In Vitro Binding of Bile Acids by Extruded Potato Peels

Mary E. Camire,* Jianxin Zhao, and Dale A. Violette

Department of Food Science and Human Nutrition, 5736 Holmes Hall, University of Maine, Orono, Maine 04469-5736

Potato peelings have nutritional value as a source of dietary fiber. Peels were extrusion-cooked at 104 or 143 °C with feed moistures of 31, 33.5, or 36%. An *in vitro* digestion procedure was developed to measure binding of bile acids to peels and other materials. Peels bound more deoxycholic than cholic acid, and extruded peels bound more of each acid than did nonextruded peels. Extrusion resulted in greater glycocholic acid binding, but there was no difference in taurocholic acid binding by extruded or nonextruded peels. Bile acid binding by cholesterol, pectin, and cellulose was similar to levels reported using radioassays. Deoxycholic acid binding was correlated with total and insoluble dietary fiber and with iron content.

INTRODUCTION

Today dietary fiber is a desirable food component due to its perceived health benefits. In recent years many food products containing oat bran were developed to take advantage of consumer interest in that material. Oat bran was shown to reduce serum cholesterol, and hence reduce risk of cardiovascular disease, in humans and rats fed diets containing oat bran (Anderson and Siesel, 1990). The soluble fiber fraction consisting of β-glucans was believed to bind cholesterol-bearing bile acids, thereby preventing bile acid resorption and stimulating conversion of serum and liver cholesterol to additional bile acids.

New food sources of dietary fiber should enjoy similar success if consumption of those sources can lead to health benefits such as reduction of cholesterol. For the past 3 years we have studied the potential benefits and drawbacks to the use of potato peels as a source of dietary fiber, as well as the employment of extrusion cooking to improve food safety. Although potato peels contain primarily insoluble fiber (Camire and Flint, 1991), we wanted to document possible effects of potato peels on bile acids. Animal studies are costly, so *in vitro* bile acid binding was chosen. Many researchers have adopted the method of Kritchevsky and Story (1974) which uses ^{14}C-labeled bile acids and detects binding by difference in radioactivity. Eastwood and Hamilton (1968), however, used a colorimetric procedure. Hoaglund (1989) recovered bile acids by HPLC. Due to the general agreement in trends among research groups employing these methods, we selected a colorimetric assay which is specific for 3α-hydroxy bile acids. We also modified the Kritchevsky and Story (1974) method by including a simulated gastric step, followed by digestion at neutral pH with pancreatin. Other researchers (Eastwood and Hamilton, 1968; Kritchevsky and Story, 1974) have incubated fiber with bile acids only in buffer within a range of pH values. The lack of acidification and enzyme action may account, in part, for differences observed between *in vitro* bile acid binding by fiber sources and cholesterol changes in animals fed those same fibers.

Our objective was threefold: to determine whether potato peels bound appreciable amounts of bile acids; to evaluate effects of extrusion-cooking conditions on bile acid binding; and to demonstrate the usefulness of our *in vitro* bile acid binding procedure.

EXPERIMENTAL PROCEDURES

Potato Peels. Steam peels from Netted Gem (Russett Burbank) potatoes were provided by McCain Foods Ltd., Florenceville, NB. Peels were deep-frozen and then dried at 100 °C for 4 h. Dried potato peels were refrozen at −3 °C to prevent mold growth and then ground in a Thomas-Wiley mill (Model 4, Philadelphia, PA) to pass a 2-mm screen.

