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In vitro binding of bile acids by bananas, peaches, pineapple, grapes, pears, apricots and nectarines ☆

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Abstract

The in vitro binding of bile acids by bananas (*Musa × paradisiaca*), peaches (*Prunus persica*), pineapple (*Ananus comosus*), grapes (*Vitis* spp.), pears (*Pyrus communis*), apricots (*Prunus armeniaca*) and nectarines (*Prunus persica, nectarina*) was determined using a mixture of bile acids secreted in human bile at a duodenal physiological pH of 6.3. Six treatments and two blank incubations were conducted, testing various fresh fruits on an equal dry matter basis. Considering cholestyramine (bile acid binding, cholesterol lowering drug) as 100% bound, the relative in vitro bile acid binding percentages on dry matter (DM), total dietary fibre and total polysaccharides basis were 2–9%, 15–101% and 10–101%, respectively. Bile acid binding, on a DM basis, for bananas was significantly ($P \leqslant 0.05$) higher and that for nectarines significantly lower than those for peaches, pineapple, grapes, pears and apricots. The bile acid bindings for peaches and pineapple were similar and significantly higher than those for grapes, pears and apricots. Binding values for grapes and pears were significantly higher than apricots. These results point to the relative health promoting potential of bananas > peaches = pineapple > grapes = pears > apricots > nectarines, as indicated by their bile acid binding on a DM basis. The variability in bile acid binding between the fruits tested maybe related to their phytonutrients, antioxidants, polyphenols, flavonoids (anthocyanins, flavonols, and proanthocyanidins), structure, hydrophobicity of undigested fractions, anionic or cationic nature of the metabolites produced during digestion or their interaction with active binding sites. Animal studies are planned to validate in vitro bile acid binding of fruits, observed herein, to their healthful potential, atherosclerosis amelioration and cancer prevention.
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Keywords: Bananas; Peaches; Pineapple; Grapes; Pears; Apricots; Nectarines; Bile acid binding

1. Introduction

Consumption of fruits as a significant portion of our daily diets has been associated with a lower risk of coronary heart disease and cancer (Block, Patterson, & Subar, 1992; Gaziano et al., 1995; Ness & Powles, 1997). USDA Food and Nutrition Information Center (2005) recommends daily active life, intake of low fat food products and consumption of vegetables and fruits. Most fruits are naturally low in fat, sodium, and calories. Fruits are important sources of many nutrients, including potassium, dietary fibre, vitamin C and folic acid and they do not contain cholesterol. Some of the fruits listed by the USDA food pyramid include apricots, bananas, grapes, nectarines, peaches, pears and pineapple. Phytonutrients in the fruits have been shown to stimulate natural detoxifying enzymes in the body and lower the risk of atherosclerosis and cancer (Ames, Shigenaga, & Hagen, 1993). Resveratrol, present in the seed and skin of grapes, prevents cancer and atherosclerosis risk (Gehm, McAndrews, Chien, & Jameso, 1997; Lin & Tsai, 1999). Toxic metabolites in the gut and secondary bile acids increase the risk of colorectal cancer (Costarelli et al., 2002). Fruits are high in health promoting phytonutrients, antioxidants, flavonoids (anthocyanins, flavonols

