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Basic nutritional investigation

Consumption of polyphenol concentrate with dietary fructo-oligosaccharides enhances cecal metabolism of quercetin glycosides in rats

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ABSTRACT

Objective: We verified the hypothesis that the consumption of polyphenol concentrate (PC), rich in quercetin and its glycosides (36 g/100 g), in association with different dietary fiber matrices, that is, an easily fermentable fructo-oligosaccharides (FOS) or non-fermentable cellulose (CEL), causes a disparate adaptive response of the cecal microbial activity in rats. This in turn facilitates further utilization of biologically active polyphenolic compounds, which are not, as usual, digested in the foregut.
Methods: Four-week experimental feeding of male Wistar rats consisted of diets containing 5% FOS or CEL, as a source of dietary fiber, with or without 0.3% addition of PC.
Results: Positive changes in rat cecum were observed resulting from the ingestion of an FOS-containing diet, such as decreased pH and increased the production of short-chain fatty acids in the digesta, compared with a CEL-containing diet. The addition of PC to the FOS diet did not eliminate the positive effects of the latter, except for a slight increase in cecal pH and a decrease in microbial glycolytic activity. However, a simultaneous increase in the cecal butyrate pool was also observed. An adaptation process of the microflora enzymatic system to dieting with PC and FOS was proven in further enhanced susceptibility of rutin (quercetin 3-O-glucorhamnoside), hyperoside (quercetin 3-O-galactoside), and quercitrin (quercetin 3-O-rhamnoside) to hydrolysis by the cecal digesta solution.
Conclusion: Especially when consumed together, PC and FOS are important dietary factors affecting the susceptibility of quercetin glycosides to microbial metabolism in the cecum. The intensification of the hydrolysis of quercetin glycosides by dietary treatments leads also to the increased metabolism of quercetin itself.

Introduction

Polyphenols are phytochemicals representing a large variety of compounds with antioxidant properties and several biological activities, especially against free radical–related diseases, such as cancers, diabetes, and cardiovascular disorders. In recent years the bioavailability of polyphenols, as the major factor influencing their activity, has been the object of intensive investigations; it varies widely among polyphenols and often depends on their chemical form occurring in food products [1]. Results of some experiments have shown that galloylation of catechins markedly decreases their small intestinal absorption [2], whereas procyanidins completely escape absorption in the foregut [1].

One of the most extensively investigated polyphenols has been quercetin, a flavonoid belonging to the class of flavonols. As a result, the safety of quercetin as food ingredient is currently well established [3]. Quercetin is a lipophilic substance with a molecular weight of 302 Da, indicating its possible crossing by enterocytes by simple diffusion; however, some investigators have suggested absorption already from the stomach [4]. In contrast, in plants and foods the major fraction of quercetin is bound with sugars constituting different glycosidic forms, which are less absorbable in the small intestine and reach the hindgut [3,5–7]. In the large intestine, micro-organisms cleave the glycosidic bonds and free quercetin can be absorbed and/or metabolized to the other potentially bioactive compounds [5,7].

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Nevertheless, it is well established that some quercetin glycosides, i.e., glucosides, are actively hydrolyzed and absorbed in the small intestine [8]. However, one recent study has shown that, when provided with dietary sources, quercetin aglycone is more bioavailable than its glucosides in humans [9], but even free quercetin is not absorbed completely in the upper part of the rat gut (ca. 60%) [10], which indicates that even in that form it may reach the hindgut.

Overall, the absorption of polyphenols and their derivatives occurs along the entire gut, and often the metabolic processes in the large intestine are predominant, which is not fully understood thus far. In contrast, the metabolism of non-digestible saccharides in the gastrointestinal tract classified, as part of dietary fiber (DF), such as cellulose (CEL) and fructo-oligosaccharides (FOS), is better recognized and characterized. Among others, non-digestible saccharides are not hydrolyzed by pancreatic enzymes in the upper part of the gut and are completely transported to the large intestine [11–13]. However, the susceptibility of CEL to the fermentative processes occurring in the hindgut is decidedly lower than that of FOS, which has been proved to possess prebiotic properties and enhance the production of short-chain fatty acids (SCFA) [12].

We verified the hypothesis that consumption of a polyphenol preparation, rich in quercetin and its glycosides, in association with different DF matrices, that is, easily fermentable FOS or non-fermentable CEL, causes a disparate adaptive response of the cecal environment in rats, and this in turn facilitates further utilization of biologically active polyphenolic compounds, i.e., rutin (quercetin 3-O-glucorhamnoside), hyperoside (quercetin 3-O-galactoside), and quercitrin (quercetin 3-O-rhamnoside), which are not digested in the upper part of the gastrointestinal tract, as is usual.

Materials and methods

Source of DF and polyphenol concentrate

The preparation of α-CEL was purchased from Sigma-Aldrich (Poznań, Poland), and FOS with a degree of polymerization 2-7 (Raftilose) was provided by BENEO-Orafti (Oreye, Belgium).

The polyphenol concentrate (PC) was obtained from apple pomace, which is a byproduct originating during commercial apple juice production. The pomace was dried at 70°C and ground. Subsequently, phenolic compounds were isolated using ethanol extraction, followed by adsorption on ion-exchange polystyrene resin, desorption by ethanol-water solution, evaporation, and freeze-drying, as described in the pending patent of Król et al. [14].

