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Dietary fiber–rich colloids from apple pomace extraction juices do not affect food intake and blood serum lipid levels, but enhance fecal excretion of steroids in rats

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Abstract

The aim of this study was to investigate the effects of colloids isolated from apple pomace extraction juices (so-called B-juices) produced by enzymatic liquefaction on food intake, levels of blood serum lipids, and fecal excretion of bile acids (BA) and neutral sterols (NS) *in vivo*. Ten male Wistar rats per group were fed diets containing either no apple dietary fiber (DF) (control), a 5% supplementation with juice colloids, or an alcohol-insoluble substance (AIS) from apples for 6 weeks. Apple DF in diets led to lower weight gain in rats fed with B-juice colloids ($P < 0.05$). For these rats, food intake was not affected but was highest with feeding AIS (10% more than control) to cover energy requirements. The supplementation of diet with apple DF from extraction juices or AIS had minor effects on blood serum lipids. In rats fed either juice colloids or AIS, up to 30% (5.31 μmol/g dry weight) and 88% (7.69 μmol/g dry weight) more primary BA were excreted in feces, respectively, as compared to that in the control group (4.10 μmol/g dry weight) ($P < 0.05$). In cecal contents, a 15% (juice colloids) to 37% (AIS) increase in primary BA was found. In contrast, concentrations of secondary BA were lower in feces of test groups ($P < 0.05$). Excretion of total BA and NS was higher in rats fed apple DF ($P < 0.05$). Our study is the first to prove that there are beneficial physiologic effects of apple DF isolated from pomace extraction juices produced by enzymatic liquefaction. These results may help to develop such innovative juice products that are rich in DF of fruit origin for diminishing the lack of DF intake.

Keywords: Apple juice colloids; Pomace extraction; Blood lipids; Bile acids; Neutral sterols

1. Introduction

As indigestible food components, dietary fibers (DF) play an important role in nutrition because of their beneficial physiological effects. They are able to influence lipid metabolism, to bind water, and to increase viscosity as well as volume of intestinal contents and fecal weight. In the intestines, DF interact with micelles via binding of bile acids (BA), inhibiting digestive enzymes and absorption of certain nutrients [1]. Furthermore, DF have advantageous physiologic effects such as reducing serum levels of cholesterol and postprandial glucose as well as intestinal transit time by enhancing gut motility. In contrast to insoluble fiber sources, soluble forms of DF such as pectin are more effective in their cholesterol-lowering properties [2–4].

In our part of the world, apples and their products are the most common consumed fruits. Besides polyphenols, apples are a good source of DF. Until today, several studies have been made on DF from apples *in vivo*, for example, pectin [5–7] and pomace [8–10] and their physiologic effects on lipid metabolism. However, DF released into juice during apple juice production has not yet been examined *in vivo.*

In addition, DF-rich juices offer a beneficial alternative of DF-poor juices. Such physiologically valuable juice products were obtained using liquefying enzymes, *e.g.,* pectinases and cellulases, for production of apple juice in a two-step process [11]. After a common pectolytic mash treatment in the first step and the separation of premium juice (A-juice), the remaining pomace was extracted a second time with cellulases and/or pectinases. Besides higher juice yields, resulting juices contained higher amounts of polyphenols and up to 10 times more DF [11–13]. DF-rich colloids from so-called pomace extraction juices (B-juices) have been structurally characterized before [14].

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Until now, no data about physiologic effects of such produced pomace extraction juices as well as their DF-containing juice colloids have been published. Therefore, we first investigated and describe the effects of corresponding B-juice colloids on lipid metabolism. In the present study, DF-rich colloids from B-juices were isolated and physiologically examined in rats. Besides blood lipid parameters, food intake, body weight, and fecal excretion of sterols were monitored. An AIS prepared from apples with almost intact cell wall structure served as a counterpart to soluble juice colloids released during enzymatic treatment of apple pomace and was also tested.

