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Physiological Effects of Extraction Juices from Apple, Grape, and Red Beet Pomaces in Rats

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In comparison to classical fruit juice processing, polyphenols and dietary fiber can be extracted from pomace by means of pectinases and cellulases. In the present study, rats were fed with such produced extraction juices from apples, grapes, and red beets as drinking fluids instead of water for 4 weeks to evaluate their physiological effects. In all test groups, the intake of extraction juices was greater as compared to control (water intake), resulting in a higher urine excretion. In the apple and grape group, pH values in feces was lower than control. Administration of extraction juices from apples increased fecal counts of *Lactobacillus* and *Bifidobacterium*. More acetate and total short-chain fatty acids appeared in intestinal contents of the apple and red beet group. Furthermore, the intestinal contents of test groups contained higher concentrations of primary bile acids, cholesterol, and cholesterol metabolites but lower concentrations of secondary bile acids. The total amount of steroids excreted by these groups was also greater than control. Quercetin and isorhamnetin appeared in urine of rats fed extraction juices from apples and grapes; in urine of the former group, phloretin was found also. Administration of the extraction juices, enriched in secondary plant metabolites and dietary fiber, resulted in beneficial nutritional effects in rats.

KEYWORDS: Extraction juices; apples; grapes; red beets; physiological effects; rats

INTRODUCTION

During the past few years, secondary plant metabolites such as polyphenols (e.g., flavonoids, including anthocyanins, or phenolcarbonic acid derivatives) and dietary fiber have become ever more important in preventive nutrition. This is probably due to the antioxidative potential of flavonoids, and quercetin in particular, in vivo (*1*−*3*). Because of their antioxidant properties, polyphenols may play a role in prevention of LDL-cholesterol oxidation, which is important in arteriosclerosis (*4*). In several in vitro and in vivo studies it was shown that polyphenols can be absorbed by different mechanisms in different parts of the gastrointestinal tract (*5*−*9*).

In most fruits such as apples (*Malus sylvestris* Mill. var. *domestica* Borkh.) and grapes (*Vitis vinifera* L.), phenolics and flavonoids and the resulting antioxidant activity are predominantly located in the peel (*10*, *11*). Thus, pomaces, skins, or kernels remaining after fruit and/or vegetable juice production were proposed as a source for the production of functional food compounds or preparations rich in secondary plant metabolites (*12*).

Most of the dietary fiber components such as pectins, hemicelluloses, and cellulose are cell wall components and remain in pomace during the usual juice production as do secondary plant metabolites. In general, dietary fibers have a high nutritional significance. Depending on their structures and functional properties, they may interact with steroids in the small intestine, resulting in higher excretion of bile acids (BA) and neutral sterols (NS) (*13*−*16*). In the large intestine, dietary fiber is fermented by the microflora under formation of short-chain fatty acids (SCFA) (*17*). Especially the SCFA butyrate is important due to its regulating effects in the cell cycle and protective properties against colon cancer (*18*, *19*). Altogether, both, secondary plant metabolites and dietary fiber components may contribute to health benefits in humans.

On the industrial scale, juices are produced by pressing mashed fruits and vegetables, with or without application of pectolytic enzyme preparations, by use of different equipment. However, most of the secondary plant metabolites and dietary fiber compounds are not transferred into the liquid phase during the dejuicing process and remain in the pomace after pressing (*20*, *21*). For approximately 25 years, so-called "total liquefaction" has been discussed as a procedure for obtaining maximal juice yield in one step by the synergetic action of cellulases and pectinases (*22*, *23*) resulting in almost complete destruction of the cell walls (liquefaction). However, the obtained juices

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have a poorer sensory quality and a higher tendency to browning. Furthermore, the total liquefaction procedure is limited because of high enzyme costs and legal restrictions (*24*).

Recently, a two-step procedure has been proposed, where a classical mash treatment (with or without pectinases) is applied in the first step before the so-called premium juice is separated. Subsequently, in the second step, the remaining pomaces are treated with water and complex enzyme preparations containing mainly pectinases, hemicellulases, and/or cellulases (*20*, *25*). The extraction juices obtained had different analytical characteristics than the premium juices, for example, higher contents of flavonoids and dietary fiber (*21*, *25*, *26*). Effects of the enzyme preparations on the cell wall material of apples have been examined in a model system under the used mash and pomace treatment. During the pomace treatment, higher concentrations of dietary fiber components including pectin were dissolved and removed from the remaining residues (*27*). The polysaccharides (colloids) isolated from the apple extraction juices consisted mainly of galacturonic acid, arabinose, and galactose and had complex structures (*24*). More primary and total BA as well as more NS were excreted in rats given diets containing 5% of such isolated colloids from apple extraction juices for 6 weeks (*28*). Furthermore, lower luminal pH values, higher cecal SCFA concentrations, and changes in microbiota were found as compared with controls (*29*).

Recently, extraction juices were prepared from apples (*25*), grapes (*21*), and red beets (*Beta vulgaris* L. var. *conditiva* Alef.) by the two-step procedure mentioned above. In contrast to most fruits and vegetables, red beets contain the betalains replacing the anthocyanins as secondary plant metabolites (*30*, *31*). The red-violet betanin is a 5-*O*-β-glucoside of the aglycon betanidin. Furthermore, the yellow betaxanthins are present in red beets. Betalain preparations are allowed as food colorants (*32*).

Most physiological experiments were made with phenolic-rich extracts from grapes, grape seed, or skin extracts (*33*−*37*), wine or wine extracts (*38*), grape juice (*39*), and apple extracts (*40*) or with isolated polyphenol preparations. Singletary et al. (*41*) found that grape juice formulation provided in the drinking water of rats at concentrations of 490 and 650 mg phenolics/dL inhibited mammary tumorigenesis.

There are very few data about the physiological effects of extraction juices obtained after enzymatic treatment of pomaces by pectinases and cellulases in humans and animals. In a previous intervention study with humans, it was shown that consumption of 1.4 L of a flavonoid- and dietary fiber-containing extraction juice from apples per day for 2 weeks resulted in several somehow beneficial physiological effects: a higher liquid intake and urine volume, higher counts of *Bifidobacterium*, higher concentrations of 3,4-diphenylacetic acid and quercetin in urine, greater concentrations of SCFA in feces, and higher fecal excretion of steroids (*42*).

In the present study, physiological effects of extraction juices from apples, grapes, and red beets, which were given to rats for a period of 4 weeks as drinking fluids instead of water, were evaluated with special emphasis on behavior, food and liquid intake, weight gain, fecal and urinary characteristics, polyphenols and betanins in urine, bacterial counts, and weights of intestinal walls and contents, as well as the concentrations of short-chain fatty acids, bile acids, and neutral sterols in the intestinal contents and in feces.

MATERIALS AND METHODS

Materials. The apple pomace extraction juice was obtained by a two-step treatment of apples in accordance with Will et al. (*20*, *25*) by use of 100 mg/kg of the enzyme preparation Pectinex Smash (Novozymes, Dittingen, Switzerland) for the apple mash treatment and 200 mg/kg of Pectinex AFP-L 4 (Novozymes) for the subsequent apple pomace extraction. The corresponding pomace extraction juice was concentrated for storage purposes and later diluted to a juice strength of 6.2 °Brix prior to administration to rats. (°Brix is an internationally used unit for juice and concentrate strength in the fruit juice industry. It is defined as grams of sucrose/100 mL of juice.).

