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Raspberry pomace alters cecal microbial activity and reduces secondary bile acids in rats fed a high-fat diet☆

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Abstract

The profile of bile acids (BA) largely depends on the enzymatic activity of the microbiota, but this can be modulated by the dietary addition of biologically active compounds, e.g., polyphenols and polyunsaturated fatty acids. The aim of this study was to examine the effect of dietary raspberry pomace as a rich source of biologically active compounds on microbial activity and the BA profile in the caecum of rats fed a high-fat diet. Wistar rats were fed the standard diet AIN-93, a high-fat diet or a modified high-fat diet enriched with 7% different types of processed raspberry pomaces produced by standard grinding and fine grinding, with or without seeds. Rats fed the high-fat diet for 8 weeks showed some disorders in liver function and cecal BA, as manifested by an increased concentration of cholesterol, total BA in the liver and cholic, deoxycholic, and β-muricholic acids in the cecal digesta. In general, irrespective of the type of raspberry pomace, these dietary preparations decreased liver cholesterol, hepatic fibroblast growth factor receptor 4, peroxisome proliferator-activated receptor alpha, cecal ammonia and favorable changed BA profile in the cecum. However, among all dietary pomaces, the finely ground preparation containing seeds had the greatest beneficial effect on the caecum by modulating bacterial activity and reducing the levels of secondary BA.

Keywords: Cholesterol; Bile acids; PPARα; Ellagitannins; Wistar rat

1. Introduction

It is well known that a high-fat diet stimulates the secretion of bile acids (BA) into the intestinal lumen, leading to a higher risk of neoplastic changes in the lower gut [1–3]. The effect of dietary fat on BA metabolism results in microbial activity that promotes the deconjugation, dehydrogenation, and dehydroxylation of primary to secondary BA in the distal small intestine and colon, thus increasing the chemical diversity of the BA [4,5]. The secondary BA are one of the factors associated with an increased risk of colon cancer [6–8]. Nevertheless, the modulation of the bacterial activity by the dietary addition of biologically active compounds, e.g., polyphenols and polyunsaturated fatty acids [9–11] might regulate the profile and concentration of the BA in the gastrointestinal tract. There is still little information regarding compounds that are able to regulate the BA profile in the gastrointestinal tract or their synthesis in the liver.

BA are synthesized from cholesterol in hepatocytes and then conjugated to glycine or taurine and released into the duodenum following the ingestion of a meal to facilitate the absorption of triglycerides, cholesterol, and lipid-soluble vitamins [12–14]. The synthesis of these compounds is regulated by at least 14 liver enzymes [15,16]. The main enzymes responsible for BA synthesis are cholesterol 7-alpha-hydroxylase (CYP7A1) and sterol 12-alpha-hydroxylase (CYP8B1) [12,15]. The hepatic expression of CYP7A1 and CYP8B1 is regulated by farnesoid X receptor (FXR), which is highly expressed in liver [17]. The activity of FXR can be regulated by BA, which are the main signaling endogenous ligands for this receptor, and through mechanisms that are dependent on the nuclear receptors small heterodimer partner (SHP) [15] and fibroblast growth factor 15/19 (FGF15/19) [18–20]. There is also molecular evidence for cross-talk between FXR and peroxisome proliferator-activated receptor α (PPARα), which is a nuclear receptor that mainly controls lipid and lipoprotein metabolism [21].

The BA synthesis in the liver seems to be modulated by biologically active compounds supplied in the diet e.g., diet enriched with ellagic acid increased expression of the CYP7A1 and CYP8B1 genes involved in bile acid synthesis [22]. Raspberries are popularly consumed fruits that are rich in biologically active compounds. These fruits are known as an excellent source of dietary antioxidants, largely due to their high

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level of phenolic compounds comprised primarily of cyanidins, anthocyanins, ellagitannins, and quercetin [23,24]. During the processing of raspberries, particularly for the production of a concentrated juice, a significant portion of the compounds, mainly fiber and polyphenols, remains in the pomace. The nutritional and health-promoting properties of these bioactive compounds are not sufficiently understood. It is worth noting that more than 80% of the raspberry pomace consists of the seeds, which include approximately 23% oil and are a rich source of essential fatty acids [25,26]. Unfortunately, seeds that have not been processed pass intact through the digestive system with all their biologically active compounds, thus reducing the biological value of raspberry pomace.