Phenolic determinations in the PC

The determinations were performed using high-performance liquid chromatography (Dionex System with a diode array detector; Dionex, Germering, Germany) coupled with a Gemini 5u C18 110A column (250 × 4.60 mm; Phenomenex, Torrance, CA, USA). Phase A consisted of 0.05% phosphoric acid in water; phase B consisted of 0.05% phosphoric acid in acetonitrile, a column temperature of 25°C, and a flow rate of 1.25 mL/min, gradient stabilization was for 5.0 min with 4% of phase B, then 5.0–12.5 min with 4–15% of phase B, 12.5–42.4 min with 15–40% of phase B, 42.4–51.8 min with 40–50% of phase B, 51.8–53.4 min with 50% of phase B, and 53.4–55.0 min with 4% of phase B. The concentrate was first dissolved in 80% methanol and then mixed 1:1 with phase A. Standards of chlorogenic acid, phlorizin, epicatechin, and rutin (quercetin 3-O-glucorhamnoside) were from Sigma-Aldrich, and procyanidin B2, quercetin, hyperoside (quercetin 3-O-galactoside), quercetin 3-O-glucoside, and quercitrin (quercetin 3-O-rhamnoside) were from Extrasynthese Co. (Lyon, France). The identification of procyanidin C1 was based on literature data of the ultraviolet spectrum, and the identification of quercetin 3-O-xyloside and quercetin 3-O-arabinoside was based on the literature and our data acquired previously with high-performance liquid chromatography/mass spectroscopy. In addition, the total phenolic content of the concentrate was determined using the Folin-Ciocalteau reagent as described previously [15].

Table 1
Proportional composition of CEL and FOS diets with or without PC

Ingredient	Without PC		With PC	
	CEL	FOS	CEL	FOS
Casein	14	14	14	14
DL-methionine	0.2	0.2	0.2	0.2
CEL preparation	5	—	5	—
FOS preparation	—	5	—	5
PC	—	—	0.3	0.3
Soybean oil	8	8	8	8
Mineral mix (recommended for AIN-93G diet)	3	3	3	3
Vitamin mix (recommended for AIN-93G diet)	1	1	1	1
Choline chloride	0.2	0.2	0.2	0.2
Maize starch	68.6	68.6	68.3	68.3

CEL, cellulose; FOS, fructo-oligosaccharides; PC, polyphenol concentrate

Animals and diets

The animal protocol used in this study was approved by the local animal care and use committee (permission no. 23/2008). The experiment was conducted in 24 adult male Wistar rats weighing 272 ± 14 g, divided into four groups of six animals each. All animals were housed individually over 4 wk under standard conditions with free access to water and semi-purified casein diets (Table 1). The source of DF (5%) in two groups was the CEL preparation, and the remaining two received the FOS preparation; one diet from each CEL and FOS group was also supplemented with the PC at the expense of maize starch (0.3%).

Sample collection and analysis

On termination of the experiment, rats were anaesthetized with sodium pentobarbital according to the recommendations for euthanasia of experimental animals. The pH of cecal digesta were measured using a microelectrode and a pH/ION meter (model 301, Hanna Instruments, Póvoa de Varzim, Portugal), and dry matter was determined at 105°C. Fresh cecal digesta was used for analysis of ammonia concentration, and determinations of enzyme activity and the concentration of SCFA were performed after storage of samples at −70°C. Ammonia was extracted and trapped in a solution of boric acid and then determined by direct titration with sulfuric acid [16]. The enzyme activity (α- and β-glucosidase, α- and β-galactosidase, β-glucuronidase) was measured by the rate of *p*- or *o*-nitrophenol released from enzymes' nitrophenyl-glucosides for 10 min [11] and expressed as micromoles of product formed per hour per gram of cecal digesta. The concentration of SCFA was measured using gas chromatography under conditions described previously [11] and calculated also as the pool and profile of SCFA. The pool of SCFA was calculated as the product of SCFA concentration (micromoles per gram) and relative digesta mass (grams per 100 g of body weight).

Preparation of cecal digesta solution and hydrolysis of selected quercetin glycosides

Cecal digesta samples were obtained from rats consuming the CEL or FOS diet with or without PC (one sample from each group consisted of digesta from two rats). Then 0.8 g of sample and 2 mL of 0.1 M cold phosphate buffer (4°C, pH 7) were mixed vigorously for 2 min and centrifuged (10 min, 6000 × *g*). The obtained supernatant was placed into a measuring flask (5 mL) at 4°C. The procedure was repeated twice with 1.5- and 1.0-mL additions of the buffer, respectively, and the flask contents were made up to the line with buffer. The prepared extract of digesta containing bacterial enzymes and no detectable levels of quercetin and its derivatives was subsequently treated under anaerobic conditions with a basal solution of polyphenol mixture, consisting of rutin (quercetin 3-O-glucorhamnoside, 120 μmol/L), hyperoside (quercetin 3-O-galactoside, 264 μmol/L), and quercitrin (quercetin 3-O-rhamnoside, 260 μmol/L). Hydrolysis of the aforementioned components was performed in quadruplicate using 1.5-mL test tubes in which 0.25 mL of the cecal digesta solution was mixed with 0.25 mL of the polyphenol mixture solution. The mixture was incubated for 1, 3, 6, and 24 h (37°C); after each period 0.5 mL of methanol was added to one of the four tubes, and the contents centrifuged for 5 min (5000 × *g*). The supernatant was then subjected to high-performance liquid chromatographic determination of quercetin and its glycosides. To do so, the Dionex System with a diode array detector was used, coupled with a 4-μm column (150 × 2.00 mm, Fusion-RP 80A, Phenomenex Synergi). Phase A consisted of 0.05% phosphoric acid in water; phase B consisted of 0.05% phosphoric acid in acetonitrile, a flow rate of 0.25 mL/min, and a temperature of 25°C; gradient stabilization was for 10 min with 4% of phase B, 0–33 min with 4–50% of phase B, 33–34 min with 50% of phase B, and 34–35 min with 4% of phase B.