After dejuicing of ground white grapes (grown at the Department of Grape-Vine Breeding of the State Research Institute Geisenheim) on a horizontal press Bucher HP-L 200 (Bucher, Niederwenigen, Switzerland), the remaining grape pomace was suspended in hot demineralized water at a ratio of 1:1 (w/v) and then extracted for 2 h at 50 °C in a temperature-controlled stirring tank in the presence of Rohapect AP-1 (AB Enzymes GmbH, Darmstadt, Germany) and Rohament CL (AB Enzymes) at a dose of 200 mg/kg each. The extraction juice was obtained by use of the Bucher HP-L 200. Further conditions of enzymatic pomace treatment were as described previously (*21*).

After dejuicing of milled red beets at 75 °C in presence of 400 mg of ascorbic acid/kg in a decanter (Flottweg, Vilsiburg, Germany), the pomace was suspended in hot demineralized water at a ratio of 1:1 (w/v) and then extracted for 2 h at 50 °C in a temperature-controlled stirring tank in the presence of Pectinex Ultra SP-L and Ultrazym AFP-L (Novozymes) at a dose of 200 mg/kg each and of 200 mg of ascorbic acid/kg. The extraction juice was obtained by use of a decanter from Flottweg. The extraction juice was concentrated in an industrial-scale multistep falling draught evaporator to 45 °Brix for storage purposes and later diluted to juice strength of 6.0 °Brix prior to administration to rats. Juice from red beets was subsequently stored frozen at −20 °C and freshly thawed prior to administration to rats.

The other extraction juices were filled in bottles, pasteurized, and stored at 4 °C until use.

Animal Experiments. Male Wistar rats (n = 48; Shoe-Wistar, Charles River, Germany) weighing approximately 250 g were kept in a temperature-controlled environment (22 ± 2 °C) with 12-h light−dark cycle and free access to food. Throughout the whole study, the rats were fed a semisynthetic control diet consisting of 63% wheat starch, 20% casein, 5% mineral mixture, 2% vitamin mixture, 5% sunflower oil, and 5% microcrystalline cellulose (*29*). After 1 week of adaptation (week 0), the rats were randomly divided into four groups of 12 animals each. The control group was maintained on drinking water, whereas the three test groups received pomace extraction juices from apples, grapes, or red beets, respectively, instead of drinking water for four weeks. The rats had free access to food and water or extraction juice during the experiment. The animal study was carried out in the Max-Rubner-Laboratory of the German Institute of Nutrition, Potsdam-Rehbruecke, Germany.

Growth of the rats and intake of food and water or extraction juice were determined weekly. For determination of steroids, the feces were collected completely for 24 h at week 4. After 4 weeks of juice administration, the rats were killed, and selected organs (e.g., jejunum, cecum, and colon) and their contents were prepared for analysis. The colon was divided into proximal and distal parts. SCFA, steroids, dry matter (DM), and pH values were analyzed in intestinal contents. The microbial counts were determined at the end of the experiment in fresh fecal samples collected directly from anus. At weeks 0, 2, and 4 of the experimental period, urine samples were collected for 16 h using metabolic cages fitted with urine/feces separators under conditions as described above. Urine was acidified with 0.1 M HCl and supplemented with 0.1% (w/v) ascorbic acid. Aliquots of the urine samples were immediately frozen and stored at −20 °C until analysis.

The experimental protocol was performed in accordance with the guidelines of the ethics committee of the Ministry of Agriculture, Nutrition and Forestry (State Brandenburg, Germany, Permission 32/48-3560-0/3).

Analytical Procedures. The total polyphenols were analyzed by use of the Folin−Ciocalteu reagent (FCR) and calculated as (+)-catechin equivalents. Individual phenolic constituents of grape extraction juice were determined by HPLC with a fluorinated reversed phase and UV/electrochemical detection according to Rechner et al. (*43*). Individual polyphenols of apple pomace extraction juice were analyzed by RP-

HPLC/DAD (*44*). Flavan-3-ols, procyanidins, and dihydrochalcones were detected at 280 nm, phenolcarbonic acids at 320 nm, and flavonols at 360 nm. To identify the phenolics in the samples, standards of the individual phenolic compounds were used. The betalains were determined by HPLC/DAD as described by Stintzig et al. (*45*). Furthermore, betaxanthins (as vulgaxanthin I) and betacyans (as betanin) were determined photometrically according to Nilsson (*46*). The antioxidant capacity of the extraction juices was determined by the Trolox equivalent antioxidant capacity (TEAC) test (*21*) and given as Trolox equivalents.

Dietary fiber was analyzed by the enzymatic−gravimetric AOAC method (*47*). Free galacturonic acid was determined in juices by use of high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC/PAD) (*24*).

Colloid concentrations were determined by size-exclusion chromatography on a Pharmacia FPLC system equipped with two HR 10/10 fast desalting columns connected in series. Both columns were packed with Sephadex G25 superfine gel material. Extraction juices were diluted (1:20) with bidistilled water. Samples were filtered and injected via a 200 μL sample loop. The eluent was 0.2 M NaCl with a flow rate of 1.0 mL/min. Substances from 5000 Da onward eluted in the void volume of the column system (V_{excl} = 5.0 mL). They were separated from low-molecular ingredients of the juices, which started to elute after about 8 mL. Both peaks were detected by use of a refractive index detector. The peak in the void volume was integrated by chromatography software and quantified with an external calibration curve with five concentrations from 100 to 1000 mg/L (*20*).

SCFA were analyzed in intestinal contents and feces by gas chromatography via a modified method of Brighenti (*48*) as described previously (*15*).

The microbial counts (total aerobes and anaerobes, coliforms, *Bacteroides*, *Lactobacillus*, *Bifidobacterium*) were determined by classical methods as described elsewhere (*29*) and are expressed as logarithm of colony-forming units (CFU)/gram.

The extraction and purification procedures from freeze-dried intestinal contents and feces materials as well as the analysis of bile acids by HPLC via precolumn derivatization with 4-bromomethyl-7-methoxycoumarin and fluorescence detection and of neutral sterols by HPTLC are given elsewhere (*16*).

Urine samples (20 mL) were analyzed by HPLC after solid-phase extraction on C_{18} cartridges (Machery-Nagel, Düren, Germany). A parallel sample was enzymatically deglucuronidated for 2 h at 37 °C with β-glucuronidase/sulfatase from *Helix pomatia* (Sigma, Seelze, Germany; 500 units/500 μL in 0.2 M sodium acetate buffer, pH 5.0) in nitrogen atmosphere prior to HPLC analysis. For analysis of flavonoids, 100 μL of urine sample were applied on an HPLC column (*49*) with a gradient consisting of 0.02 M sodium phosphate buffer (pH 3.4) and methanol/0.1 M sodium phosphate buffer (4:1 v/v; pH 3.4) at a flow rate of 0.8 mL/min. The stationary phase was a Nucelosil 100 RP-18 end-capped column (250 mm × 4.6 mm, 5 μm) from Macherey-Nagel, Düren (Germany) at 30 °C. Flavonoids were detected by use of a multielectrode coulometric detector (12-electrode CoulArray) from Esa Inc., Chelmsford, MA, with potentials between 0 and 825 mV.

Statistical Analysis. Values are means ± SEM. Data were analyzed by one-way ANOVA, and differences between the test groups and the control group were evaluated by Dunnett's *t* test. *p* values of <0.05 were considered significant.

