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Chemical Composition of Natural and Polyphenol-free Apple Pomace and the Effect of This Dietary Ingredient on Intestinal Fermentation and Serum Lipid Parameters in Rats

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ABSTRACT: Unprocessed pomace containing 61% of dietary fiber (DF) and 0.23% of polyphenols (PP) and ethanol- or ethanol/acetone-extracted pomaces containing 66% DF and 0.10% PP and 67% DF and 0.01% PP, respectively, were subjected to a 4 week study in rats. The aim of the study was assessing the advantages of dietary supplementation with the above pomaces. To measure the animal response to dietary treatments, parameters describing cecal fermentation and lipoprotein profile were assessed. The dietary use of 5% unprocessed pomace caused an increase in cecal short-chain fatty acid (SCFA) production and a decrease in blood triacylglycerols, leading to a drop in serum atherogenic index. Ethanol-extracted pomace increased the glycolytic activity of cecal microbiota and decreased cecal branched-chain fatty acid production, whereas acetone extraction led to lower cecal ammonia concentration, decreased colonic pH value, and higher HDL/total cholesterol ratio. The variations in the atherogenic index indicate flavonoids as the key pomace component in relation to blood lipid profile benefits.

KEYWORDS: dietary fiber, apple pomace, apple polyphenols, rats, model diets

INTRODUCTION

The results of epidemiological studies indicate that frequent consumption of fruits and vegetables lowers the risk of lifestyle diseases such as cardiovascular disorders and cancer.[1−3] Dietary fiber and antioxidants (vitamins C and E, carotenoids, polyphenols) are the two nutritional components with a recognized role in the prevention of chronic diseases.[4]

Dietary fiber facilitates passage of material through the digestive tract, and it decreases postconsumption blood glucose and insulin levels. The products of microbiological fiber fermentation enhance intestinal health and lower total cholesterol and LDL levels.[5] Dietary fiber significantly contributes to the prevention and treatment of obesity, arteriosclerosis, cardiovascular diseases, large intestine cancer, and diabetes.[3,6] For that reason, the recommended daily allowance of fiber at 30−40 g[3] significantly exceeds the average intake, which ranges between 10 and 25 g.[3,7]

Dietary fiber is a matrix that contains phytocomponents, including polyphenols. For this reason, products of plant origin effectively prevent the risk of disorders caused by oxidative stress, such as cancer, cardiovascular diseases, and degenerative diseases.[4] Unlike vitamins C and E, which are absorbed in the upper part of the digestive tract, polyphenols are antioxidants that are found in the bowel.[8] The presence of polyphenols in the bowel is believed to significantly minimize the risk of chronic diseases in consumers who show a preference for whole-grain products.[9] Fiber and polyphenol groups are valuable components of vegetables and fruits, including apples; nonetheless, they are consumed in insufficient quantities to deliver beneficial physiological effects. It has been scientifically proven that the daily consumption of three apples (400−600 g) results in a mere 5−8% decrease in cholesterol levels.[10] The above findings motivate research into products that deliver greater quantities of valuable nutrients than fresh fruit. Apple pomace, a byproduct of apple juice pressing, is such a product.[11] Treated and processed dietary fiber preparations obtained from fruit pomace contain at least 50% dietary fiber, and its quantity varies subject to the polyphenol content of the applied raw material.[11] The composition and the physicochemical properties of dietary fiber in products are determined by the conditions of enzymatic and chemical fruit treatment.[12]

Contemporary juice production involves mash enzymation as an essential part of the clear juice technology. The use of enzymes that degrade native pectin and reduce juice viscosity is necessary to increase juice yield and maximize production efficiency. Pectinolytic enzymes change the polysaccharide composition of the cell wall, and the pectin content of the resulting apple pomace is halved in comparison with the pomace produced without enzymation.[13] The chemical composition of such products and their beneficial effect on the digestive tract and metabolism have not yet been fully recognized. The health-promoting effects of dietary fiber vary subject to its origin and physicochemical properties, such as solubility and water-binding capacity. Those properties affect fiber swelling and its availability for intestinal microorganisms.[14] The content and composition of polyphenol fractions released from the fiber matrix in the digestive tract are an equally important, but less recognized consideration.[15] The conditions of mash enzymation influence the polyphenol content and the antioxidant activity of clear apple juice.[16] As demonstrated by unpublished results of our study, the

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use of industrial enzymes in doses recommended for mash treatment does not result in the hydrolysis of quercetin glycosides, the most abundant polyphenols in apples. The highest quercetin glycoside concentrations are found in the peel, which is why pressed pomace is a rich source of those polyphenols. The discussed compound groups demonstrate both synergic and antagonistic action in the digestive tract and the metabolic system.[17] The above findings validate further research into the content and composition of polyphenol fractions in plant material. Research results could support the production of dietary fiber preparations, including apple pomace and polyphenol extracts.[18]

The objective of this study was to assess the composition and physiological properties of dietary fiber preparations obtained from apples, which are characterized by various polyphenol concentrations. This research aimed to determine the effect of products with different polyphenol contents and compositions on the functioning of the digestive tract and the metabolic system. Two research hypotheses were verified with the use of rats as the research model: (1) industrial apple pomace, obtained in the juice production process with the involvement of pectinolytic enzymes, enhances digestive tract function,; and (2) decreased pomace pectin content does not affect the in vivo action of fiber and polyphenol complexes at the local and systemic level.

