Interactions between Plant Bioactive Food Ingredients and Intestinal Flora—Effects on Human Health

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Gut is the site of active fermentation of non-digestible dietary components (dietary fibre and prebiotics) as well as bioconversion and absorption of plant-derived phenolics. These compounds have an important role in gut fermentation by influencing the composition of microflora and fermentation metabolites, and consequently by contributing to both local and systemic effects in humans. Possibilities to enhance viability and promote growth of probiotic bacteria by non-digestible food components have been a subject to extensive scientific interest in the last ten years. Gut bacteria are known to degrade and ferment dietary fibre, producing metabolites, especially short-chain fatty acids. They also mediate a number of important consequences through their further metabolism in the liver. Current research is at quick steps increasing our understanding about the interactions between gut microbes and bioactive dietary phenolics. Absorption and metabolism of phenolic compounds occurs along the digestive tract. Those compounds not absorbed or converted earlier enter the colon, and may be converted to metabolites concomitantly with carbohydrate fermentation. All the colonic metabolites can have effects on the epithelium at the site of conversion, and also affect the colonic flora locally. When absorbed the metabolites are found in plasma and urine and can have systemic health effects. The health effects of phenolic compounds have been studied extensively, but those of the metabolites are poorly known. As strong antimicrobial agents the phenolics might also have unpredictable effects on the composition of the intestinal flora.

Key words: dietary fibre; phenolics; gut microflora; health effects

INTRODUCTION TO PLANT BIOACTIVE COMPOUNDS

Plant containing foods form an important part of our daily diet. Therefore plant constituents and their nutritional value have been intensively studied over the decades. Many of them are well known and their health effects widely characterized. These include dietary fibre, essential fatty acids and vitamins. Dietary fibre is a nutritional definition of the non-digestible part of plant food, which is not absorbed in the small intestine. Dietary fibre thus comprises a range of different compounds, the majority of which are plant cell wall polysaccharides, such as hemicellulose and cellulose. Dietary fibre, however, often also contains lignin and other phenolic constituents.

Besides essential primary metabolites (carbohydrates, lipids, proteins) higher plants synthesize a wide variety of low molecular weight compounds, the effects of which are less well characterized from the nutritional or health promoting point of view. These compounds, named as secondary metabolites are often produced in low levels, and depending greatly on the vegetative state of the plant. Many secondary metabolites, having a very complex or unique structure, play an important role as defence compounds and their production is enhanced in stress conditions (e.g. through attack of the pathogens, special climate conditions etc.). They are stored in the certain organs of the plant and are often accumulated in vacuoles of the cell.

Close to 100,000 secondary metabolites have been discovered from plant kingdom. They are characterized by an enormous chemical diversity. Their production is often strongly restricted to certain plant families. On the other hand there are a number of secondary metabolites such as plant phenolics which are abundant in many plant species. Our current understanding about the role of secondary metabolites is still rather limited. However, they are important in the survival of the plant in its ecosystem. Besides the importance for the plant itself secondary metabolites have formed centuries been of interest as flavours, fragrances, dyes, pesticides and pharmaceuticals. In functional food development secondary metabolites, or as often called phytochemicals, are playing an increasingly important role. The most widely studied groups are phytosterols, plant phenolics and glucosinolates. Edible plants do not only contain compounds which are favourable for our health
but also toxic compounds. A good example of the latter is the glycoalkaloids in potato which may occur in our food. Also in food processing and/or in gut digestion some unfavourable plant metabolites may be formed.

Already more than 5,000 flavonoids are known. They are the most abundant phenolic compounds in plant based food. Flavonoids can be divided into several classes according to the degree of oxidation of the oxygen heterocycle: flavones, flavonols, isoflavones, anthocyanidins, flavanols, proanthocyanidins and flavanones (reviewed by Robards and Antolovich (86)). Simple phenolics such as phenolic acids and more complex compounds as lignans occur in various edible plants. Lignans are essentially cinnamyl alcohol esters which through cyclization and other modifications create a wide range of structural types (61). Classification and chemical structures of dietary phenolics are presented in Fig. 1.