2. Methods and materials

2.1. Materials

Juice colloids were isolated from apple pomace extraction juices 1B and 4B produced by enzymatic liquefaction [11] using preparative ultrafiltration (Bucher, Germany, cut-off 18,000) and subsequent alcohol precipitation (1 part retentate + 5 parts 96% ethanol). Colloids were then dried at 60°C. Extraction juice 1B was produced solely by water extraction of pomace without any additional enzymes, whereas juice 4B was obtained after a pectolytic and cellulolytic pomace treatment. Alcohol-insoluble substance (AIS) was prepared from freshly harvested apples (variety Boskoop, Werder Frucht, Glindow, Germany). In portions, 45 kg of washed apples with skins and cores were crushed into small pieces in two parts of 96% ethanol using a blender and an Ultra-Turrax T25 (Jahnke & Kunkel, IKA Labortechnik, Germany) and boiled under reflux for 15 minutes. The liquid phase was removed by suction, and the remaining residue was washed with 65% ethanol and extracted again. It was then sequentially dehydrated with 65%, 80%, and 96% ethanol followed by acetone. The vacuum-dried AIS was milled to a particle size of ≤0.5 mm. The total DF content of juice colloids 1B obtained in retentate was 56.9% (54.3% soluble, 2.6% insoluble DF), of juice colloids 4B in retentate it was 80.1% (78.3% soluble, 1.8% insoluble DF), and of AIS it was 96.2% (22.9% soluble, 73.3% insoluble DF) as determined by Association of Official Analytical Chemists method [15].

2.2. Animals and diets

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 No. L8-3560-0/3). Male Wistar rats (Shoe-Wistar; Tierzucht Schönwalde, Schönwalde, Germany) having weights of 177 ± 4 g were randomly divided into four groups of 10 animals each and were kept in a temperature-controlled environment (22 ± 2°C) with 12-hour light, 12-hour dark cycle. After adapting to control diet for a period of 1 week, the three test groups were fed a diet supplemented with either DF-rich colloids isolated from extraction juice 1B or 4B or with AIS from apples for 6 weeks (Table 1). The control group rats were maintained on the control diet. Rats had free access to water and to their respective diets. Food consumption and body weight were monitored weekly.

Table 1
Composition of the experimental diets (g/kg diet)

Ingredient	Diet			
	Control	Colloids 1B	Colloids 4B	AIS
Casein*	200	200	200	200
Wheat starch†	630	580	580	580
Sunflower oil‡	50	50	50	50
Microcrystalline cellulose§	50	50	50	50
Mineral mixture¶	50	50	50	50
Vitamin mixture¶	20	20	20	20
Colloids 1B	—	50	—	—
Colloids 4B	—	—	50	—
AIS	—	—	—	50
DF content (g/100 g dry weight)‖				
Total	6.2	9.8	8.4	12.7
Soluble	0.2	4.0	1.7	2.4
Insoluble	6.0	5.8	6.7	10.3

* Hermann Kröner GmbH Co. KG, Ibbenbüren, Germany.
† Bayerische Milchindustrie eG, Landshut, Germany.
‡ Plus Vertriebs GmbH, Mühlheim/Ruhr, Germany.
§ J. Rettenmaier & Söhne GmbH & Co., Ellwangen-Holzmühle/Germany.
¶ Altromin GmbH, Lage, Germany.
Mineral mixture (mg/kg): Ca (146070.34), P (97355.04), Mg (8784.27), Na (39229.41), K (116496.45), S (10535.81), Cl (63510.38), Fe (2931.22), Mn (1733.86), Zn (387.54), Cu (85.21), I (7.50), Mo (3.31), F (70.08), Se (3.84), Co (2.06), Al (0.07). Vitamin mixture (mg/kg): vitamin A (750000 IU), vitamin D_3 (25000 IU), vitamin E (7500), vitamin K_3 (500), vitamin B_1 (1000), vitamin B_2 (1000), vitamin B_6 (750), vitamin B_{12} (1500), nicotinic acid (2500), pantothenate (2500), folic acid (500), biotin (10), choline chloride (50000) *p*-aminobenzoic acid (5000), inosit (5000), vitamin C (1000), methionine (173250).
‖ As determined by AOAC method [15].
AIS = alcohol-insoluble substance.

2.3. Sampling procedures

On weeks 0, 3, and 6 of the experimental period, blood samples were taken from eye plexus of ether-anesthetized rats, after a 16-hour fast, for analysis of serum lipids. For the determination of BA and NS in fecal samples on weeks 0, 2, 4, and 6, rats were separated and feces was collected for 24 hours. After 6 weeks of diet, rats were killed and luminal contents from cecum and colon were removed and analyzed for BA and NS as well. Wet and dry weights and pH values of intestinal contents were also determined.

