

REVIEW ARTICLE

In Vitro Bile Acid Binding of Various Vegetables

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Abstract

Health promoting potential (cholesterol lowering and cancer risk reduction) of various vegetables could be determined by evaluating their bile acid binding capacity. Bile acid binding relative to cholestyramine of sixteen vegetables was evaluated in six studies. Various cooking methods used were (boiled, steamed, sautéed, microwave, microwave with water) or raw (uncooked). Steaming resulted in highest bile acid binding values, whereas boiling the lowest. The Steamed vegetable would retain most of their phytonutrients. By steaming, bile acid binding values were: Beets 18%, Okra 16%, Eggplant 14%, Asparagus, Collard Greens, Mustard Greens and Kale 10-13%, Cauliflower, Green Beans, Carrots, Spinach, Brussels Sprouts and Broccoli 6-8%, Green Bell Pepper and Cabbage 4% and Turnips only 1.2%. With prudent, low fat diet, steamed vegetables with 6-18% bile acid binding would be desired. Consuming these steamed vegetables would result in potential lower risk of premature degenerative diseases (heart disease and cancer) and improve public health.

Keywords: Bile acid binding, Vegetables, Steaming, Sautéing, Microwave, Boiling

Introduction:

Atherosclerosis and cancer are the two leading causes of death and disability in the developed world and are increasing rapidly in the developing world [1]. These are major human health problems that are preventable with low fat diet and physical activity. High cholesterol and sedentary lifestyle are major contributors to these health problems. Bile acids are acidic steroids synthesized in the liver from cholesterol. After conjugation with glycine or taurine, bile acids are secreted into the duodenum. Dietary fat is solubilized into micelles for its absorption by bile acids. Bile acids are actively reabsorbed by the terminal ileum and undergo an enterohepatic circulation [2]. Binding bile acids and increasing their fecal excretion reduces fat absorption resulting in lowering cholesterol. Excreting bile acids is the major route for removal of cholesterol from the body [3-6]. Secondary bile acids are known to be carcinogenic [7]. The

cholestyramine (bile acid-binding, cholesterol-lowering drug) binds bile acids and cellulose does not [8-11]. These observations demonstrated positive correlations between *in vivo* and *in vitro* studies. The healthful, cholesterol-lowering (atherosclerosis amelioration) potential of various vegetables could be predicted by evaluating their *in vitro* bile acid binding.

In vitro bile acid binding without the use of labeled isotopes is an economical method for screening various food fractions to evaluate their healthful potential. Some international plant breeding companies have been using *in vitro* bile acid binding procedure [12] in their seed selections to propagate more health promoting crops, in order to reduce costly animal and human studies.

1.1 Bile Acid Binding Procedure

Kritchevsky and Story [13] have described *in vitro* bile acid binding procedure where labeled isotopes of bile acids were used. The *in vitro* bile acid binding procedure that

does not use labeled isotopes eliminating the radiation hazards and disposal costs of the isotopes. The *in vitro* bile acid binding procedure has been established by Kahlon and Chow [12] and further fine-tuned by Kahlon and Woodruff [14, 15]. 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 [16, 17]. This stock solution 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. A stock solution of 36 mmol/L was stored in a freezer maintained at -20°C . Prior to each assay, working solution 0.72 or 2.88 $\mu\text{mol/mL}$ was prepared from the stock solution, for test samples or cholestyramine,

respectively. Cellulose, a non-bile acid binding fiber, was the negative control and cholestyramine, a bile acid binding anionic resin, was the positive control. Cholestyramine is a drug that lowers cholesterol by binding bile acids. Eight replicate incubations, consisting of six substrates with bile acid mixture, one substrate blank without bile acid mixture and one bile acid mixture without the substrate, were run for each treatment. Diagram of the bile acid binding procedure is given in **Figure 1**. Each supernatant sample was analyzed in triplicate for unbound bile acids. 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 $\mu\text{mol/L}$. Individual blank substrates were subtracted, and bile acid concentrations were corrected based on the mean recoveries of bile acid mixture (positive blank).

Substrate (100 mg) + 1 mL, 0.01 N HCl
Incubate 1 hr ↓ 37⁰C (shaker bath)
+0.1 mL, 0.1 N NaOH (neutralize)
+ 4 mL bile acid mixture (0.72 μmol/mL, for cholestyramine 2.88 μmol/mL)*
**+ 5 mL (5X, 10 mg/mL in 0.1M phosphate buffer, pH 6.3) porcine pancreatin (amylase,
protease & lipase)**
Incubate 1 hr ↓ 37⁰C (shaker bath)
Transfer contents to 10 mL centrifuge tubes
Centrifuge 99,000 g ↓ 18 min, 25⁰ C
Remove supernatant (1)
Rinse incubation tubes with 5 mL, pH 6.3 phosphate buffer
Centrifuge 99,000 g ↓ 18 min, 25⁰ C
Remove supernatant (2)
Pool Supernatant (1) and (2), Store -20⁰ C
***0.1M phosphate buffer, pH 6.3 (phosphate buffer only for blank, 4X bile acid mixture
for cholestyramine)**

Figure 1. Diagram of the bile acid binding procedure

1.2 Results and Discussion:

Bile acid binding relative to cholestyramine on dry matter basis of various vegetables is given in **Table 1**. Sixteen common vegetables were studied and their bile acid binding and reported in six studies. Various

cooking methods were evaluated for their influence on bile acid binding. Cooking methods used were (boiled, steamed, sautéed, microwave, microwave with water) or raw (uncooked).