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and proanthocyanidins), and polyphenols (Reed, 2002; Sun, Chu, Wu, & Liu, 2002). The healthful, cholesterol-lowering (atherosclerosis amelioration) or detoxification of harmful metabolites (cancer prevention) potential of food fractions could be predicted by evaluating their in vitro bile acid binding, based on positive correlations found between in vitro and in vivo studies showing that cholestyramine (bile acid binding, cholesterol lowering drug) binds bile acids and cellulose does not (Daggy, O'Connell, Jerdack, Stinson, & Setchell, 1997; Kahlon & Chow, 2000; Nakamura & Matsuzawa, 1994; Suckling et al., 1991). Bile acids are acidic steroids synthesized in the liver from cholesterol. After conjugation with glycine or taurine, they are secreted into the duodenum. Bile acids are actively reabsorbed by the terminal ileum and undergo an enterohepatic circulation (Hofmann, 1977). Binding of bile acids and increasing their fecal excretion has been hypothesized as a possible mechanism by which dietary fibre lowers cholesterol (Anderson & Siesel, 1990; Lund, Gee, Brown, Wood, & Johnson, 1989; Trowell, 1975). By binding bile acids, food fractions prevent their reabsorption and stimulate plasma and liver cholesterol conversion to additional bile acids (Balmer & Zilversmit, 1974; Eastwood & Hamilton, 1968; Kritchevsky & Story, 1974; Potter, 1998). Excretion of toxic metabolites and secondary bile acids could lower the risk of cancer (Costarelli et al., 2002). Bile acid binding of grain fractions, ready to eat breakfast cereals, various fruits and dry beans has been observed to be proportional to their dry matter content (Kahlon & Woodruff, 2003a, 2003b; Kahlon & Shao, 2004; Kahlon, Smith, & Shao, 2005; Kahlon & Smith, 2006). Kahlon and Smith (2006) previously reported relative bile acid binding on a dry matter basis to be blueberries (7%), plums (6%), prunes (5%), strawberries (5%), cherries (5%), cranberries (4%) and apples (1%).

The objective of this study was to determine healthful potentials of bananas (*Musa × paradisiaca*), peaches (*Prunus persica*), pineapple (*Ananus comosus*), grapes (*Vitis* spp.), pears (*Pyrus communis*), apricots (*Prunus armeniaca*) and nectarines (*Prunus persica, nectarine*) by evaluating their in vitro bile acid binding using fruits on an equal dry matter basis, with a bile acid mixture observed in human bile at the duodenal physiological pH 6.3.

2. Materials and methods

2.1. Materials

Fresh ripe and ready to eat bananas, peaches, pineapple, grapes, pears, apricots and nectarines were obtained from a local grocery super market. All the fruits were washed and lyophilized in a Lyph-lock 18 freeze dryer (Laconco Corporation, Kansas City, MO). Freeze dried samples were ground, frozen, using dry ice in a Thomas-Wiley Mini mill (Arthur Thomas, Philadelphia, PA) to pass a 0.4 mm screen. Samples were analyzed for moisture by method 935.29 (AOAC, 1990). Cellulose, a non-bile acid binding

fibre, was the negative control and cholestyramine, a bile acid binding anionic resin (a drug that lowers cholesterol and binding bile acids), was the positive control (Sigma St. Louis, MO). Eight replicate incubations, six with bile acid mixture, one substrate blank without bile acid mixture and one bile acid mixture without substrate, were run for each treatment and control. All the fruits used for incubation on a dry matter basis were 103–110 mg and cellulose was 24 mg and cholestyramine 26 mg.

