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MINI-REVIEW

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Bioactive berry compounds—novel tools against human pathogens

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Abstract Berry fruits are rich sources of bioactive compounds, such as phenolics and organic acids, which have antimicrobial activities against human pathogens. Among different berries and berry phenolics, cranberry, cloudberry, raspberry, strawberry and bilberry especially possess clear antimicrobial effects against, e.g. *Salmonella* and *Staphylococcus*. Complex phenolic polymers, like ellagitannins, are strong antibacterial agents present in cloudberry and raspberry. Several mechanisms of action in the growth inhibition of bacteria are involved, such as destabilisation of cytoplasmic membrane, permeabilisation of plasma membrane, inhibition of extracellular microbial enzymes, direct actions on microbial metabolism and deprivation of the substrates required for microbial growth. Antimicrobial activity of berries may also be related to antiadherence of bacteria to epithelial cells, which is a prerequisite for colonisation and infection of many pathogens. Antimicrobial berry compounds may have important applications in the future as natural antimicrobial agents for food industry as well as for medicine. Some of the novel approaches are discussed.

Introduction

Berries are traditionally an important part of the Nordic diet. About 50 different berries are grown in the northern region, and about half of them are edible. The most well known and also commercially most important wild berries are lingonberry, bilberry, raspberry, cloudberry, cranberry, buckthorn berry and crowberry. In Finland the annual crops of lingonberry and bilberry has been estimated to be as large as 500 million kg and 250 million kg, respectively (Viljakainen 2003). Thus, wild berries in the Nordic region form an enormous natural source, only a fractional part of which has been advantaged so far. In North America cranberry along with blueberry are two important native fruits which are commercially grown. Around 500 million tons of cranberries are produced every year. Only 5% of the annual crop is harvested for fresh fruit, and most of it is used for processing. Cranberries are used as ingredients in over 700 products. Cranberry juice is one of the important products produced by food processing industry (Vattem and Shetty 2003). Pomace is a by-product this industry, which traditionally has been used as an ingredient in animal feed or it has been disposed into soils, where it poses (due to low pH) significant ecological problems. Thus, potential new uses of cranberry pomace are being explored (Zheng and Shetty 1998; Zheng and Shetty 2000).

Interest in the composition of berries has been intensified because of the increased awareness of their possible positive health effects. Berries are rich sources of various bioactive compounds possessing interesting biological activities. They are rich in fibre, vitamins and minerals, and especially wild berries are rich in various phenolic compounds (Viberg and Sjöholm 1996; Törrönen et al. 1997; Häkkinen et al. 1999, 2000; Vuorinen et al. 2000; Mullen et al. 2002) and organic acids (Viljakainen 2003). Bilberry, for example, has through the ages been used as a medicinal herb for treatment of diarrhoea and to improve night vision, and cranberry has been used for urinary infections (Ulltveit 1998). Antimicrobial activity of the berry compounds has attracted interest, because recent studies suggest that they may affect the behaviour of human pathogenic bacteria (Cavanagh et al. 2003; Kontiokari et al. 2003; Puupponen-Pimiä et al. 2001, 2004b; Rauha et al. 2000; Vattem et al. 2004). Some studies also indicate that bioactive berry compounds may act as new type antimicrobials, which may control a wide range of pathogens and may overcome the issue of antibiotic resistance. Sometimes comparison of the studies and results is difficult, since different authors have used different experimental settings, or the used methods are only partially reported (e.g. pH of the cultures and cell

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density of the target microorganisms). Limitations due to the poor solubility of many phenolic compounds may also cause some contradictions. For these reasons direct comparisons of the minimum inhibitory concentrations (MICs) are not appropriate. This review focuses on antibacterial properties of phenolic compounds and organic acids present in berries and their effects on human microflora.

