Available online at www.sciencedirect.com
ScienceDirect
Nutrition Research 28 (2008) 351 – 357
Nutrition Research
www.nrjournal.com

Steam cooking significantly improves in vitro bile acid binding of collard greens, kale, mustard greens, broccoli, green bell pepper, and cabbage☆

Talwinder Singh Kahlon*, Mei-Chen M. Chiu, Mary H. Chapman

Western Regional Research Center, USDA-ARS, Albany, CA 94710, USA

Received 2 October 2007; revised 25 February 2008; accepted 11 March 2008

Abstract

Bile acid binding capacity has been related to the cholesterol-lowering potential of foods and food fractions. Lowered recirculation of bile acids results in utilization of cholesterol to synthesize bile acid and reduced fat absorption. Secondary bile acids have been associated with increased risk of cancer. Bile acid binding potential has been related to lowering the risk of heart disease and that of cancer. Previously, we have reported bile acid binding by several uncooked vegetables. However, most vegetables are consumed after cooking. How cooking would influence in vitro bile acid binding of various vegetables was investigated using a mixture of bile acids secreted in human bile under physiological conditions. Eight replicate incubations were conducted for each treatment simulating gastric and intestinal digestion, which included a substrate only, a bile acid mixture only, and 6 with substrate and bile acid mixture. Cholestyramine (a cholesterol-lowering, bile acid binding drug) was the positive control treatment and cellulose was the negative control. Relative to cholestyramine, in vitro bile acid binding on dry matter basis was for the collard greens, kale, and mustard greens, 13%; broccoli, 10%; Brussels sprouts and spinach, 8%; green bell pepper, 7%; and cabbage, 5%. These results point to the significantly different ($P \leq .05$) health-promoting potential of collard greens = kale = mustard greens > broccoli > Brussels sprouts = spinach = green bell pepper > cabbage as indicated by their bile acid binding on dry matter basis. Steam cooking significantly improved the in vitro bile acid binding of collard greens, kale, mustard greens, broccoli, green bell pepper, and cabbage compared with previously observed bile acid binding values for these vegetables raw (uncooked). Inclusion of steam-cooked collard greens, kale, mustard greens, broccoli, green bell pepper, and cabbage in our daily diet as health-promoting vegetables should be emphasized. These green/leafy vegetables, when consumed regularly after steam cooking, would lower the risk of cardiovascular disease and cancer, advance human nutrition research, and improve public health.
Published by Elsevier Inc.

Keywords: In vitro; Bile acid binding; Collard greens; Kale; Mustard greens; Broccoli; Brussels sprouts; Spinach; Green bell pepper; Cabbage

Abbreviations: DM, dry matter; TDF, total dietary fiber; USDA, US Department of Agriculture.

1. Introduction

Vegetarians or those consuming vegetables as a major portion of their daily diet along with lower calories from saturated fat and animal products are at a lower risk of coronary heart disease and cancer. Vegetables are a good source of dietary fiber, antioxidants, phytonutrients, provitamins, polyphenols, and minerals. The US Department of

☆ The mention of firm names or trade products does not imply that they are endorsed or recommended by the US Department of Agriculture over other firms or similar products not mentioned.

* Corresponding author. Tel.: +1 510 559 5665; fax: +1 510 559 5777.
E-mail address: talwinder.kahlon@ars.usda.gov (T.S. Kahlon).

0271-5317/$ – see front matter. Published by Elsevier Inc.
doi:10.1016/j.nutres.2008.03.007