Berries contribute a significant amount of various phenolic compounds in the Nordic diet. Many berries are rich in flavonoids, such as flavonols. High flavonol contents are found for example in cranberry, blackcurrant and lingonberry (41). In fact, wild berries and blackcurrant contain more flavonols than vegetables and fruits commonly used. Anthocyanidins are the dominating group of flavonoids present in berries. They are good absorbers of visible light, thus appearing as colored substances, responsible for the characteristic orange/red/blue colors of berries, such as strawberries, raspberries, bilberries and red and black currants. Ellagic acid, which is present in the vacuoles of the plant cell as hydrolyzable, water soluble polymer called ellagitannin, is the main phenolic compound in berries of the family Rosaceae, genus Rubus (red raspberry, arctic Bramble, and cloudberry), and genus Fragaria (strawberry). Ellagitannins are not found in any other common foods in Finnish diet, so these berries remain the most important sources of them (42). Some berries, such as cranberry, also contain tannins, called proanthocyanidins, which are complex flavonoid polymers. These compounds give a characteristic bitter taste to many berries (7). Lingonberry, strawberry and cranberry are examples of berries rich in lignans (62).

Phenolic compounds have a variety of beneficial biological properties. They are potent antioxidants, and exhibit various other physiological activities including anti-inflammatory, antimicrobial, antiallergic, anticarcinogenic and antihypertensive activities. High flavonoid consumption is associated with reduced risk of chronic diseases like cardiovascular diseases (65, 69).

Lignans and isoflavones have been known for a long time to possess estrogenic activity and therefore they are also called phytoestrogens.

Antioxidant phenolics are present naturally in a wide range of foods, especially fruits, berries, cereals and vegetables. Isoflavones are found primarily in soya products and lignans in whole-grain cereals, legumes, vegetables and fruit (particularly berries). There is now increasing interest in the extent to which such natural antioxidants in the diet can protect the body against the ravages of free radicals. Plant phenols can strongly prevent oxidation of low density lipoproteins in vitro, and they have either alone or in combination with e.g. dietary fibre a variety of other biological effects in numerous mammalian cell systems, as well in vivo. However, we do not yet know how readily and in which form such antioxidants can be absorbed from various foods. Also the mechanism of absorption is largely unknown. The question of bioavailability is one of the key issues in the research of plant phenolic antioxidants since it is not yet confirmed whether the effective concentrations found in vitro systems really reflect physiological concentrations.

In this review we focus on gut reactions of dietary fibre and phenolic compounds of plants. We give examples of how phenolic compounds are possibly absorbed and metabolised in gut, and which type of interactions and antimicrobial properties berry phenolics have with gut microflora as well as with intestinal and food pathogens (Fig. 2).

GUT CONVERSIONS OF DIETARY FIBRE AND OLIGOSACCHARIDES

Dietary fibre (DF) is a nutritional definition for the non-digestible part of plant food. The concept of DF was defined in 1972 as the skeletal remains of plant cells that are resistant to hydrolysis by the enzymes found in man (104). Later DF was redefined as plant polysaccharides and lignin, which are resistant to hydrolysis by the digestive enzymes of man (105). According to the suggested new definition, oligosaccharides are also included in DF (2): “Dietary fibre is the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. Dietary fibre includes polysaccharides, oligosaccharides, lignin, and associated plant substances. Dietary fibres promote beneficial physiological effects including laxation, and/or blood cholesterol attenuation, and/or blood glucose attenuation.”

The bulk of dietary fibre thus consists of carbohy-
Fig. 1. Chemical structures of the main classes of dietary phenolic compounds.
Fig. 2. Reaction of carbohydrates and phenolic compounds with colonic microflora with respect to their health effects.

drates, in most cases plant cell wall polysaccharides. Recently, much emphasis has also been put to the associated bioactive compounds, so called "co-passengers" of dietary fibre. Many of these are phenolic compounds, and will be discussed separately in this article.

The amount of carbohydrates reaching the colon depends on the amount consumed. The recommended daily consumption of DF is 25–35 g. DF and oligosaccharides entering the large intestine provide a substrate for the colonic microbiota (51). Obviously, other carbohydrates that are not completely absorbed in the small intestine may also enter the colon (24, 40). In the colon, polymeric substrates are first degraded by the hydrolytic enzymes of the intestinal bacteria, and the released sugars are then fermented. The colonic microbiota and/or its activity may alter, depending on the nature of the substrate. Many different bacterial species may be needed for hydrolysis and fermentation of a single complex polysaccharide (59).