Berry phenolics and organic acids

Phenolic compounds are secondary metabolites ubiquitous in all higher plants. The role of these compounds in plants is not fully understood. However, many of them act as defence compounds, e.g. against plant pathogens, and they are often induced as a response to various stress conditions. Phenolics occur in plant tissues as simple substituted phenols, mainly as glycosides, or as complex polymerised molecules with high molecular weights. Flavonoids, phenolic acids, lignans and complex phenolic polymers (polymeric tannins) are typical for berries (Fig. 1).

Berries are rich sources of flavonoids, such as flavonols. High flavonol contents are found for example in cranberry, lingonberry and black currant (50–200 mg kg^{-1} fresh weight, Häkkinen et al. 1999). In fact, wild berries and black currant contain more flavonols than many vegetables and fruits commonly used. Anthocyanins (anthocyanidin glycosides) are the predominating group of flavonoids present in berries (up to 2,000–5,000 mg kg^{-1} fresh weight, Määttä et al. 2001). They are good absorbers of visible light, thus appearing as coloured substances, responsible for the characteristic orange/red/blue colours of berries, such as strawberries, raspberries, bilberries and red- and black currants. For example in strawberry, 44% of phenolic compounds are anthocyanins. More simple phenolic acids, such as hydroxycinnamic acids and hydroxybenzoic acids, are also common in many berries (Herrmann 1989). Chlorogenic acid, which is an ester between caffeic acid and quinic acid, is a commonly occurring compound in many berries.

Flavonoids and phenolic acids form the building blocks for polymeric tannins, which can be classified into hydrolysable and condensed tannins. Hydrolysable tannins are either gallotannins or ellagitannins. Upon hydrolysis gallotannins yield glucose and gallic acid. Ellagitannins contain one or more hydroxydiphenoyl residues which are linked to glucose as a diester in addition to gallic acid. Upon hydrolysis the hydrodiphenoyl residue undergoes lactonisation to produce ellagic acid. Berries, especially of family *Rosaceae*, genus *Rubus* (red raspberry, arctic bramble and cloudberry), are rich in ellagitannins (Häkkinen et al. 2000; Mullen et al. 2002). These berries and strawberry produce only ellagitannins based on stable glucose conformation. In addition to pentagalloylglucose, these berries contain dimeric or polymeric ellagitannins with only small amounts of monomers (Haslam 1989). All *Rubus* berries contain a dimeric form called sanquin H6 (Haddock et al. 1982; Tanaka et al. 1993). According to Tanaka et al. (1993), raspberries contain tetrameric ellagitannin, lambertianin D. Ellagitannins are not found in any other common foods in the Nordic diet, so these berries remain the most important sources of them (Häkkinen et al. 2000). Some berries, such as cranberry, also contain condensed tannins, called proanthocyanidins, which are polymers of flavan-3-ols and flavan-3,4-diols or their mixtures (Chung et al. 1998a). These compounds give a characteristic bitter taste to many berries (Bate-Smith 1973). Lingonberry, strawberry and cranberry are examples of berries rich in diphenolic compounds called lignans (10–15 mg kg^{-1} dry weight, Mazur et al. 2000).

Cultivar, seasonal and geographic origin contribute the individual characteristics of sugar and acid composition in berries. According to Viljakainen et al. (2002), the main acids of the northern region wild-berry juices are invariably citric and malic acids, even though their concentrations varied widely from one berry to another (2.9–16.2 g l^{-1} and 3.3–24.7 g l^{-1}, respectively). In addition, juices of lingonberry, cranberry, cloudberry and black currant contain benzoic acid (0.1–0.7 g l^{-1}). In lingonberry especially, benzoic acid concentration is high (up to 1.3 g l^{-1} free benzoic acid), and the pH is very low (pH 2.6–2.9). The pH of berry juices is low (2.4–3.5), which is advantageous in preventing microbial contaminations.