A two-by-three factorial experimental design was used to study the effects of final zone barrel temperature (103 or 143 °C) and feed moisture (31, 33.5, and 36%, db) on bile acid binding. Peels were extruded in a Werner & Pfleiderer ZSK 30 corotating twin-screw laboratory extruder with the following specifications: barrel bore diameter, 30.9 mm; screw outside diameter, 30.7 mm; screw length, 879 mm; and 4-mm die hole. The screw configuration has been described by Arora *et al.* (1993). A feed rate of 8.2 kg/h and screw speed of 300 rpm were held constant. Barrel temperature varied from feed end to die end as follows: 53, 57, 99, 103, 103, 101; or 53, 84, 116, 116, 130, 143, 118 °C. Water was pumped to the feed inlet to provide a peel moisture levels of 31, 33.5, or 36% (db). Duplicate samples were obtained for each combination of experimental variables. Extrudates were dried at 112 °C in a forced-air oven (Proctor and Schwartz Inc., Model K20124, Philadelphia, PA) for 15 min and then ground to pass a 2-mm screen. Particle size determinations were made on 25-g ground samples using the method of Toma *et al.* (1979), and mean particle size has been reported by Arora *et al.* (1993).

Reagents. Cholic acid, deoxycholic acid, glycocholic acid, taurocholic acid, and serum bile acid testing kit (450-A) were purchased from Sigma Chemical Co. (St. Louis, MO). Porcine pancreatin (5× USP) was purchased from ICN Biochemicals (Cleveland, OH). AACC hard red wheat bran and Sigma cellulose, cholestyramine, and pectin were also tested to provide comparisons with other studies of *in vitro* bile acid binding. All other chemicals were of ACS reagent grade, and distilled water was used throughout the study.

Bile Acid Binding Procedure. An *in vitro* digestion procedure was used. Triplicate 0.100-g samples of potato peels, bran, cellulose, pectin, and cholestyramine in 20 × 125 mm Kimax screw-capped tubes were digested with 1 mL of 0.01 N HCl for 1 h at 37 °C in a shaking water bath (Fisher Scientific Versa-bath 236). Blank tubes containing reagents only were taken through each step daily. After this simulated gastric step, samples were brought to pH 7.0 with 0.1 N NaOH. Individual bile acids were evaluated separately. Each bile acid solution contained 31.25 μmol/mL in 0.1 M phosphate buffer, pH 7.0. Bile acid solubilization was conducted using the procedure of Story and Kritchevsky (1976). Porcine pancreatin was dissolved in 0.01 M, pH 7, phosphate buffer to yield a concentration of 10 mg/mL.

The next step simulated conditions in the small intestine. Each tube was shaken for another hour at 37 °C after addition of 4 mL

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Table I. *In Vitro* Binding of Cholic and Deoxycholic Acids[a]

sample	barrel temp (°C)	feed moisture (%, db)	bound cholic acid (μmol/100 mg)	% cholic acid bound	bound deoxycholic acid (μmol/100 mg)	% deoxycholic acid bound
potato peels	104	31	8.52 ± 0.81[b] cd	6.55 ± 0.60[c]	24.95 ± 1.99[d] de	17.27 ± 1.34
potato peels	104	33.5	7.73 ± 0.24 bcd	5.43 ± 0.19	23.98 ± 1.45 d	16.89 ± 0.98
potato peels	104	36	11.46 ± 0.52 ef	8.07 ± 0.26	28.50 ± 0.44 e	18.87 ± 0.30
potato peels	143	31	9.53 ± 1.37 de	6.20 ± 0.81	17.58 ± 1.30 c	13.30 ± 1.28
potato peels	143	33.5	6.08 ± 0.35 b	4.62 ± 0.27	16.71 ± 1.12 bc	12.66 ± 0.80
potato peels	143	36	9.36 ± 1.99 d	6.80 ± 1.45	15.10 ± 2.12 bc	11.75 ± 1.63
potato peels			2.62 ± 0.41 a	1.90 ± 0.30	13.84 ± 0.26 b	10.63 ± 0.15
cholestyramine			116.88 ± 0.46 g	80.27 ± 0.15	150.24 ± 0.67 g	99.98 ± 0.00
pectin			7.34 ± 0.38 bc	5.33 ± 0.29	99.95 ± 0.45 f	70.03 ± 0.25
cellulose			ND[e]	0	0.02 ± 0.04 a	0
wheat bran[f]			11.93 ± 1.72 f	8.50 ± 1.25	23.77 ± 0.27 d	16.77 ± 0.23

