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In vitro binding of bile acids by okra, beets, asparagus, eggplant, turnips, green beans, carrots, and cauliflower ☆

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Abstract

The *in vitro* binding of bile acids by okra (*Abelmoschus esculentus*), beets (*Beta vulgaris*), asparagus (*Asparagus officinalis*), eggplant (*Solanum malongena*), turnips (*Brassica rapa rapifera*), green beans (*Phaseolus vulgaris*), carrots (*Daucus carota*), and cauliflower (*Brassica oleracea botrytis*) 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 raw vegetables on an equal dry matter basis. Considering cholestyramine (bile acid-binding, cholesterol-lowering drug) as 100% bound, the relative *in vitro* bile acid binding on dry matter (DM) and total dietary fibre (TDF) basis was 1–16% and 2–54%, respectively. Bile acid binding for okra was significantly higher than for all the other vegetables tested. For beets, binding values were significantly higher than for asparagus. Binding values for asparagus were significantly higher than for eggplant, turnips, beans green, carrots and cauliflower. These results point to the health promoting potential of okra > beets > asparagus > eggplant = turnips = green beans = carrots = cauliflower, as indicated by their bile acid binding, on a dry matter basis.
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Keywords: Okra; Beets; Asparagus; Eggplant; Turnips; Green beans; Carrots; Cauliflower; Bile acid binding

1. Introduction

Vegetarians or those consuming vegetables as a major portion of their daily diet, along with consuming fewer calories from saturated fat and animal products, are at a lower risk of coronary heart disease and cancer. USDA Food and Nutrition Information Center (2005) Food Guide Pyramid – Steps to a Healthier You (http://www.mypyramid.gov) recommends daily active life, intake of low fat food products and consumption of dark, leafy and colourful vegetables. Some of the colourful vegetables listed by the USDA food pyramid include carrots, asparagus, beets, cauliflower, eggplant, green beans, okra and turnips.

Vegetables are a good source of dietary fibre, phytonutrients, provitamins, antioxidants, polyphenols and minerals. Antioxidants in beets and green beans (Cardador-Martinez, Loarca-Pina, & Oomah, 2002; Jiratanan & Liu, 2004), prebiotics and immune protecting phytochemicals of asparagus (Diwanay, Chitre, & Patwardhan, 2004; Gautam et al., 2004; Gibson, 1998), hydroxycinnamic acid of eggplant (Whitaker & Stommel, 2003), glucosinolates of cauliflower (Kushad et al., 1999; Tian, Rosselot, & Schwartz, 2005) have been associated with health promoting effects. Phytonutrients in vegetables have been shown to stimulate natural detoxifying enzymes in the body and lower the risk of atherosclerosis and cancer (Ames, Shigenaga, & Hagen, 1993; Hecht, 1999). Raw, fresh vegetables are consumed in salads, sandwiches and/or with dips and are believed to be more nutritious than those consumed after cooking. Kahlon, Chapman, and Smith (2007)

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reported the bile acid binding, relative to cholestyramine, on dry matter basis, for raw fresh green leafy vegetables spinach (9%), kale (8%), brussels sprouts (8%), broccoli (5%), mustard greens (4%), green bell peppers (3%), cabbage (2%) and collards (2%). 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 (a 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 synthesised 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 faecal excretion has been hypothesised 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, fresh raw green vegetables, and various dry beans has been observed to be proportional to their dry matter content (Kahlon & Woodruff, 2003a; Kahlon & Shao, 2004; Kahlon, Smith, & Shao, 2005; Kahlon et al., 2007).

The objective of this study was to determine the healthful potential of okra (*Abelmoschus esculentus*), beets (*Beta vulgaris*), asparagus (*Asparagus officinalis*), eggplant (*Solanum malongena*), turnips (*Brassica rapa rapifera*), green beans (*Phaseolus vulgaris*), carrots (*Daucus carota*), and cauliflower (*Brassica oleracea botrytis*), by evaluating their *in vitro* bile acid binding using fresh colourful raw vegetables on an equal dry matter basis, with a bile acid mixture, observed in human bile under duodenal physiological pH of 6.3.