Mechanisms of antibacterial activity of berries

Currently relatively few data are available concerning the antimicrobial mechanisms of purified berry-derived compounds. However, compounds originating from other edible plants, such as tea and its catechins, have been studied intensively. Much of the recent work has focused to tea polyphenols, such as epigallocatechin gallate (EGCG) and epicatechin gallate, which have the capacity to modulate β-lactam resistance in methicillin-resistant *Staphylococcus aureus* (MRSA) (Stapleton et al. 2004; Hu et al. 2001; Hatano et al. 2003; Zhao et al. 2003). In addition, the antimicrobial properties of plant-derived essential oils have recently been reviewed by Burt (2004). Therefore this chapter concerns also some general knowledge of the antimicrobial mechanisms.

Antibacterial effects at cellular level

The cytoplasmic membrane in microbes is composed essentially of a phospholipid bilayer with embedded proteins (Maillard 2002). It is semipermeable and regulates the transfer of solutes and metabolites in and out of the cell cytoplasm. It is also associated with several important enzymes involved in various metabolic functions. The cytoplasmic membrane is often considered as the major target site for biocides (Maillard 2002). Phenol, also termed carbolic acid, and the phenol derivatives are membrane-active microbicides (Paulus 1993). Phenolics

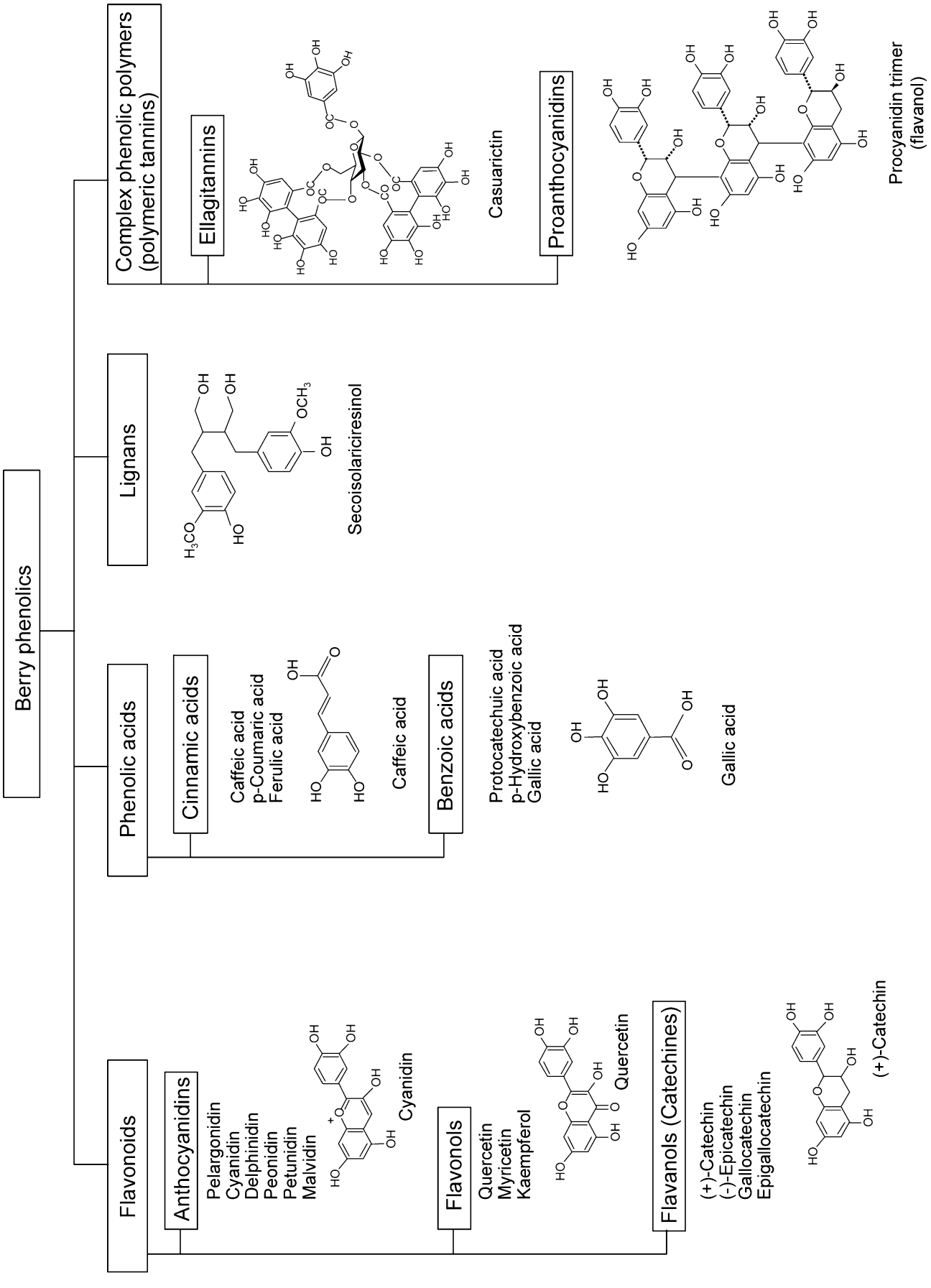


Fig. 1 Chemical structures of the main classes of phenolic compounds in berries

attack the cell wall and penetrate the cell, where they react with the cytoplasma and the cellular proteins. Walsh et al. (2003) also reported that membrane damage may account for at least part of the mode of action of thymol and eugenol, which are phenolic essential oil components. Phenolics are optimally active in acid and neutral media, i. e. in their undissociated state (Paulus 1993).

Ultee et al. (2002) studied the antimicrobial mechanisms of carvacrol, a phenolic essential oil component, against Gram-positive *Bacillus cereus*. They hypothesised that carvacrol destabilises the cytoplasmic membrane and, in addition, acts as a proton exchanger, thereby reducing the pH gradient across the cytoplasmic membrane. The resulting collapse of the proton motive force and depletion of the ATP pool eventually lead to cell death.