RESULTS

Composition of the Extraction Juices. Extraction juices produced from apples, grapes, and red beets had a DM content of 4.4 (grape) to approximately 6.0 °Brix (apple, red beet) (**Table 1**). Colloid contents were between 0.9 (grape, red beet) and 2.75 g/L (apple). Free galacturonic acid was, at 2.2 g/L, most prominent in the extraction juice from apples, as was total acid, at 3.75 g/L. This extraction juice also had the highest dietary fiber content at 2.96 g/L, followed by the extraction juice from grapes with 2.44 g/L dietary fiber. Red beet extraction juice had only 0.64 g/L dietary fiber. More than twice as many total polyphenols were determined by FCR in the grape extraction juice (1877 mg/L) as compared to that from apples (829 mg/L) and red beets (902 mg/L). The TEAC values from the juices correlated with the total polyphenols. The extraction juice from grapes was, at 23.4 mmol of Trolox/L, the juice with the highest antioxidative capacity, whereas the corresponding juices from apples and red beets had 7.9 and 7.3 mmol of Trolox/L, respectively. However, almost comparable values were measured for total polyphenols in extraction juices from apples and grapes by HPLC, which is probably due to the nonavailability of suitable reference substances for all polyphenols occurring in apples and grapes as well as due to their partial instability. In extraction juice from apples, chlorogenic acid was dominating besides epicatechin, 4-coumaroylquinic acid, the apple-characteristic phloridzin, procyanidin B2, and several quercetin-3-glycosides. In contrast, in grape extraction juice catechin and epicatechin dominated, and their reaction products procyanidin B1 and B2 were also present in higher amounts. In red beet extraction juice, 123 mg/L total betalains (70 mg/L betacyans, 53 mg/L betaxanthins) was determined.

Table 1. Composition of Extraction Juices from Apples, Grapes, and Red Beets

characteristic	extraction juice		
	apple	grape	red beet
dry matter (°Brix)[a]	6.2	4.4	6.0
colloids (g/L)	2.75	0.9	0.9
total acid (as tartaric acid; pH 7.0) (g/L)	3.75	3.0	0.5
free galacturonic acid (g/L)	2.22	0.69	0.19
dietary fiber (g/L)	2.96	2.44	0.64
total polyphenols (mg/L)	829	1877	902
antioxidative capacity (TEAC) (mmol of Trolox/L)	7.9	23.4	7.3
by HPLC (mg/L): catechin	5.4	225.3	
epicatechin (mg/L)	35.6	195.0	
cryptochlorogenic acid	3.8		
chlorogenic acid	118.9		
3-coumaroylquinic acid	7.7		
4-coumaroylquinic acid	44.4		
5-coumaroylquinic acid	4.1		
phloretin-2-xyloglucoside	4.8		
phloretin-2′-xylogalactoside	4.8		
phloridzin	93.6		
procyanidin B1	9.5	60.7	
procyanidin	78.2	46.7	
gallic acid		13.5	
p-coumaroyl glucosyltartrate		3.2	
caftaric acid		8.4	
coutaric acid		4.7	
quercetin derivatives	46.4[b]	21.1	
cis-piceid (as *trans*-resveratrol)		0.4	
total polyphenols	561	580	
betacyans (mg/L)			70
betaxanthins (mg/L)			53
total betalains (mg/L)			123

[a] °Brix is an internationally used unit for juice and concentrate strength in the fruit juice industry. It is defined as grams of sucrose/100 mL of juice. [b] In milligrams per liter: quercetin-3-rutinoside, 4.5; quercetin-3-galactoside, 12.3; quercetin-3-glucoside, 6.7; quercetin-3-xyloside, 5.2; quercetin-3-arabinoside, 1.3; and quercetin-3-rhamnoside, 16.4.

Behavior, Food and Liquid Intake, Weight Gain, and Fecal and Urinary Characteristics. The diets were well accepted by all rats. There were no treatment-related changes in the rats' behavior or appearance during the experiment. Behavior was judged by their appearance, phenotype, and pattern in comparison to the control. All rats remained healthy during the experimental period.

Table 2. Food and Liquid Intake, Weight Gain, and Urine Excretion of Rats Given Water or Extraction Juices from Apples, Grapes, or Red Beets[a]

	experimental group			
	control	apple	grape	red beet
	Food Intake (g/day)			
week 1	17.16 ± 2.12	14.11 ± 1.80	16.80 ± 3.05	16.35 ± 2.31
week 2	16.44 ± 1.47	14.64 ± 1.86[b]	14.62 ± 1.50[b]	15.25 ± 1.85
week 4	16.51 ± 2.15	14.98 ± 1.52	14.85 ± 1.53[b]	13.80 ± 1.53[c]
	Liquid Intake (g/day)			
week 0	17.14 ± 4.90	18.89 ± 4.16	18.92 ± 4.43	18.67 ± 4.60
week 2	24.04 ± 6.69	46.73 ± 10.49[d]	41.85 ± 9.29[d]	32.50 ± 4.84[d]
week 4	23.12 ± 3.70	38.52 ± 7.11[d]	49.87 ± 12.46[d]	56.87 ± 11.78[d]
	Weight Gain (g)			
week 0	249.5 ± 17.5	255.0 ± 16.3	247.8 ± 10.7	246.3 ± 13.2
week 2	291.6 ± 19.3	296.0 ± 17.1	279.6 ± 13.7	289.4 ± 20.2
week 4	322.3 ± 20.0	325.2 ± 21.4	308.1 ± 17.9	318.2 ± 25.4
	Urine Excretion (mL/16 h)			
week 2	20.47 ± 8.03	32.67 ± 14.67[b]	22.87 ± 12.49	43.75 ± 13.63[d]
week 4	15.78 ± 7.01	37.21 ± 14.33[d]	24.97 ± 11.82[b]	53.25 ± 21.48[d]

[a] Values are means ± SEM; $n = 12$. Mean values were significantly different from control group as indicated by footnotes *b–d*. [b] $p < 0.05$. [c] $p < 0.005$. [d] $p < 0.001$.

The food intake was highest in the control group. At week 4, significantly lower food intake was measured in the test groups given the extraction juice from grapes and red beets. The weight gain of the rats was not affected by the extraction juices administered (**Table 2**). The average body weight increased during the experiment by 70−73 g in the control, apple, and red beet groups but only by 60 g in the grape group ($p < 0.05$). Intake of extraction juices was significantly greater in all test groups than intake of water in the controls. This effect resulted in higher urine excretion in rats given the extraction juices (**Table 2**).

There was no difference in DM of intestinal contents or feces at the end of the experiment. Means of DM were between 20.65% and 21.22% in cecal contents, between 23.53% and 26.00% in contents of proximal colon, between 35.43% and 37.68% in contents of distal colon, and between 48.13% and 50.44% in fresh feces. The pH values in contents of proximal colon tended to be lower in the test groups (apple group, 7.19; grape group, 7.18; red beet group, 7.18) than in controls (7.34) (means; $n = 12$). However, after 4 weeks of drinking extraction juices from apples and grapes, the pH values in fresh feces material were 6.53 and 6.60, respectively, significantly lower ($p < 0.05$) as compared with the controls (6.74).

Bacterial Counts. There were only small differences in bacterial counts between all diet groups. However, significantly more *Lactobacillus* and *Bifidobacterium* were found in fresh feces of rats that had been fed extraction juice from apples. They differed from the controls by 1 order of magnitude. The administration of the extraction juice from red beets resulted also in higher counts of *Lactobacillus* (**Figure 1**).

Intestinal Walls and Contents. In groups fed the extraction juices, slight changes in weights of intestinal walls and contents were measured. Jejunum walls were heavier in the control group. There was a tendency to lower wall weights when rats received the extraction juices from grapes and red beets. The jejunal and cecal contents of the test groups were greater than those of the controls, whereas colonic contents of the grape group were lower as compared to the control group ($p < 0.005$) (**Table 3**).