Bacteriologically, the large intestine is an extremely complex ecosystem, which probably contains more than 400 different species of bacteria. During fermentation, the bacterial mass increases and consequently also the faecal bulk. The nonfermentable part of DF also adds to the faecal bulk. An increase in bulk in the large intestine results in a shorter transit time and prevents constipation. In addition DF can bind and remove potentially harmful compounds in the colon (3, 34, 36, 63). Fermentation by microbiota results in the formation of a number of end products that influence large intestine physiology and metabolism. Short-chain fatty acids (SCFA) produced during fermentation reduce the pH and alter the balance of microbiota in the colon. SCFA are metabolized in different ways.

Acetic, propionic and butyric acids are the main SCFA that are formed during the colonic fermentation of carbohydrates. SCFA are formed in different molar ratios depending on the substrates available. SCFA are absorbed and metabolized in different ways that may have important implications for human health. Acetic acid is absorbed, transferred via circulation and me-
tabolized in muscle, kidney, heart and brain tissues. Propionic acid is metabolized in the liver where it, among other things, may suppress cholesterol synthesis. Butyric acid is metabolized by the colonic epithelium; it regulates cell growth and differentiation, and thus it has been proposed to assist in maintaining a healthy mucosa (26).

In addition to SCFA, hydrogen and carbon dioxide, as primary gas products, and methane or hydrogen sulphide, as secondary gas products, are produced. In the case of methanogenic bacteria, methane is also formed as an end product. Gases are excreted in breath or as flatus. Excessive or rapid formation of gases may cause flatulence and intestinal pain.

PREBIOTIC EFFECTS OF CARBOHYDRATES

Prebiotic is a concept launched in 1995: a prebiotic is a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health (38). In 1999, it was proposed to modify the definition of a prebiotic effect to the following: a prebiotic effect is a food-induced increase in numbers and/or activity predominately of bifidobacteria and lactic acid bacteria in the human intestine (107). The definition incorporates the bifidobacteria and the lactic acid bacteria because they are considered good markers of a well-balanced intestinal flora. The health aspect is omitted from the definition, because, to date, little information is available which could support such a statement (107).

Any dietary ingredient that reaches the colon, e.g. non-digestible carbohydrates, is a candidate prebiotic. The Consensus Report from the year 1999 (107) contains results from the European-Commission-funded project on non-digestible oligosaccharides. The prebiotic effect of non-digestible oligosaccharides has been studied by means of both in vitro and human in vivo experiments. The only prebiotic effect that has been fully demonstrated is the selective stimulation of growth of bifidobacteria. Of the candidate prebiotics, the inulin-type fructans have been most thoroughly investigated. According to the above mentioned Consensus Report, there is strong evidence, based on many different in vivo studies, that β (2-1) type fructans fulfil the prebiotic criteria. A limited number of human feeding studies indicate that galacto-oligosaccharides may have a prebiotic effect, and a few animal studies indicate that soybean oligosaccharides may be prebiotics as well. Very little scientific information on the prebiotic effect of xylo-oligosaccharides and pyroextrins is available (107).

Many commercial carbohydrates and oligosaccharides have been reported to be bifidogenic (11–13, 56, 58). Most of the studies have been made with inulin, fructo-oligosaccharides or transgalacto-oligosaccharides. Okazaki et al. (74) reported that xylo-oligosaccharides can selectively promote the growth of bifidobacteria. Esteves et al. (35) produced oligomers from brewery’s spent grain, a by-product of the brewing industry that has a high xylan content. B. longum fermented these oligosaccharides, even though the overall growth was poor compared with that obtained with oligofructose. Lactulose may be considered as a bifidogenic or lactogenic factor capable of promoting the growth of probiotic microflora in the colon (6, 89).

In the large intestine, prebiotics, in addition to their selective effects on bifidobacteria and lactobacilli, influence many aspects of bowel function through fermentation. SCFA are a major product of prebiotic breakdown, but as yet, no characteristic pattern of fermentation acids has been identified. Through stimulation of bacterial growth and fermentation, prebiotics affect bowel habit and are mildly laxative (27).

ABSORPTION AND METABOLISM OF BIOACTIVE PHENOLIC COMPOUNDS

Phenolic compounds are absorbed from the intestinal tract of humans and are excreted either unchanged or as metabolites in the urine or faeces. Metabolism takes place either in tissues or for the fraction of non-absorbed and fraction of re-excreted in the bile, by the action of colonic bacteria. The structural diversity of phenolics influences their absorption and bioavailability, which is shown by varying quantities of phenolics found intact in urine (91). Low recovery values were observed for quercetin (about 1%) and anthocyanins (1.5–5.1%) and higher values for catechins, isoflavones and anthocyanidins (3–27%) (93).