2. Materials and methods

Fresh okra, beets, asparagus, eggplant, turnips, green beans, carrots, and cauliflower were obtained from a local grocery supermarket. All the fresh vegetables were washed and lyophilised 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 through a 0.4 mm screen. Samples were analysed for total dietary fibre by method 991.16 (AOAC, 2005), nitrogen by method 990.03 (AOAC, 1990) with a Vario Macro Elemental Analyser (Elementar Analysen systeme GmbH, Hanau, Germany), crude fat with petroleum ether by the accelerated solvent extractor (ASE 200 Dionex Corp., Sunnyvale, CA) as described by Berrios, Camara, Torija, and Alonso (2002), ash by method 942.05 (AOAC, 1990) and 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 binds bile acids), was the positive control; both were obtained from 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 of the vegetables used for incubation on dry matter basis weighed 97–101 mg; cellulose weighed 24 mg and cholestyramine 26 mg.

3. Bile acid binding procedure

The *in vitro* bile acid binding procedure was a modification of that of 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 the 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 at −20 °C and diluted to the working solution (0.72 μmol/ mL) just prior to each assay.

Six replicates of 97–101 mg dry matter of okra, beets, asparagus, eggplant, turnips, green beans, carrots, and cauliflower, 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 0.01 N HCl for one hour in a 37 °C shaker bath. After this acidic incubation which simulated 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. 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 one hour 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* in a 75-Ti rotor at 39 K for 18 min at 25 °C in an ultracentrifuge (model L-60, Beckman, Palo Alto, CA). Supernatant was removed into a second set of labelled tubes. An additional 5 mL of phosphate buffer was used to rinse out the incubation tube and added to the centrifuge tube, which was vortexed and centrifuged as before. Supernatant was removed and combined with the previous supernatant. Aliquots of pooled supernatant were frozen at −20 °C for bile acids analysis. Bile acids were analysed using Trinity Biotech bile acids procedure No. 450 (Trinity Biotech Distribution, St Louis, MO) using a Ciba-Corning Express Plus analyser (Polestar Labs, Inc., Escondido, CA). Each sample was analysed in triplicate. Values were determined from a standard curve obtained by analysing 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 okra, beets, asparagus, eggplant, turnips, green beans, carrots, and cauliflower 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.

4. Results and discussion

Composition of the okra, beets, asparagus, eggplant, turnips, green beans, carrots, and cauliflower is given in Table 1. Both cellulose and cholestyramine were considered as 100% total dietary fibre. There was wide variation in the total dietary fibre (21–46%), protein (5–34%), fat (0.2–2.0%) and ash (7–13%) content of these vegetables. Carbohydrate (55–86%) was determined by difference: percent carbohydrates = [100 − (protein + crude fat + ash)]. On an equal dry matter (DM) basis, bile acid binding was significantly higher for cholestyramine than all the vegetables tested (Table 2). Bile acid binding of okra was significantly ($P \leqslant 0.05$) higher than the other vegetables tested. There was significant higher bile acid binding for beets than asparagus. Eggplant, turnips, green beans, carrots and cauliflower bound bile acids similarly and significantly lower than asparagus. Cholestyramine bound 92% of the bile acids. These values are similar to previously reported observations (Kahlon & Chow, 2000). Cholestyramine bound glycocholate and taurocholate 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.

Table 1
Composition of okra (*Abelmoschus esculentus*), beets (*Beta vulgaris*), asparagus (*Asparagus officinalis*), eggplant (*Solanum malongena*), turnips (*Brassica rapa rapifera*), green beans (*Phaseolus vulgaris*), carrots (*Daucus carota*), and cauliflower (*Brassica oleracea botrytis*), dry matter (DM) basis

Sample	DM %				
	Total dietary fibre[a]	Protein[b]	Fat[b]	Ash[b]	carbohydrate[c]
Okra	46.3	18.7	0.8	9.0	71.5
Beets	20.7	19.3	0.2	8.2	72.3
Asparagus	28.0	33.8	1.7	9.4	55.1
Eggplant	36.9	15.5	0.9	7.9	75.7
Turnips	37.1	21.2	0.4	12.5	65.9
Green beans	29.6	19.1	1.2	7.4	72.3
Carrots	22.7	5.4	1.6	7.5	85.5
Cauliflower	27.1	26.9	2.0	10.3	60.8
Cholestyramine	100.0	–	–	–	–
Cellulose	100.0	–	–	–	–

[a] $n = 6$.
[b] $n = 3$.
[c] carbohydrates = [100 − (protein + crude fat + ash)].