MATERIALS AND METHODS

Apple Pomace Preparation. Fiber and polyphenol preparations were obtained from industrial apple pomace produced in the 2007/2008 season in a modern juice production line at ALPEX (Łęczeszyce, Poland). Industrial pomace was subjected to three treatments to obtain natural apple fiber (AP): (1) convection drying at 70 °C; (2) screening on 3 × 3 mm sieves to separate seeds; and (3) grinding in a ball mill to a particle size below 0.5 mm. The fiber yield reached 78% on dried pomace.

Dietary fiber with average polyphenol content (APE) was obtained by two-step ethanol pre-extraction of ground natural fiber (granulation 0.5−2 mm). A 2 h extraction was performed using 15% ethanol solution (ratio 1:4) at 40 °C with the involvement of a previously described method.[18] In the second step of the procedure, a 50% ethanol solution was applied to perform the extraction (ratio 1:4). The residue was dried and ground in a similar manner as for natural fiber.

Postextraction fiber (APA) was obtained by removing alcohol-insoluble polyphenols (procyanidins) from APE fiber, which was extracted with 70% acetone in a 1:5 ratio. Extraction was carried at 25 °C for 24 h, and the fiber was dried in a convection dryer at ≤60 °C.

Composition Analysis of Apple Fiber and Polyphenol Preparations. *Protein, fat, dry matter, and ash content* were determined by AOAC methods:[19] protein content of fruit products, AOAC 920.152; fat (ether extract of plants), AOAC 930.09; dry matter−ash content of fruit, AOAC 940.26. *Total dietary fiber in foods* was determined according to AOAC 985.29. *Insoluble dietary fiber in food and food products* was determined according to AOAC 991.42. *Soluble dietary fiber in food and food products* was calculated as follows: soluble dietary fiber (SDF) = total dietary fiber (TDF) − insoluble dietary fiber (IDF). *NSP determination* was peformed through analysis by GC as alditol acetates after acid hydrolysis according to the method proposed by Englyst and Cummings.[20] The content of uronic acids was determined spectrophotometrically by using the *m*-hydroxydiphenyl assay as described in ref 21 with galacturonic acid as the external standard. *Simple sugars, sorbitol, and sucrose* were determined by adding 40 mL of water to 1.5 g of the sample with 0.1 g of $CaCO_3$, and the mixture was boiled for 3 min; it was cooled and qualitatively transferred to a 50 mL volumetric flask. The sample was filtered and desalted in an anion exchange column (2 parts of Amberlite IRA 400 anion exchanger to 1 part of Amberlite IR 120 cation exchanger). After centrifugation at 4800*g* for 5 min, the solution was analyzed in a Knauer HPLC set (Berlin, Germany) with an RI detector and a Bio-Rad Aminex HPX-87C column, 300 × 7.8 mm (Phenomenex, Torrance, CA), with a flow rate of 0.5 mL/min at a temperature of 85 °C. Glucose, fructose, sucrose, and sorbitol (Sigma, St. Louis, MO) were used as standards. *For polyphenol analysis by HPLC*, the extraction was carried as follows: 4 mL of the solvent (70% methanol) was added to 0.5 g of the sample, and the mixture was sonified for 15 min. After centrifugation at 10000*g*, the extract was separated, and the residue was twice extracted with 70% methanol (3 mL). The collected extracts were made up to 10 mL. The methanol extract was analyzed by HPLC as previously described[18] in a HPLC system with a DAD detector (Dionex, Sunnyvale, CA) and a Gemini 5u C18 110A column, 50 × 4.60 mm (Phenomenex). Eluent A was 0.05% phosphoric acid in water, and eluent B was 0.05% phosphoric acid in acetonitrile. The gradient program, applied at the flow rate of 1.25 mL/min, was as follows: stabilization with 4% B for 5 min, followed by 4−15% B for 5.00 to 12.50 min, 15−40% B for 12.5−42.40 min, 40−50% B for 42.40−51.80 min, 50% B for 51.80−53.40 min, and 50−4% B for 53.40−55.00 min. The column temperature was 25 °C. The polyphenol composition of the preparation was identified by comparison with the available standards: epicatechin, *p*-coumaric acid, chlorogenic acid, and phloridzin (all from Sigma-Aldrich

Table 1. Composition (Percent) of Experimental Diets

component	experimental group			
	C	AP	APE	APA
casein	20.0	20.0	20.0	20.0
methionine	0.3	0.3	0.3	0.3
soybean oil	10.0	10.0	10.0	10.0
cellulose	5.0			
apple pomace		5.0		
apple pomace after ethanol extraction			5.0	
apple pomace after acetone extraction				5.0
cholesterol	0.5	0.5	0.5	0.5
mineral mixture AIN-93G[a]	3.5	3.5	3.5	3.5
vitamin mixture AIN-93G[b]	1.0	1.0	1.0	1.0
fructose	30.0	30.0	30.0	30.0
maize starch	29.7	29.7	29.7	29.7