[a] Dry weight basis. [b] Mean ± standard deviation ($n = 6$); Tukey's HSD = 2.01 ($P < 0.05$). [c] (Bile acid bound/available bile acid) × 100. [d] Mean ± standard deviation ($n = 6$); Tukey's HSD = 3.69 ($P < 0.01$). [e] None detected. [f] Mean particle size higher than those of peels (Arora *et al.*, 1993).

of bile acid solution and 5 mL of pancreatin solution. Samples were then quantitatively transferred to 50-mL plastic centrifuge tubes and centrifuged for 10 min at 26890*g* in a Sorvall RC2-B centrifuge. Supernatants were removed by Pasteur pipets to a second set of tubes. An additional 5 mL of phosphate buffer was added to the centrifuge tubes containing the samples to flush bile acids physically trapped within samples, as recommended by Eastwood *et al.* (1976). Tubes were vortex-mixed and centrifuged as before. Supernatants were removed and pooled with the original supernatants.

The Sigma bile acid method is linear to 0.2 μmol/mL, which is below the concentration for the blanks (31.25 μmol/mL). Therefore, 0.1 mL of supernatant was brought to 5 mL with water. For each sample and reagent blank, 0.2 mL was pipetted into each of three tubes—control blank, sample, and sample blank. Sample tubes received 0.5 mL of test reagent [nicotinamide adenine dinucleotide (NAD), nitro blue tetrazolium salt (NBT), diaphorase, and 3α-hydroxysteroid dehydrogenase] and blanks received the same reagents without the dehydrogenase. Tubes were incubated at 37 °C for 5 min. The reaction was stopped by the addition of 0.1 mL of 1.33 M phosphoric acid, and then absorbance at 530 nm was read in a Beckman DU-64 spectrophotometer.

A standard curve (0–12.5 μmol/mL) was prepared daily for each bile acid studied by treating each standard to the colorimetric procedure and then drawing a line of best fit (Cricket Graph, version 1.3, Malvern, PA). The amount of bile acid bound was determined as micromoles per 100 mg of sample (dry weight) by the difference between the reagent blank and sample

$$\text{binding} = \frac{(A_{\text{blank}} - A_{\text{sample}}) \times 1.5}{\text{sample wt } (1 - \text{mc})} \quad (1)$$

where A is the absorbance at 530 nm and mc is the sample moisture content.

Binding of cholic and deoxycholic acids was measured in triplicate for nonextruded peels, each duplicate extrusion run for each of the six extrusion conditions, cholestyramine, pectin, cellulose, and AACC wheat bran. Binding of conjugated bile acids was measured in triplicate for nonextruded peels, peels extruded at either barrel temperature with 36% feed moisture, and cholestyramine.

Peel Composition. Moisture was determined in triplicate by loss in weight of 1-g samples after 16 h at 102 °C. Insoluble fiber and soluble dietary fiber were analyzed in triplicate per extrusion duplicate with AACC Method 32-07 (AACC, 1990). For mineral analysis, 2-g samples were ashed in duplicate at 575 °C for 16 h. Ash was dissolved in 10 mL of concentrated HCl and then evaporated to near dryness. The residue was redissolved in 20 mL of 2 N HCl and diluted to 100 mL with water after filtration through Whatman 541 paper. Solutions were analyzed for minerals with a Jarrel atomic absorption spectrophotometer.

Statistical Analyses. Bile acid binding, dietary fiber, and mineral content were analyzed with the multivariate general linear hypothesis ANOVA program (SYSTAT, Evanston, IL). Differences between barrel temperature–feed moisture interactions were tested with Tukey's honest significant difference (HSD) test at $P < 0.05$. Such interactions were treated as one factor to compare extruded samples with nonextruded peels, cholestyramine, pectin, cellulose, and wheat bran. The HSD test was also applied to these factors. Pearson's correlation coefficient was used to determine relationships between bile acid binding and dietary fiber and iron content.

