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THE INHIBITORY EFFECT OF POLYPHENOLS ON HUMAN GUT MICROBIOTA

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The intestinal microbiota (IM) is responsible for metabolism of many compounds provided in the diet, such as polyphenols, increasing their bioavailability. However, there are remarkably few studies investigating the influence of polyphenols on the composition and activity of the gut microbial community. This study evaluated the influence of the polyphenols naringenin (N), naringin (NR), hesperetin (H), hesperidin (HR), quercetin (Q), rutin (QR), and catechin (CAT) on the growth of human IM representatives (*Bacteroides galacturonicus*, *Lactobacillus* sp., *Enterococcus caccae*, *Bifidobacterium catenulatum*, *Ruminococcus gauvreauii*, *Escherichia coli*). Polyphenols were added to liquid medium at a final concentration of 20, 100 or 250 μg/ml (for Q concentrations were 4, 20 or 50 μg/ml) and their impact on the IM was assessed by measurement of the turbidity after 24-h culture. The minimal inhibitory concentration (MIC) for polyphenols that inhibited bacteria was estimated. CAT had no impact on the tested IM. Q had the strongest impact on *R. gauvreauii*, *B. galacturonicus* and *Lactobacillus* sp. (MIC 20–50 μg/ml) growth, whilst its rutinoside had no impact. NR and HR had no impact, but their aglycones N and H inhibited growth of almost all analyzed bacteria (MIC ≥250 μg/ml). We conclude that flavonoid aglycones, but not their glycosides, may inhibit growth of some intestinal bacteria. This means that polyphenols probably can modulate the IM and indirectly interfere with their own bioavailability.

Key words: *aglycones, flavonoid glycosides, growth inhibition, intestinal microbiota, minimal inhibitory concentration, polyphenols*

Abbreviations: CAT – (+)-catechin; H – hesperetin (5,7,3'-trihydroxy-4'-methoxyflavanone); HR – hesperidin (hesperetin-7-rutinoside); N – naringenin (4',5,7-trihydroxyflavanone); NR – naringin (naringenine-7-rhamnosidoglucoside); PP – pure polyphenols; Q – quercetin; QR – rutin (quercetin-3-O-rutinoside)

INTRODUCTION

Polyphenols are the most common group of plant based bioactive compounds. They provide the flavor and color to fruits and vegetables, but can also influence human health because of their antioxidant properties, free-radical scavenging activity and antimicrobial properties. In plant tissues, they are present mainly in the form of glycosides (*i.e.* attached to sugars), although occasionally they are found as aglycones.

Growing interest in a polyphenol rich diet has been observed in recent years. It is believed that these compounds can protect against various diseases, *e.g.* cancers, cardiovascular diseases, diabetes, and some immunological disorders (1, 2). However, other studies demonstrate no effect or even suggest potential harm (3, 4). Many polyphenols can inhibit the growth of microorganisms, both bacteria and fungi, as well as viruses (2, 5, 6), so their influence on various pathogens has been thoroughly examined. The inhibitory effect of ponciretin, hesperetin, naringenin and diosmetin on growth of *Helicobacter pylori* (7) was proved. (+)-Catechin significantly inhibited growth of *Clostridium histolyticum* (8). The growth of *Clostridium* spp. was decreased significantly by a tannin-rich diet or red wine polyphenols, while *Bacteroides* and *Lactobacillus* were stimulated (9, 10).

There are remarkably few studies investigating the influence of polyphenols on the composition and activity of the nonpathogenic gut microbial community. Most of the studies performed in recent years have concerned the bioavailability of polyphenols and their metabolism by human and bacterial enzymes in the gut. The microbial bioconversion capacity of each individual human being influences the final metabolites produced and impacts their bioavailability. Therefore, the composition of human intestinal microbiota (IM) can modulate the polyphenol impact on host health. The composition as well as the proportions of different species that form the IM is highly diverse (11). All individuals have their own unique profile of species, which can be compared to a fingerprint only. On the basis of 16S rRNA analysis of IM samples taken from different individuals it was revealed that, despite the great diversity of bacterial species, the majority (98% of all species) belong to only four bacterial divisions, *Firmicutes* (64%), *Bacteroidetes* (23%), *Proteobacteria* (8%) and *Actinobacteria* (3%), whereas other minor taxonomic divisions are quite diverse (12, 13). Representatives of the genera *Bifidobacterium*, *Eubacterium*, *Lactobacillus*, and *Bacteroides* constitute a large majority (14), but many others species, including pathogenic ones, can be found in the human intestine. There are about 10^{12} bacteria per gram of colonic content, which is the highest accumulation of microorganisms in the environment that has ever been noted (15).