Table 1. *In Vitro* Bile Acid Binding of Vegetables as Colestyramine % (dry matter basis)

Vegetable	Raw ^a	Raw ^b	Raw ^c	Raw ^f	Steamed ^c	Steamed ^d	Steamed ^e	Boiled ^e	Sautéed ^e	Micro-wave ^f	Micro-Wave + water ^f
Spinach	8.6					7.8					
Kale	8.2		10.5	10.5		13.1	12.2	11.1	13.9	13.0	12.2
Brussels Sprouts	7.8					7.9					
Broccoli	4.6		5.0	5.0		9.5	6.7	6.3	9.3	8.9	6.7
Mustard Greens	4.3		8.1	8.1		12.8	11.2	8.2	14.3	12.6	11.8
Green Bell Pepper	2.5		1.7	1.7		6.7	1.9	3.5	4.9	2.2	2.5
Cabbage	2.4		1.0	1.0		4.8	3.9	2.8	5.9	3.8	2.9
Collard Green	2.1		8.5	9.3		13.1	9.5	7.9	8.6	8.8	9.2
Okra		15.9			16.0						
Beets		11.2			17.5						
Asparagus		3.7			9.8						
Eggplant		1.3			13.7						
Turnips		1.0			1.2						
Green Beans		1.0			7.3						
Carrots		0.7			7.5						
Cauliflower		0.6			6.1						

^aData from [18] *Food Chemistry* 100:1531-1536 (2007).

^bData from [19] *Food Chemistry* 103:676-680 (2007).

^cData from [20] *Nutrition Research* 27:750-755 (2007).

^dData from [21] *Nutrition Research* 28:351-357 (2008).

^eData from [22] *Food and Nutrition Sciences* 3:951-958 (2012).

^fData from [23] *Vegetos* 25:29-36 (2012).

Cooking methods used were (boiled, steamed, sautéed, microwave, microwave with water) or raw (uncooked). Some of the cooking methods were repeated 2-3 times in these studies. With the same cooking method bile acid binding values for Kale and Mustard Greens were similar [21, 22, 23]. However, there was over 3-fold variability in the bile acid binding of steamed Green Bell Pepper [21, 22]. This variability in the bile acid binding was possibly

due to differences in maturity, growing conditions and/or seasonal variability in Green Bell Pepper. Similar amount of total dietary fiber (29-30 mg) was incubated to determine bile acid binding of asparagus, carrots, cauliflower and turnips [20]. The bile acid binding of these vegetable varied 5-8 folds. Data suggested that bile acid binding was not related to total dietary fiber content of these

vegetables. Bile acid binding based on dry matter basis appears to be the best estimate.

In snacks and salad, it is quite common to consume spinach, cauliflower, broccoli, carrots and beets as fresh uncooked. Spinach and Brussels Sprouts were observed with similar bile acid binding as uncooked [18] or steamed [21]. Similar bile acid binding for raw compared with steamed was observed for Okra in two studies [19, 20]. It is quite common that vegetables are boiled before consuming. It is obvious that some of the desired nutrients are lost in the boiled water. However, in the boiling process some cell structures are changed for easy digestion and possibly result in making additional bile acid binding sites available. Bile acid binding was similar for Kale, Mustard Greens and Collard Greens raw vs boiled [22]. Sautéing improved bile acid binding compared with steaming for Broccoli, Mustard Greens and Cabbage [22]. However, oil content is increased by Sautéing vegetables. Microwave cooking with or without added water is a quick

and convenient method. Bile acid binding of Mustard Greens was higher by microwave cooking than boiling [22, 23]. In terms of vegetables cooking methods that results in decreasing order of bile acid binding was steamed, sautéed, microwaved, microwaved with water and boiled. Data suggest that boiling these vegetables should be least desired. Many of the desirable phytonutrients are leached into water with boiling and would be lost unless the boiling water is used.

The vegetables are mostly cooked before consuming. The Steamed vegetable would retain most of the desired nutrients. Summarizing our bile acid binding studies [18, 20, 21] Dr. Michael Gregor [24] reported in [NutritionFacts.org](https://nutritionfacts.org), the best bile acid binding vegetables in <https://nutritionfacts.org/video/which-vegetable-binds-bile-best/>.