Table II. *In Vitro* Binding of Conjugated Bile Acids[a]

sample	extrusion conditions	bound glycocholic acid (μmol/100 mg)[b]	bound taurocholic acid (μmol/100 mg)
potato peels	104 °C, 36% mc	7.23 ± 0.58 b	8.30 ± 1.58 a
potato peels	143 °C, 36% mc	8.14 ± 0.73 b	7.05 ± 1.09 a
potato peels	not extruded	3.80 ± 0.52 a	7.77 ± 1.71 a
cholestyramine	not extruded	97.34 ± 0.84 c	102.57 ± 0.25 b

[a] Mean ± standard deviation ($n = 6$). Values followed by different letters within a column are significantly different (Tukey's HSD, $P < 0.01$). [b] Dry weight basis.

RESULTS

Cholic Acid. Barrel temperature, feed moisture, and the interaction of these factors significantly affected ($P < 0.001$, $R^2 = 0.739$) binding of cholic acid by extruded potato peels. Peels extruded at 104 °C and 36% moisture bound the greatest amount among extruded peels (Table I). All extruded peels bound more cholic acid than did nonextruded peels or cellulose. Cholestyramine bound 80% of available cholic acid (Table I). Binding by pectin was significantly lower ($P < 0.05$) than binding by peels extruded at 104 °C and 36% moisture or at 143 °C and 31% moisture.

Deoxycholic Acid. All materials in this study bound a greater percentage of deoxycholic than cholic under the same conditions (Table I). Feed moisture had no effect on binding of deoxycholic acid, but barrel temperature and temperature–moisture interaction were significant ($P < 0.001$) factors. The coefficient of determination (R^2) was 0.935 for this model. All peels extruded at 104 °C had significantly ($P < 0.01$) greater binding; peels processed at 143 °C were not different from nonextruded peels (Table I). Cellulose bound a negligible amount of deoxycholic acid, while cholestyramine bound over 99% of the acid.

Conjugated Bile Acids. Potato peels extruded under both conditions bound significantly ($P < 0.01$) more glycocholic acid than did nonextruded peels (Table II). However, no difference was found among peels for binding of taurocholic acid. Cholestyramine bound more taurocholic than glycocholic acid.

Dietary Fiber. Total dietary fiber was significantly ($P < 0.05$) lower in samples extruded at the higher barrel temperature (Table III). Insoluble fiber decreased significantly ($P < 0.05$) in extruded peels (Table III), while soluble fiber increased in all extruded samples but that

Table III. Dietary Fiber Content of Potato Peels[a]

barrel temp (°C)	feed moisture (%, db)	total dietary fiber (%, db)	insoluble fiber (%, db)	soluble fiber (%, db)
104	31	49.88 ± 0.70 b	45.20 ± 0.26 d	4.68 ± 0.48 b
104	33.5	48.72 ± 0.74 b	44.35 ± 0.31 c	4.38 ± 0.56 b
104	36	49.22 ± 0.13 b	45.15 ± 0.19 d	4.08 ± 0.30 b
143	31	47.15 ± 0.34 a	42.52 ± 0.30 a	4.62 ± 0.52 b
143	33.5	47.40 ± 0.50 a	43.30 ± 0.36 b	4.10 ± 0.32 b
143	36	46.70 ± 0.68 a	42.95 ± 0.35 ab	3.75 ± 0.39 ab
not extruded		50.00 ± 0.14 b	47.20 ± 0.14 e	2.80 ± 0 a

[a] Mean ± standard deviations ($n = 6$). Values followed by different letters within a column are significantly different (Tukey's HSD, $P < 0.05$).