2.2. Bile acid binding procedure

The in vitro bile acid binding procedure was a modification of that by Camire, Zhao, and Violette (1993), as previously reported (Kahlon & Chow, 2000). The stock bile acid mixture was formulated with glycocholic bile acids, (providing 75%) and taurine-conjugated bile acids (25%) of the bile acids, based on the composition of human bile (Carey & Small, 1970; Rossi, Converse, & Hoffman, 1987). This mixture contained glycocholic acid (9 mmol/l), glycochenocholic acid (9 mmol/l), glycodeoxycholic acid (9 mmol/l), taurocholic acid (3 mmol/l), taurochenocholic acid (3 mmol/l) and taurodeoxycholic acid (3 mmol/l) in pH 6.3, 0.1 M phosphate buffer. This stock solution of 36 mmol/l was stored in the −20 °C freezer and diluted to the working solution (0.72 μmol/ml) just prior to each assay. Six replicates of 103–110 mg dry matter of bananas, peaches, pineapple, grapes, pears, apricots and nectarines, cholestyramine 26 mg and cellulose 24 mg were tested. One substrate blank, one positive blank (2.88 μmol bile acid mixture per incubation) and six treatment replicates were weighed into 16 × 150 mm glass, screw-capped tubes. Samples were digested in 1 ml of 0.01 N HCl for 1 h in a 37 °C shaker bath. After this acidic incubation simulating gastric digestion, the sample pH was adjusted to 6.3 with 0.1 ml of 0.1 N NaOH. To each test sample was added 4 ml of bile acid mixture working solution (0.72 μmol/ml) in a 0.1 M phosphate buffer, pH 6.3. A phosphate buffer (4 ml, 0.1 M, pH 6.3) was added to the individual substrate blanks. After the addition of 5 ml of porcine pancreatin (5×, 10 mg/ml, in a 0.1 M phosphate buffer, pH 6.3; providing amylase, protease and lipase for digestion of samples), tubes were incubated for 1 h in a 37 °C shaker bath. Mixtures were transferred to 10 ml centrifuge tubes (Oak Ridge 3118-0010 Nalgene, Rochester, NY) and centrifuged at 99,000*g* for 18 min at 25 °C in an ultracentrifuge. Incubation tubes were rinsed with 5 ml phosphate buffer and centrifuged as before. Aliquots of pooled supernatant were frozen at −20 °C for bile acids analysis. Bile acids were analyzed using Trinity Biotech bile acids procedure No. 450 (Trinity Biotech Distribution, St. Louis, MO) using a Ciba-Corning Express Plus analyzer (Polestar Labs, Inc., Escondido, CA). Each sample was analyzed in triplicate. Values were determined from a standard curve obtained by analyzing Trinity Biotech bile acid calibrators (No. 450-11) at 5, 25, 50, 100 and 200 μmol/l. Individual substrate blanks were subtracted, and bile acid concentrations

were corrected based on the mean recoveries of bile acid mixture (positive blank). The effect of treatment was tested using Lavene's test for homogeneity; least square means were calculated. Dunnett's one-tailed test was used for comparison of cholestyramine, as well as cellulose, against all treatments, and differences among bananas, peaches, pineapple, grapes, pears, apricots and nectarines were tested for significance with Tukey's test for comparison of all possible pairs of means (SAS Institute, Cary, NC). A value of $P \leqslant 0.05$ was considered the criterion of significance.

3. Results and discussion

Compositions of the bananas, peaches, pineapple, grapes, pears, apricots and nectarines are given in Table 1. Both cellulose and cholestyramine were considered as 100% total dietary fibre and polysaccharides. There was wide variation in the dietary fibre, polysaccharides and protein contents of these fruits. Total dietary fibre, polysaccharide and protein values, on a dry matter, basis for the fruits tested were bananas 10%, 42% and 4%, peaches 14%, 10% and 8%, pineapple 10%, 25% and 4%, grapes 5%, 5% and 3%, pears 19%, 35% and 2%, apricots 15%, 14% and 10% and nectarines 14%, 21% and 9%, respectively. In the fruits tested, fat and mineral values ranged from 1% to 3% and 2% to 6%, respectively.

On an equal dry matter (DM) basis, bile acid binding was significantly higher for cholestyramine and significantly lower for cellulose than those all the fruits tested (Table 2). Bile acid binding, for bananas, was significantly ($P \leqslant 0.05$) higher and that, for nectarines, significantly lower than those for peaches, pineapple, grapes, pears and apricots. The bile acid binding for peaches and pineapple were similar and their values were significantly higher than those of grapes, pears and apricots. Binding values for grapes and pears were similar and significantly higher than those for apricots. Cholestyramine bound 93% of the bile acids. These values are similar to the previously reported observations (Kahlon & Chow, 2000). Cholestyramine bound glycocholate and taurocholate by 87 and

93%, respectively (Sugano & Goto, 1990). In our study, cholestyramine binding to the mixture of bile acids was similar to that observed for taurocholate by Sugano and Goto (1990). Story and Kritchevsky (1976) reported 81% bile acid binding by cholestyramine using 50 mg of substrate and 50 μmol of bile acids. Higher bile acid binding by cholestyramine in our studies may be due to the use of physiological pH and/or a higher substrate to bile acid ratio.