[a] AIN-93G,[23] per kg mix, g: calcium carbonate anhydrous (40.04% Ca), 357; potassium phosphate monobasic (22.76% P, 28.73% K), 196; potassium citrate and tripotassium monohydrate (36.16% K), 70.78; sodium chloride (39.34% Na, 60.66% Cl), 74; potassium sulfate (44.87% K, 18.39% S), 46.6; magnesium oxide (60.32% Mg), 24; ferric citrate (16.5% Fe), 6.06; zinc carbonate (52.14% Zn), 1.65; sodium metasilicate·$9H_2O$ (9.88% Si), 1.45; manganous carbonate (47.79% Mn), 0.63; cupric carbonate (57.47% Cu), 0.3; powdered sucrose, 221.026; chromium potassium sulfate·$12H_2O$ (10.42% Cr), 0.275; mg: boric acid (17.5% B), 81.5; sodium fluoride (45.24% F), 63.5; nickel carbonate (45% Ni), 31.8; lithium chloride (16.38% Li), 17.4; sodium selenate anhydrous (41.79% Se), 10.25; potassium iodate (59.3% I), 10; ammonium paramolybdate·$4H_2O$ (54.34% Mo), 7.95; ammonium vanadate (43.55% V), 6.6. [b] AIN-93G,[23] g/kg mix: nicotinic acid, 3.0; calcium pantothenate, 1.6; pyridoxine-HCl, 0.7; thiamin-HCl, 0.6; powdered sucrose, 974.655; riboflavin, 0.6; folic acid, 0.2; biotin, 0.02; vitamin B_{12} (cyanocobalamin, 0.1% in mannitol), 2.5; IU/g: vitamin E (*all-rac*-α-tocopheryl acetate, 500), 15.0; vitamin A (*all-trans*-retinyl palmitate, 500000), 0.8; vitamin D_3 (cholecalciferol, 400000), 0.25; vitamin K-1 (phylloquinone), 0.075.

Corp., St. Louis, MO) at 280 nm; quercetin rutinoside (Sigma); quercetin glucoside, quercetin galactoside, quercetin rhamnoside, and quercetin (all from Extrasynthese, Genay, France) at 360 nm; cyanidin glucoside (Extrasynthese) at 520 nm. *Total procyanidins (PC)* were determined by extracting the residue remaining after the methanol extraction of flavonoids, hydroxycinnamic acids, and dihydrochalcones sequentially with 70% acetone using the method applied to extract procyanidins. Total procyanidins were calculated by summing up procyanidin results in a vanillin test using methanol and acetone extracts. Total procyanidins were determined according to the previously described vanillin test.[22]

All analyses were performed at least in duplicate.

Rats and Diets. The experimental animals were used in observance of European guidelines for the care and use of laboratory animals upon the approval of the Ethical Committee for Animal Experimentation in Northeastern Poland. The experiment was performed on 32 male Wistar rats aged approximately 4 weeks (body weight = 265.3 ± 2.8 g). The experimental diets were administered for 4 weeks to eight rats per each group housed individually in plexiglass cages.

The administered diets had similar contents of protein (casein supplemented with methionine), fat (soybean oil), and minerals and vitamins (AIN-93G mixtures)[23] and similar contents of fiber, which originated from different sources: cellulose (control diet) and the analyzed fiber and polyphenol preparations (Table 1). The compared fiber preparations, including natural (AP), flavonoid-reduced (APE), and flavonoid-deprived preparations (APA), were administered in the amount of 5% of air-dried feed. Experimental diets and tap water were administered ad libitum. The animals were maintained under standard conditions at the temperature of 21−22 °C, relative air humidity of 50−70%, with intensive room ventilation (15×/h) and a 12 h lighting regimen. Individual body weights and food intakes were recorded.

Sample Collection and Analysis. After 4 weeks, the rats were anesthetized using sodium pentobarbitone. Blood samples were collected from the caudal vena cava. Serum samples were prepared by centrifugation at 1500*g* for 15 min at 4 °C, and they were stored at −40 °C for further analyses. After laparotomy, selected parts of the digestive tract (small intestine, cecum, colon) were removed and weighed. Directly after euthanasia (ca. 10 min), ileal, cecal, and colonic pH values were measured, tissue samples were collected to determine dry matter, ammonia, and SCFA contents, and the remaining material was frozen at −70 °C for the determination of protein content and microbial enzyme activity. The viscosity of the ileal digesta was measured. The ileal, cecal, and colonic walls were flushed clean with ice-cold saline, blotted on filter paper, and weighed to determine tissue weight. The liver and kidneys were separated, cleaned with ice-cold saline, blotted on filter paper, weighed, frozen in liquid nitrogen, and stored at −70 °C for subsequent assays of thiobarbituric acid reactive substances (TBARS).

Analysis of Gastrointestinal Function Parameters. Cecal pH was measured using a microelectrode and a pH/ION meter (model 301, Hanna Instruments, Vila do Conde, Portugal). The dry matter content of fresh digesta was determined at 105 °C. Ammonia was extracted and trapped in a boric acid solution, and it was analyzed by direct titration with sulfuric acid.[24] Short-chain fatty acid (SCFA) concentrations were measured by gas chromatography with the involvement of a previously described method.[25] Cecal SCFA pools were calculated as the product of SCFA concentrations and cecal digesta mass. The activity of microbial enzymes (α- and β-glucosidase, α- and β-galactosidase, β-glucuronidase) was measured on the basis of the rate of *p*-nitrophenol and *o*-nitrophenol release from nitrophenyl glucosides, and it was expressed in terms of micromoles of the product formed per hour per gram of cecal digesta or per gram of digesta.[26]

The viscosity of ileal digesta was determined as follows: small intestinal digesta was collected, mixed with a vortex, and centrifuged at 10000*g* for 10 min. The supernatant fraction (0.5 mL) was placed in a Brookfield LVDV-II+ cone−plate rotational viscometer (CP40; Brookfield Engineering Laboratories Inc., Stoughton, MA), and the viscosity of all samples was measured at the fixed temperature of 37 °C and a shear rate of 60 s^{-1}. Viscosity values were recorded as apparent viscosity.