Agriculture (USDA) Food and Nutrition Information Center, through the Food Guide Pyramid—Steps to a Healthier You (http://www.mypyramid.gov), recommends the consumption of dark leafy and colorful vegetables and low-fat food products along with daily active life and maintaining desirable body weight [1]. Some of the vegetables listed by the USDA food pyramid include collard greens, kale, mustard greens, broccoli, and spinach. Isothiocyanates of the cruciferous vegetables in the *Brassica* family have been shown to protect against various types of cancers [2-5]. Sulforaphane, indole-3-carbinol, glucaric acid, and other isothiocyanates are antioxidants and potent stimulators of natural detoxifying enzymes in the body. These compounds are believed to be responsible for the lowered risk of atherosclerosis and cancer [6,7]. Toxic metabolites in the gut and secondary bile acids increase the risk of colorectal cancer [8]. Atherosclerosis and cancer are 2 leading causes of death and disability in the developed world and are increasing rapidly in the developing world. These are the major human nutrition problems and are preventable with diet and physically active lifestyle. 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 [9-12]. Bile acids are acidic steroids synthesized in the liver from cholesterol. After conjugation with glycine or taurine, bile acids are secreted into the duodenum. Bile acids are actively reabsorbed by the terminal ileum and undergo an enterohepatic circulation [13]. Binding of bile acids and increasing their fecal excretion have been hypothesized as a possible mechanism by which food fractions lower cholesterol [14-16]. The bile acids are needed for the absorption of dietary fat from the gastrointestinal tract. The dietary fat is a precursor of cholesterol synthesis in the body. By binding bile acids, food fractions prevent their reabsorption and stimulate plasma and liver cholesterol conversion to additional bile acids [17-20]. Excretion of toxic metabolites and secondary bile acids could lower the risk of cancer [8]. The bile acid binding of grain fractions, ready-to-eat cereals, and various dry beans has been observed to be proportional to their dry matter (DM) content [21-24]. Steam cooking beets, eggplants, asparagus, carrots, green beans, and cauliflowers significantly improved in vitro bile acid binding compared with the values obtained for these vegetables uncooked [25]. In vitro bile acid binding of the following vegetables has been reported: uncooked spinach, 9%; kale, 8%; Brussels sprouts, 8%; broccoli, 5%; mustard greens, 4%; green bell peppers, 3%; cabbage, 2%; and collard greens, 2% [26]. Vegetables are normally cooked before their consumption; how cooking would influence bile acid binding of these green/leafy vegetables is the subject of this report.

The objective of this study was to evaluate the healthful potential of steam-cooked collard greens (*Brassica oleracea acephala*), kale (*Brassica oleracea acephala*), mustard greens (*Brassica juncea*), broccoli (*Brassica oleracea italica*), Brussels sprouts (*Brassica oleracea gemmifera*), spinach (*Spinacia oleracea*), green bell pepper (*Capsicum annuum*), and cabbage (*Brassica oleracea capitala*) as determined by their bile acid binding on equal DM basis, with a bile acid mixture under human duodenal physiological pH of 6.3.

2. Methods and materials

Fresh collard greens, kale, mustard greens, broccoli, Brussels sprouts, spinach, green bell pepper, and cabbage were obtained from a local grocery supermarket. All the vegetables were washed, chopped to size (Table 1), and stream cooked in a double boiler (Duralon nonstick 2-qt, 6-in diameter pot with 2-qt stainless steam insert with a lid; Meyer, Vallejo, CA) (any 3-piece steamer would be adequate) to the tenderness as ready to be eaten. The steam cooking time was mustard greens, 20 minutes; kale and collard greens, 15 minutes; and broccoli, Brussels sprouts, spinach, green bell pepper, and cabbage, 10 minutes (Table 1). All the cooked vegetables were dried to a constant weight at 68°C for 48 hours in a steam-heated forced-air food dryer (Proctor 062; Proctor & Schwartz, Inc, Horsham, Pa). Dry samples were ground in a Thomas-Wiley Mini mill (Arthur Thomas, Philadelphia, Pa) to pass a 0.4-mm screen. Total dietary fiber (TDF) of the test samples was determined by method 985.29 [27]. A brief description of the TDF method is as follows: Duplicate test portions of dried foods, fat extracted if containing more than 10% fat, were gelatinized with Termarnyl (heat-stable α-amylase; Megazyme International, Wicklow, Ireland), and then enzymatically digested with protease and amyloglucosidase to remove protein and starch. Four volumes of ethyl alcohol were added to

Table 1
DM content of steam-cooked collard greens (*B oleracea acephala*), kale (*B oleracea acephala*), mustard greens (*B juncea*), broccoli (*B oleracea italica*), Brussels sprouts (*B oleracea gemmifera*), spinach (*S oleracea*), green bell pepper (*C annuum*), and cabbage (*B oleracea capitala*)