In Gram-negative species, an outer-membrane (OM) is a fairly effective barrier for hydrophobic substances and macromolecules, and a set of multidrug resistance pumps (MDRs) extrudes amphipathic toxins across the outer membrane (OM) (Poole 2004; Tegos et al. 2002; Nikaido 2003). OM permeability is regulated by hydrophilic channels known as porins, which generally exclude the entry of hydrophobic substances. However, it is possible to specifically weaken the OM by various agents that disintegrate the LPS layer. Such agents are collectively termed as permeabilisers. Certain essential oil components, such as small terpenoid and phenolic compounds, have been reported to be active upon OM (Helander et al. 1998). The components are small molecules with a generally hydrophobic character, but their bactericidal properties clearly demonstrate their membrane pertubing or membrane rupturing characteristics (Brul and Coote 1999; Burt 2004). Helander et al. (1998) reported that the phenolic components thymol and carvacrol were both bactericidal and had OM-disintegrating properties. At VTT we have studied the effects of phenolic extracts derived from raspberry, strawberry and cloudberry on the OM permeability of *Salmonella*, utilising a fluorescent-probe (a nonpolar hydrophobic probe, 1-*N*-phenylnaphtylamine) uptake assay (Alakomi et al. 2003; Helander and Mattila-Sandholm 2000). We found that cloudberry and raspberry, but not strawberry, extract clearly caused permeabilisation of *Salmonella* (unpublished results).

Barber et al. (2000) studied the antimicrobial intermediates of the general phenylpropanoid- and lignin-specific pathways. They reported that hydroxycinnamaldehydes were the most effective, possessing both antifungal and antibacterial activity. Hydroxycinnamic acids were generally antibacterial (mean MIC, 3.0 mM). Hydroxycinnamic acids, due to their propenoid side chain, are less polar than the corresponding hydroxybenzoic acids, and this property might facilitate the transport of these molecules across the cell membrane (Campos et al. 2003). Polarity of the molecules only partially explains that the different effects of the phenolic acids and equilibrium between solubility in both lipid and aqueous phases might be necessary to ensure the inhibitory activity of hydroxycinnamic acids on bacterial growth (Campos et al. 2003).