Short-Chain Fatty Acids. Significantly more acetate was found in cecal contents of rats fed the extraction juices [apple and red beets ($p < 0.05$); grape ($p = 0.06$)] for 4 weeks as compared to the control group. There were no differences in propionate and butyrate concentration among all groups (**Figure 2**). More total SCFA were measured in the apple group ($p < 0.05$) as well as in the grape and red beet group ($p \leq 0.09$). Because the cecum is the major site of fermentation, the highest SCFA concentrations were measured in that part of the gut. Due to subsequent absorption of SCFA during further gut passage, the concentrations of SCFA decreased continuously from cecum to feces in all groups. Thus, in contents of the proximal colon, concentrations of total SCFA were 131 μmol/g DM in the control group and 145 μmol/g DM in the red beet group (data not shown). Higher concentrations of acetate and total SCFA appeared in the contents of distal colon if rats were fed the extraction juices from apples and red beets ($p < 0.05$) (**Figure 2**). The means of total SCFA were only between 55 and 75 μmol/g DM in these groups. For the former group, also significantly more acetate and total SCFA were found in fresh feces samples ($p < 0.05$). In feces, between 23 and 38 μmol/g DM total SCFA were present (data not shown).

The molar proportion between acetate, propionate, and butyrate was for all intestinal contents of the control group quite similar (approximately 63:23:14), whereas in the test groups a molar proportion of approximately 68:20:12 was found. However, in fresh feces materials of all groups, the molar proportion between the three major SCFA was approximately 81:14:5 on average.

It should furthermore be mentioned that additional small amounts of *n*- and *iso*-valerates were present in the intestinal contents and feces as a result of bacterial degradation of certain amino acids.

Bile Acids and Neutral Sterols. At the end of the experiment, the concentrations of steroids (BA and NS) were determined in cecal and colonic contents and in feces.

In the cecal contents, a broad spectrum of individual BA was found (**Figure 3**). Besides cholic acid (CA), deoxycholic acid (DCA), chenodeoxycholic acid (CDCA), lithocholic acid (LCA), and their metabolites such as 7-ketodeoxycholic acid (KDCA), 12-ketolithocholic acid (KLCA), hyodeoxycholic acid (HDCA), ursodeoxycholic acid (UDCA), and α- and β-muricholic acid (MCA), several tauroconjugates such as taurocholic acid (TCA), taurodeoxycholic acid (TDCA), and taurochenodeoxycholic acid (TCDCA) were present in intestinal contents of the rats.

In cecal contents, the proportion of tauroconjugated BA was 14.62% in the control group and 10.39−11.16% in the test groups ($p < 0.005$). During the passage toward the lower intestinal tract, the BA were completely deconjugated by bacterial enzymes. Therefore, in the colonic contents, the proportion of tauroconjugates decreased to 4.19% in the control group and to 1.70−2.45% in the test groups ($p < 0.005$). As expected, exclusively free BA were present in feces.

In general, the concentrations of CA, CDCA, αMCA, and βMCA were greater in the cecal contents of rats that had been given the extraction juices as compared to the controls (**Figure 3**). These BA belong to the so-called primary BA, having a hydroxyl (or keto) group at C-atom 7 of the steroid nucleus. On the other hand, concentrations of the secondary bile acids DCA and HDCA were lower in the test groups that are missing that particular hydroxyl (or keto) group at C-atom 7 of the steroid nucleus. In contrast, the concentrations of LCA, a further secondary BA, increased in rats fed the extraction juices (**Figure 3**). Similar effects were observed in the colonic contents; the concentrations of individual primary BA were greater and those of secondary BA (including LCA) were lower in rats that consumed the extraction juices instead of water (data not

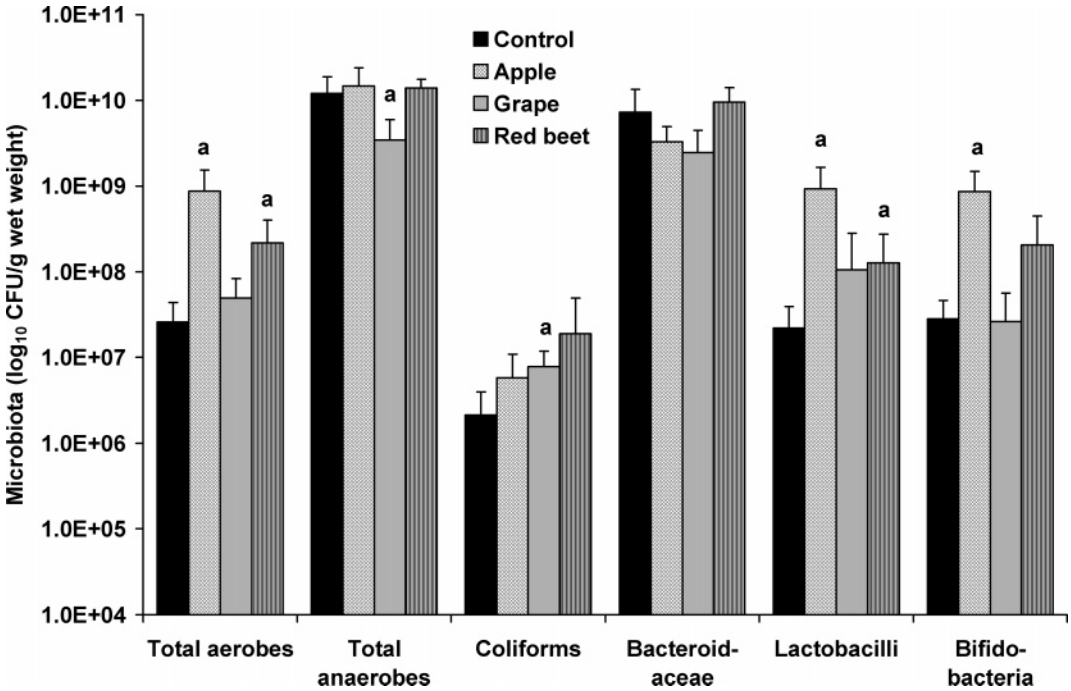

Figure 1. Microbial counts [logarithm of colony-forming units (CFU)/gram of wet weight] in fresh fecal material of rats given water or extraction juices from apples, grapes, or red beets for 4 weeks. Values are means ± SEM; $n = 12$. Mean values were significantly different from control group: (a) $p < 0.05$.

Table 3. Wet Weights of Intestinal Walls and Contents of Rats Given Water or Extraction Juices from Apples, Grapes, or Red Beets for 4 Weeks[a]

	experimental group			
	control	apple	grape	red beet
	Intestinal Walls			
jejunum	4.79 ± 0.436	3.74 ± 0.645[d]	3.27 ± 0.376[d]	3.45 ± 0.432[d]
cecum	1.01 ± 0.577	1.01 ± 0.134	0.85 ± 0.117	0.87 ± 0.083
colon	1.09 ± 0.209	1.21 ± 0.152	0.83 ± 0.192[b]	0.96 ± 0.177
	Intestinal Contents			
jejunum	1.74 ± 0.335	2.49 ± 0.433[d]	2.05 ± 0.398	2.24 ± 0.366[c]
cecum	3.67 ± 1.002	4.85 ± 1.009[b]	3.87 ± 0.536	4.30 ± 0.869
colon	2.54 ± 0.740	2.85 ± 1.019	1.31 ± 0.807[c]	2.39 ± 0.765

[a] Values, given in grams, are means ± SEM; $n = 12$. Mean values were significantly different from control group as indicated by footnotes *b–d*. [b] $p < 0.05$. [c] $p < 0.005$. [d] $p < 0.001$.

shown). The concentrations of BA in feces at week 4 are shown in **Figure 4**. In general, test groups were characterized by significantly higher concentrations of individual primary BA and significantly lower concentrations of individual secondary BA as compared to the controls. These differences were most prominent in rats fed the extraction juice from apples.