2.4. Analytical procedures

Triglycerides, total cholesterol, and HDL- and LDL-cholesterol were determined enzymatically in blood serum

Table 2
Effect of colloids isolated from apple pomace extraction juices produced by enzymatic liquefaction and of alcohol-insoluble substance (AIS) from apples on pH values and total wet and dry weight (g) of intestinal contents in rats

Diet	Cecum			Colon*		
	pH Value	Wet Weight	Dry Weight	pH Value	Wet Weight	Dry Weight
Control	7.2 ± 0.1	2.81 ± 0.40	0.72 ± 0.11	6.8 ± 0.1	0.75 ± 0.40	0.41 ± 0.16
Colloids 1B	6.9 ± 0.2†	3.76 ± 0.46†	0.91 ± 0.10†	6.6 ± 0.1†	0.94 ± 0.35	0.45 ± 0.10
Colloids 4B	7.0 ± 0.2†	3.60 ± 0.51†	0.88 ± 0.14†	6.6 ± 0.1†	1.15 ± 0.26†	0.59 ± 0.12†
AIS	6.6 ± 0.2†	6.51 ± 0.99†	1.40 ± 0.34†	6.3 ± 0.1†	0.81 ± 0.44	0.35 ± 0.17

Values are means ± SD for 8–10 animals per group.
* Data refer to distal colon.
† Different from control ($P < 0.05$).

samples using commercial kits (Olympus Diagnostica GmbH, Hamburg, Germany). BA and NS were analyzed by high-performance liquid chromatography and high-performance thin-layer chromatography, respectively. Preparation of fecal samples for analysis of BA and NS as well as analytical conditions were performed as previously described [16].

2.5. Statistical analysis

Results are expressed as mean ± SD. Data were analyzed by an unpaired Student's t test. Values of $P < 0.05$ were considered to be significant.

3. Results

3.1. Food intake and body weight

During the entire 6-week experimental period, food intake was 22.3 ± 1.6 g/day, 20.4 ± 4.3 g/day, 20.2 ± 4.8 g/day, and 24.0 ± 2.8 g/day for rats fed the control diet, juice colloids 1B or 4B, and AIS, respectively. Body weight increased in all groups (mean 180 ± 19 g). Weight gain was highest in the control group (207 ± 31 g) and lowest in the group given juice colloids 4B (156 ± 19 g). Test groups fed diets supplemented with juice colloids 1B and AIS increased their body weight of 170 ± 26 g and 189 ± 17 g within 6 weeks.

3.2. Total intestinal contents and pH values

In rats fed with apple DF, higher amounts of cecal contents were found, which differed in wet and dry weights from those of control animals ($P < 0.05$) (Table 2). Supplementation with colloids 4B also resulted in increased contents in the distal colon. DF from apples led to lower luminal pH values in the cecum and colon ($P < 0.05$). The lowest pH values were measured in animals given the AIS-containing diet.

3.3. Serum lipids

A 5% dietary supplementation with apple DF from extraction juices or AIS for 6 weeks had minor effects on serum lipid levels. Only in rats fed the diets with colloids isolated from extraction juice 4B did total cholesterol and HDL-cholesterol slightly decrease to 1.65 ± 0.30 mmol/L and 0.58 ± 0.07 mmol/L, respectively, as compared to those of control animals (total cholesterol 2.09 ± 0.34 mmol/L, HDL-cholesterol 0.70 ± 0.06 mmol/L), whereas LDL-cholesterol slightly increased (Table 3). HDL-cholesterol levels of the test group fed a diet supplemented with 1B colloids were comparable to those of the group given 4B colloids (0.63 ± 0.07 mmol/L). However, serum lipid levels remained within the normal range for all groups.