Source	Cut to size [a]	Steamed (min)	DM (%) [b]
Collard greens	0.75 to 1.0 in crosswise	15	8.53
Kale	0.75 to 1.0 in crosswise	15	9.80
Mustard greens	0.75 to 1.0 in crosswise	20	5.40
Broccoli	1.0- to 2.0-in florets	10	8.29
Brussels sprouts	Cut in half	10	12.91
Spinach	0.75 to 1.0 in crosswise	10	6.55
Green bell pepper	0.5-in slices	10	6.68
Cabbage	1.0- to 1.5-in pieces	10	8.45

[a] 600 to 800 g of fresh vegetables was washed, trimmed and sliced, and steamed in a double boiler; DM was determined after drying at 68°C for 48 hours in a food dehydrator.

[b] n = 3.

precipitate soluble dietary fiber. Total residue was filtered and washed successively with 78% ethyl alcohol, 95% ethyl alcohol, and acetone. After drying, residue was weighed. One duplicate was analyzed for protein, and another was incinerated at 525°C and ash was determined. TDF = weight residue − weight (protein + ash).

Samples were analyzed for nitrogen by method 990.03 [28], with a Virio Macro Elemental Analyser (Elementar Analysensysteme GmbH, Hanau, Germany); crude fat with petroleum ether by accelerated solvent extractor (ASE 200; Dionex Corp, Sunnyvale, Ca); ash by method 942.05 [28]; and moisture by method 935.29 [28]. Cholestyramine, a bile acid binding anionic resin (a drug that lowers cholesterol and binding bile acids), was the positive control treatment, and cellulose (a non–bile acid binding fiber) was the negative control. Both were obtained from Sigma (St Louis, Mo).

2.1. Bile acid binding procedure

In vitro bile acid binding procedure was a modification of that by Camire et al [29], as previously reported [22]. The stock bile acid mixture was formulated, with glycocholic bile acids providing 75% and taurine-conjugated bile acids providing 25% of the bile acids, based on the composition of the human bile [30,31]. 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 before each assay. Eight replicate incubations—6 substrates with bile acid mixture, 1 substrate blank without bile acid mixture, and 1 bile acid mixture without substrate—were run for each of the 8 vegetables and 2 control treatments. Each treatment replicate was weighed into 16 × 150–mm glass, screw-capped tube. Samples were digested in 1 mL 0.01 N hydrochloric acid (HCl) for 1 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 sodium hydroxide (NaOH). To each test sample, 4 mL of bile acid mixture working solution was added. 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 the digestion of samples), tubes were incubated for 1 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 minutes at 25°C in an ultracentrifuge (model L-60; Beckman, Palo Alto, Calif). The supernatant was removed into a second set of labeled 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 analyzed by the Trinity Biotech bile acids procedure no. 450-A (Trinity Biotech Distribution, St Louis, MO) using a Ciba-Corning Express Plus analyzer (Polestar Labs, Inc, Escondido, Calif). 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 blank substrates were subtracted, and bile acid concentrations were corrected based on the mean recoveries of bile acid mixture (positive blank).

2.2. Statistical analysis

Data are presented as means ± SEM. Before accepting analysis of variance results, Lavene test was used to check for homogeneity of variances among treatments. Because variances were considered homogenous from test results, analysis of variance was used to test for significant differences among treatments. Dunnett 1-tailed test was computed with treatments compared to cholestyramine as a positive control and compared to cellulose as a negative control. For comparing noncontrol treatments to each other, Tukey test for comparison of all possible pairs of means was used. SAS Proc GLM (SAS Institute, Carey, NC) was used for all statistical analysis and testing [32,33]. A value of $P \leq .05$ was considered the criterion of significance.

3. Results

The bite-size cut pieces of green/leafy vegetables, steam cooking time, and percentage DM as ready to be eaten are given in Table 1. Dry matter content of steam cooked was highest for Brussels sprouts (13%) and lowest for mustard greens (5%). The composition of the collard greens, kale, mustard greens, broccoli, Brussels sprouts, spinach, green bell pepper, and cabbage is given in Table 2. Both cellulose and cholestyramine were considered as 100% TDF. Total dietary fiber and protein values on DM basis for the steam-cooked vegetables were collard greens, 29 and 32%; kale, 31 and 28%; mustard greens, 38 and 30%; broccoli, 29 and 32%; Brussels sprouts, 29 and 26%; spinach, 25 and 42%; green bell pepper, 33 and 17%; and cabbage, 27 and 23%, respectively. On DM basis, fat and mineral content in the cooked vegetables tested was 1% to 4% and 7% to 18%, respectively. The values for raw vegetables obtained from the same grocery store are shown in parenthesis [26].