The bile acid binding of all sixteen vegetables as steamed is given in Figure 2.

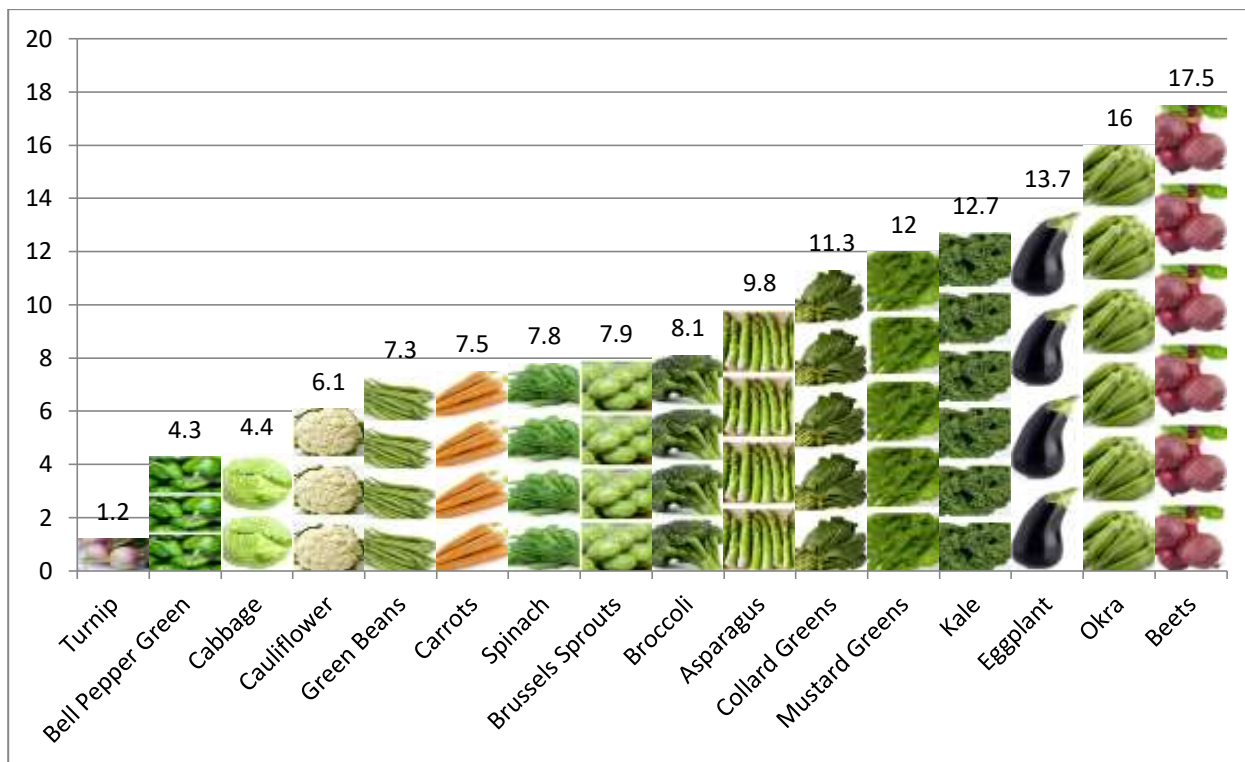


Figure 2. *In Vitro* Bile Acid Binding of Steamed Vegetables Cholestyramine% (dm basis).

By steaming, bile acid binding values for various vegetables were: Beets 18%, Okra 16%, Eggplant 14%, Asparagus, Collard Greens, Mustard Greens and Kale 10-13%, Cauliflower, Green Beans, Carrots, Spinach, Brussels Sprouts and Broccoli 6-8%, Green Bell Pepper and Cabbage 4% and Turnips only 1.2%. Various steamed vegetables with 6-18% bile acid binding when consumed regularly, would result in potential lowering the risk of premature degenerative diseases (heart disease and cancer) and improve public health.

1.3 Conclusion:

Health promoting potential of various vegetables was determined by evaluating their bile acid binding capacity relative to cholestyramine (a cholesterol lowering drug). Cooking methods used were (boiled, steamed, sautéed, microwave, microwave with water) or raw (uncooked) in six studies. Steaming vegetables resulted in the highest bile acid binding and boiling with the lowest. The Steamed vegetable would retain most of the nutritional potential. Boiling results in leaching

of the valuable nutrients. Consuming regularly steamed Beets, Okra, Eggplant, Asparagus, Collard Greens, Mustard Greens, Kale, Cauliflower, Green Beans, Carrots, Spinach, Brussels Sprouts and Broccoli with 6-18% bile acid binding would be desired. With prudent low fat diet, consuming these steamed vegetables would result in potential lower risk of premature degenerative diseases (heart disease and cancer) and improve public health.

Conflict of Interest: The authors declare no conflict of interest with any products or statements in this review manuscript.

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