Table IV. Iron Content of Potato Peels[a]

barrel temp (°C)	feed moisture (%, db)	iron (ppm)
104	31	882 ± 30 b
104	33.5	859 ± 68 b
104	36	908 ± 7 b
143	31	1167 ± 25 d
143	33.5	1072 ● 22 c
143	36	1075 ± 25 c
not extruded		638 ± 14 a

[a] Mean ± standard deviations ($n = 6$). Values followed by different letters within a column are significantly different (Tukey's HSD, $P < 0.05$).

extruded at 143 °C and 36% moisture. The amount of deoxycholic acid bound by all peels was correlated with insoluble fiber ($r = 0.84, P < 0.01$) and total fiber ($r = 0.75$, $P < 0.01$). Soluble fiber was not correlated with binding of either bile acid.

Minerals. No differences were found between nonextruded and extruded peels for aluminum, calcium, copper, magnesium, and potassium. Significant ($P < 0.05$) differences were discovered for iron (Table IV). For extruded peels, iron content correlated with deoxycholic acid binding ($r = -0.80$). When nonextruded peels were included for correlation, iron still correlated with the amount of deoxycholic acid bound ($r = -0.86$) but was not correlated with binding of cholic acid.

DISCUSSION

Greater binding of deoxycholic acid than cholic acid has been reported by many researchers, including Story and Kritchevsky (1976) and Pandolf and Clydesdale (1992). Kern *et al.* (1978) proposed that less cholic acid, a trihydroxy bile acid, was bound than dihydroxy bile acids because hydrophobic interactions are involved with binding. The minimal binding by cellulose and high binding by cholestyramine agree with results obtained by Gallaher and Schneeman (1986) with rat intestinal contents. Wheat bran had a larger particle size (Arora *et al.*, 1993), which may account for its higher binding (Mongeau and Brassard, 1982). In this study, deoxycholic binding was correlated with mean particle size ($r = 0.898$), but nonextruded peels had greater mean particle size (423 μm) than extruded peels (380–410 μm; Arora *et al.*, 1993).

Extrusion may shear branches from insoluble fiber molecules to yield smaller, soluble compounds. Such soluble fiber would still have a composition similar to that of the insoluble form but would not have the ability to bind or physically entrap bile acids. The reduction in total dietary fiber may be due to formation of compounds too small to be recovered by the method. Eastwood and Hamilton (1968) attributed the bile acid binding of plant materials to their lignin content, but previously we found that Klason lignin decreased upon extrusion of potato peels (Camire and Flint, 1991). Peels darkened during extrusion (Arora *et al.*, 1993), presumably due to Maillard reaction products which may bind cholic and chenodeoxycholic acids *in vitro* but did not reduce plasma cholesterol in rats fed these compounds (Ragot *et al.*, 1992).

Increased iron content in extruded peels was expected as a result of screw wear during extrusion, particularly of high-fiber materials (Fairweather-Tait *et al.*, 1987). Pandolf and Clydesdale (1992) reported increased *in vitro* bile acid binding by dietary fiber samples when ferrous sulfate heptahydrate was added prior to incubation with bile acids. Since a negative relationship was found between mineral content and bile acid binding in potato peels, iron may alter bile acid binding sites or, more likely, the same shear forces which favor screw wear may degrade fiber, preventing adequate bile acid absorption.

CONCLUSIONS

Extrusion-cooking parameters affect the ability of potato peels to bind bile acids under simulated digestion conditions. While the mechanism for increased bile acid binding due to extrusion may involve formation of Maillard reaction products and screw wear, further study of binding of bile salts and lipid micelles should be investigated with extruded food materials which are used commercially in the manufacture of breakfast cereals and snacks. Colorimetric evaluation of bile acid binding agrees with results obtained by other researchers for standard materials without the safety and waste disposal problems incurred with the use of radioactive-labeled bile acids. *In vitro* bile acid determination using this colorimetric method offers a relatively inexpensive and time-saving screening opportunity prior to *in vivo* testing.

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