This study proposes two solutions — which have not been described previously in vivo — that may increase the health potential of the raspberry pomace in terms of the modulation of BA synthesis in the liver and the BA profile in the gut: (1) Separation of the seeds from the pulp fraction, thereby also increasing the concentration of biologically active compounds from the pulp that are readily available to the organism. (2) Fine grinding of the native pomace, damaging the seed coat and thus increasing the availability of the accumulated valuable seed compounds. The aim of this study was to examine whether dietary raspberry pomaces with or without seeds and their grinding level may favorably alter cecal microbial enzymatic activity and BA profiles in rats fed a high-fat diet.

2. Materials and methods

2.1. Preparation of raspberry pomaces

Dried raspberry press cake was obtained from a fruit transformation plant in the Masovia region in Poland. Four preparations were evaluated. Native press cake was characterized by a particle diameter smaller than 1.25 mm. To obtain other preparations, the press cake was ground up and sieved as follows. Fine-ground native press cake (containing seeds) was prepared using a Blixer 3 blender (Robot Coupe, France) with solid CO_2 at −78.5°C to obtain particles smaller than 0.65 mm. To prepare seedless press cake, the material was milled in a laboratory ball mill (own built, Łódź University of Technology, Poland) for 1 h and sieved to a diameter smaller than 0.8 mm and larger than 0.65 mm. To obtain the nonstandard-ground fraction, a portion of the seedless press cake was further milled on the ball mill until granulation smaller than 0.65 mm was achieved.

2.2. Chemical composition of the raspberry pomaces

Dry matter, ash, crude protein, crude fat and total dietary fiber (TDF) were determined according AOAC official methods [27]: 920.151; 940.26; 920.152; 930.09; and 985.29. Carbohydrates were determined according to the following formula: carbohydrate = total solids – (protein + fat + ash). The proximate compositions of the raspberry pomaces are presented in Table 1. For the ellagitannin (ET) and anthocyanin (AC) measurements, the samples of raspberry pomaces and known standards were diluted with 50% (v/v) methanol, filtered through PTFE filters (0.45 μm) and introduced into high-performance liquid chromatography (HPLC) systems.

2.2.1. Quantification of Ellagitannins

The content of ETs was determined using a Smartline chromatograph (Knauer, Berlin, Germany) composed of degasser (Manager 5000), binary pump (P1000), an autosampler (3950), a thermostat and a detector PDA (2800). The ETs were separated on a 250 × 4.6 i.d., 5-μm, Gemini C18 110A column (Phenomenex) by gradient elution with 0.05% (v/v) phosphoric acid in water (solvent A) and 83:17 (v/v) acetonitrile:water with 0.05% phospohoric acid (solvent B). The column temperature was set at 35°C, the flow rate was 1.25 mL/min, and the gradient program was as follows: 0–5 min, 5% (v/v) B; 5–10 min, 5–15% (v/v) B; 10–35 min, 15–40% (v/v) B; 35–40 min, 40–73% (v/v) B; 40–44 min, 73% (v/v) B; 44–46 min, 73–5% (v/v) B; 46–54 min, 5% (v/v) B. The injection volume was 20 μl. Data were collected using the ClarityChrom version 3.0.5.505 program (Knauer, Berlin, Germany). ETs were detected at 250 nm, and the standard curves generated using lambertianin C, sanguiin H-6, and ellagic acid were applied for quantification. Standards of lambertianin C and sanguine H-6 were isolated from raspberry extract as described by Sójka et al. (2013) [28]. Ellagic acid standard was purchased from Extrasynthese.