Table 3
Effect of colloids isolated from apple pomace extraction juices produced by enzymatic liquefaction and of alcohol-insoluble substance (AIS) from apples on serum lipids in rats

Serum Lipids (mmol/L)	Diet			
	Control	Colloids 1B	Colloids 4B	AIS
Triglycerides				
Week 0	0.93 ± 0.35	0.88 ± 0.26	0.99 ± 0.25	1.03 ± 0.32
3	0.76 ± 0.26	0.95 ± 0.26	1.00 ± 0.25	0.77 ± 0.17
6	0.95 ± 0.43	1.04 ± 0.27	1.08 ± 0.28	0.69 ± 0.25
Total cholesterol				
Week 0	2.14 ± 0.38	2.38 ± 0.50	2.12 ± 0.31	2.23 ± 0.36
3	2.14 ± 0.49	1.86 ± 0.31	1.62 ± 0.21	1.96 ± 0.36
6	2.09 ± 0.34	1.86 ± 0.27	1.65 ± 0.30*	1.86 ± 0.25
HDL				
Week 0	0.68 ± 0.13	0.83 ± 0.12	0.82 ± 0.07	0.69 ± 0.11
3	0.70 ± 0.06	0.71 ± 0.09	0.65 ± 0.08	0.67 ± 0.09
6	0.70 ± 0.06	0.63 ± 0.07*	0.58 ± 0.07*	0.70 ± 0.12
LDL				
Week 0	1.37 ± 0.27	0.81 ± 0.30	0.95 ± 0.26	1.16 ± 0.37
3	1.15 ± 0.28	1.15 ± 0.47	1.35 ± 0.37	0.87 ± 0.48
6	1.06 ± 0.43	1.02 ± 0.46	1.50 ± 0.29*	1.07 ± 0.19

Values are means ± SD for 10 animals per group.
* Different from control after 6 weeks of respective diet ($P < 0.05$).

Table 4
Effect of colloids isolated from apple pomace extraction juices produced by enzymatic liquefaction and of alcohol-insoluble substance (AIS) from apples on fecal excretion of bile acids (BA) after 6 weeks of diet

Intestinal content/Diet	Primary BA μmol/g Dry Weight								Secondary BA μmol/g Dry Weight				
	TCA	CA	7KDCA	TCDCA	CDCA	UDCA	α-MCA	β-MCA	TDCA	DCA	12KLCA	LCA	HDCA
Cecum													
Control	0.84	0.72	0.03	0.11	0.57	0.15	1.26	1.29	0.76	0.58	0.09	1.58	2.13
Colloids 1B	0.78*	0.81*	0.04	0.11	0.62*	0.16	1.49*	1.73*	0.70*	0.71*	0.09	1.93*	1.60*
Colloids 4B	0.78*	0.79*	0.04	0.11	0.59	0.18*	1.53*	1.68*	0.75	0.75*	0.10	1.86*	1.64*
AIS	0.86	0.96*	0.05*	0.11	0.65*	0.16	1.91*	2.09*	0.80	0.87*	0.11	1.52	1.65*
Colon†													
Control	0.16	0.34	0.09	0.06	0.42	0.19	1.63	1.23	0.15	1.85	0.86	1.77	2.78
Colloids 1B	0.09*	0.83*	0.20*	0.07	0.65*	0.18	1.88*	1.67*	0.09*	1.40*	0.73*	1.79	2.25*
Colloids 4B	0.12*	0.87*	0.18*	0.04	0.65*	0.22	1.90*	1.46*	0.07*	1.44*	0.76*	1.77	2.57*
AIS	0.11*	1.43*	0.35*	0.03*	0.74*	0.17	2.68*	1.98*	0.05*	1.44*	0.74*	1.46*	1.63*
Feces													
Control	n.d.	0.42	0.11	n.d.	0.44	0.20	1.69	1.24	n.d.	2.06	0.94	1.88	2.82
Colloids 1B	n.d.	0.83*	0.20*	n.d.	0.61*	0.17*	1.98*	1.52*	n.d.	1.61*	0.97	1.71*	2.50*
Colloids 4B	n.d.	0.76*	0.19*	n.d.	0.67*	0.21	2.03*	1.45*	n.d.	1.77*	0.89	1.72*	2.75*
AIS	n.d.	1.51*	0.34*	n.d.	0.92*	0.14*	2.83*	1.95*	n.d.	1.52*	0.87*	1.42*	1.85*

Values are means for 10 animals.
* Different from control ($P < 0.05$).
† Data refer to distal colon. n.d. = not detected. TCA = taurocholic acid; CA = cholic acid; 7KDCA = 7-ketodeoxycholic acid; TCDCA = taurochenodeoxycholic acid; CDCA = chenodeoxycholic acid; UDCA = ursodeoxycholic acid; MCA = muricholic acid; TDCA = taurodeoxycholic acid; DCA = deoxycholic acid; 12KLCA = 12-ketolithocholic acid; LCA = lithocholic acid; HDCA = hyodeoxycholic acid.