Table 2
In vitro bile acid binding by okra (*Abelmoschus esculentus*), beets (*Beta vulgaris*), asparagus (*Asparagus officinalis*), eggplant (*Solanum malongena*), turnips (*Brassica rapa rapifera*), green beans (*Phaseolus vulgaris*), carrots (*Daucus carota*), and cauliflower (*Brassica oleracea botrytis*) on equal weight, dry matter (DM) basis[A,B]

Sample	Bile acid binding (μmol/100 mg DM)	Bile acid binding relative to cholestyramine, %
Okra	1.61 ± 0.04^{b}	15.9 ± 0.4^{b}
Beets	1.13 ± 0.02^{c}	11.2 ± 0.2^{c}
Asparagus	0.38 ± 0.02^{d}	3.7 ± 0.2^{d}
Eggplant	0.14 ± 0.01^{e}	1.3 ± 0.1^{e}
Turnips	0.10 ± 0.03^{e}	1.0 ± 0.3^{e}
Green beans	0.10 ± 0.03^{e}	1.0 ± 0.3^{e}
Carrots	0.07 ± 0.02^{e}	0.7 ± 0.2^{e}
Cauliflower	0.07 ± 0.01^{e}	0.6 ± 0.1^{e}
Cholestyramine	10.14 ± 0.09^{a}	100.0 ± 0.9^{a}
Cellulose	0.18 ± 0.02^{e}	1.8 ± 0.2^{e}

[A] Mean ± SEM within a column with different superscript letters differ significantly ($P \leqslant 0.05$), $n = 6$.
[B] The dry matter used for incubation was all the vegetables was 97–101 mg, cholestyramine and cellulose 24–26 mg.

Assigning a bile acid binding value of 100% to cholestyramine, the relative bile acid binding on dry matter basis for the test samples was okra 16%, beets 11%, asparagus 4%, and 1% by eggplant, turnips, green beans, carrots and cauliflower. Bile acid binding for okra, beets and asparagus were significantly different from each other and their values were significantly higher than eggplant, turnips, green beans, carrots and cauliflower. Relative bile acid binding on dry matter basis was okra > beets > asparagus > eggplant = turnips = green beans = carrots = cauliflower. The differences in bile acid binding between various vegetables tested may relate to their phytonutrients (antioxidants, hydroxycinnamic acid, glucosinolates, isothiocyanates, flavonoids, chlorophyll, tannins, and micro-elements), hyrophobicity or active binding sites.

Previously reported relative bile acid binding for green leafy vegetables on dry matter basis was spinach 9%, kale 8%, brussels sprouts 8%, broccoli 5%, mustard greens 4%, green bell peppers 3%, cabbage 2% and collards 2% (Kahlon et al., 2007). The bile acid binding for okra

(16%) and beets (11%) observed herein suggest that these vegetables may be more healthful than spinach, kale, brussel sprouts, broccoli, mustard greens and collards. Bile acid binding for asparagus (4%) observed herein was similar to that previously reported for broccoli (5%) and mustard greens (4%) and those for eggplant, turnips, green beans, carrots and cauliflower were similar to that reported for cabbage and collards (Kahlon et al., 2007).

On dry matter basis, bile acid binding of okra and beets is very encouraging, which could be an indicator of their significantly higher healthful potential than asparagus, eggplant, turnips, green beans, carrots and cauliflower. Bile acid binding on dry matter basis has been reported in ready-to-eat cereals, cereal fractions, dried beans and fresh raw vegetables (Kahlon & Woodruff, 2003a, 2003b; Kahlon & Shao, 2004; Kahlon et al., 2005; Kahlon et al., 2007). Binding bile acids and preventing their recirculation results in reduced fat absorption, excretion of cancer-causing toxic metabolites and cholesterol utilisation to synthesise more bile acids. This is believed to be the mechanism by which food fractions lower cholesterol and prevent cancer. Evaluating healthful properties of various other vegetables and food fractions would be desirable, by testing their bile acid binding on dry matter basis.

