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In vitro binding of bile acids by blueberries (*Vaccinium* spp.), plums (*Prunus* spp.), prunes (*Prunus* spp.), strawberries (*Fragaria X ananassa*), cherries (*Malpighia punicifolia*), cranberries (*Vaccinium macrocarpon*) and apples (*Malus sylvestris*) ☆

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Abstract

The in vitro binding of bile acids by blueberries (*Vaccinium* spp.), plums (*Prunus* spp.), prunes (*Prunus* spp.), strawberries (*Fragaria X ananassa*), cherries *(Malpighia punicifolia*) cranberries (*Vaccinium macrocarpon*) and apples (*Malus sylvestris*) 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 to testing various fresh raw 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 on dry matter (DM), total dietary fiber (TDF) and total polysaccharides (PCH) basis was for blueberries 7%, 47% and 25%; plums 6%, 53% and 50%; prunes 5%, 50% and 14%; strawberries 5%, 23% and 15%; cherries 5%, 37% and 5%; cranberries 4%, 12% and 7%; and apple 1%, 7% and 5%, respectively. Bile acid binding on DM basis for blueberries was significantly ($P \leqslant 0.05$) higher than all the fruits tested. The bile acid binding for plums was similar to that for prunes and strawberries and significantly higher than cherries, cranberries and apples. Binding values for cherries and cranberries were significantly higher than those for apples. These results point to the relative health promoting potential of blueberries > plums = prunes = strawberries = cherries = cranberries > apples as indicated by their bile acid binding on DM basis. The variability in bile acid binding between the fruits tested maybe related to their phytonutrients (antioxidants, polyphenols, hydroxycinnamic acids, flavonoids, anthocyanins, flavonols, proanthocyanidins, catechins), structure, hydrophobicity of undigested fractions, anionic or cationic nature of the metabolites produced during digestion or their interaction with active binding sites. Inclusion of blueberries, plums, prunes, strawberries, cherries and cranberries in our daily diet as health promoting fruits should be encouraged. Animal studies are planned to validate in vitro bile acid binding of fruits observed herein to their potential of atherosclerosis amelioration (lipid and lipoprotein lowering) and cancer prevention (excretion of toxic metabolites). Published by Elsevier Ltd.

Keywords: Blueberries; Plums; Prunes; Strawberries; Cherries; Cranberries; Apples; 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 (Bazzano et al., 2002; Block, Patterson, & Subar, 1992; Doll, 1990; Dragsted, Strube, & Larsen, 1993; Gaziano et al., 1995; Johnsen et al., 2003; Joshipura et al., 2001; Key et al., 1998; Law & Morris, 1998; Liu et al., 2000; Ness & Powles, 1997; Rashidkhani, Lindblad, & Wolk, 2005; Reed, 2002; Strandhagen, Hansson, Bosaeus, Isaksson, & Eriksson, 2000; van't Veer, Jansen, Klerk, & Kok, 2000). The 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 vegetables and fruits. Most fruits are

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naturally low in fat, sodium, and calories. Fruits are important sources of many nutrients, including potassium, dietary fiber, vitamin C, folic acid and they do not contain cholesterol. Some of the fruits listed by the USDA food pyramid include apples, strawberries, blueberries, cherries, plums and prunes. Phytonutrients in these fruits have been shown to stimulate natural detoxifying enzymes in the body and lower the risk of atherosclerosis and cancer (Ames, Shigenaga, & Hagen, 1993). Prevention of urinary tract infection and kidney stone formation with cranberry juice has been reported (Kessler, Jansen, & Hesse, 2002; Kontiokari et al., 2001). Laxative effects of prunes is believed to be due to its sorbitol, neochlorogenic and chlorogenic acids (Stacewicz-Sapuntzakis, Bowen, Hussain, Damayanti-Wood, & Farnsworth, 2001). 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; blueberries, plums, prunes, strawberries, cranberries and cherries are rich in antioxidants, hydroxycinnamic acids, flavonoids (anthocyanins, flavonols, and proanthocyanidins), while apples contain polyphenols and catechins (Reed, 2002; Sun, Chu, Wu, & Liu, 2002). The 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 fiber 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 as well as various dry beans has been observed to be proportional to their dry matter content (Kahlon & Woodruff, 2003a; Kahlon & Shao, 2004; Kahlon, Smith, & Shao, 2005). Bile acid binding of dark green leafy and colorful vegetables indicating their health promoting potential, has been reported (Kahlon, Chapman, & Smith, 2006). Relative to cholestyramine on an equal dry matter basis bile acid binding of various ready to eat breakfast cereals was 9.4% for wheat, 8.6% for oats, 2.8% for rice and 2.1% for corn (Kahlon & Woodruff, 2003b).