3.4. BA in intestinal contents and feces

In fecal samples, a wide spectrum of BA were found, which consisted mainly of α- and β-muricholic acid (MCA), hyodeoxycholic acid (HDCA), deoxycholic acid (DCA), and lithocholic acid (LCA). Although the pattern of BA was found similar for all animal groups, distinct differences in individual BA were detected (Table 4). Apple colloids isolated from extraction juices as well as the AIS caused an increase in the excretion of primary BA after 6 weeks of diet ($P < 0.05$). In feces of rats fed with either juice colloids 1B or 4B and AIS 30% (5.31 μmol/g dry weight) and 88% (7.69 μmol/g dry weight) more primary BA were found, respectively, as compared to control (4.10 μmol/g dry weight) (Fig. 1). Similar proportions were obtained for primary BA in colonic contents. In cecal contents, ~15 and 37% higher amounts of primary BA were detected for juice colloid– and AIS-containing diets, respectively. The main primary BA consisted of α- and β-MCA, cholic acid (CA), and chenodeoxycholic acid (CDCA). Tauro-conjugated BA were present only in luminal contents of cecum (15–17%) and colon (1.5–3%), especially conjugates of CA, CDCA, and DCA. In contrast, the microbial transformation of primary into secondary BA (DCA, LCA, HDCA) was reduced in feces of all three test groups (colloids 1B: −12%; colloids 4B: −7%; AIS: −25%) ($P < 0.05$). Percentages of secondary BA in feces were in correspondence with those found in colonic samples. In cecum, a decrease in secondary BA of ~1–2% (juice colloids) and 4% (AIS) was observed. However, excretion of total BA was also higher in the test groups ($P < 0.05$) (Fig. 2). With isolated B-juice colloids in diet higher concentrations of total BA were excreted (~3% for 1B, 5% for 4B), even ~13% more with the AIS-containing diet. In all rat groups, ~70% of the excreted BA belonged to the chenodeoxycholic acid family and 30% to the cholic acid family.

3.5. NS in feces and intestinal contents

As a second group of steroids, NS were determined in feces and intestinal contents. Levels of total and individual NS in feces of control animals remained stable during the entire experimental period (~20 μmol/g dry weight),

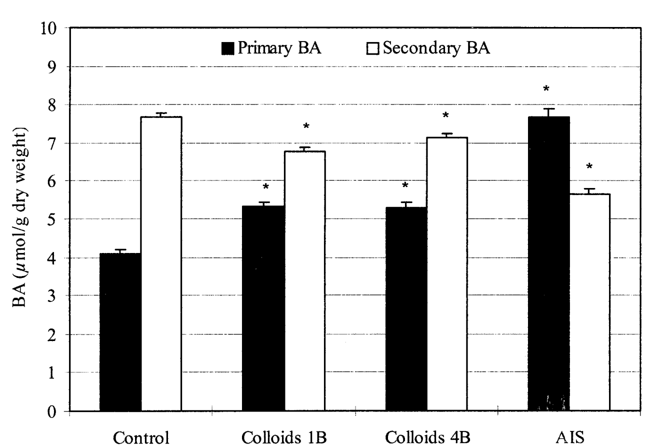

Fig 1. Excretion of primary and secondary bile acids (BA) in feces of rats fed control or 5% apple dietary fiber (DF) diet (colloids 1B or 4B from extraction juices or alcohol-insoluble substance [AIS]) for 6 weeks. *Different from control ($P < 0.05$).