Except for the cells lining gastrointestinal tract, all other cells in the body are only exposed to the flavonoids mainly as metabolites and degradation products, and concentrations in human plasma rarely exceeds 1 μM (47). For most flavonoids absorbed from the small intestine, the plasma concentrations rapidly decrease (elimination half-life period of 1–2 hr). Thus maintenance of high concentration in plasma requires repeated ingestion of phenolic compounds. However, the half-life of metabolites formed by the colonic microflora is longer due to long residence time of polyphenols in the colon. For example, more than 2 days are required for enterodiol and enterolactone (phytoestrogen metabo-
lies) to reach the baseline concentrations in plasma after consumption of flaxseed (68).

The form of phenolic compound seems to affect intestinal absorption. The most widely discussed parameters, reviewed by Scalbert et al. (92), are glycosylation, molecular weight and esterification. Most of the flavonoids present in foods are glycosylated, and this has a great impact on their absorption. The first step in the metabolic pathway of phenolics is enzymatic removal of the sugar moiety. This can occur in the cells of the gastrointestinal tract mucosa or by enzymes secreted by colonic microflora. Németh et al. (67) have recently shown that deglycosylation by small intestinal epithelial cell β-glucosidases (lactase phlorizin hydrolase and cytosolic β-glucosidase) is a critical step in the absorption and metabolism of flavonoid glycosides in general. There is also additional indication for interaction with Na-dependent glucose transporter (SGLT1) prior to hydrolysis by β-glucosidase (20, 111). It has been suggested by Németh et al. (67) that significant variation in β-glucosidase activity between individuals may be a factor determining variation in flavonoid bioavailability. Anthocyanin glycosides, such as cyanidin-3-glycoside or the corresponding galactoside, are however not deglycosylated by β-glucosidases. Thus it can be expected that anthocyanins are absorbed more poorly than other dietary flavonoids in the upper intestine. McGhie et al. (64) recently reported that anthocyanin glycosides from berry fruits are absorbed and excreted unmetabolized by both humans and rats. They suggested that the nature of the sugar conjugate and the phenolic aglycon are both important determinants of anthocyanin absorption and excretion.

Absorption of phenolic compounds also depends on their molecular weight. Two classes of high molecular weight phenolics which are very poorly absorbed in the gut are the tea theaflavins (MW = 568) and the proanthocyanidins (MW = 578) (31, 32). Absorption of catechins and caffeic acid is influenced by esterification. Absorption of galloylated tea catechins was considerably lower compared to non-galloylated catechins (113). Also caffeic acid was much better absorbed than chlorogenic acid (its ester with quinic acid) (76). Pfört et al. (80) recently reported of potential stomach absorption of flavonoids, with yet unclear mechanism.

All phenolic compounds are during metabolism conjugated to form O-glucuronides, sulphate esters and O-methyl ethers. This conjugation takes place first in gut barrier. Conjugated compounds are then transported to the liver. The liver can extend the conjugation of the compound by adding a sulphate group, a methyl group, a glucuronide group or all of them. The addition of glucuronide and sulphate groups increases the circulatory elimination time and a methyl group probably also decreases toxicity. Almost all circulating polyphenols are glucuronidated and/or sulphated, and no free aglycones are found in plasma (8, 60).

**GUT CONVERSIONS OF PHENOLIC COMPOUNDS**

*Flavonoids and Phenolic Acids*

Glycosylated phenolic compounds, which are not hydrolysed and absorbed in the upper intestine, enter the colon from lumen, and conjugated phenolic compounds via entero-hepatic circulation (93). The primary step in the colon is deconjugation and de-esterification of phenolic compounds, which results in formation of aglycones or free phenolic acids. Colon bacteria have a number of deconjugative enzyme activities e.g. β-D-glucuronidases, β-D-glucosidases and α-L-rhamniosidases, the action of which is shown as liberation of aglycones of flavonoids from their glycosides and glucuronides (4, 10, 55).