The objective of this study was to determine health promoting properties of blueberries (*Vaccinium* spp.), plums (*Prunus* spp.), prunes (*Prunus* spp.), strawberries (*Fragaria X ananassa*), cherries (*Malpighia punicifolia*) cranberries (*Vaccinium macrocarpon*) and apple (*Malus sylvestris*) 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 under duodenal physiological pH of 6.3.

2. Materials and methods

2.1. General

Blueberries, plums, prunes (plums, dried), strawberries, cherries, cranberries and apples 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, 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 fiber, was the negative control and cholestyramine, a bile acid binding anionic resin, 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 dry matter basis were 103–107 mg and cellulose 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 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 in the −20 °C freezer and diluted to the working solution (0.72 μmol/ml) just prior to each assay. Six replicates of 103–107 mg dry matter of blueberries, plums, prunes, strawberries, cherries, cranberries and apples, 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 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 0.1M phosphate buffer, pH 6.3. A phosphate buffer (4 ml, 0.1M, pH 6.3) was added to the individual substrate blanks. After the addition of 5 ml of porcine pancreatin (5x, 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* 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 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 tube. 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 blueberries, plums, prunes, strawberries, cherries, cranberries and apples 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

Composition of the blueberries, plums, prunes, strawberries, cherries, cranberries and apples is given in Table 1. Both cellulose and cholestyramine were considered as 100% total dietary fiber and polysaccharides. There was wide variation in the dietary fiber and polysaccharides content of these fruits. Total dietary fiber and polysaccharide values on dry matter basis for the fruits tested were blueberries 15% and 29%, plums 11% and 12%, prunes 10% and 37%, strawberries 22% and 33%, cherries 13% and 90%, cranberries 36% and 63%, and apples 17% and 24%, respectively. On a dry matter basis protein content in the fruits tested was 2–7%, both fat and mineral 1–4%.

On an equal dry matter (DM) basis, bile acid binding was significantly higher for cholestyramine than all the fruits tested (Table 2). Bile acid binding of blueberries was significantly ($P \leqslant 0.05$) higher than all the fruits tested. The bile acid binding for plums was similar to that for prunes and strawberries and significantly higher than cherries, cranberries and apples. Binding values for cherries and cranberries were significantly higher than those for apples. Cholestyramine bound 93% 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.

Assigning a bile acid binding value of 100% to cholestyramine, the relative bile acid binding on a dry matter basis for the test samples of fruits was blueberries 7%, plums 6%, prunes 5%, strawberries 5%, cherries 5%, cranberries 4% and apples 1%. Bile acid binding for blueberries was significantly higher and for apples significantly lower than plums, prunes, strawberries, cherries and cranberries. Bile acid binding for plums was significantly higher than that for cherries and cranberries. Relative bile acid binding on dry matter basis was blueberries > plums = prunes = strawberries = cherries = cranberries > apples. The differ-

Table 1
Composition of blueberries (*Vaccinium* spp.), plums (*Prunus* spp.), prunes (*Prunus* spp.), strawberries (*Fragaria X ananassa*), cherries (*Malpighia punicifolia*), cranberries (*Vaccinium macrocarpon*) and apples (*Malus sylvestris*), dry matter (DM) basis

Source	DM %						
	Carbohydrate	Total dietary fiber	Sugar	Polysaccharides	Protein	Fat	Minerals
Blueberries	91.8	15.2	63.1	28.7	4.7	2.1	1.5
Plums	89.4	11.0	77.7	11.7	5.5	2.2	2.9
Prunes (plums, dried)	92.5	10.3	55.2	37.3	3.2	0.6	3.8
Strawberries	84.9	22.1	51.5	33.4	7.4	3.3	4.4
Cherries	89.5	12.8	NL	89.5	4.7	3.5	2.3
Cranberries	94.8	35.7	31.4	63.4	3.0	1.0	1.2
Apples	95.6	16.6	72.0	23.7	1.8	1.2	1.3
Cholestyramine	–	100.0	–	100.0	–	–	–
Cellulose	–	100.0	–	100.0	–	–	–

Data for the fruits from USDA National Nutrient Database for Standard Refer (2004) (http://www.nal.usda.gov/fnic/foodcomp/search/); Polysaccharides = Carbohydrate – Sugar; NL, not listed.