Metabolic Index Analysis. Glucose, cholesterol, and triacylglycerol (TG) concentrations in the serum and the activity of alanine aminotransferase (ALT) and asparagine aminotransferase (AST) were determined using Alpha Diagnostics (Warsaw, Poland) and Pointe Scientific (Warsaw, Poland) commercial kits. TBARS were determined in line with the method described in ref 27.

The results were processed statistically by Duncan's multiple-range test at the significance level of $p \leq 0.05$ using Statistica 6.0 (StatSoft Corp., Kraków, Poland) software.

Table 2. Chemical Composition of Fiber−Phenolic Preparations (% w/w)

	preparation[a]		
ingredient	AP	APE	APA
dry matter	95.4 a	92.9 b	91.8 b
crude ash	1.1 a	1.8 b	1.6 b
crude protein	5.7 b	7.0 a	7.2 a
ether extract	2.0	2.5	2.0
total dietary fiber (TDF)	61.1 c	66.6 b	67.2 a
soluble dietary fiber (SDF)	7.6	8.7	8.7
other carbohydrates	*25.3* a	*14.9* b	*13.8* c
polyphenolic compounds[b]	0.23 a	0.10 b	0.01 c

[a] AP, natural apple pomace; APE, apple pomace extracted with ethanol; APA, apple pomace extracted with ethanol and acetone. Values marked with different letters are statistically different at $p < 0.05$. [b] As a total of HPLC and the vanillin test.

RESULTS

Composition of Fiber and Polyphenol Preparations. Processed industrial apple pomace (preparation AP) contained 61.1% of dietary fiber and 0.23% of polyphenolic compounds (Table 2). The applied ethanol and ethanol−acetone decreased polyphenol concentrations in the pomace to 0.10% (preparation APE) and 0.01% (preparation APA), respectively, whereas the content of dietary fiber increased to 66.6 and 67.2%, respectively. Preparations AP, APE, and APA were characterized by similar protein (6−7%), ash (1.1−1.8%), and fat (2−2.5%) contents.

Low molecular weight carbohydrates (glucose, fructose, sorbitol) had an estimated 10% share and other soluble carbohydrates a 15.8% share of the initial preparation (AP) (Table 3). None of the above fractions were found in preparations extracted with ethanol and ethanol−acetone. All preparations had a similar polysaccharide composition (Table 3), and the predominant fractions were neutral sugars (16.8−19.2%), lignin (14.8−17%), and uronic acids (8.4−10.5%). The main neutral sugars were arabinose, galactose, and xylose, followed by glucose and mannose. Rhamnose and fructose had the smallest share of the analyzed fractions.

Apple pomace (AP) contained the following polyphenolic compounds: proanthocyanidin (113 mg/100 g), quercetin glycoside (rutinoside, galactoside, glucoside, xyloside, arabinoside, rhamnoside) (62 mg/100 g), quercetin (4.5 mg/100 g), phloridzin (42 mg/100 g), and chlorogenic acid (7 mg/100 g) (Table 4). Preparation APE contained 93 mg/100 g of proanthocyanidin,

Table 3. Carbohydrate Composition of Fiber−Polyphenolic Preparations (% w/w)

	preparation[a]		
ingredient	AP	APE	APA
low molecular weight carbohydrates			
glucose	0.7	nd	nd
fructose	8.1	nd	nd
sorbitol	0.6	nd	nd
other soluble carbohydrates	15.8	nd	nd
total NSP	18.2	18.0	21.0
NSP composition			
rhamnose	0.5	0.5	0.5
fructose	0.5	0.6	0.7
arabinose	5.1	4.9	5.6
xylose	3.6	4.3	4.6
mannose	1.3	1.1	1.4
galactose	4.6	5.1	5.8
glucose	2.5	1.5	2.4
uronic acid	11.0 a	9.0 b	10.3 b
lignin	17.8	21.1	16.2

[a] AP, natural apple pomacel; APE, apple pomace extracted with ethanol; APA, apple pomace extracted with ethanol and acetone. nd, not detected. Values marked with different letters are statistically different at $p < 0.05$.

Table 4. Phenolic Composition of Fiber−Polyphenolic Preparations (mg/100 g)

	preparation[a]		
	AP	APE	APA
polyphenol composition (HPLC method)			
chlorogenic acid	6.9	1.5	0.4
procyanidin C1	2.1	0.0	0.0
quercetin rutinoside	0.9	0.0	0.0
quercetin galactoside	20.6	2.4	0.1
quercetin glucoside	3.0	0.6	0.1
quercetin xyloside	8.1	0.9	0.2
quercetin arabinoside	14.7	1.7	0.3
quercetin rhamnoside	12.2	1.7	0.3
quercetin glycoside 1	1.4	0.0	0.0
quercetin glycoside 2	0.8	0.0	0.0
quercetin	4.5	0.9	0.2
phloridzin	42.0	1.0	0.0
total	117.0	10.7	2.0
proanthocyanidins (vanillin test)	113.0	93.0	5.0

[a] AP, natural apple pomace; APE, apple pomace extracted with ethanol; APA, apple pomace extracted with ethanol and acetone. 0.0, not detected at detection limit below 0.02 mg/100 g.