Aglycones of flavonoids are further catabolised to smaller phenolic acid derivatives by C-ring fission. Ring fission metabolism in the colon has been shown to occur in flavonols (e.g. quercetin), flavanols (e.g. catechins) and flavones (e.g. hesperetin). Hydroxyphenyl acetic acids are formed from myricetin and quercetin (39, 55, 115); hydroxyphenyl propionic acids from catechins e.g. procyanidins (30) and phenylpropionic acid, cinnamic acid and benzoic acids from chlorogenic acid (75). Intestinal metabolism of phenolic acids e.g. hydroxycinnamates includes a number of reactions: ester bond cleavage, reduction, O-methylation, decarboxylation and catabolical formation of carbon dioxide. Hippuric acid, a glycine-conjugate, and its 3-hydroxyderivative are considered as non-specific biomarkers of the hydroxycinnamates (85, 94).

Pathways for quercetin (flavonol), (+)-catechin (flavanol) and hesperetin (flavanone) metabolism in vivo have been proposed by Hollman and Katan (46), including bacterial and tissue metabolites found in plasma or urine. In vitro faecal fermentation methods have been applied to study the bioconversion of phenolic compounds by colonic bacterial flora (4, 30). Modern methods of analysis have made the identification and quantification of metabolites possible (30, 66, 112). Thus combining information from in vivo and in vitro studies distinction between overall metabolism by ileum, liver and colon and colonic metabolism alone can be made and the role of colonic flora on the metabo-
lism of phenolic compounds can be defined.

Phytoestrogens

Phytoestrogens have been shown to bind to estrogen receptors competing with estradiol at the receptor complex, yet failing to stimulate a full estrogenic response after binding to nuclear. Thus they may be protective against hormone-related cancers. This anti-estrogenic function has been shown for daidzein and genistein, abundant in soy products, and enterodiol and enterolactone, mammalian metabolites of plant lignans (9).

Genistein has been shown to be metabolized to p-ethyl phenol and daidzein to equol. In an anaerobic incubation with human fecal flora dihydrogenistein and dihydrodaidzein were identified as major metabolites for genistein and daidzein (19), respectively, but further investigation with labelled genistein showed that end-products of this compound were 2-(4-hydroxyphenyl)-propanoic acid and 1,3,5-trihydroxybenzene. Dihydrogenistein and 6’-OH-O-desmethylangolensin were identified as intermediate metabolites (22, 23). Thus the colonic metabolism of isoflavonoids include several steps and still needs further investigation.

Plant lignans are diphenolic compounds abundant in cereals, berries and fruit (61). Lignan content is highest in the outer layers of rye kernel, where the DF content is also highest (70). Secoisolariciresinol and matairesinol were considered to be the most important plant lignans in rye, but recently new lignans have been found and in addition syringaresinol, pinoresinol, lariciresinol and isolariciresinol have been quantitated in rye (44). Syringaresinol content in rye bran is almost ten fold compared to its secoisolariciresinol and matairesinol contents (45). So far, the reported plant lignan content of flax includes only secoisolariciresinol and matairesinol (102), but still flax is by far the highest source of plant lignans due to the high content of secoisolariciresinol.

Intestinal microflora transform plant lignans to mammalian ones. Bioconversion of plant lignans to enterodiol and enterocolactone is a complex phenomenon including several steps and most likely actions by diverse bacterial species in the colon. Facultative aerobes are capable of deglycosylating, dehydroxylating and demethylating secoisolariciresinol to enterodiol and subsequently oxidating to enterolactone. Also, matairesinol is known to be demethylated and dehydroxylated to enterolactone (96). The efficiency of enterolactone conversion of pure lignans found in rye has been shown to vary during fermentation with human fecal flora in vitro (45). Also, efficiency of enterolactone formation shows a large interindividual variation, which is caused by differences in the activities of the bacterial flora (1, 87). Carbohydrates may have a role in lignan conversion, as well. Evidence from an in vitro fermentation model suggests that the conversion of plant lignans to their mammalian metabolites is enhanced by the presence of high-carbohydrate substrate (18). DF intake also had a positive correlation to excretion of enterodiol in humans (87).