On an equal DM basis, bile acid binding was significantly higher for cholestyramine and significantly lower for cellulose than for all the steam-cooked vegetables tested (Table 3). The binding values were similar for collard greens, kale, and mustard green and significantly ($P \leq .05$) higher than for broccoli, Brussels sprouts, spinach, green bell pepper, and cabbage. Values were similar for Brussels sprouts and spinach and those of spinach and green bell pepper, and these values were significantly lower than that for broccoli and significantly higher than that for cabbage. Assigning a

Table 2
Composition of steam-cooked and raw[a] collard greens (*B oleracea acephala*), kale (*B oleracea acephala*), mustard greens (*B juncea*), broccoli (*B oleracea italica*), Brussels sprouts (*B oleracea gemmifera*), spinach (*S oleracea*), green bell pepper (*C annuum*), and cabbage (*B oleracea capitala*), DM basis

Source	TDF[b]	Protein[c]	Fat[c]	Minerals[c]	Carbohydrate[d]
	DM (%)				
Collard greens	28.7 (36.3)	31.6 (21.3)	3.1 (5.8)	12.3 (16.3)	53.0 (56.6)
Kale	30.7 (36.8)	27.5 (33.8)	4.0 (11.8)	11.8 (15.8)	56.7 (38.6)
Mustard greens	37.6 (34.0)	29.5 (40.9)	1.6 (3.9)	10.7 (16.7)	58.2 (38.5)
Broccoli	29.1 (31.1)	31.8 (33.2)	2.8 (5.4)	8.9 (10.3)	56.5 (51.2)
Brussels sprouts	29.0 (34.1)	25.7 (29.0)	1.2 (4.1)	6.6 (9.5)	66.5 (57.4)
Spinach	25.2 (27.1)	42.3 (38.2)	3.3 (4.6)	17.5 (28.1)	36.9 (29.0)
Green bell pepper	32.7 (26.5)	16.6 (16.9)	2.9 (3.7)	8.0 (10.5)	72.5 (68.9)
Cabbage	27.3 (29.9)	22.8 (23.1)	0.9 (4.4)	8.4 (10.5)	67.9 (62.0)
Cholestyramine	100.0	–	–	–	–
Cellulose	100.0	–	–	–	–

The values for raw vegetables are in parentheses.
[a] Data from Kahlon et al [26]. Raw vegetables were obtained from the same local grocery supermarket.
[b] n = 6.
[c] n = 3.
[d] Carbohydrates = [100 − (protein + crude fat + ash)].

bile acid binding value of 100% to cholestyramine, the relative bile acid binding on DM basis for the test samples of steam-cooked vegetables was collard greens, kale, and mustard greens, 13%; broccoli, 10%, Brussels sprouts and spinach, 8%; green bell pepper, 7%; and cabbage, 5%. Relative bile acid binding on DM basis was collard greens = kale = mustard greens > broccoli > Brussels sprouts = spinach = green bell pepper > cabbage.

The bile acid binding on equal TDF basis is shown in Table 4. Cholestyramine bound bile acids significantly higher and cellulose bound bile acids significantly lower than did the various steam-cooked vegetables tested. On TDF basis considering cholestyramine as 100% bound, bile acid binding values were collard greens, 46%; kale, 43%; mustard greens, 34%; broccoli, 33%; Brussels sprouts, 27%; spinach, 31%; green bell pepper, 21%; and cabbage, 18%. Bile acid binding values on TDF basis among various steam-cooked vegetables tested were collard greens > kale > mustard greens = broccoli > spinach > Brussels sprouts > green bell pepper > cabbage. Bile acid binding values for collard greens were significantly higher and those for cabbage were significantly lower than those for all the other cooked vegetables tested. Except for mustard green and broccoli, where values were similar, there were significant differences in the bile acid binding values of all the cooked green/leafy vegetables tested.

