. Cancer* **1997**, *27*, 14–21.
- Jacobs, D. R.; Pereira, M. A.; Meyer, K. A.; Kushi, L. H. Fiber from whole grains, but not refined grains, is inversely associated with all-cause mortality in older women: The Iowa Women's Health Study. *J. Am. Coll. Nutr.* **2000**, *19*, 326S–330S.
- Rieckhoff, D.; Trautwein, E. A.; Malkki, Y.; Erbersdobler, H. F. Effects of different cereal fibers on cholesterol and bile acid metabolism in the Syrian golden hamster. *Cereal Chem.* **1999**, *76*, 788–795.
- LaRusso, N. The role of bile acids in intestinal absorption of cholesterol. In *Bile Acids in Gastroenterology*; Barbara, L., Dowling, R. H., Hofmann, A. F., Roda, E., Eds.; MTP Press Ltd.: Boston, 1983; pp 183–191.
- Hofmann, A. Intestinal absorption of bile acids and biliary constituents. The intestinal component of the enterohepatic circulation and the integrated system. In *Physiology of the Gastrointestinal Tract*, 3rd ed.; Johnson, L. R., Ed.; Raven Press: New York, 1994; pp 1845–1865.
- Sayar, S.; Jannink, J. L.; White, P. J. In vitro bile acid binding of flours from oat lines varying in percentage and molecular weight distribution on  $\beta$ -glucan. *J. Agric. Food Chem.* **2005**, *53*, 8797–8803.
- Huang, C. M.; Dural, N. H. Adsorption of bile acids on cereal type food fibers. *J. Food Proc. Eng.* **1995**, *18*, 243–266.
- Kahlon, T. S.; Chow, F. I. In vitro binding of bile acids by rice bran, oat bran, wheat bran, and corn bran. *Cereal Chem.* **2000**, *77*, 518–521.
- Camire, M. E.; Zhao, J.; Violette, D. A. In vitro binding of bile acids by extruded potato peels. *J. Agric. Food Chem.* **1993**, *41*, 2391–2394.
- Pandolf, T.; Clydesdale, F. M. Dietary fiber binding of bile acid through mineral supplementation. *J. Food Sci.* **1992**, *57*, 1242–1245.
- Camire, M. E.; Dougherty, M. P. Raisin dietary fiber composition and in vitro bile acid binding. *J. Agric. Food Chem.* **2003**, *51*, 834–837.

- (18) Eastwood, M. A.; Hamilton, D. Studies on the adsorption of bile salts to nonabsorbed components of the diet. *Biochim. Biophys. Acta* **1968**, *152*, 165–173.
- (19) Story, J. A.; White, A.; West, L. G. Adsorption of bile acids by components of alfalfa and wheat bran in vitro. *J. Food Sci.* **1982**, *47*, 1276–1279.
- (20) Kay, R. M.; Strasberg, S. M.; Petrunka, C. N.; Wayman, M. Differential adsorption of bile acids by lignins. In *Dietary Fibers: Chemistry and Nutrition*; Inglett, G. E., Falkehag, S. I., Eds.; Academic Press: New York, 1979; pp 57–65.
- (21) Kahlon, T. S.; Woodruff, C. L. In vitro binding of bile acids by rice bran, oat bran, barley and  $\beta$ -glucan enriched barley. *Cereal Chem.* **2003**, *80*, 260–263.
- (22) Miller, S. S.; Wood, P. J.; Pietrzak, L. N.; Fulcher, R. G. Mixed linkage beta-glucan, protein content, and kernel weight in *Avena* species. *Cereal Chem.* **1993**, *70*, 231–233.
- (23) Perez-Jimenez, J.; Saura-Calixto, F. Literature data may underestimate the actual antioxidant capacity of cereals. *J. Agric. Food Chem.* **2005**, *53*, 5036–5040.
- (24) Peterson, D. M.; Emmons, C. L.; Hibbs, A. H. Phenolic antioxidants and antioxidant activity in pearling fractions of oat groats. *J. Cereal Sci.* **2001**, *33*, 97–103.
- (25) Theander, O.; Westerlund, E. A.. Studies on dietary fiber. 3. Improved procedures for analysis of dietary fiber. *J. Agric. Food Chem.* **1986**, *34*, 330–336.
- (26) Manthey, F. S.; Hareland, G. A.; Huseby, D. J. Soluble and insoluble dietary fiber content and composition in oat. *Cereal Chem.* **1999**, *76*, 417–420.
- (27) Wu, Y. V.; Sexon, K. R.; Cluskey, J. E.; Inglett, G. E. Protein isolate from high-protein oats: preparation, composition and properties. *J. Food Sci.* **1977**, *42*, 1383–1386.
- (28) AACC, American Association of Cereal Chemists. Method 44-15 A. *Approved Methods of the AACC*, 9th ed.; The Association: St. Paul, MN, 1995.
- (29) AACC, American Association of Cereal Chemists. Method 32-23, Method 76-13, Method 30-25, and Method 32-21. *Approved Methods of the AACC*, 10th ed.; The Association: St. Paul, MN, 2000.
- (30) Miller, H. E.; Rigelhof, F.; Marquart, L.; Prakash, A.; Kanter, M. Antioxidant content of whole grain breakfast cereals, fruits and vegetables. *J. Am. Coll. Nutr.* **2000**, *19*, 312S–319S.
- (31) Story, J. A.; Kritchevsky, D. Comparison of the binding of various bile acids and bile salts in vitro by several types of fiber. *J. Nutr.* **1976**, *106*, 1292–1294.
- (32) Cluskey, J. E.; Wu, Y. V.; Wall, J. S.; Inglett, G. E. Oat protein concentrates from a wet-milling process: preparation. *Cereal Chem.* **1973**, *50*, 475–481.
- (33) Wu, Y. V.; Cluskey, J. E.; Wall, J. S.; Inglett, G. E. Oat protein concentrates from a wet-milling process: composition and properties. *Cereal Chem.* **1973**, *50*, 481–488.
- (34) Amrein, T. M.; Graenicher, P.; Arrigoni, E.; Amado, R. In vitro digestibility and colonic fermentability of aleurone isolated from wheat bran. *Lebensm.-Wiss. U.-Technol.* **2003**, *36*, 451–460.
- (35) Wood, P. J.; Arrigoni, E.; Miller, S. S.; Amadò, R. Fermentability of oat and wheat fractions enriched in  $\beta$ -glucan using human fecal inoculation. *Cereal Chem.* **2002**, *79*, 445–454.
- (36) Ibanoglu, S.; Ainsworth, P.; Hayes, G. D. In vitro protein digestibility and content of thiamin and riboflavin in extruded tarhana, a traditional Turkish cereal food. *Food Chem.* **1997**, *1–2*, 141–144.
- (37) Dahlin, K.; Lorenz, K. Protein digestibility of extruded cereal grains. *Food Chem.* **1993**, *48*, 13–18.

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