7.3 mg/100 g of quercetin glycoside, 0.9 mg/100 g of quercetin, 1.0 mg/100 g of phloridzin, and 1.5 mg/100 g of chlorogenic acid. Preparation APA contained only 5.0 mg/100 g of proanthocyanidin, 1.0 mg/100 g of quercetin glycoside, 0.4 mg/100 g of chlorogenic acid, and no quercetin.

Animal Growth and Gastrointestinal Functioning. The applied diets with various compositions did not have a significant influence on the final body weights of rats after 4 weeks of the experiment (Table 5). No differences in ileal tissue and digesta were recorded. In comparison with the control diet, the presence of fiber−phenolic preparations in the diets significantly increased ileal viscosity, and the presence of APE additionally lowered the pH of the digesta ($p < 0.05$). The lowest cecal mass values were reported in control, followed by significantly higher values in the AP group ($p < 0.05$) and average values in the remaining group. In rats fed diets containing apple dietary fiber, numerically higher levels of cecal digesta mass were found, but the noted values did not show significant differences in comparison with the control group. Significantly higher hydration levels ($p < 0.05$) and a lower pH of cecal digesta were observed in the group of rats fed apple preparations in comparison with control. In the group fed the APA preparation, the ammonia content of cecal digesta was lower than in the remaining groups.

The glycolytic activity of cecal microflora was characterized by significant diversity, subject to diet composition and the analyzed enzyme (Table 6). The APE group demonstrated the highest α-glucosidase activity, which differed significantly from those of the AP group and control ($p < 0.05$). In comparison with the control diet, a significantly higher α-glucosidase activity was also observed in the APA group.

The APE group was characterized by the highest β-glucosidase activity, but the observed differences were significant only in comparison with the control group ($p < 0.05$). The highest level of α-galactosidase activity was noted in the cecal digesta of the APE group, followed by the APA group. In both cases, the noted values differed significantly from control ($p < 0.05$). Significant variations in β-galactosidase activity were determined between the control and APA groups, whereas groups C and AP differed with regard to their β-glucuronidase activity levels.

In comparison with the control group, the supplementation of rat diets with apple pomace significantly increased the concentrations of the quantitatively most important SCFA (acetic, propionic, butyric acids) as well as the total SCFA production in the cecum (Table 6). Significant differences were observed between selected groups with regard to branched acids (isobutyric and isovaleric acid). The average content of isobutyric and isovaleric acid in APE and APA groups was significantly reduced ($p < 0.05$) in comparison with the control and AP groups. With regard to the SCFA profile, that is, the quantitative proportion of the most important fatty acids, the AP group was marked by a significantly lower share of acetic acid and a higher share of propionic and butyric acid in comparison with the remaining groups.

Blood Biochemical Indicators and the Antioxidant Status of Selected Organs. The applied diets influenced glucose concentrations and lipid fractions of the blood (Table 7). Lower glucose levels were observed in the AP group ($p < 0.05$) in comparison with control. In the remaining groups, glucose concentrations were similar to or only numerically lower than in control. In comparison with the control group, rats fed apple pomace showed lower total blood triacylglycerols and lower total blood cholesterol levels. The difference was only numerical in the APE group, whereas significant variations were noted in the remaining groups.

With regard to the quantitative ratio of triacylglycerols to total cholesterol (log TG/HDL-C), significant differences were determined between groups C and AP, whereas the average values noted in the remaining groups were within the levels found in

Table 5. Final Body Weight and Gastrointestinal Function Parameters in Rats

	experimental group[a]			
	C	AP	APE	APA
final body weight, g	349.90 ± 9.80	343.10 ± 13.20	346.30 ± 6.20	343.90 ± 9.60
small intestine				
tissue, g/100 g mc	2.00 ± 0.12	1.97 ± 0.05	2.00 ± 0.13	1.88 ± 0.05
digesta, g/100 g mc	0.23 ± 0.03	0.21 ± 0.03	0.23 ± 0.04	0.29 ± 0.03
viscosity, mPa s	2.23 ± 0.06 b	2.97 ± 0.21 a	2.93 ± 0.22 a	3.50 ± 0.29 a
pH	7.01 ± 0.05 a	6.88 ± 0.05 a	6.62 ± 0.13 b	6.91 ± 0.07 a
cecum				
tissue, g/100 g mc	0.23 ± 0.01 b	0.27 ± 0.01 a	0.26 ± 0.02 ab	0.27 ± 0.02 ab
digesta, g/100 g mc	0.78 ± 0.04	0.87 ± 0.05	0.87 ± 0.08	0.83 ± 0.05
dry matter, %	25.20 ± 0.91 a	18.30 ± 0.35 b	19.40 ± 0.56 b	19.70 ± 0.42 b
pH	7.11 ± 0.01 a	6.96 ± 0.07 b	6.89 ± 0.02 b	6.91 ± 0.03 b
ammonia, mg/100 g of digesta	0.35 ± 0.02 a	0.32 ± 0.01 a	0.31 ± 0.02 a	0.25 ± 0.05 b
colon				
tissue, g/100 g mc	0.44 ± 0.02	0.40 ± 0.02	0.45 ± 0.03	0.44 ± 0.02
digesta, g/100 g mc	0.36 ± 0.07	0.24 ± 0.07	0.31 ± 0.04	0.28 ± 0.02
pH	6.85 ± 0.04 a	6.74 ± 0.05 ab	6.70 ± 0.08 ab	6.65 ± 0.06 b