The bile acid binding calculated for steam-cooked green/leafy vegetables as ready to be eaten is given in Table 5. Bile

Table 3
In vitro bile acid binding by steam-cooked collard greens (*B oleracea acephala*), kale (*B oleracea acephala*), mustard greens (*B juncea*), broccoli (*B oleracea italica*), Brussels sprouts (*B oleracea gemmifera*), spinach (*S oleracea*), green bell pepper (*C annuum*), and cabbage (*B oleracea capitala*), on equal weight, DM basis

Treatment	Bile acid binding (μmol/100 mg DM)	Bile acid binding relative to cholestyramine (%)
Collard greens	1.40 ± 0.0^{b}	13.1 ± 0.1^{b}
Kale	1.40 ± 0.01^{b}	13.1 ± 0.1^{b}
Mustard greens	1.36 ± 0.01^{b}	12.8 ± 0.1^{b}
Broccoli	1.02 ± 0.01^{c}	9.5 ± 0.1^{c}
Brussels sprouts	0.84 ± 0.01^{d}	7.9 ± 0.1^{d}
Spinach	$0.83 \pm 0.01^{d,e}$	$7.8 \pm 0.1^{d,e}$
Green bell pepper	0.72 ± 0.01^{e}	6.7 ± 0.1^{e}
Cabbage	0.51 ± 0.03^{f}	4.8 ± 0.2^{f}
Cholestyramine	10.69 ± 0.08^{a}	100.0 ± 0.7^{a}
Cellulose	0.08 ± 0.08^{g}	0.8 ± 0.7^{g}

Mean ± SEM within a column with different superscript letters (a to g) differ significantly ($P \leq .05$; n = 6). The DM used for incubation for all the vegetables was 100 to 102 mg; cholestyramine, 25 mg; and cellulose, 26 mg.

Table 4
In vitro bile acid binding by steam-cooked collard greens (*B oleracea acephala*), kale (*B oleracea acephala*), mustard greens (*B juncea*), broccoli (*B oleracea italica*), Brussels sprouts (*B oleracea gemmifera*), spinach (*S oleracea*), green bell pepper (*C annuum*), and cabbage (*B oleracea capitala*), on equal TDF basis

Treatment	Bile acid binding (μmol/100 mg TDF)	Bile acid binding relative to cholestyramine (%)
Collard greens	4.90 ± 0.03^{b}	45.8 ± 0.3^{b}
Kale	4.55 ± 0.02^{c}	42.6 ± 0.2^{c}
Mustard greens	3.62 ± 0.03^{d}	33.9 ± 0.3^{d}
Broccoli	3.50 ± 0.03^{d}	32.8 ± 0.3^{d}
Brussels sprouts	2.91 ± 0.04^{f}	27.2 ± 0.4^{f}
Spinach	3.31 ± 0.03^{e}	31.0 ± 0.3^{e}
Green bell pepper	2.20 ± 0.03^{g}	20.6 ± 0.3^{g}
Cabbage	1.87 ± 0.09^{h}	17.5 ± 0.9^{h}
Cholestyramine	10.69 ± 0.08^{a}	100.0 ± 0.7^{a}
Cellulose	0.08 ± 0.08^{i}	0.8 ± 0.7^{i}

Mean ± SEM within a column with different superscript letters (a to i) differ significantly ($P \leq .05$; n = 6). The TDF (mg) used for incubation was collard greens, 29; kale, 31; mustard greens, 38; broccoli, 29; Brussels sprouts, 29; spinach, 26; green bell pepper, 33; cabbage, 27; cholestyramine, 25; and cellulose, 26 mg.

Table 5
In vitro bile acid binding by steam-cooked collard greens (*B oleracea acephala*), kale (*B oleracea acephala*), mustard greens (*B juncea*), broccoli (*B oleracea italica*), Brussels sprouts (*B oleracea gemmifera*), spinach (*S oleracea*), green bell pepper (*C annuum*), and cabbage (*B oleracea capitala*), on 100 g steam cooked as-is basis

Treatment	Bile acid binding (μmol/100 g as is)	Calculated bile acid binding relative to cholestyramine (%) *
Collard greens	119.8 ± 0.8^b	11.5^b
Kale	136.7 ± 0.6^a	13.1^a
Mustard greens	73.5 ± 0.6^e	7.0^e
Broccoli	84.6 ± 0.8^d	8.1^d
Brussels sprouts	108.9 ± 1.6^c	10.4^c
Spinach	54.6 ± 0.5^f	5.2^f
Green bell pepper	48.1 ± 0.7^g	4.6^g
Cabbage	43.1 ± 2.2^h	4.1^h

Mean ± SEM within a column with different superscript letters (a to h) differ significantly ($P \leq .05$; n = 6). The DM used for incubation for all the vegetables was 100 to 102 mg.