Intestinal microbiota plays an important, if not crucial, role in the metabolism of chemicals delivered with food. Moreover, microbiota plays a protective role by occupying intestinal surfaces and creating a milieu that prevents invasion of pathogens (*e.g.*, by production of antimicrobial compounds). Metabolism of those bacteria causes the breakdown of indigestible compounds during anaerobic fermentation, such as resistant starch and plant polysaccharides, which results in short chain fatty acids production. Other examples are vitamin K and B12 production or amino acids synthesis (11). A balance between the host immune system and the commensal gut microbiota is crucial for maintaining health. When this balance is disturbed (dysbiosis), the host-microbe relationship can progress towards a disease state (11, 14).

Human gut microbiota is involved in the metabolism of nutrients and other compounds that are supplied in a diet. It is believed that polyphenols not absorbed in the stomach reach the colon, and they undergo hydrolysis in small intestine. In this process aglycones and oligomers are released by microbial glycosidases and esterases, enhancing their absorption (16). Products of bacterial metabolism can further be metabolized to various derivatives. Although some reactions of bacterial metabolism may improve the bioavailability and activity of polyphenolic compounds, other metabolites may be harmful for human cells. Hence, apart from inter-individual variation in daily intake of polyphenols, inter-individual differences in the composition of the human microbiota may lead to differences in bioavailability and bioefficacy of polyphenols and their metabolites (17, 18).

It is particularly important to study interactions between polyphenols and microbiota and their relevance to human health, as polyphenols or their metabolites can exert a negative impact on intestinal bacteria. Hence, the aim of the study was to evaluate the influence of some flavonoids: naringin, naringenin (aglycone), hesperidin, hesperetin (aglycone), rutin, quercetin (aglycone) and catechin on the growth of human intestinal bacteria. Flavonoids used in the experiments were chosen because of their relatively significant consumption with diet. Quercetin is one of the most abundant flavonoids present in fruits and vegetables, especially in onion, pepper and tea. Foods with the highest rutin content are buckwheat, dark berries, apricots, cherries, citrus fruit, green pepper and Rooibos tea. The naringin, naringenin, hesperidin and hesperetin are abundant in citrus fruits, such as oranges, mandarins, grapefruits, and lemons. Good sources of catechin are tea, chocolate, vinegar, red wine, grapes, peaches, apples, and berries. Strains proposed in the study belong to the most important genera of human microbiota, and, according to the provider information, have all been isolated from the human intestine.