Table 2
In vitro bile acid binding by blueberries (*Vaccinium* spp.), plums (*Prunus* spp.), prunes (*Prunus* spp.), strawberries (*Fragaria X ananassa*), cherries (*Malpighia punicifolia*), cranberries (*Vaccinium macrocarpon*) and apples (*Malus sylvestris*) on equal weight, dry matter (DM) basis[A,B]

Treatment	Bile acid binding	
	(μmol/100 mg DM)	Relative to Cholestyramine, %
Blueberries	0.73 ± 0.02^{b}	7.1 ± 0.2^{b}
Plums	0.60 ± 0.01^{c}	5.8 ± 0.1^{c}
Prunes (plums, dried)	0.53 ± 0.06^{cd}	5.1 ± 0.6^{cd}
Strawberries	0.52 ± 0.03^{cd}	5.1 ± 0.3^{cd}
Cherries	0.49 ± 0.03^{d}	4.8 ± 0.3^{d}
Cranberries	0.43 ± 0.04^{d}	4.1 ± 0.4^{d}
Apples	0.12 ± 0.01^{e}	1.2 ± 0.1^{e}
Cholestyramine	10.29 ± 0.05^{a}	100.0 ± 0.4^{a}
Cellulose	0.07 ± 0.02^{e}	0.7 ± 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 fruits was 103–107 mg, cholestyramine and cellulose 24–26 mg.

Table 3
In vitro bile acid binding by blueberries (*Vaccinium* spp.), plums (*Prunus* spp.), prunes (*Prunus* spp.), strawberries (*Fragaria X ananassa*), cherries (*Malpighia punicifolia*), cranberries (*Vaccinium macrocarpon*) and apples (*Malus sylvestris*) on equal total dietary fiber (TDF) basis[A,B]

Treatment	Bile acid binding	
	(μmol/100 mg TDF)	Relative to Cholestyramine, %
Blueberries	4.82 ± 0.11^{b}	46.8 ± 1.1^{b}
Plums	5.49 ± 0.12^{b}	53.3 ± 1.2^{b}
Prunes (plums, dried)	5.14 ± 0.58^{b}	49.9 ± 5.6^{b}
Strawberries	2.36 ± 0.13^{d}	22.9 ± 1.3^{d}
Cherries	3.85 ± 0.25^{c}	37.4 ± 2.4^{c}
Cranberries	1.19 ± 0.12^{e}	11.6 ± 1.2^{e}
Apples	0.75 ± 0.04^{e}	7.3 ± 0.4^{e}
Cholestyramine	10.29 ± 0.05^{a}	100.0 ± 0.4^{a}
Cellulose	0.07 ± 0.02^{f}	0.7 ± 0.2^{f}

[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 blueberries 16, plums 12, prunes 11, strawberries 23, cherries 14, cranberries 38, apples 17, cholestyramine 26, and cellulose 24 mg.

ences in bile acid binding between various fruits tested may relate to their phytonutrients (antioxidants, polyphenols, hydroxycinnamic acids, flavonoids, anthocyanins, flavonols, proanthocyanidins, catechins), hyrophobicity or active binding sites.

On a dry matter basis, bile acid binding of 4–7% for blueberries, plums, prunes, strawberries, cherries, and cranberries is very encouraging and could indicate health promoting benefits of these fruits. These bile acid binding values are comparable to those reported for fresh green vegetables, broccoli 5%, and mustard greens 4%, under similar conditions (Kahlon et al., 2006). Similarly 4–5% relative bile acid binding for oat bran (cereal with US-FDA approved for label health claim for lowering cholesterol) has been reported (Kahlon & Chow, 2000; Kahlon & Woodruff, 2003a). Bile acid binding on a dry matter basis has been reported as a relative health benefit of ready to eat cereals, cereal fractions and dried beans (Kahlon & Woodruff, 2003a, 2003b; Kahlon & Shao, 2004; Kahlon et al., 2005). Binding bile acids and preventing 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 health properties of various fruits and food fractions would be desirable by testing their bile acid binding on a dry matter basis.

The bile acid binding on an equal total dietary fiber (TDF) basis is shown in Table 3. Cholestyramine bound bile acids significantly higher than the various fruits tested. On a TDF basis, considering cholestyramine as 100% bound to bile acids, then binding values were blueberries 47%, plums 53%, prunes 50%, strawberries 23%, cherries 37%, cranberries 12%, and apples 7%. The bile acid binding for blueberries, plums and prunes was similar and significantly higher than cherries, strawberries, cranberries and apples. Bile acid binding values for strawberries were significantly lower than those for cherries and significantly higher than cranberries and apples. Bile acid binding values on TDF basis among various fruits tested were blueberries = plums = prunes > cherries > strawberries > cranberries = apples. Significant differences in the bile acid binding between blueberries and plums or prunes on an equal dry matter basis was eliminated due to 28–32% lower dietary fiber in plums and prunes than blueberries (Table 1). Similar bile acid binding of cherries 5% and cranberries 4% on DM basis and significant difference in their values on TDF basis is due to 64% lower TDF in cherries than cranberries.