* Calculated bile acid binding using binding data for kale as 13.1% binding relative to cholestyramine (Table 3), relative bile acid binding = (μmol/100 g as is)(13.1)/136.7.

acid binding was highest for kale (137 μmol/100 g as-eaten) and lowest for cabbage (43 μmol/100 g). Relative to cholestyramine, bile acid binding values were calculated based on values for kale as 13.1% (Table 3). There were significant differences in the bile acid binding values of the various green/leafy vegetables tested. Relative bile acid binding values as-eaten were kale > collard greens > Brussels sprouts > broccoli > mustard greens > spinach > green bell pepper > cabbage.

4. Discussion

Steam cooking resulted in loss of minerals and lipid in all the vegetables tested. Cooking resulted in an increase in TDF in mustard greens and green bell pepper, whereas it decreased in all the other vegetables tested. The protein content increased by cooking collard greens and spinach; however, values decreased in all the other vegetables tested. The variable changes in TDF and protein content may be due to the redistribution of relative proportions of nutrients. Comparison of raw vs steam-cooked vegetables suggested that there were losses in lipid and mineral contents. Similar loss in lipid and mineral content with steam cooking of beets, okra, eggplant, asparagus, carrots, green beans, cauliflower, and turnips has been previously reported [34].

Cholestyramine bound 93% of the bile acids. These values are similar to the previously reported observations [12,35]. Story and Kritchevsky [36] 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 ratio of substrate to bile acid. Previously reported bile acid binding by uncooked vegetables was spinach, 9%; kale and Brussels sprouts, 8%; broccoli, 5%; mustard greens, 4%; peppers green, 3%; and cabbage and collard greens, 2% [26]. Significantly higher bile acid binding by collard greens, kale, mustard greens, broccoli, green bell pepper, and cabbage by steam cooking compared with their uncooked binding values suggests that these vegetables are more healthful after steam cooking. The differences in bile acid binding between various vegetables tested may relate to their phytonutrients (antioxidants, chlorophyll, flavonoids, sulforaphane, indole-3-carbinol, glucaric acid and isothiocyanates, microelements, and tannins), hydrophobicity, or active binding sites. On DM basis, 5% to 13% relative bile acid binding by the steam-cooked green/leafy vegetables tested indicates their high healthful potential. Relative bile acid binding of 5% to 9% for oat bran, oat bran ready-to-eat cereals, and barley fractions (cereals with US Food and Drug Administration approval for label health claim for lowering cholesterol) have been reported [12,21,22]. How other cooking methods influence the bile acid binding of vegetables needs to be evaluated.

The bile acid binding of oat bran, whole barley flour and β-glucan enriched barley fractions [21], ready-to-eat cereals [22], and various dry beans [23,24] has also been related to their DM content. Evaluating the healthful properties (cholesterol lowering and excretion of toxic metabolites) of various vegetables and food fractions as they are normally consumed would be desirable by testing their bile acid binding on DM basis.

Previously, Kahlon et al [26] reported bile acid binding on TDF basis for uncooked spinach, 32%; kale, 22%; Brussels sprouts, 23%; broccoli, 15%; mustard greens, 13%; peppers green, 9%; cabbage, 8%; and collard greens, 6%. Cooking resulted in a significant increase in bile acid binding for collard greens, kale, mustard greens, broccoli, green bell pepper, and cabbage. The amount of TDF used per incubation for collard greens, broccoli, and Brussels sprouts were the same; however, their bile acid binding values were significantly different. These data suggest that bile acid binding was not related to the TDF content, in agreement with previous reports that bile acid binding of various dry beans was not related to the TDF content [23,24]. The significantly higher bile acid binding for all the cooked vegetables tested herein, except for spinach and Brussels sprouts, is very encouraging because these vegetables are normally consumed after cooking. Data suggest that steam-cooked vegetables are more healthful than those consumed raw (uncooked). The bile acid binding for kale for 100 g (as-eaten basis) was the highest of all the green/leafy vegetables. There were significant differences in binding values among all the vegetables tested. Highest DM (13%) was in the Brussels sprouts per 100 g as-eaten basis; however, its bile acid binding values were significantly lower those of than kale and collard greens. Data suggest that bile acid binding potential seems to be related to unique phytonutrients that would be determined in subsequent studies.