Scalbert (1991) reviewed the proposed mechanisms to explain the antimicrobial activity of tannins include inhibition of extracellular microbial enzymes, deprivation of the substrates required for microbial growth or direct action on microbial metabolism through inhibition of oxidative phosphorylation. Complexation of metal ions in bacterial growth environment by tannins could also be a possible mechanism for their antimicrobial properties. The mode of action of tannins probably depends on the individual microorganism. In addition to this, Scalbert (1991) reviewed toxicity of tannin for fungi, bacteria and yeasts, and compared the toxicity to related lower-molecular-weight phenols. Kolodzeij et al. (2003) evaluated the antimicrobial activity of a total of 27 tannins and related compounds and observed weak to moderate antibacterial activities. Akiyama et al. (2001) examined the antibacterial action of several tannins on plasma coagulation by *S. aureus*, and the conventional chemotherapy combined with tannic acid below the MIC. They found that all tannins inhibited coagulation at concentrations below the MIC, and suggested that mechanism in antibacterial action was inhibition of fibrin formation by *S. aureus*. Thus, tannic acid may be useful adjuvant agent for treatment of *S. aureus* skin infections in addition to β-lactam antibiotics.

Alberto et al. (2001) observed growth inhibition of *Lactobacillus hilgardii* with high gallic acid concentrations (1,000 mg l^{-1}), which they suggested resulted from the toxic effect. It has been suggested that at lower concentrations, phenolic compounds serve as oxygen scavengers and reduce the redox potential, thus enhancing the growth of some lactic acid bacteria (Alberto et al. 2001; Campos et al. 2003). In addition, the metabolism of certain hydroxycinnamic acids can be a possible explanation for the beneficial effect of these compounds on growth of *Lb. hilgardii* (Campos et al. 2003).

The efflux pumps contribute in a major way to the resistance of microorganisms to antibiotics and biocides (Levy 2002; Poole 2004). Tegos et al. (2002) reported that use of multidrug pump inhibitors uncover activity of plant antimicrobials. Recently it has been reported that phenolic compounds, such as flavonoids, have the ability to inhibit MDR in bacteria (Belofsky et al. 2004). Roccaro and co-workers (2004) found that the major green tea catechin, epagallocatechin-gallate, dramatically enhanced tetracycline activity against tetracycline-resistant staphylococcal isolates. The phenomenon was caused by impairment of tetracycline efflux pump activity and increased intracellular retention of the drug. Flavonoids have also shown direct activity against methicillin-resistant *S. aureus* (MRSA) (Nanayakkara et al. 2002). Lately the effect of gallic acid and gallic acid derivatives on drug metabolising enzymes has also been studied (Ow and Stupans 2003).

According to Kubo and co-workers (2002) alkyl gallates, which are currently permitted for use as antioxidant additives in food (Aruoma et al. 1993), show antibacterial activity against Gram-positive bacteria, including MRSA strains. This antimicrobial activity has been shown to be due to interaction of the amphipathic tail

of these molecules with the cytoplasmic membrane, resulting in an inhibition of oxygen consumption and disruption of the membrane-located respiratory chain (Kubo et al. 2003).