MATERIAL AND METHODS

Bacteria cultures

Pure cultures of 6 bacteria species - *Bacteroides galacturonicus* (DSM 3978), *Lactobacillus* sp. (DSM 20059), *Enterococcus caccae* (DSM 19114), *Bifidobacterium catenulatum* (DSM 16992), *Ruminococcus gauvreauii* (DSM 19829) and *Escherichia coli* (DSM 1116) - were purchased from DSMZ (Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures, Germany). Cultures were conducted using appropriate media (suggested by DSMZ) for a given strain, at 37°C, under microaerophilic (*E. caccae*, *E. coli* and *Lactobacillus* sp.) or anaerobic conditions (*B. galacturonicus*, *B. catenulatum* and *R. gauvreauii*). However, for experiments with polyphenols, Trypticase Soy Yeast Extract Medium (TSYM) was used for *E. caccae*, *R. gauvreauii* and *B. galacturonicus*. The TSYM medium was prepared by dissolving Trypticasein Soy Broth (30 g/l) and yeast extract (3 g/l) in distilled water, pH 7.0–7.2. *Lactobacillus* was cultured in de Man-Rogosa Sharpe (MRS) medium, while *E. coli* was cultured in Nutrient Broth. The composition of *Bifidobacterium* Medium was (per liter): tryptic digest of casein (10.00 g, Sigma-Aldrich, Germany), yeast extract (5.00 g), beef extract (5.00 g), soy peptone (5.00 g), glucose (10.00 g), K_2HPO_4 (2.00 g), $MgSO_4 \times 7\ H_2O$ (0.20 g), $MnSO_4 \times H_2O$ (0.05 g), Tween 80 (1.00 ml, Sigma-Aldrich), NaCl (5.00 g), cysteine-HCl × H_2O (0.50 g, Sigma-Aldrich), resazurin (1 mg, Sigma-Aldrich), and 40 ml of salt solution. The composition of salt solution was (per liter): $CaCl_2 \times 2\ H_2O$ (0.25 g), $MgSO_4 \times 7\ H_2O$ (0.50 g), K_2HPO_4 (1.0 g), KH_2PO_4 (1.00 g), $NaHCO_3$ (10.00 g), NaCl (2.00 g). All bacteriological media were purchased from Biocorp (Warsaw, Poland) and other chemicals, if not stated otherwise, were from POCH Spolka Akcyjna (Gliwice, Poland).

Bacterial suspensions

Overnight bacterial cultures were dissolved in appropriate medium to give a final concentration of about 1.5×10^8 colony forming unit/ml (0.5 McFarland standard more than pure medium for this bacteria). The number of cells was evaluated by the turbidity measurement using a portable densitometer (DEN-1B, BIOSAN, Latvia).

Polyphenols

Naringenin (4',5,7-trihydroxyflavanone, N) and its glycoside naringin (naringenine-7-rhamnosidoglucoside, NR), hesperetin (5,7,3'-trihydroxy-4'-methoxyflavanone, H) and its glycoside hesperidin (hesperetin-7-rutinoside, HR), quercetin (Q) and its glycoside rutin (quercetin-3-O-rutinoside, QR), and (+)-catechin (CAT) were provided by Sigma-Aldrich (Germany). Pure polyphenols (PP) were dissolved in pure (100%) dimethyl sulfoxide (DMSO) to obtain stock concentrations of 25 mg/ml. PP stocks were kept in a refrigerator until analysis. The final concentration of polyphenols in medium was 20, 100 or 250 μg/ml. In some media, quercetin precipitated at concentration of 250 μg/ml; moreover, it totally inhibited growth of some bacteria at dose 100 μg/ml. Therefore, the more diluted quercetin stock was used (5 mg/ml). The volume of quercetin stock added to the medium was the same as in the case of other polyphenols so final concentrations of quercetin used in experiments were 4, 20 and 50 μg/ml, respectively.

Assessment of polyphenols' impact on intestinal bacteria

Experiments were performed in broth appropriate for bacteria species. In screw-capped probes with butyl-rubber stoppers, 4.9 ml of medium containing the appropriate amount of PP (the final concentration of 20, 100 and 250 μg/ml or 4, 20 and 50 μg/ml for Q) was inoculated with 0.1 ml of bacterial suspension. The range of polyphenols concentration in the cultures was established at a level similar to that which we can determine in the foodstuffs (19, 20). Samples were mixed, flushed with CO_2, sealed with a butyl-rubber stopper and closed with a screw cap. All experiments with anaerobes were performed in an anaerobic chamber under CO_2. The turbidity of the bacterial cultures was measured after 24 hours of incubation at 37°C and the number of cells was calculated.