Similar amounts of TDF per incubation was used for blueberries (16 mg), cherries (14 mg) and apples (17 mg) and their bile acid binding differed significantly suggest that bile acid binding was not related to the TDF content of the fruits tested. This is in agreement with previous reports that bile acid binding is not related to the TDF content for green leafy vegetables spinach, kale, brussels sprouts, broccoli, mustard greens, peppeps green, cabbage, and collards (Kahlon & Smith, 2005) and for black eye bean, garbanzo, kidney bean, lima bean, moth bean and soybean (Kahlon & Shao, 2004; Kahlon et al., 2006). The variability in bile acid binding between the fruits tested may be related their phytonutrients (antioxidants, anthocyanins, flavonoids, anthocyanidins, catechins), 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 was blueberries 25%, plums 50%, prunes 14%, strawberries 15%, cherries 5%, cranberries 7%, and apples 5%. Bile acid binding for blueberries was significantly lower than plums and significantly higher than all of the other fruits tested. Binding values for prunes and strawberries

Table 4
In vitro bile acid binding by blueberries (*Vaccinium* spp.), plums (*Prunus* spp.), prunes (*Prunus* spp.), strawberries (*Fragaria X ananassa*), cherries (*Malpighia punicifolia*), cranberries (*Vaccinium macrocarpon*) and apples (*Malus sylvestris*) on equal total polysaccharides basis[A,B]

Treatment	Bile acid binding	
	(μmol/100 mg Polysaccharides)	Relative to Cholestyramine, %
Blueberries	2.55 ± 0.06^{c}	24.8 ± 0.6^{c}
Plums	5.12 ± 0.11^{b}	49.8 ± 1.1^{b}
Prunes (plums, dried)	1.42 ± 0.16^{d}	13.8 ± 1.5^{d}
Strawberries	1.56 ± 0.09^{d}	15.2 ± 0.8^{d}
Cherries	0.55 ± 0.04^{e}	5.4 ± 0.3^{e}
Cranberries	0.67 ± 0.07^{e}	6.5 ± 0.71^{e}
Apples	0.53 ± 0.03^{e}	5.1 ± 0.3^{e}
Cholestyramine	10.29 ± 0.05^{a}	100.0 ± 0.4^{a}
Cellulose	0.07 ± 0.02^{f}	0.7 ± 0.2^{f}

[A] Mean ± SEM within a column with different superscript letters differ significantly ($P \leqslant 0.05$), $n = 6$.
[B] The polysaccharides (mg) used for incubation was blueberries 30, plums 12, prunes 40, strawberries 35, cherries 96, cranberries 67, apples 24, cholestyramine 26, and cellulose 24 mg.

were similar and significantly higher than for cherries, cranberries and apples. Bile acid binding on PCH basis for the various fruits tested was plums > blueberries > prunes = strawberries > cherries = cranberries = apples. The amount of PCH used for plums (12 mg) was 50% as much as that for apples (24 mg) and their bile acid binding was 10 fold higher (50 vs. 5%) than that for apples, data suggest that bile acid binding was not related to the PCH content of the fruits tested.

In conclusion, relative to cholestyramine, the in vitro bile acid binding on a DM basis was for blueberries 7%, plums 6%, prunes 5%, strawberries 5%, cherries 5%, cranberries 4% and apples 1%. These results point to the health promoting potential of blueberries > plums = prunes = strawberries = cherries = cranberries > apples as indicated by their bile acid binding on DM basis. The variability in bile acid binding between the fruits tested may be related their phytonutrients (antioxidants, polyphenols, hydroxycinnamic acids, flavonoids, anthocyanins, flavonols, proanthocyanidins, catechins), structure, hydrophobicity of undigested fractions, anionic or cationic nature of the metabolites produced during digestion or their interaction with active binding sites. Inclusion of blueberries, plums, prunes, strawberries, cherries and cranberries in our daily diet as health promoting fruits should be encouraged. Animal studies are planned to explore the relative potential for atherosclerosis amelioration (lowering lipids and lipoprotein) and cancer prevention (increased excretion of toxic metabolites and secondary bile acids) and other health promoting properties of the fruits in this study.

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