In conclusion, relative to cholestyramine, the in vitro bile acid binding on DM basis was the following: collard greens, kale, and mustard greens, 13%; broccoli, 10%; Brussels sprouts and spinach, 8%; green bell pepper, 7%; and cabbage, 5%. These results point to the significant differences in the health-promoting potential of steam-cooked collard greens = kale = mustard greens > broccoli > Brussels sprouts = spinach = green bell pepper > cabbage as indicated by their bile acid binding on DM basis. Compared with raw vegetables, steam cooking resulted in significantly higher bile acid binding by collard greens, kale, mustard greens, broccoli, green bell pepper, and cabbage. Data suggest that the healthful potential of these vegetables is significantly increased by steam cooking. Because bile acid binding was evaluated on DM obtained after drying cooked vegetables at 68°C for 48 hours to a constant weight, any effect on binding values due to prolonged drying time would be the subject of future investigations. A much larger-capacity (7- to 15-fold) in vitro incubation setup would be required to evaluate the bile acid binding of steam-cooked vegetables on as-is basis. Inclusion of steam-cooked collard greens, kale mustard greens, broccoli, green bell pepper, and cabbage in our daily diet as healthful vegetables should be encouraged. Atherosclerosis and cancer are the leading public health and nutrition research problems that are preventable by appropriate diet and lifestyle modifications. These green/leafy vegetables, when consumed regularly after steam cooking, would lower the risk of cardiovascular disease and cancer, advance human nutrition research, and improve public health.

Acknowledgment

The expert statistical assistance of Linda C. Whitehand, Consulting Statistician, WRRC, USDA-ARS, Albany, Ca, is greatly appreciated.

