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Raisin Dietary Fiber Composition and in Vitro Bile Acid Binding

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Raisins are dried grapes that are popular shelf-stable snacks. Three commercially important types of raisins were studied: sun-dried (natural), artificially dried (dipped), and sulfur dioxide-treated (golden) raisins. Dietary fiber composition was analyzed by AACC method 32-25. Polysaccharides were hydrolyzed, and the resulting sugars were analyzed by colorimetric and gas chomatographic methods. Fructans were measured with a colorimetric kit assay. Total dietary fiber values agreed with published values, with pectins and neutral polysaccharides of mannose and glucose residues predominating. Dipped raisins had over 8% fructans. No fructans were found in fresh grapes. Raisin types varied in their ability to bind bile acids in vitro. Coarsely chopped raisins bound more bile than did finely chopped or whole raisins.

KEYWORDS: Raisin; dietary fiber; fructooligosaccharide; bile acid

INTRODUCTION

Many Americans consume inadequate amounts of dietary fiber and too few servings of fruits (*1*). Increased consumption of fiber and fruit is associated with reduced risks for cardiovascular disease and perhaps certain cancers. Most Americans consume far less than the recommended dietary fiber level of 25−38 g/d. Increased awareness of the health benefits of common foods such as raisins might encourage some individuals to increase their consumption of these fruits. Although the exact role of dietary fiber in prevention of these diseases is a contentious issue, dietary advice to consume fiber-rich foods still appears to be valid. Blackwood and others (*2*) have recently reviewed the physicochemical properties of dietary fiber and their relationship to health.

Raisins are dried Thompson seedless grapes (*Vitis vinifera* L). In the United States, raisins are produced only in the state of California. The U.S. per capita annual consumption of raisins is approximately 3.26 kg (*3*). The total dietary fiber content of raisins is 4.36 g/100 g, according to the USDA Nutrient Database (*4*). Increased raisin consumption could provide consumers with dietary fiber and phytochemicals in a portable, compact, and shelf-stable form. Three types of raisins are economically important in the United States. Natural raisins are sun-dried and account for the majority of raisins produced and consumed. Dipped raisins are dried artificially and have a higher moisture content than do natural raisins. Golden raisins are treated with sulfur dioxide to preserve the light golden color.

Eastwood and Hamilton (*5*) demonstrated that fibers could bind bile acids, thereby causing their excretion in the feces. Kritchevsky (*6*) reviewed the topic of in vitro bile binding by fibers. The continual depletion of bile in this manner is thought to reduce serum cholesterol levels by diverting the cholesterol for manufacture of bile acids. Other mechanisms may also play a role in the ability of a fiber-rich diet to protect against heart disease. Excessive bile in the colon may increase risks for bowel cancer; thus, moderation should be considered.

Raisins may offer other cardiovascular benefits. Fructans, also known as fructooligosaccharides (FOS), in raisins may be fermented to short-chain fatty acids (*7*), thereby inhibiting cholesterol synthesis (*8*). Both the American Association of Cereal Chemists (AACC) (*9*) and the Food and Nutrition Board (*10*) definitions include fructans as components of dietary fiber. Compounds in this group, which includes inulin, are soluble in aqueous ethanol and thus are not recovered in AACC and Association of Official Analytical Chemists (AOAC) dietary fiber methods. Fructans in raisins have previously been reported only by a commercial laboratory.

Raisins may also contribute to cardiovascular health maintenance due to their antioxidant content. Raisins should contain the same phenolic acid and flavonoid antioxidants as those found in grapes, but losses occur with drying. Golden raisins retain the highest amount of hydroxycinnamic acids, approximately 112 ppm (*11*). Raisins are among the richest fruit sources of the isoflavones daidzein and genistein, with 1840 μg of these compounds per kilogram wet weight (*12*). The objectives of this study were to characterize the dietary fiber composition of the commercially important raisin types and to evaluate the in vitro bile acid binding capacity of raisins.