[a] C, control diet with cellulose; AP, diet with natural apple pomace; APE, diet with apple pomace extracted with ethanol; APA, diet with apple pomace extracted with ethanol and acetone. Values marked with different letters are statistically different at $p < 0.05$.

Table 6. Activity of Bacterial Enzymes in Cecal Digesta and SCFA Concentrations

	experimental group[a]			
	C	AP	APE	APA
enzyme activity, μmol/h/g				
α-glucosidase	8.99 ± 0.74 c	9.63 ± 0.90 bc	12.6 ± 0.7 a	11.7 ± 0.8 ab
β-glucosidase	3.85 ± 0.17 b	4.39 ± 0.27 ab	5.19 ± 0.43 a	4.54 ± 0.48 ab
α-galactosidase	6.26 ± 0.84 c	7.63 ± 0.41 bc	9.77 ± 0.83 a	8.38 ± 0.72 ab
β-galactosidase	33.70 ± 3.90 b	38.90 ± 3.50 ab	40.60 ± 1.50 ab	44.70 ± 3.80 a
β-glucuronidase	14.50 ± 1.80 a	10.10 ± 0.80 b	12.10 ± 1.10 ab	13.10 ± 1.30 ab
SCFA, μmol/g				
acetic	46.90 ± 2.50 b	58.10 ± 3.70 a	60.60 ± 1.40 a	61.20 ± 2.60 a
propionic	13.90 ± 0.80 c	19.80 ± 0.40 a	15.60 ± 0.70 b	17.60 ± 0.60 a
isobutyric	1.48 ± 0.08 a	1.52 ± 0.07 a	1.18 ± 0.09 b	1.21 ± 0.06 b
butyric	7.26 ± 0.51 d	10.9 ± 1.0 a	9.19 ± 0.55 b	8.85 ± 0.42 c
isovaleric	1.29 ± 0.06 a	1.14 ± 0.05 a	0.91 ± 0.04 b	0.84 ± 0.06 b
valeric	1.18 ± 0.06	1.32 ± 0.08	1.20 ± 0.07	1.28 ± 0.07
total SCFA	72.00 ± 3.40 b	92.70 ± 4.40 a	88.70 ± 1.90 a	90.90 ± 3.00 a
SCFA, μmol/100 g BW[b]	56.10 ± 4.20 b	79.50 ± 3.30 a	76.80 ± 7.00 a	75.20 ± 3.00 a
SCFA profile, μmol/100 μmol				
C_2	65.10 ± 1.00 ab	62.40 ± 1.40 b	68.30 ± 1.00 a	67.10 ± 0.92 a
C_3	19.40 ± 0.60 b	21.60 ± 0.90 a	17.60 ± 0.60 b	19.40 ± 0.51 b
C_4	10.10 ± 0.50 b	11.70 ± 0.90 a	10.30 ± 0.50 ab	9.80 ± 0.52 b

[a] C, control diet with cellulose; AP, diet with natural apple pomace; APE, diet with apple pomace extracted with ethanol; APA, diet with apple pomace extracted with ethanol and acetone. Values marked with different letters are statistically different at $p < 0.05$. [b] BW, body weight.

groups C and AP. Similar LDL fraction concentrations and comparable aminotransferase activity levels were noted in all groups.

None of the applied dietary ingredients affected rat liver mass and liver TBARS levels. Significantly lower TBARS levels in the kidneys of rats fed apple pomace were noted in comparison with control.

DISCUSSION

Apple pomace applied in the discussed experiment contained hemicelluloses and pectins typical of apple fiber.[13,28] Industrial apple pectins, which are often used in experiments on animals, are extracted from apple pomace by diluted acid solutions. They are then purified, concentrated, precipitated by ethanol, dried,

Table 7. Biochemical Blood Parameters and Concentrations of Thiobarbituric Acid Reactive Substances (TBARS) in Selected Organs