The medium without addition of PP inoculated with the same amount of bacteria was a positive control. The "double blank" (the medium without bacteria and without polyphenols) was made, to check if the 24-h incubation in 37°C influences the medium turbity. Pure DMSO at concentrations ranging from 0.08 to 1.0% was used as a control for the potential toxicity

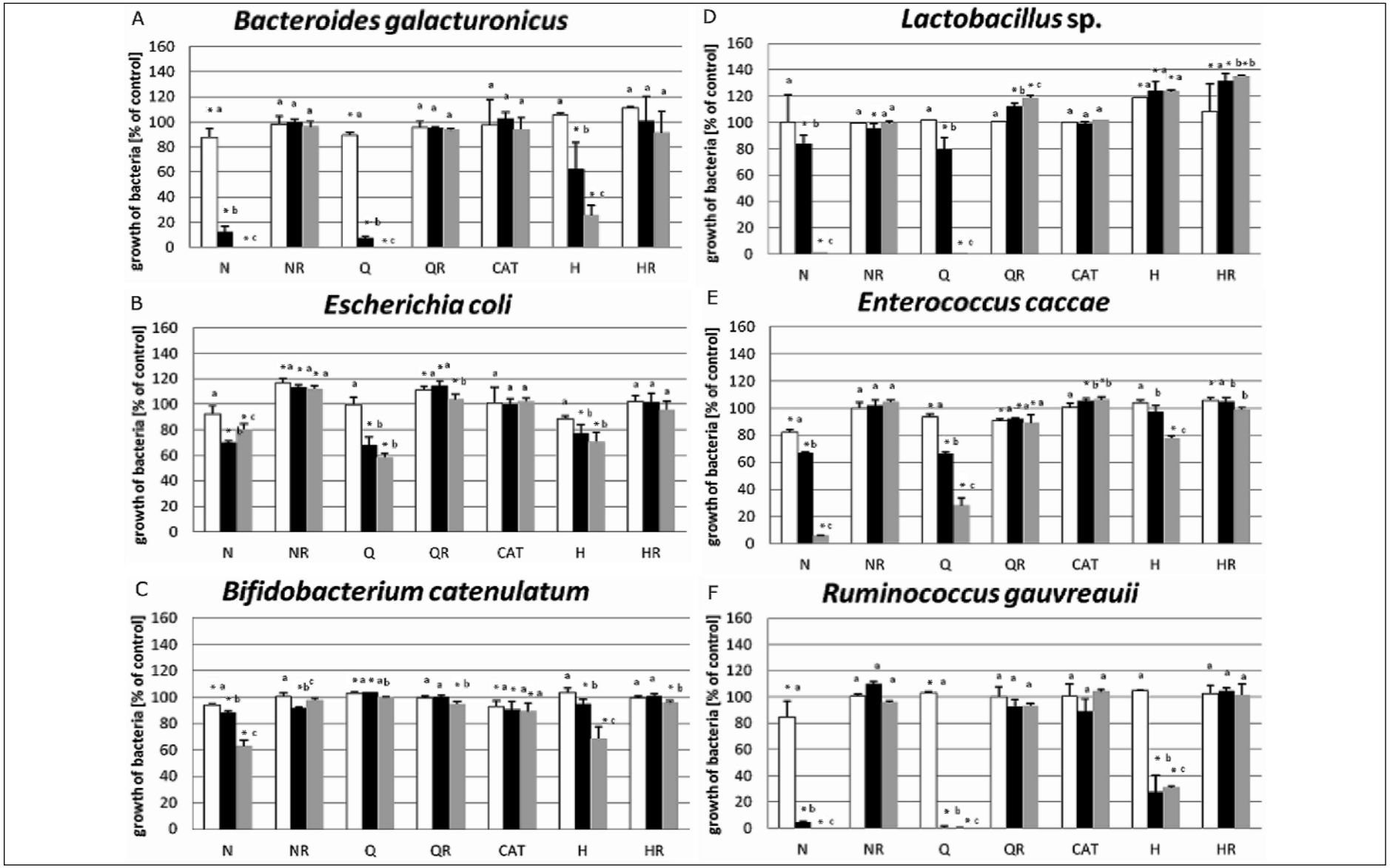

Fig. 1. The impact of naringenin (N), naringin (NR), quercetin (Q), rutin (QR), catechin (CAT), hesperetin (H), and hesperidin (HR) on the growth of A) *Bacteroides galacturonicus*, B) *Escherichia coli*, C) *Bifidobacterium catenulatum*, D) *Lactobacillus* sp., E) *Enterococcus caccae*, and F) *Ruminococcus gauvreauii.* Polyphenol concentrations: (□) **20 µg/ml (4 µg/ml for Q), (■) 100 µg/ml (20 µg/ml for Q), (▩) 250 µg/ml (50 µg/ml for Q). An asterisk * means a statistically significant difference ($p<0.05$) as compared to the sample without the polyphenol (control). The same letters (a, b, c,…) at the bars representing different concentrations of the same polyphenol indicate no statistically significant differences ($p<0.05$) between results.**