AMICROBIAL PROPERTIES OF BERRY PHENolics

The antimicrobial activities of the naturally occurring phenolics from olives, tea, and wine are well documented (109). Research on berry phenolics is rapidly increasing area, because of the high contents and high diversity of phenolic compounds present in many berries and their central role in the diet. So far, only little information is available of antimicrobial properties of the berry phenolics. Only cranberry has been extensively studied. The antibacterial properties of cranberry juice have been known for a long time, and the effect may be associated with inhibition of E. coli adherence to mucosal surfaces by cranberry juice (95). It has been suggested that proanthocyanidins (condensed tannins) are responsible for this antiadhesion property (48, 50). Recent studies with mice fed cranberry proanthocyanidins indicate that a bioactive proanthocyanidin metabolite is present in urine, or properties of the urine are altered by the proanthocyanidins in such a way that adhesion is inhibited (49). Zafiri et al. (116) have speculated that cranberry compounds could also be active in the colon. The metabolites of proanthocyanidins (e.g. released oligomers) could act on the colonic bacterial receptors making them not capable to bind any more to the uroepithelium and proliferate (43). Burger et al. (15) recently reported that a high-molecular-weight constituent of cranberry juice inhibited adhesion of Helicobacter pylori to immobilized human mucus, erythrocytes and cultured gastric epithelial cells. Different isolates of H. pylori differed in their affinity to the cranberry juice constituents. They suggested that cranberry juice may also inhibit adhesion of bacteria to the stomach in vivo, and may prove useful for the prevention of stomach ulcer that is caused by H. pylori. In addition to their bacterial antiadherence activity, tannin molecules exhibit other types of antimicrobial activity through the inhibition of enzyme production, substrate availability, and microbial metabolism (90).

We have studied the antimicrobial activity of 17 pure
phenolic compounds representing flavonoids and phenolic acids, and eight extracts from common Finnish berries against selected Gram-positive and Gram-negative bacterial species, including probiotic bacteria and the intestinal pathogen Salmonella (82). In general, berry extracts inhibited the growth of Gram-negative Salmonella and Escherichia strains but not Gram-positive lactobacillus species, whereas the flavonol myricetin inhibited the growth of all lactic acid bacteria derived from the human gastrointestinal tract flora but did not affect the Salmonella. The number of hydroxyl groups in the B ring in flavonols and flavones seems to be associated with the antimicrobial activity against lactic acid bacteria. No other structure-activity relationship was found. Cloudberry, raspberry and strawberry extracts were strong inhibitors of Salmonella. Sea buckthorn berry and blackcurrant showed the least activity against Salmonella and Escherichia strains (82). In our recent studies antimicrobial activity of berries has been tested against a variety of intestinal pathogens and food poisoning bacteria, such as Listeria monocytogenes, L. innocua, Staphylococcus aureus, Bacillus cereus, Campylobacter jejuni and Clostridium perfringens (manuscript in preparation). C. jejuni and C. perfringens were sensitive to several berry extracts, cloudberry being the most efficient. All tested berry extracts inhibited very efficiently the growth of S. aureus and B. cereus. However, Listeria strains were not sensitive at all to berries or berry extracts. Isolated ellagitannin fraction from cloudberry was very efficient against S. aureus.

Recently Rauha et al. (84) studied antimicrobial effects of some berry extracts against food poisoning bacteria. They found that the widest bactericidal activity was expressed by berries belonging to the genus Rubus (cloudberry and raspberry) which are rich in ellagitannins. Ellagic acid has been reported to exhibit a dose-dependent inhibitory effect (IC50 = 1 nm) on Helicobacter pylori isolated from peptic ulcer patients (21). Also, ellagitannins extract inhibit a range of pathogenic organisms including Vibrio cholerae, Shigella dysenteriae and Campylobacter spp. (90, 100).

Our results showed that intestinal pathogens were selectively inhibited by bioactive berry phenolics, and inhibition was not restricted only to Gram-negative bacteria. We also suggest that inhibitory effects of berry extracts may not be due to simple phenolics but to more complex phenolic polymers such as ellagitannins. The antimicrobial activity of berry extracts is evidently a synergistic effect of various phenolic compounds, many of which are still unidentified. Also, other bioactive compounds in plant extracts, alone or in combination with phenols, might be responsible for the antimicrobial effects.

LOCAL HEALTH EFFECTS OF GUT REACTIONS

The undigestible dietary plant compounds interacting with intestinal flora in the large intestine can influence health by two main principles: 1) by having local effects, either directly due to their chemical and physical properties or their influence on microorganisms, or due to the metabolites formed in fermentation, and 2) by systemic effects on body function due to absorption and distribution to target organs by blood circulation. Studying of both types of effects is very difficult. In the case of local effects in the gut, monitoring fermentation and its effects on human gut is not possible. Faecal analyses can be made, but their reflectance of the true gut reactions is limited. This has led to the fact that our understanding is mainly based on studies in animal models, as well as in vitro experiments.