The concentrations of primary, secondary, and total BA in cecal and colonic contents as well as in feces are summarized in **Table 4**. On a DM basis, the concentrations of total luminal BA increased from cecum to feces in all groups. The administration of the extraction juice from apples resulted in approximately 9% higher excretion of total BA ($p < 0.005$). In the case of extraction juice from grapes, 5% more total BA was excreted ($p < 0.05$). The extraction juice from red beets was less effective. However, all extraction juices were able to decrease the proportion of secondary BA and to increase the proportion of primary BA in intestinal contents and feces.

The proportion between BA belonging to the cholic acid family (CA, DCA, KDCA, and KLCA) and those belonging to the chenodeoxycholic acid family (CDCA, LCA, αMCA, βMCA, HDCA, and UDCA) was not altered by the drinking fluids administered. Approximately 32% of the BA belonged to cholic acid family in all groups.

Furthermore, cholesterol and its metabolites (coprostanol, coprostanone, and cholestanone) were analyzed in intestinal contents and feces. A greater concentration of total NS appeared in the cecal contents of rats fed the extraction juices as compared to the controls ($p < 0.001$). Their respective concentrations were in detail as follows (micromoles/gram of dry matter): control group 15.6 ± 1.10; apple group 19.3 ± 1.08; grape group 18.5 ± 1.16; red beet group 16.8 ± 1.26. Cholesterol was the dominating NS (approximately 73%) followed by coprostanol. The concentration of cholesterol metabolites was lower than that of cholesterol (**Figure 5**). During the passage toward the lower intestinal tract, a great proportion of cholesterol was enzymatically converted into its metabolites. Thus, its molar proportion was 33% in colonic contents of the control group and 28−30% in the test groups. The major cholesterol metabolite was the NS coprostanol in that part of the gut (micromoles/gram of dry matter): control group 13.3 ± 0.48; apple group 17.9 ± 0.30; grape group 16.1 ± 0.39; red beet group 16.0 ± 2.02. Concentrations of total NS in the cecum were 20.5 μmol/g DM in the control group and 23.6−26.1 μmol/g DM in the test groups ($p < 0.001$). The concentrations of individual NS in feces are shown in **Figure 5**. As coprostanol was the dominating NS (more than 64%), the proportion of cholesterol metabolites increased up to 67% in the control group and up to 70−72% in the test groups. Significantly higher total concentrations of NS were found in test groups that had been fed extraction juice from apples (26.1 ± 0.41 μmol/g DM), grapes (24.0 ± 0.41 μmol/g DM), and red beets (23.6 ± 2.06 μmol/g DM) ($p < 0.001$).

NS were excreted by rats to a higher extent than BA. Approximately 35% of the excreted steroids were BA. Overall, the concentration of total steroids (sum of BA and NS) was significantly higher in all test groups ($p < 0.001$) (micromoles/

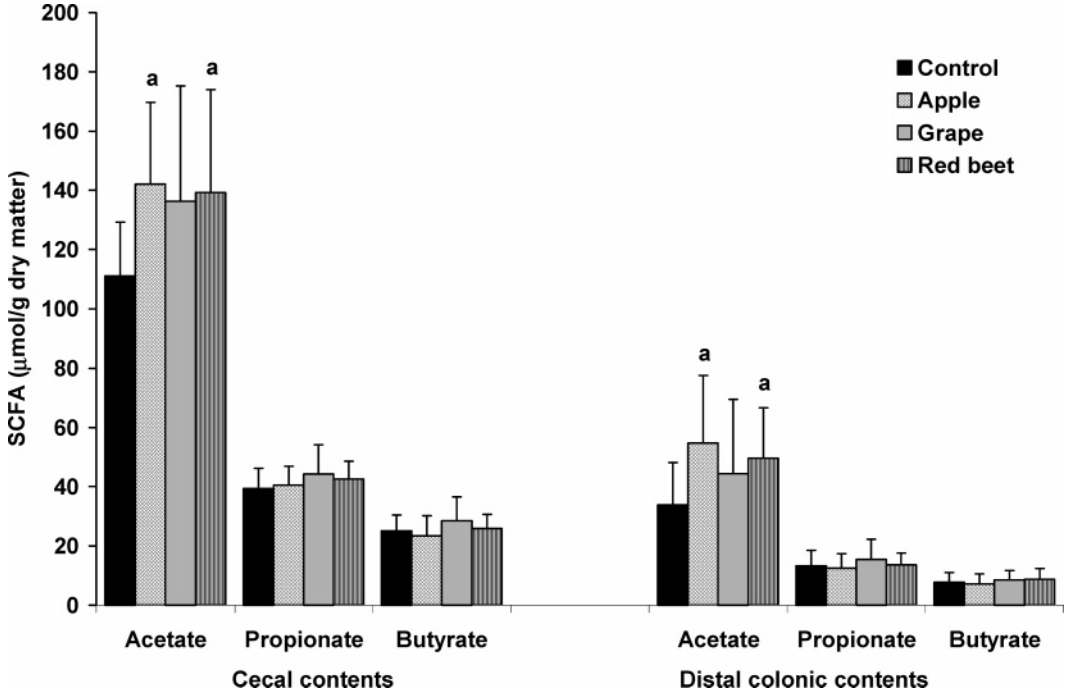

Figure 2. Short-chain fatty acids (SCFA) (micromoles/gram of dry matter) in cecal and distal colonic contents of rats given water or extraction juices from apples, grapes, or red beets for 4 weeks. Values are means ± SEM; $n = 12$. Mean values were significantly different from control group: (a) $p < 0.05$.

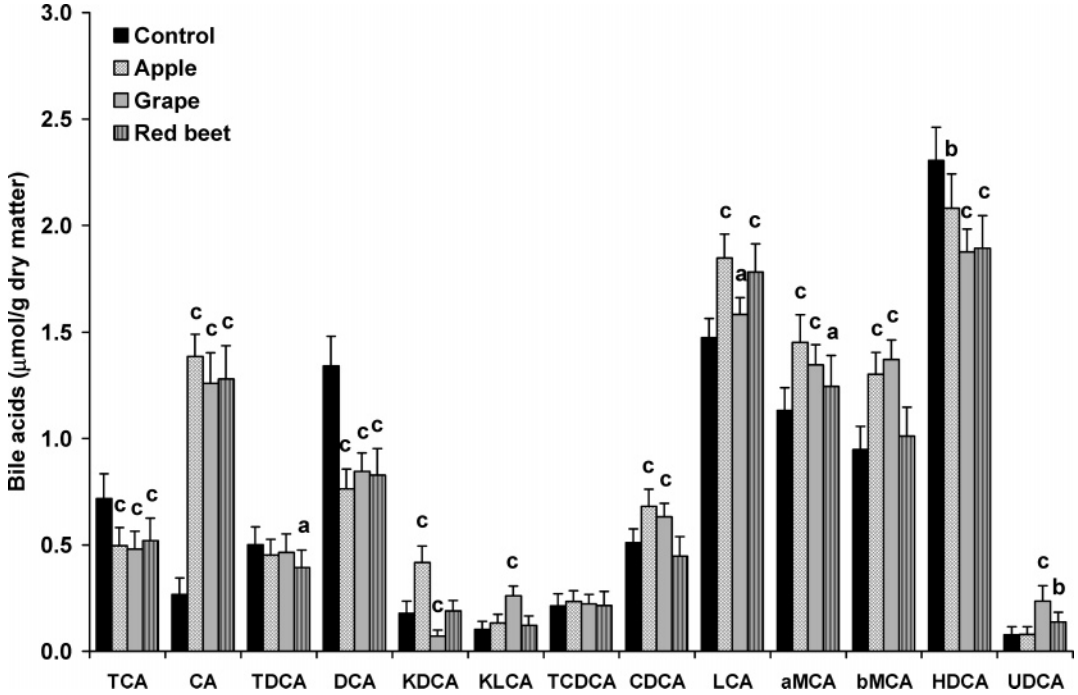

Figure 3. Bile acids (micromoles/gram of dry matter) in cecal contents of rats given water or extraction juices from apples, grapes, or red beets for 4 weeks. Values are means ± SEM; $n = 12$. Mean values were significantly different from control group: (a) $p < 0.05$; (b) $p < 0.005$; (c) $p < 0.001$. Abbreviations of bile acids are defined in the text.

gram of dry matter): control group 35.0 ± 1.31; apple group 42.4 ± 1.52; grape group 40.6 ± 1.28; red beet group 38.8 ± 1.78. In summary, the extraction juice from apples was most effective concerning the excretion of steroids.