2.2.2. Quantification of flavan-3-ols (FLs)

The contents of FLs, i.e., the sum of proanthocyanidins and catechins, were determined using the method described by Sójka et al. (2013) [28]. For separation, the same column and conditions were used. For this analysis, the Shimadzu system equipped with a pump (LC-20 AD), a degasser (DGU-20A5R), an autosampler (SIL-20ACHT), a thermostat (CTO-10ASUP), and a detector (RF-10AXL), and LabSolutions Lite version 5.52 software was used.

2.2.3. Quantification of anthocyanins

HPLC coupled to a DAD and an electrospray ion (ESI) trap mass spectrometer was used to identify ACs. The HPLC system was equipped with a SCM1000 membrane solvent degasser (ThermoQuest, San Jose, CA, USA), a binary high-pressure gradient pump (1100 Series; Agilent Technologies, Santa Clara, CA, USA), an autosampler, and a column oven (Surveyor Series, Thermo-Finnigan, San Jose, CA, USA). A Gemini C18 110A 250 mm×4.6 mm i.d. (Phenomenex) 5-μm column was used. The column temperature was 30°C, and the injection volume was 10 μl. Chromatographic data were collected using Xcalibur software, version 1.2 (Thermo-Finnigan, San Jose, CA, USA). The solvents and the gradient used for AC separations were as follows: solvent A, 0.25% (v/v) formic acid in water; solvent B, 0.25% (v/v) formic acid in acetonitrile, with a flow rate of 12 mL/min; the gradient program (time in min – % (v/v) was as follows B): 0–5, 2–5, 32–20, 37–70, 42–70, 45–5, and 55–5. The MS system coupled to HPLC was an LCQ DECA ion trap mass spectrometer (Thermo-Finnigan, San Jose, CA, USA) equipped with an ESI source, which was used in negative mode. The phenol content was quantified using a KNAUER Smartline chromatograph (Berlin, Germany). Details about equipment, separation as well as quantification of the phenol content were previously described by Sójka et al. (2015) [29]. The polyphenolic compositions of the raspberry pomaces are presented in Table 2.

2.3. Animal study

This study was conducted in strict accordance with the recommendations of the National Ethic Commission (Warsaw, Poland). All procedures and experiments complied with the guidelines and were approved by the Local Ethic Commission of the University of Warmia and Mazury (Olsztyn, Poland, Permit Number: 68/2014) with respect to animal experimentation and the care of the animals under study, and all efforts were made to minimize suffering. The nutritional experiment was performed using 48 male Wistar rats, which were allocated to 6 groups of 8 animals each that were housed individually in plastic cages. The initial body weight was comparable among groups (154±6.5 g on average). For 8 weeks, each group was fed a modified version of the semi-purified rodent diet recommended by Reeves (1997) [30] (details provided in Supplemental Table 1). Group C was fed a standard diet for laboratory rodents that consisted of 6% fat and 5% fiber (AIN-93 diet), and group HF received a high-fat diet

Table 1
Chemical composition of the raspberry pomaces, %

	Raspberry pomace, standard granulation	Raspberry pomace, fine granulation	Raspberry pomace seedless fraction, standard granulation	Raspberry pomace seedless fraction, fine granulation
Dry matter	93.13±0.00	93.77±0.05	93.23±0.01	93.70±0.02
Ash	1.68±0.00	1.75±0.02	2.74±0.01	2.73±0.05
Protein	11.20±0.31	11.65±0.12	19.64±0.60	20.38±0.14
Fat	11.44±0.10	12.46±0.17	9.19±0.05	9.09±0.08
Carbohydrates *	65.90	64.72	55.26	54.58
TDF	64.76±0.15	61.98±0.06	48.90±0.49	47.00±0.40
SDF	0.92	0.46	1.80	3.13
LM	1.14	2.74	6.36	7.58
Total polyphenols	2.91±0.04	3.19±0.05	6.40±0.00	6.92±0.04

TDF, total dietary fiber; SDF, soluble dietary fiber; LM, low-molecular carbohydrates.
* Carbohydrates = dry matter–(ash + protein + fat + polyphenolics).