The local effects are important for the maintenance of a balanced gut microflora, i.e., for control of the growth of intestinal bacteria, and for the mucosal health. The role of non-digestible carbohydrates in selectively promoting growth of specific bacteria, as well as the potential for selective inhibition of bacterial growth, was already described above. Also the fermentation rate of carbohydrates is important, and is reflected for example in the formation of gases. Very fast fermentation may cause flatulence. Carbohydrate fermentation has also been shown important with respect to colorectal mucosa and colon cancer. Especially the fermentation end product butyrate has been shown to have an important protective effect (14, 78, 110). The butyrate is rapidly taken up from the gut lumen and is metabolised in the colonocytes. This is important also to take into consideration that the large bowel has an important role in the immune defense system. The cellular effects of butyrate include cell maturation, cell differentiation and apoptosis (101). Recently it was also shown in pigs that the concentration of butyrate in the portal vein was influenced by the production rate in the large intestine (5).

Colorectal cancer is supposed to be developed as a result of a series of mutations in genes controlling cell division, apoptosis and DNA repair. The current research tools offer improving tools to learn about the interactions between the environment and genes in the colon, and to learn about the mechanisms of diet-related control mechanisms (53, 83). Dietary fibre has been considered protective partly due to its capability.
to adsorb carcinogens, but more recently the presence of phenolic compounds (such as ferulic acid) or other phytochemicals that are released from plant cells inside the gut has been suggested to play a role, too (37).

Recently research into the role of phytochemicals in preventing the process of colon cancer formation has increased, but the picture is far from clear. For example lignans have been suggested to play a role in preventing colon cancer in animal experiments (73), but recent results with flaxseed did not support cancer protective effects by lignan precursors (106). The oxidation reactions and their prevention by dietary antioxidant phenolics has been pointed out also in connection with mucosal cell proliferation and programmed death, relevant for the colon carcinogenesis process. Especially phytate has been referred to as one candidates behind the potentially protective effects of cereal fibres (77). Flavonoids, showing complex effects on cellular reactions, have also been studied for their modulation of apoptosis and mitosis. As reviewed by Johnson (53), the positive indications so far have to be further verified before conclusions about dietary flavonoids with respect to colon cancer can be drawn. More research is also needed into the effects of metabolites of these compounds, and into the local gut concentrations reachable by dietary exposure.

**SYSTEMIC HEALTH EFFECTS OF GUT REACTIONS**

It is becoming increasingly clear that microbial reactions in the gut have a great impact on the protective effects of bioactive food components against many diseases, cancers, cardiovascular diseases and urinary tract infections.

Fermentation of carbohydrates always produces systemic effects, in addition to the possible prebiotic effect. The effects of short chain fatty acids formed during fermentation on lipid metabolism, calcium absorption and colon cancer have been studied. The results are mainly preliminary and partly incompatible. However, many of the results support each other by showing improved calcium absorption using fructo-oligosaccharides (25, 71). Recently, much emphasis has also been put to the phenolic compounds associated with dietary fibre. Their role in the health benefits related to various dietary fibre sources is not yet well understood.

Plant phenolics have a variety of biological effects in numerous mammalian cell systems and in in vitro tests. However, most of the bioactivity studies so far have been carried out with small number of individual flavonoid aglycones, more than conjugated compounds, with pharmacological, not daily intake doses. It is also not known whether the effective concentrations found in in vitro systems really reflect physiological concentrations. The biological properties of microbial metabolites of phenolic compounds have rarely been studied. This is due to lack of information of the metabolites and mechanisms of action. The intestinal microbial flora and its consistency apparently play a very central role in the bioavailability and metabolism of plant phenolics, the mechanisms of which are largely unknown. It is not well known which part of the plasma metabolites are absorbed by the small intestine and which part by the colon after degradation by microflora. The large interindividual variation in bioavailability of phenolic compounds could be explained by variation in microflora composition (93).