The bile acid binding on equal total dietary fibre (TDF) basis is shown in Table 3. Cholestyramine bound bile acids to a significantly higher degree than the various fresh colourful vegetables tested. On TDF basis, considering cholestyramine as 100% bound, bile acid binding values were okra 34%, beets 54%, asparagus 13%, eggplant 4%, turnips 3%, green beans 3%, carrots 3%, and cauliflower 2%. The bile acid binding for beets was significantly higher than for the other vegetables tested herein. Bile acid binding for asparagus was significantly lower that that for okra and significantly higher than eggplant, turnips, green beans, carrots and cauliflower. Bile acid binding values on TDF basis among the various vegetables tested were beets > okra > asparagus > eggplant = turnips = green beans = carrots = cauliflower. Higher bile acid binding for okra and beets on TDF and DM basis observed herein suggest that these vegetables may be more health-promoting than spinach, Brussels sprouts, broccoli and mustard greens. The value for bile acid binding was higher for okra than beets on equal dry matter basis but lower on a TDF basis due to 44% lower dietary fibre in beets than okra. More than twenty-fold variability in the bile acid binding among various green vegetables tested indicated that bile acid binding is not related to the TDF content, in agreement with previous reports (Kahlon & Shao, 2004; Kahlon et al., 2005; Kahlon et al., 2007). The variability in the bile acid binding of colourful fresh raw vegetables may relate to their phytonutrients (antioxidants, hydroxycinnamic acid, glucosinolates, isothiocyanates, flavonoids, chlorophyll, tannins, and micro-elements) hyrophobicity, anionic, cationic, physical and chemical structure or active binding sites.

Table 3
In vitro bile acid binding by okra (*Abelmoschus esculentus*), beets (*Beta vulgaris*), asparagus (Asparagus officinalis), eggplant (*Solanum malongena*), turnips (*Brassica rapa rapifera*), green beans (*Phaseolus vulgaris*), carrots (*Daucus carota*), and cauliflower (*Brassica oleracea botrytis*) on equal total dietary fibre (TDF) basis [A,B]

Sample	Bile acid binding (μmol/100 mg TDF)	Bile acid binding relative to cholestyramine, %
Okra	3.47 ± 0.08^c	34.2 ± 0.8^c
Beets	5.45 ± 0.10^b	53.8 ± 1.0^b
Asparagus	1.35 ± 0.08^d	13.4 ± 0.8^d
Eggplant	0.37 ± 0.02^e	3.6 ± 0.2^e
Turnips	0.28 ± 0.08^e	2.8 ± 0.8^e
Green beans	0.34 ± 0.10^e	3.4 ± 1.0^e
Carrots	0.31 ± 0.10^e	3.1 ± 1.0^e
Cauliflower	0.24 ± 0.05^e	2.4 ± 0.5^e
Cholestyramine	10.14 ± 0.09^a	100.0 ± 0.9^a
Cellulose	0.18 ± 0.02^e	1.8 ± 0.2^e

[A] Mean ± SEM within a column with different superscript letters differ significantly ($P \leqslant 0.05$), $n = 6$.
[B] The TDF (mg) used for incubation was okra 46, beets 21, asparagus 28, eggplant 37, turnips 37, green beans 30, carrots 23, cauliflower 27, cholestyramine 26, and cellulose 24 mg.

The chemical composition of TDF of various vegetables tested was not found by literature search nor was it determined; therefore, it is not possible to speculate that variability in binding bile acids may relate to the differences in polysaccharides present in their dietary fibre fractions. Since vegetables were tested as uncooked, cooking may alter binding sites or fibre molecules. Such effects should be explored to evaluate the healthful potential of various vegetables.

In conclusion, relative to cholestyramine, the *in vitro* bile acid binding on an equal DM and TDF basis were for okra 16% and 34%, beets 11% and 54%, asparagus 4% and 13%, eggplant 1% and 4%, turnips 1% and 3%, green beans 1% and 3%, carrots 1% and 3%, and cauliflower 1% and 2%. On dry matter basis bile acid binding was okra > beets > asparagus > eggplant = turnips = beans green = carrots = cauliflower. Relative bile acid binding on DM basis may indicate their relative health-promoting potential. The differences in bile acid binding between the fresh colourful vegetables tested may relate to the variability in their phytonutrients (antioxidants, hydroxycinnamic acid, glucosinolates, isothiocyanates, flavonoids, polyphenols, and micro-elements), non-protein composition, structure, hydrophobicity of undigested fractions, anionic or cationic nature of the metabolites produced during digestion or their interaction with active binding sites. Studies are planned to evaluate how cooking methods impact the bile acid binding of vegetables, and also animal studies to relate *in vitro* bile acid binding of the vegetables observed herein to their healthful potential in atherosclerosis amelioration (lowering lipids and lipoprotein) and cancer prevention (excretion of toxic metabolites).