Furthermore, very small amounts of phytosterols were also present in feces (data not shown).

Polyphenols and Betanins in Urine. The excretion of polyphenols was evaluated in 16-h urine samples at weeks 2 and 4 (**Figure 6**). Rats that had been fed apple extraction juice consumed on average 3.30 and 2.72 μmol of quercetin and quercetin glycosides/day at weeks 2 and 4, respectively. Approximately 0.7% and 1.1% of that amount was excreted via urine, respectively. Similarly, when the extraction juice from grapes was consumed, 1.45 and 1.13 μmol of quercetin and quercetin glycosides/day were taken up on an average by the rats at week 2 and 4, respectively, and approximately 1.2% and 1.7% of these plant polyphenols was excreted in urine, respectively.

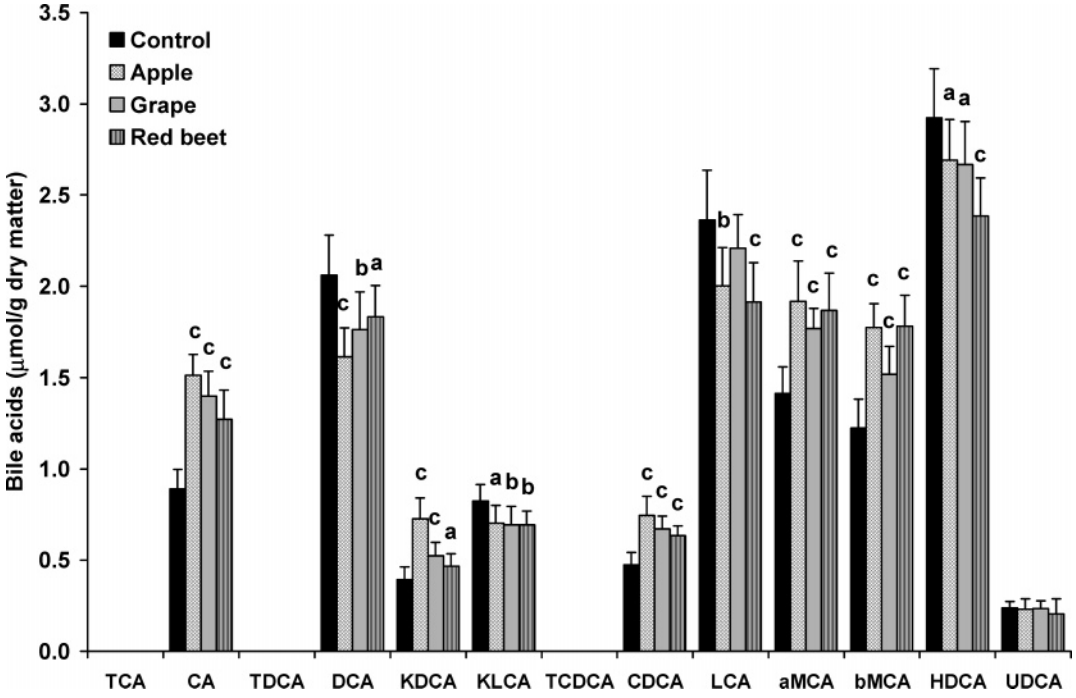

Figure 4. Bile acids (micromoles/gram of dry matter) in fresh fecal materials of rats given water or extraction juices from apples, grapes, or red beets for 4 weeks. Values are means ± SEM; $n = 12$. Mean values were significantly different from control group: (a) $p < 0.05$; (b) $p < 0.005$; (c) $p < 0.001$. Abbreviations of bile acids are defined in the text.

Table 4. Primary, Secondary, and Total Bile Acids (BA) in Cecal and Colonic Contents as Well as in Feces of Rats Given Water or Extraction Juices from Apples, Grapes, or Red Beets for 4 Weeks[a]

	experimental group			
	control	apple	grape	red beet
		Cecal Contents		
primary BA	4.04 ± 0.391	6.05 ± 0.387[d]	5.62 ± 0.280[d]	5.05 ± 0.439[d]
secondary BA	5.73 ± 0.231	5.28 ± 0.240[d]	5.03 ± 0.216[d]	5.02 ± 0.164[d]
total BA	9.77 ± 0.507	11.32 ± 0.524[d]	10.65 ± 0.337[c]	10.07 ± 0.567
		Colonic Contents		
primary BA	4.38 ± 0.380	6.43 ± 0.328[d]	5.84 ± 0.245[d]	5.83 ± 0.353[d]
secondary BA	7.27 ± 0.218	6.46 ± 0.370[d]	6.63 ± 0.197[d]	6.29 ± 0.112[d]
total BA	11.65 ± 0.394	12.89 ± 0.638[d]	12.47 ± 0.289[d]	12.11 ± 0.364
		Feces		
primary BA	4.64 ± 0.385	6.91 ± 0.378[d]	6.12 ± 0.230[d]	6.23 ± 0.432[d]
secondary BA	8.17 ± 0.246	7.01 ± 0.406[d]	7.34 ± 0.315[d]	6.83 ± 0.287[d]
total BA	12.81 ± 0.419	13.92 ± 0.628[d]	13.45 ± 0.436[b]	13.06 ± 0.677

[a] Values, given in micromoles per gram of dry matter, are means ± SEM; $n = 12$. Mean values were significantly different from control group as indicated by footnotes *b–d*. [b] $p < 0.05$. [c] $p < 0.005$. [d] $p < 0.001$.

A part of absorbed quercetin is methylated to isorhamnetin. In **Figure 6,** it is shown that approximately 1 nmol of isorhamnetin was excreted in the 16-h urine by the apple and grape group at week 2 and increased at week 4. Isorhamnetin excreted is related to approximately 0.2% of the consumed quercetin and quercetin glycosides.

Furthermore, the dihydrochalcone phloretin was present in the 16-h urine of the apple group (14 and 11 nmol at weeks 2 and 4, respectively). Flavanols such as (+)-catechin and its polymers were not found in urine samples, suggesting that they had not been absorbed. But, small amounts of some polyphenol metabolites such as 3.4-dihydroxyphenyl acetic acid and homovanillic acid, released by the bacterial transformation of polyphenols, appeared in urine of rats that received extraction juices from apples and grapes.

In contrast, no polyphenols were found in rats given the extraction juice from red beets as none were detected by HPLC in the juice as well. However, in most rats of this group the secondary plant metabolite betanin appeared in urine. The highest concentrations of betanin were found at week 2 (on average 29 nmol, approximately 0.5% of the consumed amount) (data not shown).