Many health effects of the phenolics are believed to be based on antioxidant activity. The intact polyphenol concentrations in plasma are often very low and thus do not increase the plasma antioxidant capacity. However, the metabolites seem to contribute to increased antioxidant capacity of plasma. Cassidy et al. (17) have shown that equol (colonic metabolite of daidzein) may be three to four times more abundant in plasma than the parent isoflavone. Many of the aromatic acids formed in the colon still bear free phenolic groups and retain part of the reducing capacity of the parent molecule. Measurement of the total antioxidant capacity of plasma after the consumption of polyphenol-rich food has been the focus of several studies (33, 114). The results suggest that more phenolic compounds are present, largely in the form of unknown metabolites, formed in the tissues or by the colonic microflora, and more significantly they contribute to the antioxidant capacity.

In addition to antioxidant activity, various biological activities have been reported for the bacterial metabolites of the phenolics, which might have systemic effects. Shutt and Cox (99) showed higher activity of equol to estrogen receptors than daidzein. Metabolites of rutin and quercetin, 3,4-dihydroxyphenylacetic acid and 4-hydroxyphenylacetic acid were more active in inhibition of platelet aggregation than the parent compounds (55). Bacterial transformations of anthocyanins affect mutagenicity of the ingested compound, since metabolites have been demonstrated to be non-mutagenic (54).

Health effects of plant lignans metabolites have attracted a lot of interest recently. Plasma enterolactone levels have been associated with a lower risk of several diseases e.g. acute coronary events, breast and prostate
cancers (1, 29, 52, 81, 108). Also, a chemopreventive effect of plant lignans on colon and prostate cancers has been shown in animal experiments (16, 28, 73, 88). Especially, flax has been associated with a decrease of both the number and size of tumors in the proximal ileum of Apc<sup>Min</sup> mice (72, 103). Whether this chemopreventive effect is due to unconverted phenolics in the ileum or enterolactone formation by bacterial bacteria, is to be elucidated. The importance of the microflora in the metabolism of lignans and isoflavones has been well demonstrated. Antibiotics administration prevents production of the metabolites (96), and individuals without an intact colon have low plasma and urinary lignan levels (79). The fact that long-term medication for urinary infections shows a possible elevated risk of future breast cancer in premenopausal women (57) also pointed out the importance of an active gut microflora. Also, efficiency of enterolactone formation shows a large interindividual variation, which is caused by differences in the activities of the bacterial flora (1, 87).

Carbohydrates may have a role in lignin conversion, as well. Evidence from an in vitro fermentation model suggests that the conversion of plant lignans to their mammalian metabolites is enhanced by the presence of high-carbohydrate substrate (18). DF intake also has a positive correlation to excretion of enterodiol in humans (87). Thus complex interactions between the colonic environment and factors modulating it contribute to significant variation in serum and urinary phytoestrogen levels among individuals (68). Plant phenolics, as antimicrobial agents, may selectively suppress the growth of some intestinal bacteria, and consequently influence the bacterial population dynamics. This has been shown in vitro with the berry phenolics, which selectively inhibit the growth of intestinal pathogens (82). Phenolic compounds seem to affect the growth of intestinal microbes in many mechanisms, yet not well understood. Antiadhesion is one known mechanism of action, as shown by inhibition of adhesion of <i>E. coli</i> bacteria to uropathelial cells by cranberry proanthocyanidins. Antiadhesion therapy of microbial diseases will be in general a very promising approach in the future (97, 98), and thus the role of various berry phenolics and their colonic metabolites might offer interesting subject for antiadhesion studies. Development of alternative regimens for prevention and treatment of antibiotic resistance bacterial infections will also be very important topic in the future. Thus, utilization of antimicrobial activity of phenolic compounds may offer many new possibilities not only in functional foods, but also in drug development.

In conclusion, increased understanding of interactions between non-digestible carbohydrates, phenolic compounds, gut microflora and the host is needed to properly evaluate their role in prevention of diseases. Potential health effects of phenolic compounds are well known according to in vitro studies, and future research is focusing on the health effects of intestinal metabolites. Evaluation of the health effects in vivo is complex because of the heterogeneity and of the different molecular structures, and the scarcity of data on bioavailability. The phenolic compounds. Also insufficient methods are available to measure e.g. oxidative damage in vivo and the measurement of the objective endpoint remains difficult. Identification of the metabolites and evaluation of their plasma and tissue levels, is a prerequisite for understanding their role in prevention of specific diseases.

REFERENCES


130  141–151.
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