References

Ames, B. N., Shigenaga, M. K., & Hagen, T. M. (1993). Oxidants, antioxidants, and the degenerative diseases of aging. *Proceedings of the*

National Academic Science of the United States of America, 90, 7915–7922.

Anderson, J. W., & Siesel, A. E. (1990). Hypocholesterolemic effects of oat products. In I. Furda & C. J. Brine (Eds.), *New developments in dietary fiber: Physiological, physiochemical, and analytical aspects* (pp. 17–36). New York: Plenum Press.

AOAC. (1990). *Official methods of analysis of the association of official analytical chemists* (15th ed.). Arlington, VA: The Association.

AOAC. (2005). *Official methods of analysis of the association of official analytical chemists* (18th ed.). Arlington, VA: The Association.

Balmer, J., & Zilversmit, D. B. (1974). Effect of dietary roughage on cholesterol absorption, cholesterol turnover and steroid excretion in the rat. *Journal of Nutrition, 104*, 1319–1328.

Berrios, J. D. J., Camara, M., Torija, M. E., & Alonso, M. (2002). Effect of extrusion cooking and sodium carbonate addition on the carbohydrate composition of black bean flours. *Journal of Food Processing and Preservation, 26*, 113–128.

Camire, M. E., Zhao, J., & Violette, D. A. (1993). *In vitro* binding of bile acids by extruded potato peels. *Journal of Agricultural and Food Chemistry, 41*, 2391–2394.

Carey, M. C., & Small, D. M. (1970). The characteristics of mixed micellar solutions with particular reference to bile. *American Journal of Medicine, 49*, 590–608.

Cardador-Martinez, A., Loarca-Pina, G., & Oomah, B. D. (2002). Antioxidant activity in common beans (*Phaseolus vulgaris* L.). *Journal of Agricultural and Food Chemistry, 50*, 6975–6980.

Costarelli, V., Key, T. J., Appleby, P. N., Allen, D. S., Fentiman, I. S., & Sanders, T. A. (2002). A prospective study of serum bile acid concentrations and colorectal cancer risk in post-menopausal women on the island of Guernsey. *British Journal of Cancer, 86*, 1741–1744.

Daggy, B. P., O'Connell, N. C., Jerdack, G. R., Stinson, B. A., & Setchell, K. D. (1997). Additive hypocholesterolemic effect of psyllium and cholestyramine in the hamster: Influence on fecal sterol and bile acid profiles. *Journal of Lipid Research, 38*, 491–502.

Diwanay, S., Chitre, D., & Patwardhan, B. (2004). Immunoprotection by botanical drugs in cancer chemotherapy. *Journal of Ethnopharmacology, 90*, 49–55.

Eastwood, M. A., & Hamilton, D. (1968). Studies on the adsorption of bile acids to non-absorbed components of diet. *Biochimica et Biophysica Acta, 152*, 165–173.

Gautam, M., Diwanay, S., Gairola, S., Shinde, Y., Patki, P., & Patwardhan, B. (2004). Immunoadjuvant potential of *Asparagus racemosus* aqueous extract in experimental system. *Journal of Ethnopharmacology, 91*, 251–255.

Gibson, G. R. (1998). Dietary modulation of the human gut microflora using prebiotics. *British Journal of Nutrition, 80*, S209–S212.

Hecht, S. S. (1999). Chemoprevention of cancer by isothiocyanates, modifiers of carcinogen metabolism. *Journal of Nutrition, 129*, 768S–774S.

Hofmann, A. F. (1977). The enterohepatic circulation of bile acids in man. *Clinics in Gastroenterology, 6*, 3–24.

Jiratanan, T., & Liu, R. H. (2004). Antioxidant activity of processed table beets (*Beta vulgaris var*, *conditiva*) and green beans (*Phaseolus vulgaris* L.). *Journal of Agricultural and Food Chemistry, 52*, 2659–2670.

Kahlon, T. S., & Chow, F. I. (2000). *In vitro* binding of bile acids by rice bran, oat bran, wheat bran and corn bran. *Cereal Chemistry, 77*, 518–521.