DISCUSSION

In the present study, nutritional effects of extraction juices from apples, grapes, and red beets were evaluated in rats. During the last years, a two-step procedure for production of fruit and vegetables juices was investigated: After separation of the so-called classic premium juices, the pomaces were diluted with water and then treated with a complex commercial enzyme mixture (pectinases and cellulases) before separation of the so-called extraction juices (*20*, *25*). Under the conditions applied, higher concentrations of secondary plant metabolites and of dietary fiber components were transferred into the corresponding extraction juices (*21*, *25*, *26*). The composition of secondary plant metabolites and polysaccharides in such extraction juices were reported previously (*21*, *24*). In Germany and Europe, the use of cellulases is not allowed for fruit juices, whereas cellulases are allowed for vegetable juices according to German guidelines. This means that the extraction juices from apple and grape pomace are not fruit juices but products "sui generis".

Apple and grape products are used worldwide in great amounts in human nutrition. Both are excellent sources of flavonoids and other secondary plant metabolites. Furthermore, red beets were chosen as a vegetable source material for the production of extraction juice in this study. The major secondary plant metabolites of red beets are different from those of most fruits and vegetables. They contain the betalains, which can also act as antioxidants (*31*, *50*). Polyphenol-rich grape seed and grape skin extracts had no toxicologic significance in a subchronic study with rats (*33*). Likewise, Shoji et al. (*51*)

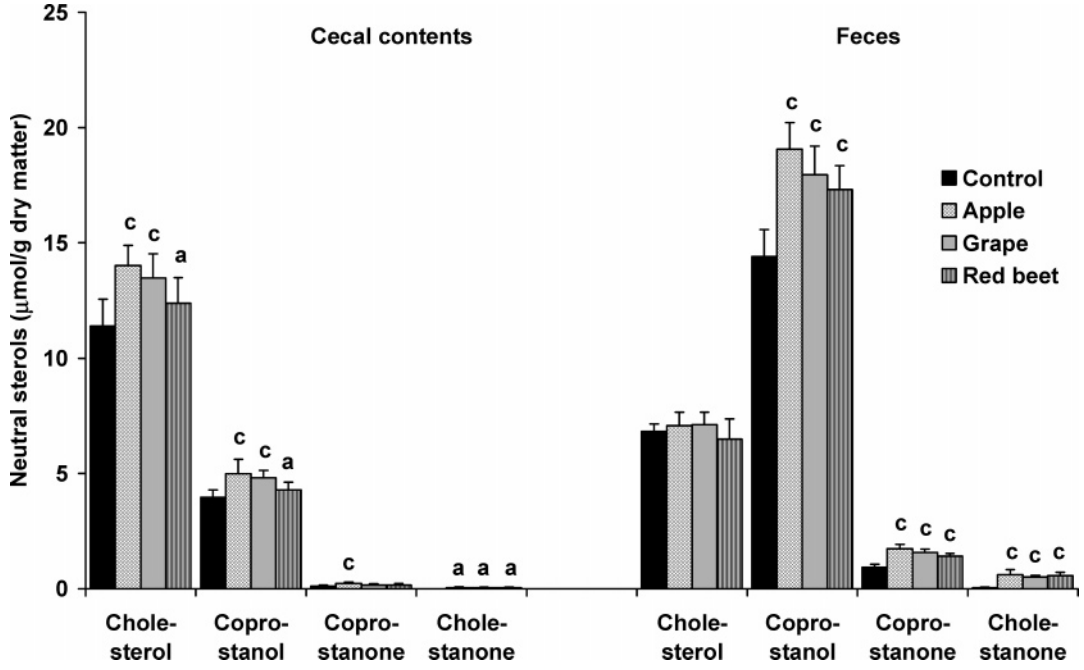

Figure 5. Neutral sterols (micromoles/gram of dry matter) in cecal contents and in fresh fecal materials of rats given water or extraction juices from apples, grapes, or red beets for 4 weeks. Values are means ± SEM; $n = 12$. Mean values were significantly different from control group: (a) $p < 0.05$; (c) $p < 0.001$.

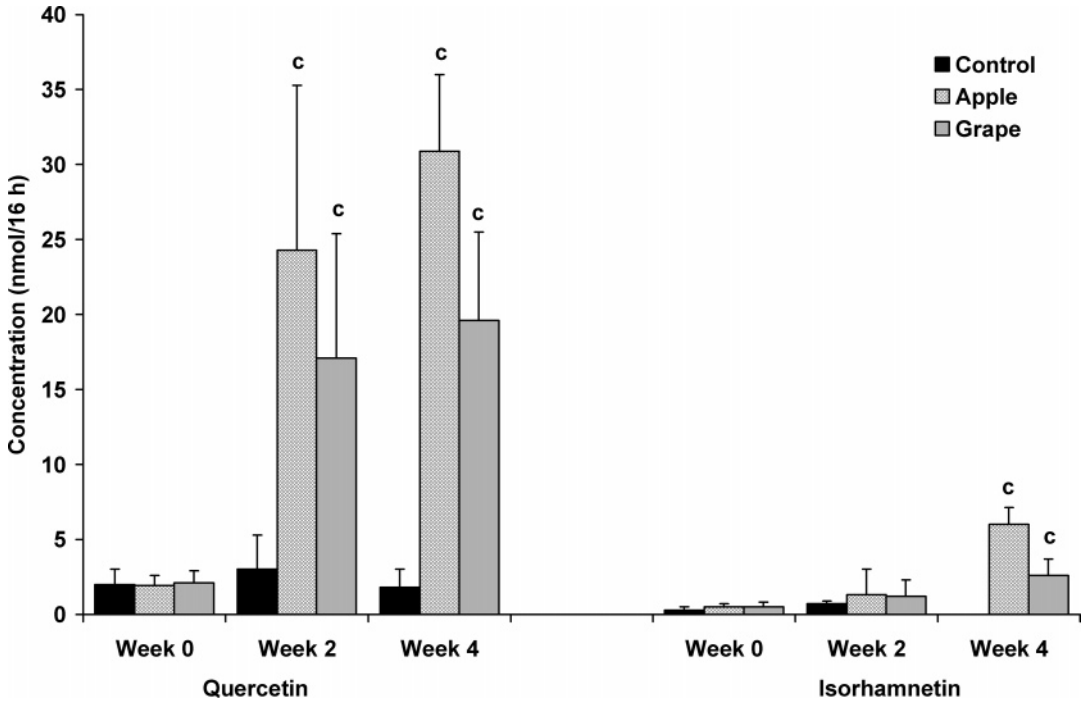

Figure 6. Total amounts of quercetin and isorhamnetin (nanomoles) in 16-h urine of rats given water or extraction juices from apples and grapes for 4 weeks. Values are means ± SEM; $n = 12$. Mean values were significantly different from control group: (c) $p < 0.001$.

confirmed that consumption of a commercial polyphenol extract is safe and nontoxic at average dietary levels.

Several effects of extraction juice administration on food and liquid intake of the rats were observed in the present study. Food intake tended to be lower in the test groups, especially at the end of the experimental period as compared to the controls. But the weight gain was comparable between all groups. On the other hand, the intake of the extraction juices was greater than the water intake by the control group, resulting also in a higher urine excretion in the test groups. Rats of the test groups probably compensated the higher caloric intake from the extraction juices by a reduced food intake. A similar effect was observed in experiments with clear and cloudy apple juices given with 1,2-dimethylhydrazine-treated rats (*52*). Nakamura and Tonogai (*53*) found a dose-dependent decrease of weight gain in rats given grape seed polyphenols.

Administration of the extraction juice from grape had no influence on the microbiota. However, in the apple group, an increase in *Lactobacillus* and *Bifidobacterium* counts was found ($p < 0.05$). An increase in *Bifidobacterium* was also observed in humans given an extraction juice from apples for 2 weeks (*42*) or given tea polyphenols (*54*).