	experimental group[a]			
	C	AP	APE	APA
serum parameters				
glucose, mmol/L	12.70 ± 0.60 a	9.20 ± 0.70 b	12.60 ± 0.90 a	10.90 ± 0.98 ab
TG, mmol/L	3.65 ± 0.37 a	2.26 ± 0.31 b	2.92 ± 0.23 ab	2.69 ± 0.26 b
TC, mmol/L	3.15 ± 0.38 a	2.54 ± 0.21 b	2.80 ± 0.13 ab	2.55 ± 0.12 b
HDL-C, mmol/L	1.20 ± 0.09	1.16 ± 0.08	1.28 ± 0.06	1.24 ± 0.12
HDL-C profile, % of TC	39.00 ± 1.60 b	46.80 ± 3.40 ab	45.90 ± 2.20 ab	49.80 ± 5.50 a
LDL-C, mmol/L	1.23 ± 0.25	0.93 ± 0.17	0.94 ± 0.09	0.77 ± 0.14
log (TG/HDL-C)	0.47 ± 0.04 a	0.27 ± 0.06 b	0.35 ± 0.03 ab	0.33 ± 0.07 ab
ALT, U/L	24.30 ± 2.60	23.40 ± 1.70	20.50 ± 1.80	24.80 ± 2.40
AST, U/L	130.00 ± 7.00	144.00 ± 10.00	140.00 ± 11.00	144.00 ± 7.00
liver				
mass, g/100 g BW	4.03 ± 0.24	3.68 ± 0.08	3.68 ± 0.10	3.66 ± 0.06
TBARS, nmol/g	51.40 ± 2.10	52.90 ± 1.00	53.90 ± 1.90	54.80 ± 1.40
kidney				
mass, g/100 g BW	0.61 ± 0.02	0.59 ± 0.01	0.58 ± 0.01	0.58 ± 0.01
TBARS, nmol/g	136.00 ± 6.00 a	111.00 ± 6.00 b	108.00 ± 6.00 b	101.00 ± 12.00 b

[a] C, control diet with cellulose; AP, diet with natural apple pomace; APE, diet with apple pomace extracted with ethanol; APA, diet with apple pomace extracted with ethanol and acetone. Values marked with different letters are statistically different at $p < 0.05$.

and standardized. Hemicelluloses are not found in pectins produced with the use of the above method.

Polyphenol compound extractions had an insignificant effect on the composition of the carbohydrate fraction, and the noted values were consistent with the results of our previous research.[13] The polyphenol fraction comprised polyphenols typical of apple pomace, with a relatively low content of chlorogenic acid and proanthocyanidins and quercetin glycoside levels similar to those reported by other authors.[11,29] Ethanol extraction removed 90% of flavonoids other than proanthocyanidins and chlorogenic acid as well as 18% of proanthocyanidins. After sequential acetone extraction, only 3% of the initial polyphenol content remained. The high effectiveness of polyphenol extraction was in line with the results of other experiments where methanol and acetone were used to quantify the polyphenol content of apple fruits,[30] separate apple polyphenols and apple dietary fiber,[31,32] and remove harmful amygdalin from apple seeds.[33]

The results of selected experiments indicate that diets supplemented with apple pectins decrease the body mass of rats.[34,35] In this experiment, no differences were observed in the body mass of rats fed cellulose and apple pomace with various polyphenol contents, pointing to the similar nutritive value of experimental diets. When compared to the control diet, the apple pomace containing diets would have a small, but greater, metabolizable energy density based on uptake of SCFA by the body tissues, but these small differences between groups turned out to be indifferent as to the final body weight of rats. The noted results are consistent with previous findings, indicating that a moderate content of various nondigestible saccharides (5−7%) does not lead to changes in diet uptake or body mass.[17,36,37]

According to various authors, an increase of the fiber content of a diet, in particular the soluble fraction content, increases the viscosity of the small intestine.[38,39] The above reaction is caused by pectins.[40] The above findings were validated by our experiment, where an increase in the small intestinal viscosity of rats fed apple pomace did not affect digesta weight in the small intestine or further sections of the digestive tract.

Diet supplementation with apple pomace increased the moisture content of intestinal digesta, as demonstrated by the results of another experiment, where the addition of apple pectin increased the hydration of rat feces.[41] In this study, a decrease in the small intestinal pH of rats fed apple pomace was also observed. The above could be attributed to the initiation of the fermentation process in the upper part of the digestive tract, which is observed when the viscosity of the digesta is increased.[42]

Nondigestible saccharides are known to increase the content of substrate for bacterial fermentation the in colon, leading to an increase in digesta and intestinal tissue.[43,44] The above generally results from intensified fermentation, which increases the content of SCFA[45] and decreases the pH of the digesta.[40] A lower pH of colonic digesta supports the colonization of beneficial microflora and inhibits the growth of harmful microorganisms, including proteolytic bacteria.[46] Aprikian et al.[34] reported that a 5% addition of apple pectins or a 10% addition of freeze-dried apples increased cecal digesta, whereas a beneficial decrease in digesta pH was observed only upon the combined use of both dietary supplements. In this experiment, diet supplementation with apple pomace extracted with ethanol and acetone decreased intestinal pH but without an accompanying increase in digesta. Similar results were reported for an experiment in which diet supplementation with 3.3% pectin did not increase cecum mass,[47] but a 7% pectin dose provoked the effect. In yet another experiment, the 5% addition of alcohol-insoluble apple extract increased the wet mass of cecal contents by >2-fold.[35]

Intensive saccharide fermentation is accompanied by the microbiological degradation of proteins in the small intestine, and the above may raise nitrogen levels (mainly ammonia) in the digesta.[48] When ammonia concentrations exceed the bacteria's processing capacity, they exert a harmful effect on intestinal mucosa, leading to microcirculation disorders, surface injuries of

epithelial cells,[49] and overloaded liver metabolism.[50] In this experiment, apple pomace supplementation did not increase the ammonia content of cecal digesta or the concentrations of branched fatty acids (isobutyric and isovaleric acid), which are mostly the product of amino acid degradation.[51]

In the discussed experiment, the use of natural apple pomace significantly inhibited the level of β-glucuronidase activity in the gut microflora. Our findings stand in contrast to the results of other studies in which apple pectin had a stimulating effect on β-glucosidase and β-glucuronidase activity.[41,47] High levels of β-glucuronidase activity have been found to increase the risk of cancer because the enzyme's deconjugative properties support the transformation of xenobiotics into toxic substances.[52] For this reason, a drop in microbial β-glucuronidase activity may be beneficial for intestinal and overall health.