Table 1. Minimum inhibitory concentration (MIC) of polyphenols against *Bacteroides galacturonicus* (BAC), *Lactobacillus* sp. (LCB), *Enterococcus caccae* (EN), *Bifidobacterium catenulatum* (BF), *Ruminococcus gauvreauii* (RUM), and *Escherichia coli* (EC).Values are expressed as μg/ml.

Polyphenol	BAC	LCB	EN	BF	RUM	EC
Naringenin	250	250	>250	>250	250	>250
Naringin	NI	NI	NI	NI	NI	NI
Quercetin	50	50	>50	>50	20	>50
Rutin	NI	NI	>250	NI	>250	NI
Hesperidin	NI	NI	NI	NI	NI	NI
Hesperetin	>250	NI	>250	>250	>250	>250
Catechin	NI	NI	NI	>250	NI	NI

NI – no inhibitory effect observed in analyzed range of polyphenols.

action of polyphenols solvent. As the addition of polyphenols to different media changed the medium color and turbidity, blank samples (medium with appropriate concentration of PP without bacteria) were also prepared. The value obtained for the blank was each time subtracted from the value obtained for the sample turbidity, taking into account the kind of medium and the concentration of polyphenol. The obtained results were expressed as % of positive control in order to facilitate the comparison between different bacteria species.

Minimal inhibitory concentration

The minimal inhibitory concentration (MIC) of polyphenols was considered as the lowest concentration which inhibited bacterial growth (when the turbidity of bacterial culture with polyphenol after 24 h equaled the turbidity of the blank sample) and was expressed as μg/ml.

Statistical analysis

All experiments were performed in a minimum of three replications and the turbidity of each sample was measured a minimum of three times, rotating along the main tube axis to avoid errors caused by non-ideal shape of the tube. The results are shown as the arithmetic mean (±standard deviation). A single-factor analysis of variance test (ANOVA) with a *post hoc* Tukey test was applied to perform a statistical analysis. A Kolmogorov-Smirnov test was applied to examine the normality of distribution. The differences were considered statistically significant at $p<0.05$.

RESULTS

The impact of naringenin (N), naringin (NR), hesperetin (H), hesperidin (HR), quercetin (Q), rutin (QR), and (+)-catechin (CAT) on particular bacteria species is presented in *Fig. 1A-1F*. The polyphenols (N, NR, HR, H, QR, CAT) were used at a final concentration of 20, 100 or 250 μg/ml, except quercetin, which was applied at a concentration of 4, 20 or 50 μg/ml. In the experiments the maximum amount of DMSO (solvent) added to bacteria did not exceed 1% and there was no toxic effect observed of such DMSO concentrations (data not shown). The turbidity of "double blank" (medium without PP and without bacteria) did not changed after 24-hours incubation at 37°C.

When comparing the results of all experiments it can be clearly demonstrated that naringenin and quercetin slowed down the growth of all analyzed intestinal bacteria or even completely inhibited it, and the inhibitory effect was dose-dependent. On the other hand, their glycosides (naringin and rutin, respectively) had no inhibitory effect, and in some cases exerted a stimulatory effect. The same observation was made for hesperetin and its rutinoside hesperidin, although in this case the impact of aglycone was weaker, but also dose-dependent. Catechin (existing only as aglycone) did not influence the growth of most analyzed bacteria. Only *B. catenulatum* was slightly inhibited (MIC >250 μg/ml), while *E. caccae* was stimulated by higher doses of catechin.