MATERIALS AND METHODS

Materials. Natural, dipped, and golden raisins were obtained from Victor Packing Inc. (Madera, CA) in 13.6-kg plastic-lined cardboard boxes. Boxes were kept refrigerated at 4 °C. Fresh Thompson seedless grapes were also purchased locally for the fructan analysis. The sticky

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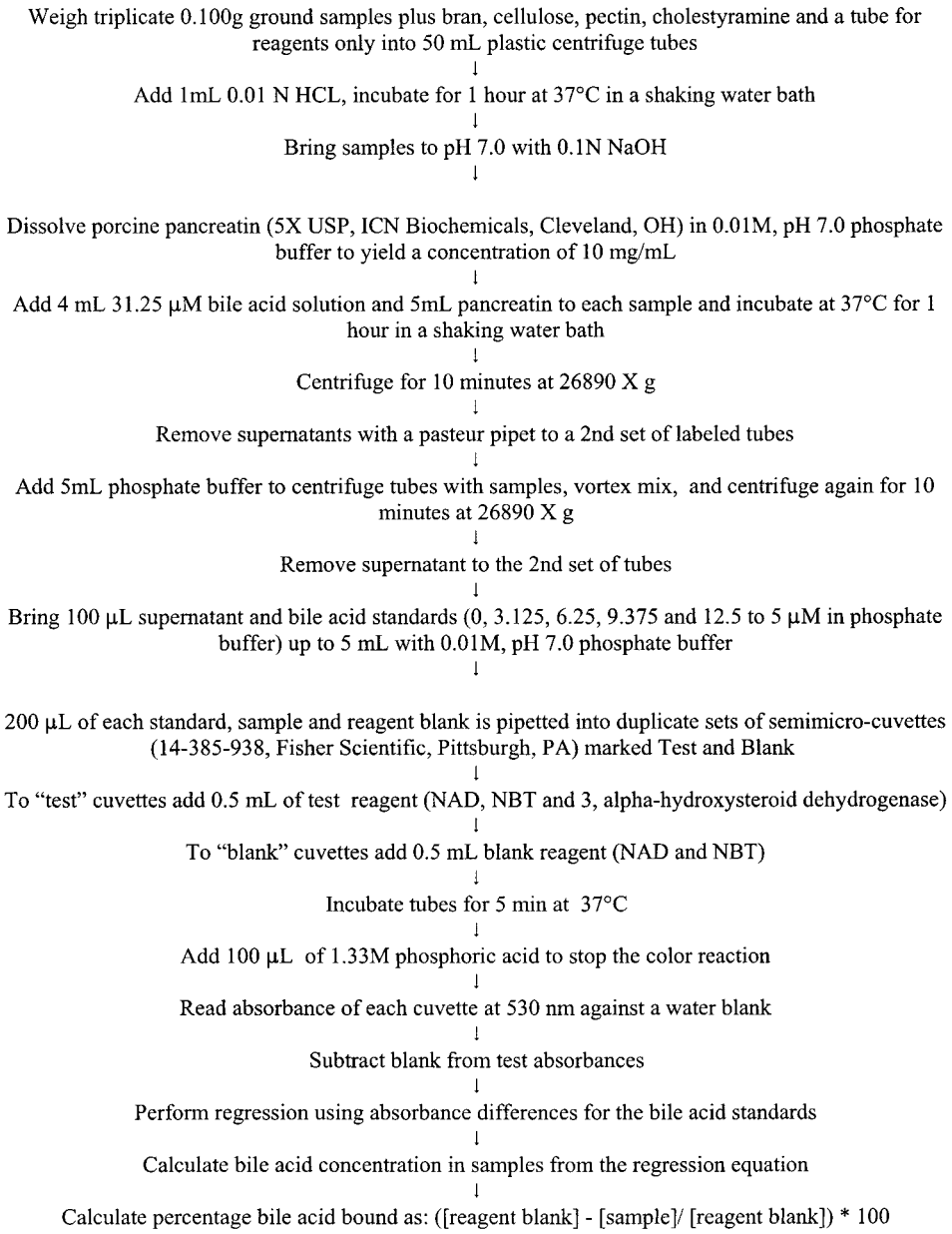

Figure 1. Flowchart for in vitro bile acid binding assay.

nature of the fruits limited options for particle size reduction. Cold raisins were chopped with a stainless steel knife on a plastic cutting board and then pressed manually through a 2.06-mm (US No. 10) screen.

Dietary Fiber Analyses. AACC Method 32-25 (*13*), as modified by Camire et al. (*14*), was used to characterize raisin dietary fiber in duplicate. Reducing sugars were measured by both colorimetric (*15*) and gas chromatographic methods (*13*). The detection limit was 0.01%.

Fructan Analysis. Oligofructans and fructan polymers were measured with a Fructan Assay Procedure (Megazyme Intl. Ireland Ltd., Wicklow, Ireland) based on AACC method 32-32. Sucrose is hydrolyzed and removed with reducing sugars before fructans are enzymatically digested (*16*). The resulting fructose units are reacted with *p*-hydroxybenzoic acid hydrazide to form a colored complex that absorbs at 410 nm. Six replicate measurements were made for each raisin type.