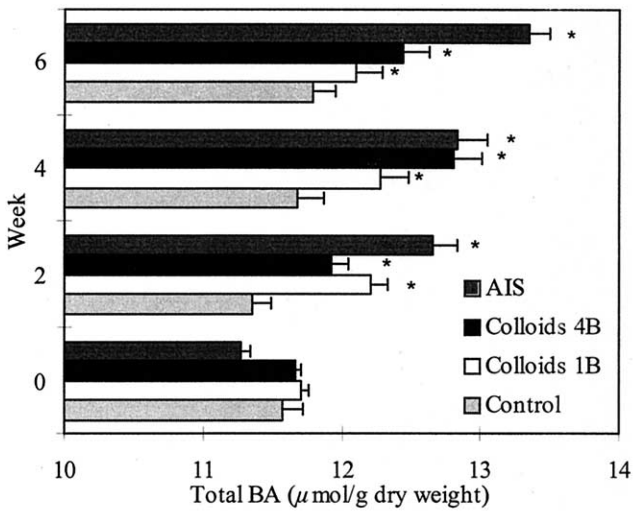

Fig 2. Excretion of total bile acids (BA) in feces of rats fed control or 5% apple dietary fiber (DF) diet (colloids 1B or 4B from extraction juices or alcohol-insoluble substance [AIS]) for 6 weeks. *Different from control ($P < 0.05$).

whereas excretion of NS increased in test groups fed with apple DF ($P < 0.05$) (Table 5). During intestinal passage, cholesterol was microbially converted into coprostanol, which was the dominating NS in colonic and fecal material. Higher coprostanol concentrations of 21–27% were found in feces of test groups as compared to control. Absolute concentrations of cholesterol, cholestanon, and coprostanon were also raised in intestinal samples of apple DF groups; however, to a lesser extent. Almost no cholestanon was detected in cecal contents. In the cecum, ~75% of NS belonged to cholesterol, in colon 29–38% and in feces 25–29%.

4. Discussion

In this study, we first describe the physiologic effects of DF-rich colloids isolated from apple pomace extraction juices. Juice colloids contained ~57% and ~80% DF which had mainly soluble character according to the AOAC method [15]. Most of these soluble DF components belonged to the pectin fraction [14]. Besides isolated colloids from extraction juices tested, an AIS prepared from apples was investigated representing the counterpart of intact cell wall architecture. It contained mainly insoluble DF (~73%).

Apple DF in experimental diets led to lower weight gain, which was significant for animals fed with B-juice colloids ($P < 0.05$). Similar results were observed in Wistar rats fed a guar gum diet consisting of 75% soluble DF [17]. Lower weight gain with apple DF was also shown in obese Zucker rats [18]. When feeding AIS highest food intake was observed (10% more than control) for covering energy requirements. This diet contained almost twice as much DF than control, mainly consisting of insoluble cell wall material such as cellulose. In rats, a higher food consumption for diets supplemented with cellulose was also reported in previous studies [19,20]. However, a ~55 (colloids 1B) or 36% (colloids 4B) higher DF content in diets did not affect food intake. Bravo *et al.* [10] found no difference in food consumption as well as in weight gain in rats when given a diet

Table 5
Effect of colloids isolated from apple pomace extraction juices produced by enzymatic liquefaction and of alcohol-insoluble substance (AIS) from apples on fecal excretion of neutral sterols (NS) after 6 weeks of diet

Intestinal Content/Diet	μmol/g Dry Weight				
	Cholesterol	Coprostanol	Cholestanon	Coprostanon	Total NS
Cecum					
Control	13.99 ± 0.60	4.08 ± 0.15	n.d.	0.26 ± 0.06	18.33 ± 0.65
Colloids 1B	14.43 ± 0.44	4.49 ± 0.36*	n.d.	0.24 ± 0.05	19.16 ± 0.59*
Colloids 4B	14.40 ± 0.37	4.42 ± 0.14*	0.01 ± 0.01	0.23 ± 0.05	19.06 ± 0.34*
AIS	15.18 ± 0.54*	4.69 ± 0.16*	0.01 ± 0.01	0.29 ± 0.05*	20.17 ± 0.44*
Colon†					
Control	7.45 ± 0.10	9.98 ± 0.20	0.21 ± 0.04	1.99 ± 0.08	19.63 ± 0.24
Colloids 1B	7.53 ± 0.24	12.23 ± 0.34*	0.46 ± 0.08*	3.15 ± 0.17*	23.37 ± 0.51*
Colloids 4B	7.76 ± 0.16*	12.32 ± 0.23*	0.51 ± 0.08*	2.83 ± 0.14*	23.42 ± 0.31*
AIS	7.31 ± 0.17	13.45 ± 0.21*	0.66 ± 0.06*	3.40 ± 0.18*	24.82 ± 0.19*
Feces					
Control	5.82 ± 0.08	11.37 ± 0.66	0.65 ± 0.04	2.49 ± 0.08	20.33 ± 0.70
Colloids 1B	6.42 ± 0.12*	14.06 ± 0.62*	0.65 ± 0.03	3.05 ± 0.08*	24.18 ± 0.67*
Colloids 4B	6.31 ± 0.15*	13.74 ± 0.71*	0.73 ± 0.03*	3.08 ± 0.09*	23.86 ± 0.77*
AIS	6.35 ± 0.16*	14.40 ± 0.51*	0.81 ± 0.04*	3.59 ± 0.19*	25.15 ± 0.59*