Yoda et al. (2004) examined antimicrobial activity of the major green tea polyphenol, EGCG, against various *Staphylococcus* strains and Gram-negative rods. MICs were much higher for Gram-negative rods (≥800 μg/ml) compared to *S. aureus* strains (50–100 μg/ml). This suggested that the structure of the bacterial cell wall and the different affinities of EGCG with the various cell wall components are responsible for the different susceptibilities. Zhao et al. (2001b) studied 25 clinical isolates of MRSA and found that EGCG and β-lactams attach the same site: peptidoglycan on the cell wall. EGCG synergises the activity of β-lactams against MRSA, owing to interference with the integrity of the cell wall through direct binding to peptidoglycan. Another hypothesis to explain β-lactam resistance-modulating activity is that the molecules interact with cytoplasmic membrane and membrane lipids (Caturla et al. 2003). However, in a recent study Zhao et al. (2003) observed no restoration of antimicrobial activity by the combination of β-lactams and EGCG against Gram-negative rods. The differences of combinational effects were confirmed to be related to the cellular locations of the enzymes.

Zhao et al. (2001a) have reported an interesting phenomenon: tea EGCG blocks or significantly diminishes the transfer of the conjugative R plasmid in *Escherichia coli*. Conjugative plasmid-mediated resistance to antibiotics, especially among enteric bacteria, is one of the major causes of the widespread increase in resistant bacteria.

Effects of pH on antibacterial activity of berries

Many parameters govern the survival and growth of microorganisms in food, one of which is pH. The microbicidal acids belong to the membrane-active substances, which damage the inner cell membrane. The mode of action of antimicrobially active acids is based on the ability of their undissociated forms to interact with or to pass through the membrane of the microbial cell, which normally is negatively charged. Thus, the membrane acts as a barrier for the negatively charged forms of acids. In their undissociated state (in pH below the pK_a value of the acid), the acids may alter the membrane permeability of the microbial cell and interfere with many enzymatic processes in the cell (Doores 1993). The pK_a of most of organic acids lies between pH 3 and pH 5. Since the cytoplasmic pH is generally higher than that of the growth medium, the weak acid dissociates, releasing the proton and leading to acidification of the cytoplasm (Cotter and Hill 2003). However, to protect themselves from the challenge posed by low-pH environments, microbes have developed different acid tolerance mechanisms (reviewed, e.g. by Cotter and Hill 2003 and Audia et al. 2001). The OM of Gram-negative bacteria is able to exclude external hydrophobic molecules, and experiments have shown that Gram-negative bacteria are inherently resistant to many external agents (Paulus 1993). Weak organic acids, like citric and lactic acid, in addition to their antimicrobial property due to the lowering of the pH, also function as permeabilisers of the Gram-negative bacterial OM. Therefore they may increase the effects of other antimicrobial substances (Brul and Coote 1999; Alakomi et al. 2000).

Friedman and Jürgens (2000) demonstrated that caffeic, chlorogenic and gallic acids are not stable at high pH and that the pH- and time-dependent spectral transformations are not reversible. Pure phenolic acids, such as hydroxycinnamic acids, have been recently shown both bactericidal and bacteriostatic activity against several strains of *Listeria monocytogenes*. The nature of the antimicrobial effect was contingent upon the medium pH. For example, all the hydroxycinnamic acids were bactericidal at pH 4.5, and only bacteriostatic at higher pH. In contrast, chlorogenic acid inhibited the growth of *L. monocytogenes* only at pH 6.5 (Wen et al. 2003). Medium pH also affects the antimicrobial activity of caffeic, *p*-coumaric and ferulic acids towards *E. coli* and *S. aureus*. Inhibition of both species increased as pH of the bacterial growth medium decreased from pH 7.0 to pH 5.0 (Herald and Davidson 1983). Thus, when evaluating the antimicrobial efficacy of phenolic compounds either in food matrices or in human body, pH is a very important parameter to be considered.

Bacterial antiadhesion activity of berries

Bacterial adherence to mucosal surfaces is generally considered to be an important prerequisite for colonisation and infection. Adherence of bacteria to epithelial cells has been shown to correlate with virulence in infections of the genitourinary tract, gastrointestinal tract and respiratory tract. In the view of the importance of bacterial adherence in colonisation and infection, attempts have been made to block the attachment of harmful bacteria to mucosal surfaces.