Kahlon, T. S., & Woodruff, C. L. (2003a). *In vitro* binding of bile acids by rice bran, oat bran, barley and β-glucan enriched barley. *Cereal Chemistry, 80*, 260–263.

Kahlon, T. S., & Woodruff, C. L. (2003b). *In vitro* binding of bile acids by various ready to eat breakfast cereals. *Cereal Foods World, 48*, 73–75.

Kahlon, T. S., & Shao, Q. (2004). *In vitro* binding of bile acids by soy bean (*Glycine max*), black eye bean (*Vigna unguiculata*), garbanzo (*Cicer arietinum*) and lima bean (*Phaseolus lunatus*). *Food Chemistry, 86*, 435–440.

Kahlon, T. S., Smith, G. E., & Shao, Q. (2005). *In vitro* binding of bile acids by kidney bean (*Phaseolus vulgaris*), black gram (*Vigna mungo*), bengal gram (*Cicer arietinum*) and moth bean (*Phaseolus aconitifolins*). *Food Chemistry, 90*, 241–246.

Kahlon, T. S., Chapman, M. H., & Smith, G. E. (2007). In vitro binding of bile acids by spinach, kale, brussels sprouts, broccoli, mustard greens, green bell peppers, cabbage and collards. *Food Chemistry, 100*, 1531–1536.

Kritchevsky, D., & Story, J. A. (1974). Binding of bile salts *in vitro* by nonnutritive fiber. *Journal of Nutrition, 104*, 458–462.

Kushad, M. M., Brown, A. F., Kurilich, A. C., Juvik, J. A., Klein, B. P., Wallig, M. A., et al. (1999). Variation of glucosinolates in vegetable crops of Brassica oleracea. *Journal of Agricultural and Food Chemistry, 47*, 1541–1548.

Lund, E. K., Gee, J. M., Brown, J. C., Wood, P. J., & Johnson, I. T. (1989). Effect of oat gum on the physical properties of the gastrointestinal contents and on the uptake of D-galactose and cholesterol by rat small intestine *in vitro*. *British Journal of Nutrition, 62*, 91–101.

Nakamura, T., & Matsuzawa, Y. (1994). Drug treatment of hyperlipoproteinemia: bile acid-binding resins. *Nippon Rinsho, 52*, 3266–3270.

Potter, S. M. (1998). Soy protein and cardiovascular disease: The impact of bioactive components in soy. *Nutrition Reviews, 56*, 231–235.

Rossi, S. S., Converse, J. L., & Hoffman, A. F. (1987). High pressure liquid chromatography analysis of conjugated bile acids in human bile: Simultaneous resolution of sulfated and unsulfated lithocholyl amidates and the common conjugated bile acids. *Journal of Lipid Research, 28*, 589–595.

Story, J. A., & Kritchevsky, D. (1976). Comparisons of binding of various bile acids and bile salts *in vitro* by several types of fiber. *Journal of Nutrition, 106*, 1292–1294.

Suckling, K. E., Benson, G. M., Bond, B., Gee, A., Glen, A., Haynes, C., et al. (1991). Cholesterol lowering and bile acid excretion in the hamster with cholestyramine treatment. *Atherosclerosis, 89*, 183–190.

Sugano, M., & Goto, S. (1990). Steroid binding peptides from dietary proteins. *Journal of Nutrition Science and Vitaminology, 36*, S147–S150.

Tian, Q., Rosselot, R.A., Schwartz, S.J. (2005). Quantitative determination of intact glucosinolates in broccoli, broccoli sprouts, Brussels sprouts, and cauliflower by high-performance liquid chromatography-electrospray ionization-tandem mass spectrometry. *Analytical Biochemistry* 2005; Jun 15. [Epub ahead of print].

Trowell, H. C. (1975). Refined foods and disease. In D. P. Burkitt & H. C. Trowell (Eds.) (pp. 195–226). London: Academic Press.

USDA, Food and Nutrition Information Center. (2005). Food Guide Pyramid. Steps to a Healthier You. (http://www.mypyramid.gov).

Whitaker, B. D., & Stommel, J. R. (2003). Distribution of hydroxycinnamic acid conjugates in fruit of commercial eggplant (*Solanum melongena* L.) cultivars. *Journal of Agricultural and Food Chemistry, 51*, 3448–3454.