There were only minor effects of the extraction juices on intestinal walls and contents. In the upper gut portions of the test groups, greater amounts of luminal contents were found than in controls, whereas no differences were observed for colonic contents. It was shown in several studies that the presence of dietary fiber in the diet and, therefore, also in the gut may result in higher masses of intestinal walls in rats due to thicker gut walls and changes in the proliferation behavior leading to healthier mucosa as an adaptation to higher viscosity of feces and/or as a result of an increase in luminally available SCFA (especially in the cecum). Furthermore, the motility increases since much more gut material must be transported through the lower intestinal tract.

In the present study, more acetate and total SCFA were detected in cecal contents of the test groups as well as in contents of distal colon and in feces of the apple group as a result of microbial fermentation of the dietary fiber components and polyphenols present in the extraction juices. Sembries et al. (*29*) found an increase of total SCFA, acetate, and propionate in cecal contents of rats fed diets containing 5% isolated colloids from extraction juices from apples. It is well-known that pectin polysaccharides, the major dietary fiber components in such extraction juices (*24*), are fermented preferentially with the formation of acetate (*15*, *55*, *56*). High concentrations of acetate and butyrate were also produced during fermentation of quercetin-3-glucoside with *Eubacterium ramulus* isolated from human feces (*57*). Therefore, in this case both dietary fiber and polyphenols may be responsible for the enhanced formation of SCFA. Likewise, Aprikian et al. (*56*) found an increase in the cecal SCFA pool in rats given apple pectin, a high polyphenol apple cider apple extract, or both. After application of the lyophilized apple material, significantly more butyrate was present in cecal contents of rats compared with controls (*58*).

Because of the higher concentrations of SCFA in the intestinal contents of the test groups, lower pH values were measured. This effect was also observed during experiments with rats that were fed diets containing colloids isolated from extraction juices (*29*) or pectin (*59*).

Due to interactions between BA and dietary fiber components in the small intestine, more BA were transported toward the lower parts of the intestinal tract in the test groups, resulting in higher concentrations of BA in the respective intestinal contents as found in the present study. As a consequence, more BA were excreted in these groups as well. However, the proportion of secondary BA was lower in the test groups. Secondary BA are products of microbial conversions (deconjugation, dehydroxylation, etc.). Additionally, the excretion and transport of NS through the intestinal tract was enhanced in rats that received extraction juices and resulted in an increase in cholesterol metabolites such as coprostanol. There are only few and contradictory data on the effects of polyphenol-enriched diets on the excretion of steroids in feces (*60*). Most studies were conducted with tea polyphenols. Thus, Nakamura and Tonogai (*53*) indicated that administration of 1 g of grape seed polyphenol/kg resulted in increased fecal excretion of BA and NS in normal rats. Aprikian et al. (*56*) found lower BA excretion in rats fed diets containing apple pectin, but excretion of cholesterol, coprostanol, and total steroids was increased in these rats. In another study, the same group (*58*) showed that administration of 15% lyophilized apple in the diet, containing 0.3% cholesterol, resulted in higher excretion of cholesterol and total steroids. Higher concentrations of BA and NS were also found in rats fed pectin (*59*). On the other hand, application of diets containing 5% colloids from apple extraction juice resulted in enhanced excretion of BA and NS in rats (*29*). However, the contents of polyphenols, pectin, or pectin-containing diets were certainly greater in the discussed study than in the present study. Recently, Sembries et al. (*42*) showed in a pilot study with healthy humans, however, that consumption of 1.4 L/day of an extraction juice from apples for 2 weeks resulted also in higher excretion of total and primary BA as well as of NS.

Intake, bioavailability, and excretion of polyphenols in humans were recently reviewed by Scalbert and Williamson (*61*). The daily intake has been calculated at 1 g/day. Polyphenol bioavailability is affected by chemical−structural and biochemical factors. Polyphenols not absorbed or excreted in the bile reach the lower parts of the intestinal tract, where they are extensively metabolized by the microflora into various biologically active aromatic acids (e.g., derivatives of phenylpropionic, phenylacetic, and benzoic acids) (*62*, *63*). Schneider et al. (*57*) isolated *Eubacterium ramulus* from human feces that was capable of degrading the aromatic ring system of quercetin-3-glucoside in vitro with the formation of 3,4-dihydroxyphenylacetic acid and SCFA. 3,4-Dihydroxyphenylacetic acid was also found in feces of gnotobiotic rats associated with *E. ramulus* after administration of quercetin-3-glucoside (*64*). The oral intake of flavonoids resulted in a dramatic increase of the fecal *E. ramulus* population in humans (*65*).

The urinary excretion of quercetin is small (*66*) but significant as shown by Young et al. (*67*). After consumption of 750−1500 mL of apple juice for 1 week, humans excreted in urine 0.29−0.47% of the plant metabolites consumed. Kanner et al. (*50*) calculated an amount of 0.5−0.9% of the ingested betanin in human urine after administration of 120 mg of betanin. In an older study, 3% of orally administered betanin appeared in urine of rats (*68*). Recently, Frank et al. (*69*) found a recovery of 0.28% of the consumed betalains in urine of healthy humans. In the present study, also low amounts of polyphenols and betalains were detected in urine. DuPont et al. (*70*) indicated that phloretin was excreted in the urine of humans after administration of cider and suggested that low doses of quercetin are extensively methylated in humans. In a study with humans who consumed extraction juice from apples, more quercetin and 3,4-dihydroxyphenylacetic acid were found in urine (*42*).

Recently, Barth et al. (*52*) reported that cloudy apple juice decreased the DNA damage, hyperproliferation, and aberrant crypt foci development in the distal colon of rats treated with 1,2-dimethylhydrazine. These effects were lower if clear apple juice was administered. Both apple juices contained comparable concentrations and types of monomeric and polymeric polyphenols, but the concentration of pectin was 4-fold higher in the cloudy juice. Therefore, it was speculated that additive and synergistic effects between polyphenols and pectins may be responsible for the high cancer-preventive properties of the cloudy apple juice. The extraction juices used during the present study also contained both polyphenols and dietary fiber components in higher concentrations. In some studies, not juices but higher concentrated polyphenol and dietary fiber materials were administered to rats. Thus, application of dietary fiber- and polyphenol-rich materials from grape pomaces resulted in an increased excretion of fat and protein in rats and in lower total and LDL cholesterol concentrations in hypercholesterolemic rats (*71*). Likewise, Aprikian et al. (*56*) showed in rats that apple pectin and polyphenols were more effective on the large intestine fermentation and lipid metabolism when fed combined together than when fed separately, suggesting that some interactions between the dietary fiber and polyphenol components of apples may exist.

In conclusion, the administration of extraction juices from apples, grapes, and red beets had several beneficial nutritional effects in rats. The extraction juice from apples seems to be the most effective of the three extraction juices tested here since most effects were observed in rats that were fed this juice. The dietary fiber- and flavonoid-enriched extraction juices may be a base for development of healthy and innovative products. Further studies with human subjects are needed to confirm our findings and to improve the developmental work for such innovative fruit juice products. Beside pectinases, the use of cellulases might be advantageous also for the fruit juice industry.

ABBREVIATIONS USED

BA, bile acid; CA, cholic acid; CDCA, chenodeoxycholic acid; CFU, colony-forming unit; DCA, deoxycholic acid; DM, dry matter; HDCA, hyodeoxycholic acid; KDCA, 7-ketodeoxycholic acid; KLCA, 12-ketolithocholic acid; LCA, lithocholic acid; MCA, muricholic acid; NS, neutral sterol; SCFA, short-chain fatty acid; TCA, taurocholic acid; TCDCA, taurochenodeoxycholic acid; TDCA, taurodeoxycholic acid; UDCA, ursodeoxycholic acid.

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