The estimated MIC values of polyphenols that inhibited the growth of intestinal bacteria are summarized in *Table 1*. The lowest MIC value was demonstrated for quercetin (20–50 μg/ml). There was no correlation observed between the response to the polyphenol and physiological characteristic of bacteria (anaerobic or aerobic).

DISCUSSION

In many *in vivo* and *in vitro* experiments the health-promoting properties of pure polyphenol compounds have been shown (1). However, many of them despite their presence in large amounts in the diet cannot develop biological activity because of low bioavailability or metabolic changes they are subjected to in the human body. There have been many studies assessing the effectiveness of polyphenol absorption in the digestive tract or determining pathways of particular compounds' metabolism by intestinal microorganisms. The bioavailability of polyphenols depends, among other things, on molecule dimensions, level of polymerization, presence and kind of sugar in the molecule and on the compound's hydrophobicity (21). Polyphenol glycosides constitute about 80% of all polyphenolic compounds in plant tissues, while aglycones are rather rare (22). Glycosylation has a significant influence on the uptake of polyphenols. Flavonoid aglycones express hydrophobic character and can be transported through biological membranes on the basis of passive diffusion (23). Flavonoid glycosides generally do not undergo acid hydrolysis in the stomach and are carried to the intestine in their native form, where they are hydrolyzed by intestinal microbiota (21, 24).

In recent years, one of the most important goals of studies has been to determine the impact of intestinal microbiota (IM) on the bioavailability of polyphenols, but only a few studies have investigated the converse relationship. In the present study, the inhibition of intestinal bacteria growth by some polyphenols was observed. Generally, aglycones were harmful to the bacteria while their glycosides did not have any effect. This implies that polyphenols can indirectly influence their uptake from the diet and this impact is dependent on the form of polyphenol.

Quercetin and its glycosides are among the most common polyphenols found in the human diet. Because of their abundance, they have received much attention from researchers. The main sources of quercetin glycosides in human diet are red wine, onion (mainly glucosides), and tea (mainly rutinoside), but they are also present in berries and apples (24). Quercetin has been proven to be an excellent antioxidant that also possesses anti-cancer, anti-inflammatory, anti-proliferative and gene expression changing capacities (1, 25). In consequence, quercetin is a popular diet supplement that supports the immunological system. However, according to the so-called "nutritional paradigm", when the element is given in excess, it reaches the toxicity limit (26). The daily intake of quercetin from food sources ranges from 5 to 40 mg but can reach up to 500 mg/day when consumption of fruits and vegetables is high, especially when the peel of the fruit is consumed (27). Various

supplements containing quercetin are available at pharmacies, and the daily dose recommended is usually about 500 mg of quercetin aglycone. To date, toxic effects of quercetin have only been observed *in vitro* and were most likely associated with the formation of oxidation products of quercetin (quinones) that impair the function of several critical enzymes (25). Consequently, during prolonged quercetin supplementation care should be taken because of the possible side effects. Supplements usually contain only the aglycone form of quercetin, whereas food components normally contain high amounts of various quercetin derivatives that might have a better biological availability than the aglycone itself. Hollman *et al*. (28) proved that quercetin glucosides are absorbed faster and more efficiently than aglycone, and the quercetin rutinoside (rutin) was less rapidly absorbed.

In the study, it was found that rutin had no inhibitory influence on the analyzed intestinal bacteria (*Fig. 1A-1F*). In the case of *Lactobacillus*, slight stimulation of growth was even observed (*Fig. 1D*). In contrast, its aglycone quercetin exerted an inhibitory effect (except on *Bifidobacterium catenulatum*), especially strong on *Ruminococcus gauvreauii* (with MIC 20 µg/ml), *Bacteroides galacturonicus* and *Lactobacillus* (MIC 50 µg/ml). This inhibitory impact was strongly dose-depended. It should be highlighted that although quercetin itself (aglycone) is not a normal dietary component, it can be generated by some intestinal bacteria from quercetin glycosides and hence influence other microbiota living in the colon (21).

In the study of Sanchez-Patan (29), the extract of wine phenolics (rich in quercetin, flavan-3-ols and anthocyanins) had no influence on the *Lactobacillus*/*Enterococcus* spp., *Bacteroides* spp., *Bifidobacterium* spp., and members of the domain *Bacteria*. Only a slight inhibition in the *Clostridium histolyticum* group was observed after 30 and 48 hours but these changes were not significant.