Assigning a bile acid binding value of 100% to cholestyramine, the relative bile acid bindings on a dry matter basis for the fruits tested were bananas (9%), peaches (6%), pineapple (6%), grapes (5%), pears (5%), apricots (3%) and nectarines (2%). Bile acid binding for bananas was significantly higher and those for nectarines significantly lower than that for peaches, pineapple, grapes, pears and apricots. Bile acid binding for peaches and pineapple were similar and significantly higher than those for grapes, pears and apricots. Values for grapes and pears were similar and significantly higher than apricots. Relative bile acid binding on DM basis was: bananas > peaches = pineapple > grapes = pears > apricots > nectarines. The differences in bile acid binding between various fruits tested may relate to their phytonutrients, antioxidants, polyphenols, flavonoids (anthocyanins, flavonols, proanthocyanidins), hyrophobicity or active binding sites.

On a dry matter basis, bile acid binding of 5%–9% for bananas, peaches, pineapple, grapes and pears is very encouraging, which could be an indicator of their health promoting potential. Similar (5%–7%) bile acid binding values for blueberries, plums, prunes, strawberries and cherries have previously been reported (Kahlon & Smith, 2006). Similarly 5%–9% relative bile acid binding for oat bran and oat bran ready to eat cereal (cereal with US-FDA approved for label health claim for lowering cholesterol) has been reported (Kahlon & Chow, 2000; Kahlon & Woodruff, 2003b). Bile acid binding, on a dry matter basis, has been reported as relative healthful potential of ready-to-eat cereals, cereal fractions, dried beans and various fruits (Kahlon & Woodruff, 2003a, 2003b; Kahlon & Shao, 2004; Kahlon et al., 2005; Kahlon & Smith, 2006).

Table 1
Composition of bananas (*Musa × paradisiaca*), peaches (*Prunus persica*), pineapple (*Ananus comosus*), grapes (*Vitis* spp.), pears (*Pyrus communis*), apricots (*Prunus armeniaca*) and nectarines (*Prunus persica, nectarina*), dry matter (DM) basis

Source	Carbohydrate	Total dietary fibre	Sugar	Polysaccharides	Protein	Fat	Minerals
	DM (%)						
Bananas	91.0	10.4	48.7	42.3	4.3	1.3	3.3
Peaches	85.7	13.5	75.4	10.3	8.2	2.2	3.9
Pineapple	93.3	10.3	68.4	24.9	4.0	0.9	1.8
Grapes	91.7	4.8	86.9	4.8	3.4	1.9	3.0
Pears	94.9	19.0	60.2	34.7	2.3	0.7	2.0
Apricots	81.5	14.7	67.7	13.8	10.3	2.9	5.5
Nectarines	85.0	13.7	63.6	21.4	8.5	2.6	3.9
Cholestyramine	–	100	–	100	–	–	–
Cellulose	–	100	–	100	–	–	–

Data for the fruits from USDA National Nutrient Database for Standard Reference Release 17 (2004) (http://www.nal.usda.gov/fnic/foodcomp/search/); Polysaccharides = Carbohydrate − Sugar.

Table 2
In vitro bile acid binding by bananas (*Musa × paradisiaca*), peaches (*Prunus persica*), pineapple (*Ananus comosus*), grapes (*Vitis* spp.), pears (*Pyrus communis*), apricots (*Prunus armeniaca*) and nectarines (*Prunus persica, nectarina*) on equal weight, dry matter (DM) basis[A,B]