References

[1] USDA, Food and Nutrition Information Center. Food guide pyramid. Steps to a healthier you. http://www.mypyramid.gov, 2005.
[2] Steinmetz KA, Potter JD. Vegetables, fruits, and cancer prevention: a review. J Am Diet Assoc 1996;96:1027-39.
[3] Zhang Y, Talalay P, Cho CG, Posner GH. A major inducer of anticarcinogenic protective enzymes from broccoli: isolation and elucidation of structure. Proc Natl Acad Sci 1992;89:2399-403.
[4] Zhang Y, Kensler TW, Cho CG. Anticarcinogenic activities of sulforaphane and structurally related synthetic norbornyl isothiocyanates. Proc Natl Acad Sci 1994;91:3147-50.
[5] Fahey JW, Zhang Y, Talalay P. Broccoli sprouts: an exceptionally rich source of inducers of enzymes that protect against chemical carcinogens. Proc Natl Acad Sci 1997;94:10367-72.
[6] Ames BN, Shigenaga MK, Hagen TM. Oxidants, antioxidants, and the degenerative diseases of aging. Proc Natl Acad Sci 1993;90:7915-22.
[7] Hecht SS. Chemoprevention of cancer by isothiocyanates, modifiers of carcinogen metabolism. J Nutr 1999;129:768S-74S.
[8] Costarelli V, Key TJ, Appleby PN, Allen DS, Fentiman IS, Sanders TA. A prospective study of serum bile acid concentrations and colorectal cancer risk in post-menopausal women on the island of Guernsey. Br J Cancer 2002;86:1741-4.
[9] Suckling KE, Benson GM, Bond B, Gee A, Glen A, Haynes C, et al. Cholesterol lowering and bile acid excretion in the hamster with cholestyramine treatment. Atherosclerosis 1991;89:183-90.
[10] Nakamura T, Matsuzawa Y. Drug treatment of hyperlipoproteinemia: bile acid–binding resins. Nippon Rinsho 1994;52:3266-70.
[11] Daggy BP, O'Connell NC, Jerdack GR, Stinson BA, Setchell KD. Additive hypocholesterolemic effect of psyllium and cholestyramine in the hamster: influence on fecal sterol and bile acid profiles. J Lipid Res 1997;38:491-502.
[12] Kahlon TS, Chow FI. In vitro binding of bile acids by rice bran, oat bran, wheat bran and corn bran. Cereal Chem 2000;77:518-21.
[13] Hofmann AF. The enterohepatic circulation of bile acids in man. Clin Gastroenterol 1977;6:3-24.
[14] Trowell HC. In: Burkitt, Trowell, editors. Refined foods and disease. London: Academic Press; 1975. p. 195-226.
[15] Lund EK, Gee JM, Brown JC, Wood PJ, Johnson IT. 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. Br J Nutr 1989;62:91-101.
[16] Anderson JW, Siesel AE. Hypocholesterolemic effects of oat products. In: Furda I, Brine CJ, editors. New developments in dietary fiber: physiological, physiochemical, and analytical aspects. New York: Plenum Press; 1990. p. 17-36.
[17] Eastwood MA, Hamilton D. Studies on the adsorption of bile acids to non-absorbed components of diet. Biochim Biophys Acta 1968;152:165-73.
[18] Balmer J, Zilversmit DB. Effect of dietary roughage on cholesterol absorption, cholesterol turnover and steroid excretion in the rat. J Nutr 1974;104:1319-28.
[19] Kritchevsky D, Story JA. Binding of bile salts in vitro by nonnutritive fiber. J Nutr 1974;104:458-62.
[20] Potter SM. Soy protein and cardiovascular disease: the impact of bioactive components in soy. Nutr Rev 1998;56:231-5.
[21] Kahlon TS, Woodruff CL. In vitro binding of bile acids by rice bran, oat bran, barley and *b*-glucan enriched barley. Cereal Chem 2003;80:260-3.
[22] Kahlon TS, Woodruff CL. In vitro binding of bile acids by various ready to eat breakfast cereals. Cereal Foods World 2003;48:73-5.
[23] Kahlon TS, Shao Q. 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 Chem 2004;86:435-40.
[24] Kahlon TS, Smith GE, Shao Q. 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 Chem 2005;90:241-6.
[25] Kahlon TS, Chiu MM, Chapman M. Steam cooking significantly improves in vitro bile acid binding of beets, eggplant, asparagus, carrots, green beans and cauliflower. Nutr Res 2007;27:750-5.
[26] Kahlon TS, Chapaman MH, Smith GE. In vitro binding of bile acids by spinach, kale, Brussel sprouts, broccoli, mustard greens, green peppers, cabbage and collards. Food Chem 2007;100:1531-6.
[27] AOAC. Official methods of analysis of the Association of Official Analytical Chemists. 17th ed. Arlington, VA: The Association; 2000. p. 78E-80E [Chap. 45].
[28] AOAC. Official methods of analysis of the Association of Official Analytical Chemists. 15th ed. The Association: Arlington, VA. 1990, p. 70, 725, 1st Suppl. P 83.
[29] Camire ME, Zhao J, Violette DA. In vitro binding of bile acids by extruded potato peels. J Agric Food Chem 1993;41:2391-4.
[30] Carey MC, Small DM. The characteristics of mixed micellar solutions with particular reference to bile. Am J Med 1970;49:590-608.

[31] Rossi SS, Converse JL, Hoffman AF. 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. J Lipid Res 1987;28:589-95.
[32] Steel RGD, Torrie JH. Principles and procedures of statistics. New York: McGraw-Hill; 1960.
[33] SAS online Doc 9.1.3 Carey, NC; SAS institute Inc., 2004.
[34] Kahlon TS, Chiu MM, Chapman M. Steam cooking significantly improves in vitro bile acid binding of beets, eggplant, asparagus, carrots, green beans and cauliflower. Nutr Res 2007;27:750-5.
[35] Sugano M, Goto S. Steroid binding peptides from dietary proteins. J Nutr Vitaminol 1990;36:S147-50.
[36] Story JA, Kritchevsky D. Comparisons of binding of various bile acids and bile salts in vitro by several types of fiber. J Nutr 1976;106:1292-4.