The antibacterial mechanism of polyphenols has not been fully elucidated, but several mechanisms have been proposed. First of all, polyphenols can bind to bacterial cell membranes, disturbing membrane function and, consequently, inhibiting cell growth (30). Bacteria contain metalloenzymes, while flavonoids can form strong complexes with heavy metals (31). This can cause various metabolic perturbations (enzyme inhibition, impaired functions of ion channels) or lead to iron deficiency in the gut and hence affect sensitive bacterial populations (1, 10). Among other proposed mechanisms, enzyme inhibition and reactive oxygen generation by epigallocatechin gallate or DNA gyrase inhibition by quercetin are mentioned.

The results of the present study can add another side effect of excessive consumption of aglycones - changes in the composition of physiological microbiota in the human intestine, which may not be beneficial.

It has been documented in previous studies that some species of bacteria from *Lactobacillus*, *Streptococcus* and *Enterococcus* genera may produce relatively high amounts of hydrogen peroxide (32). H_2O_2 can act as antibacterial factor but it may be also involved in pathomechanism of irritable bowel disease (IBD) by perpetuating the inflammatory reaction and increasing apoptosis and necrosis. The changes in the composition of physiological microbiota can be undesirable also in situation when they cause the reduction of probiotics level. Probiotics are known to be useful in treatment of many disorders, for example they can alleviate the symptoms of colitis (33).

Similar results were obtained for flavanones (hesperetin, hesperidin, naringin, naringenin). Naringenin strongly inhibited the growth of *Ruminococcus gauvreauii*, *Bacteroides galacturonicus* and *Lactobacillus* sp., and hesperetin influenced *Bifidobacterium catenulatum*, *R. gauvreauii* and *B. galacturonicus*. Neither glycoside of naringenin (naringin) nor glycoside of hesperetin (hesperidin) had an impact on the analyzed intestinal bacteria. Our results are similar to those of Mandalari *et al*. (34). They found that the deglycosylation caused by the enzyme preparation Pectinase 62L significantly increased the antimicrobial potency of *Citrus* flavonoids. This means that the activity potential of flavanones is dependent on the sugar presence/absence in the moiety.

Other factor influencing the inhibitory activity of flavonoids' aglycones may be structure of their skeleton. Catechin belongs to flavan-3-ols and has the same hydroxylation pattern as quercetin (OH groups at C3, C5, C7, C3', and C4'). However, quercetin contains also the C2–C3 double bond and 4-carbonyl group in the C ring. Of this, only quercetin had inhibitory influence. Flavanones (hesperetin and naringenin) have 4-keto group while their C ring is saturated (no double bond C2–C3), but they also exerted inhibitory effect. Those results can suggest that 4-carbonyl group in the C ring is critical for the inhibitory activity of aglycones.

The polyphenols that are most well-absorbed in humans are isoflavones and gallic acid, followed by catechins, flavanones, and quercetin glucosides, with different kinetics. The least well-absorbed polyphenols are the proanthocyanidins, the galloylated tea catechins, and the anthocyanins (21). Aglycones are absorbed more rapidly than glycosides. Bugianesi *et al*. (35) demonstrated that the peak plasma concentration (C_{max}) values for flavanone metabolites were measured about 5 hours after the ingestion of citrus fruits rich in glycosides, while it was only 2 hours after the ingestion of tomato paste, which contains naringenin aglycone. It is believed that aglycones are absorbed more rapidly than glycosides, because they are absorbed in the small intestine, while glycosides need time to reach the colon and must be hydrolyzed by cecal microbiota before absorption of the released aglycones in the colon (21). Looking at the results of this study, another explanation is possible. The slower or poorer uptake of some polyphenols may also be caused by their inhibitory effect on intestinal microbiota. Aglycones released by microbiota in the colon can inhibit bacterial activity and consequently reduce polyphenol metabolism by IM. Normally, aglycones do not reach the colon as they are absorbed in the earlier fragments of the gut.