Values are means ± SD of 10 animals per group.
* Different from control ($P < 0.05$).
† Data refer to distal colon.
n.d. = not detected.

with a 10% supplementation of apple pulp or without additional DF.

A 5% supply of DF from apples in experimental diets did not affect lipid levels in serum. Only levels of cholesterol had the property to decrease with these diets. Being soluble DF, pectin has serum cholesterol–lowering properties [2], also in normal lipidemic rats [6,7]. On the other hand, a 5% or 7% pectin supplementation of diet was sufficient to decrease cholesterol in serum and liver, whereas in alimental hypercholesterolemic rats fed diets with 5% pomace from apples [8] or from tomato or grapes [9] almost no effects were observed except for cholesterol in liver. Serum cholesterol levels only decreased when adding 15% of pomace.

Feeding DF from apples led to a higher water binding of chymus, especially in rats fed with AIS, as indicated by dry weights of cecal contents. In contrast to our findings, feeding 10% apple pulp from apples resulted in higher dry and wet weights of feces as well [10]. In the lower parts of intestine such as the colon, only minor differences in dry and wet weights were detected.

Furthermore, excretion of primary BA (CDCA, CA, αMCA, βMCA) in feces was increased after the apple DF diet for 6 weeks ($P < 0.05$), whereas concentrations of secondary BA (DCA, HDCA) decreased ($P < 0.05$). Secondary BA are discussed as potential promoters in colon cancerogenesis [21]. Because of bacterial DF fermentation, the activity of responsible converting enzymes is reduced at lower fecal pH values, *e.g.*, the conversion of CDCA into LCA by 7α-dehydroxylase [22]. However, at the main site of bacterial fermentation in rats—the cecum-, lower pH values were obtained with DF from apples. Additionally, higher amounts of total BA were found in feces of these animals ($P < 0.05$). Distribution of BA was ~70% CDCA family and ~30% CA family. The soluble DF component pectin is known to interact with BA under conditions present in the small intestine [23] resulting in an enhanced transport of BA into the lower intestinal parts. Pectin increases fecal excretion of BA, influences hepatic synthesis of BA and cholesterol, and finally reduces levels of serum cholesterol [1,6,7,24]. During passage through intestine, tauro-conjugated BA were completely deconjugated by enzymes of the intestinal microflora. Only in contents of the upper gut parts, some tauro-conjugates were found.

Similar effects were observed for NS. Total NS and coprostanol concentrations were higher in rats fed apple DF-containing diets. Pectin from apples is known to increase cholesterol excretion in feces [7,24]. As a result of microbial transformations, greater amounts of its metabolites coprostanon (cecum, colon) and cholestanon (colon) were found in intestinal contents and feces of rats with apple DF diets. Absorption of BA and NS is partially inhibited due to interactions between DF (pectin) and BA. As a consequence, the pool of BA as well as lipid metabolism lack cholesterol, which is mobilized from depot or synthesized *de novo* [25]. Therefore, reduction of serum cholesterol constitutes a positive side effect of consuming DF-rich diets.

In this study, we found several *in vivo* effects of DF isolated from apple pomace extraction juices produced by enzymatic liquefaction in rats. Our study provides results on beneficial physiologic effects on cholesterol metabolism, which make such DF-rich extraction juices promising healthy and innovative fruit products. Furthermore, apple pomace extraction juices produced with the use of cellulases are enriched with DF of apple origin and may help to diminish the lack of daily recommended DF intake of at least 30 g.

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