Treatment	Bile acid binding (μmol/100 mg DM)	Bile acid binding relative to cholestyramine (%)
Bananas	0.90 ± 0.04^B	8.8 ± 0.4^B
Peaches	0.60 ± 0.03^c	5.8 ± 0.3^c
Pineapple	0.59 ± 0.02^c	5.7 ± 0.2^c
Grapes	0.50 ± 0.02^d	4.9 ± 0.2^d
Pears	0.47 ± 0.01^d	4.5 ± 0.1^d
Apricots	0.31 ± 0.02^e	3.0 ± 0.2^e
Nectarines	0.21 ± 0.02^f	2.1 ± 0.2^f
Cholestyramine	10.3 ± 0.05^A	100.0 ± 0.4^A
Cellulose	0.07 ± 0.02^g	0.7 ± 0.2^g

[A] Means ± SEM within a column with different superscripts differ significantly ($P \leqslant 0.05$), $n = 6$.
[B] The dry matter used for incubation, for all the fruits was 103–110 mg, cholestyramine and cellulose, 24–26 mg.

Binding of bile acids and prevention of their recirculation results in reduced fat absorption, excretion of cancer causing toxic metabolites and cholesterol utilization to synthesize more bile acids. This is believed to be the mechanism by which food fractions lower cholesterol and prevent cancer. Evaluating healthful properties of various fruits and food fractions would be useful by testing their bile acid binding on a dry matter basis.

The bile acid binding, on equal total dietary fibre (TDF) basis, is shown in Table 3. Cholestyramine bound bile acids significantly more and cellulose significantly less than the various fruits tested. On a TDF basis considering cholestyramine as 100% bound, bile acid binding values were: bananas (85%), peaches (43%), pineapple (55%), grapes (101%), pears (24%), apricots (21%) and nectarines (15%). There were significant differences between the bile acid binding values of grapes, bananas, pineapple, peaches, pears and nectarines. Relative bile acid binding values, on a TDF basis among various fruits tested were: grapes >

bananas > pineapple > peaches > pears = apricots = nectarines. Similar bile acid binding of grapes to that of cholestyramine is due to very low (5%) TDF in grapes compared to 100% in cholestyramine (Table 1). Bile acid binding of bananas was 53% higher than that of pineapple, with both using 11 mg of TDF per incubation. Data suggest that bile acid binding was not related to the TDF content of the fruits tested. This is in agreement with a previous report that bile acid bindings of blueberries, plums, prunes, strawberries, cherries, cranberries and apples were not related to their TDF contents (Kahlon & Smith, 2006). Similar observations have been reported for black eye bean, garbanzo, kidney bean, lima bean, moth bean and soybean (Kahlon & Shao, 2004; Kahlon et al., 2005). The variability in bile acid binding between the fruits tested maybe related their phytonutrients, antioxidants, polyphenols, flavonoids (anthocyanins, flavonols, proanthocyanidins), hyrophobicity, anionic, cationic, physical and chemical structure or active binding sites.

Bile acid binding of various fruits, based on equal total polysaccharides (PCH), is shown in Table 4. On a PCH basis, considering cholestyramine as 100% bound relative bile acid binding were bananas (21%), peaches (56%), pineapple (23%), grapes (101%), pears (13%), apricots (22%) and nectarines 10%. Bile acid binding for peaches was significantly lower than that of grapes and significantly higher than those of bananas, pineapple, pears, apricots and nectarines. Bile acid binding for bananas, pineapple and apricots were similar and significantly higher than that for pears and nectarines. Bile acid bindings on a PCH basis for the various fruits tested were grapes > peaches > bananas = pineapple = apricots > pears = nectarines. Similar bile acid binding for grapes to those for cholestyramine is due to only 5% as much PCH in grapes compared with cholestyramine (Table 1). The bile acid binding by bananas was only 37% as much as that for peaches, even though four-fold as much (44 mg) PCH was used for bananas com-

Table 3
In vitro bile acid binding by bananas (*Musa × paradisiaca*), peaches (*Prunus persica*), pineapple (*Ananus comosus*), grapes (*Vitis* spp.), pears (*Pyrus communis*), apricots (*Prunus armeniaca*) and nectarines (*Prunus persica, nectarina*) on equal total dietary fibre (TDF) basis[A,B]