Although a significant increase in the glycolytic activity of gut microflora was not observed, diet supplementation with apple pomace elevated SCFA concentrations and total SCFA production in cecal digesta. Our findings indicate that despite enzymatic depectinization during juice production, apple pomace contains significant amounts of substances that ferment more readily than cellulose. According to Henningsson et al.,[45] pectins are the most fermentation susceptible fiber components in apples. The above explains why monomers from nonstarch apple polysaccharides (xylose, galactose, glucose) were found in significantly larger quantities than uronic acid, the main pectin monomer, in animal feces. The presented results are consistent with other authors' findings,[34,35,45] and they suggest that, similarly to apple pectins, fiber preparations obtained from industrial apple pomace stimulate fermentation processes in the lower gastrointestinal tract of rats.

Previous experiments indicate that the physiological properties of nondigestible saccharides may be modified by linked polyphenols and that one of the effects may be an increase in large intestinal digesta.[34,53] The above can be largely attributed to the fact that when combined with dietary fiber in relatively large quantities, polyphenols reach the large intestine to affect the physicochemical properties of digesta and gut health.[17,25] Ethanol extraction applied in our experiment decreased the polyphenol content of the fiber preparation from 0.23 to 0.10%, magnifying the differences in the physiological parameters of the digestive tract relative to control; that is, it beneficially decreased digesta pH, increased the glycolytic activity of cecal microflora, and decreased the share of branched fatty acids in total SCFA. The use of acetone extraction did not cause further changes in the physiological properties of apple fiber. The above indicates that unlike the decrease in flavonoid concentrations, the removal of most proanthocyanidins had no significant effect on the functioning of the digestive tract compared.[54] Such variations result from differences in the physiological properties of polyphenols that are easy and difficult to extract (flavonoids and proanthocyanidins, respectively). Flavonoids are released in the upper part of the digestive tract, and they lower the digestibility of dietary proteins by reacting with proteolytic enzymes.[55] As a result, the amount of protein that reaches the colon and is subjected to microbiological digestion is increased, and branched-chain fatty acids are among the products of this process.[51]

Dietary fiber is known to deliver various health benefits by reducing the concentrations of serum lipids, including cholesterol, and postprandial glucose.[5] In our experiment, dietary fiber was found to deliver such an effect in rats fed natural apple pomace. The lowest atherogenic index of plasma (log TG/HDL-C) was observed in this group of animals, and the parameter is highly correlated with coronary heart disease and diabetes risk.[56] The use of extracted apple pomace resulted in a less profound decrease in serum lipid levels and the atherogenic index, although the analyzed preparations had similar contents of soluble fiber. Hypocholesterolemic activity is largely a measure of the physiological effect of soluble dietary fiber.[57] An experiment investigating rats fed diets supplemented with dried apples (10%) demonstrated such an effect.[58] In the cited experiment, the use of dried apples also decreased the concentrations of lipid oxidation products in blood. In our study, the examined fiber preparations did not influence the antioxidant status of the liver, where dietary polyphenols are metabolized for the most part.[59] A decrease in TBARS was, however, observed in the kidneys, suggesting that the excreted products of polyphenol metabolism demonstrated antioxidant properties.[60]

It can be concluded that pomace obtained during apple juice production with the use of pectin-degrading enzymes after seed removal contains >60% of dietary fiber, <10% of available carbohydrates, and 0.23% of polyphenols, with a significant share (30%) of quercetin glycosides. In comparison with cellulose, the above source of dietary fiber had a beneficial effect on digestive tract functioning (increasing ileal digesta hydration and increasing cecal SCFA concentrations and pools) by decreasing triacylglycerol and glucose levels as well as the atherogenic index of plasma. The polyphenol content of pomace, mainly readily extractable flavonoids, had a minor effect on the physiological properties of the resulting dietary fiber preparation. The removal of this polyphenol fraction decreased the pH of intestinal digesta, increased the glycolytic activity of cecal microflora, and decreased the share of branched fatty acids in total SCFA, indicating a drop in the proteolytic activity of microflora. The supplement containing apple polyphenols (AP) was characterized by a more advantageous lipid profile in the atherogenic index.

The results of this study demonstrate that pomace obtained from industrially produced apple juice is a good source of dietary fiber and that a decrease in the polyphenol content of apple fiber improves local interactions in the digestive tract and decreases metabolic overload.

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ABBREVIATIONS USED

AP, apple dietary fiber; APA, apple dietary fiber extracted with ethanol and acetone; APE, apple dietary fiber extracted with

ethanol; ALT, alanine aminotransferase; AST, asparagine aminotransferase; C, cellulose; HDL, high-density lipoproteins; LDL, low-density lipoproteins; IDF, insoluble dietary fiber; TBARS, thiobarbituric acid reactive substances; TC, total cholesterol; TDF, total dietary fiber; TG, triacyglycerols; SCFA, short-chain fatty acids.

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