Bile Acid Binding. Cholestyramine resin (C 4650, Sigma, St. Louis, MO) and hard red wheat bran (American Association of Cereal Chemists, St. Paul, MN) were also evaluated for bile acid binding ability. Individual bile acids were purchased from Sigma: cholic, deoxycholic, glycocholic, and taurocholic acids. Porcine bile extract (Sigma B 8631) was used as a source of bile acids in two experiments. Each bile acid was tested separately (**Figure 1**). A serum bile acid testing kit (Sigma 450) was used for the assay (*17*). Since the concentration of serum bile acids is low, the samples in this project were diluted to fall within the range of the test kit. The advantage of the kit is that no radioactive-labeled bile acids are needed, making the procedure more rapid and less cumbersome than previously used procedures. The supernatant of raisins taken through the procedure without added bile acids had negligible absorbance.

Chewing raisins reduces their particle size, but the effectiveness of chewing varies with each person. Natural raisins were prepared for the assay as described in the flowchart, but some were coarsely chopped and pressed through a 6.35-mm screen. Whole raisins, wheat bran, and cholestyramine were also tested. Porcine bile extract was used instead of individual bile acids. Bile standards were 0, 0.00125, 0.0025, 0.00375, and 0.005 g/mL in phosphate buffer. Due to the relatively large size of the whole raisins, the sample size used was approximately 1 g. Reagents volumes were adjusted: 5 mL of 0.1 N HCl for the

Table 1. Raisin Dietary Fiber Composition (Grams per 100 g)[a]

	soluble fiber			insoluble fiber				
raisin type	uronic acids	neutral PS[b]	total	uronic acids	neutral PS	lignin	total	total fiber
natural	0.74	0.68	1.42	2.06	1.56	.01	3.63	5.05
dipped	0.88	0.64	1.52	2.23	1.61	.01	3.85	5.37
golden	1.07	0.69	1.76	1.67	1.62	nd	3.29	5.05

[a] Average of three determinations. [b] Polysaccharide residues.

Table 2. Individual Polysaccharide Residues in Raisins as Percentage of Fresh Weight

	soluble	insoluble	
raisin type	mannose	glucose	mannose
dipped	0.64	0.92	0.68
golden	0.69	0.46	0.35
natural	0.67	0.89	0.68

gastric simulation step, 10 mL of pancreatin solution with 8 mL of bile solution for the simulated small intestine step, and 7 mL of buffer for the second centrifugation step.

Natural raisins were also tested at three levels, 0.100, 0.200, and 0.500 g, in order to estimate any dose effect. Bile extracts in the concentrations used for the particle size assay were used. Reagent volumes were the same as those in the flowchart. The amount of bile bound per gram was also calculated.

Statistical Analyses. The General Linear Model of SYSTAT version 9 (SPSS, Chicago, IL) was used to compare samples. Differences among sample means were compared using Tukey's test with a probability level of 0.05.

RESULTS AND DISCUSSION

Soluble fiber accounted for about 30% of total fiber, but golden raisin has slightly more soluble fiber (**Table 1**). Total fiber values agree with USDA fiber levels. No differences were found in lignin and pectin (as uronic acids) among the types of raisins (**Table 1**). Lignin levels were very low. Insoluble fractions contained more neutral polysaccharides and uronic acids than did the soluble fractions. Mannose was the primary sugar identified by gas chromatography in the soluble fiber fraction. Insoluble fiber contained more glucose than mannose residues (**Table 2**). Glucose was reported as the major sugar in grape pomace fiber, with approximately equal but low levels of fucose, arabinose, xylose, mannose, and galactose (*18*). Sequential dips of NaOH, citric acid, and potassium metabisulfite prior to drying solubilized grape pectins and nonstarch polysaccharides (*19*); the sulfur dioxide-treated golden raisins may have undergone a similar transformation.

Inulin and fructans are not absorbed in the small intestine (*20*); thus, these compounds could make important contributions to colonic health. We found fructans in all types of raisins (**Figure 2**). Dipped raisins had more fructans than the other types of raisins. Fresh grapes had no detectable fructans, suggesting that processing may influence fructan development from sugars in the grapes. Adding fructans to total fiber values nearly doubles the fiber content.