Treatment	Bile acid binding (μ mol/100 mg TDF)	Bile acid binding relative to cholestyramine (%)
Bananas	8.73 ± 0.37^B	84.8 ± 3.6^B
Peaches	4.44 ± 0.20^d	43.1 ± 1.9^d
Pineapple	5.70 ± 0.16^c	55.4 ± 1.6^c
Grapes	10.4 ± 0.48^A	101 ± 4.6^A
Pears	2.45 ± 0.05^e	23.8 ± 0.5^e
Apricots	2.14 ± 0.16^{ef}	20.8 ± 1.5^{ef}
Nectarines	1.54 ± 0.13^f	15.0 ± 1.2^f
Cholestyramine	10.3 ± 0.05^A	100 ± 0.4^A
Cellulose	0.07 ± 0.02^g	0.7 ± 0.2^g

[A] Means ± SEM within a column with different superscripts differ significantly ($P \leqslant 0.05$), $n = 6$.
[B] The TDF (mg) used for incubation was bananas (11), peaches (15), pineapple (11), grapes (5), pears (20), apricots (16), nectarines (15), cholestyramine (26), and cellulose (24) mg.

Table 4
In vitro bile acid binding by bananas (*Musa × paradisiaca*), peaches (*Prunus persica*), pineapple (*Ananus comosus*), grapes (*Vitis* spp.), pears (*Pyrus communis*), apricots (*Prunus armeniaca*) and nectarines (*Prunus persica, nectarina*) on equal total polysaccharides basis[A,B]

Treatment	Bile acid binding (μ mol/ 100 mg polysaccharides)	Bile acid binding relative to cholestyramine (%)
Bananas	2.14 ± 0.09^c	20.8 ± 0.9^c
Peaches	5.79 ± 0.26^B	56.2 ± 2.5^B
Pineapple	2.37 ± 0.07^c	23.0 ± 0.7^c
Grapes	10.4 ± 0.48^A	101 ± 4.6^A
Pears	1.34 ± 0.03^d	13.0 ± 0.3^d
Apricots	2.27 ± 0.17^c	22.1 ± 1.6^c
Nectarines	0.99 ± 0.08^d	9.6 ± 0.8^d
Cholestyramine	10.3 ± 0.05^A	100 ± 0.4^A
Cellulose	0.07 ± 0.02^e	0.7 ± 0.2^e

[A] Means ± SEM within a column with different superscripts differ significantly ($P \leqslant 0.05$), $n = 6$.
[B] The polysaccharides (mg) used for incubation were: bananas (44), peaches (11), pineapple (26), grapes (5), pears (37), apricots (15), nectarines (23), cholestyramine (26), and cellulose (24) mg.

pared with that for peaches (11 mg), suggesting that bile acid binding is not related to the PCH content of the fruits tested. This is in agreement with previous report that bile acid binding was not related to PCH content of blueberries, plums, prunes, strawberries, cherries, cranberries and apples (Kahlon & Smith, 2006).

In conclusion, relative to cholestyramine, the in vitro bile acid binding on DM basis was for bananas (9%), peaches (6%), pineapple (6%), grapes (5%), pears (5%), apricots (3%) and nectarines (2%). These results indicate that the relative health promoting potentials are: bananas > peaches = pineapple > grapes = pears > apricots > nectarines, as indicated by their bile acid binding on a DM basis. The differences in bile acid binding between various fruits tested may relate to their phytonutrients, antioxidants, polyphenols, flavonoids (anthocyanins, flavonols, proanthocyanidins), structures, hydrophobicity of undigested fractions, anionic or cationic natures of the metabolites produced during digestion or their interaction with active binding sites. Inclusion of bananas, peaches, pineapple, grapes and pears in our daily diet, as health-promoting fruits should be encouraged. Animal studies are planned to explore relative potential for atherosclerosis amelioration (lowering lipids and lipoprotein) and cancer prevention (excretion of toxic metabolites, secondary bile acids) and other healthful properties of the fruits studied herein.

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