It is interesting that some dietary compounds can change the end products of bacterial metabolism, which in turn can modify the expression of mucin genes and proteins leading to an increase in the mucus layer thickness. Viscous mucus covers the intestinal wall, disables bacterial movements, and protects epithelial cells from contact with bacteria (36). In some cases the mucus barrier can be impaired which results in direct contact of bacteria (both pathogenic and non-pathogenic) with the intestinal wall and, in consequence, in prolonged inflammation. The chronic intestinal inflammation is the result of a deregulation of intestinal homeostasis with a host's loss of tolerance towards normal gut microbiota. Polyphenols that have antioxidant properties appear to be a promising candidate to prevent and treat gut disorders and diseases caused by inflammation.

Many natural compounds, including plant polyphenols, have been widely used because of their strong antimicrobial properties against food-borne pathogens, and therefore they can be applied as novel preservatives in the food industry (16). The antibacterial properties of polyphenols were analyzed earlier, but mainly against human pathogens or microorganisms that cause food spoilage. Naringenin and quercetin were effective inhibitors of *S. aureus* growth and *S. typhimurium* adherence to Caco-2 enterocytes, while phloridzin and rutin enhanced the adherence of the probiotic *L. rhamnosus*. Those results suggest that polyphenols have the potential to alter gut microecology and, by affecting the total number of beneficial microbiota in the gut, may confer positive gut health benefits (37). In our previous study, we demonstrated that

some antioxidants common in plant raw material exert an inhibitory effect on probiotic strains of bacteria (38). This study confirmed that some polyphenols can exert a negative, instead of beneficial, effect by inhibition of intestinal microbiota.

The knowledge that polyphenols exert a health-promoting effect led to increased consumption of various polyphenols *e.g.*, quercetin, citrus flavonoids, and catechins. As the amount of their aglycones in food is usually very low, causing low bioavailability, nutritional supplements are becoming more popular. The concentration of polyphenols in such supplements is several times higher than in a typical diet. Excessive amounts of polyphenols reaching the colon may inhibit the growth of beneficial microbiota, which is responsible for bioconversion of polyphenols and enhancing the bioavailability of those compounds. Therefore, dietary supplementation with aglycones may exert a negative effect on human health instead of supporting it. In this study, *Bifidobacterium catenulatum* was fairly resistant to the used polyphenols, but *Lactobacillus* sp. (a common probiotic) was strongly inhibited by naringenin and quercetin at concentrations of 250 and 50 µg/ml, respectively. A similar effect was observed for *Ruminococcus gauvreauii*, *Bacteroides galacturonicus* and *Enterococcus caccae*, which are members of the commensal human microbiota. In the study only some representatives of several intestinal bacteria genera were examined. However, the information obtained should encourage food producers to carry out broader studies on the effects of supplements they provide.

A balance between the host and the gut microbiota is crucial for maintaining health and depends on many mechanisms. When this balance is disturbed (dysbiosis), the host-microbe relationship may progress towards a disease state (11, 14). Recent studies suggest that specific members of the gut microbiota play a functional role in inflammatory bowel disease (IBD), Crohn's disease, colorectal cancers, obesity, and allergies, and this could have clinical significance (11, 13, 39-44). Moreover, the existence of a brain-gut-microbiota axis has been also postulated (45). The gut bacteria help to keep bidirectional contact between the components of the brain and gut axis. The dysregulation of those mechanisms (for example by stress) has important clinical implications in the upper gastrointestinal tract and lead to the development of a broad array of gastrointestinal diseases such as peptic ulcer disease, IBD, irritable bowel syndrome, and food allergy.

The activity of commensal intestinal microbiota is also essential for increasing the bioavailability of polyphenols. Although polyphenols in plants are mainly in the form of glycosides, the majority of them must be metabolized to aglycones before absorption. However, aglycones released by bacterial metabolism may inhibit the growth and activity of bacteria present in the intestine. In this way they indirectly interfere with the bioavailability of polyphenolic compounds. This implies that the polyphenols present in the diet modulate the intestinal microbiota and thus affect their own bioavailability.

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