As expected, cholestyramine bound a considerable portion (>75%) of each of the individual bile acids. The three types of raisins bound comparable percentages of cholic acid, while wheat bran bound significantly less (**Figure 3**). Golden raisins bound only a trace amount of deoxycholic acid. Binding of glycholic acid, a conjugated acid, followed the same trend as for cholic acid (**Figure 4**). In contrast, golden raisins bound a

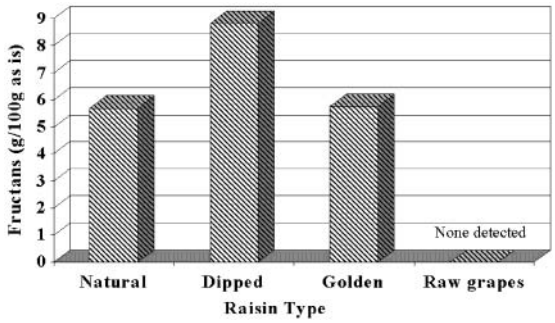

Figure 2. Fructan content of raisins and grapes (as-is basis).

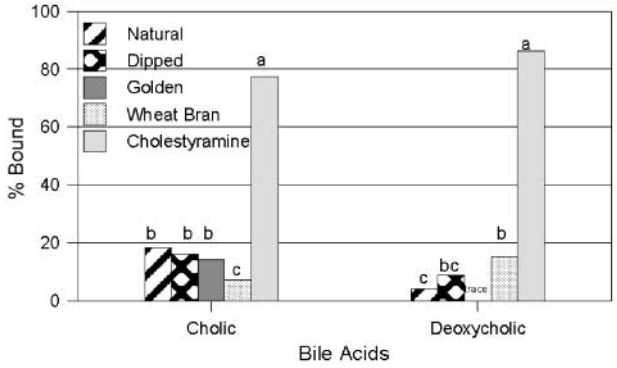

Figure 3. Simple bile acid binding by raisins and other materials. Different letters within each bile acid indicate significant differences ($p \leq 0.05$).

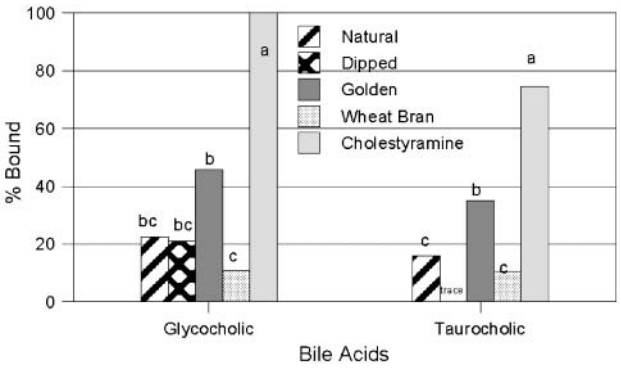

Figure 4. Conjugated bile acid binding by raisins and other materials. Different letters within each bile acid indicate significant differences ($p \leq 0.05$).

significantly higher percentage of taurocholic acid compared to natural raisins and wheat bran. Our findings for cholestyramine and bran generally agree with those reported using radiolabeled bile acids (*21*).

Coarsely chopped natural raisins bound more bile than did finely chopped or whole raisins (**Figure 5**). Bile salt binding decreased as cereal particle size was reduced (*22*); therefore, we expected that larger raisin fragments would have higher binding. The skin of whole raisins may have been a barrier to bile acid binding. The amount of raisins used in the assay had no effect on either the percentage bile bound or the amount bound per gram. Large standard deviations may have obscured treatment effects.

These findings suggest that raisins can provide more dietary fiber in the diet than was previously believed. The carbohydrate and phenolic composition of raisins may have potential benefits for cardiovascular health.

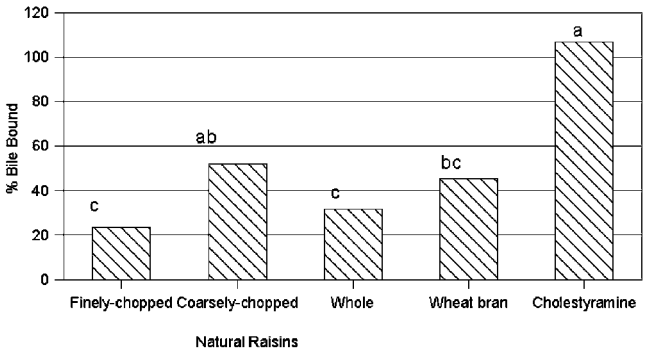

Figure 5. Percent bile binding by chopped and whole natural raisins, wheat bran, and cholestyramine. Different letters within each bile acid indicate significant differences ($p \leq 0.05$).

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