Bacteriostatic effects of berries due to antiadhesion have been demonstrated so far only with cranberry. The antibacterial properties of cranberry juice have been known for a long time, and the effects have been associated with inhibition of *E. coli* adherence to uroepithelium and multiplying by cranberry juice (Sobota 1984; Zafriri et al. 1989; Ofek et al. 1991; Reid et al. 2001). Recent research has demonstrated that a bacterial antiadhesion mechanism is responsible. Proanthocyanidins (condensed tannins) with unique molecular structures have been isolated from cranberry fruit that exhibit potent bacterial antiadhesion activity (Howell et al. 1998 and Howell 2002). Reed et al. (2003) compared differences in structure and bacterial antiadhesion activity of cranberry proanthocyanidins to proanthocyanidins from other foods, such as grape and apple juices. They found that only cranberry juice elicited bacterial antiadhesion activity in human urine after consumption. Cranberry proanthocyanidins are also compositionally different from proantho-

cyanidins from other foods, characterised by a series of oligomers based on a repeating unit structure of catechin with one or more A-type linkages. The A-type linkages in cranberry proanthocyanidins are associated with antiadhesion activity (Reed et al. 2003). Mice-feeding experiments with cranberry proanthocyanidins suggest 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 (Howell et al. 2001). Zafriri et al. (1989), have given an interesting hypothesis 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 bacteria not capable to bind any more to the uroepithelium and proliferate (Harmand and Blanquet 1978).

High-molecular-weight constituents of cranberry juice have recently shown to inhibit adhesion of *Helicobacter pylori* to immobilised human mucus, erythrocytes and cultured gastric epithelial cells. Different isolates of *Helicobacter pylori* differed in their affinity to the cranberry juice constituents (Burger et al. 2002). It was 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*.

Inhibitory effects of a high-molecular-weight constituent of cranberry on the adhesion of oral bacteria, such as *Streptococcus mutans*, have recently been demonstrated (Weiss et al. 2002). The high-molecular-weight material from blueberry also inhibited adhesion, although its activity was weaker. In preliminary clinical test these cranberry constituents also reduced *S. mutans* counts in saliva. Thus antiadhesion activity of cranberry juice also has a potential for altering the oral microflora, resulting in improved oral hygiene.

Before these findings related to antiadhesion activities of berry phenolics on harmful bacteria in gastrointestinal tract may lead to new therapeutic strategies, it must be verified that influence on colonisation is selective to pathogenic strains and colonisation of beneficial bacteria is not inhibited. To our knowledge there are no reports so far on any antiadhesion activity of berry compounds on beneficial gastrointestinal bacteria, such as lactic acid bacteria. In our laboratory such experiments with various purified phenolic fractions of berries are just going on.

Antibacterial activity of berry fruits

The antimicrobial activities of the naturally occurring phenolics from tea, olives and wine have been widely studied (Ikigai et al. 1993; Toda et al. 1989; Vivas et al. 1997). However, there is very little information about antimicrobial capacity of phenolics present in berries. We have extensively studied the antimicrobial effects of Nordic berries, focusing to the effects of berry compounds on human gastrointestinal bacteria. Antibacterial 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 a variety of intestinal pathogens and food poisoning bacteria, have been determined (Puupponen-Pimiä et al. 2001, 2004a, b).

According to our results, the degree of hydroxylation might affect the antibacterial activity of pure phenolic compounds. Flavonol myricetin (three hydroxyl groups in the B ring) was the only compound which showed strong inhibitory effects on the growth of lactic acid bacteria derived from the human gastrointestinal tract, as well as Gram-positive *Enterococcus faecalis* and *Bifidobacterium lactis*. Gram-negative *Salmonella* was not affected by myricetin. The other flavonols were more lipophilic in nature (less hydroxyl groups in the B ring than in myricetin) and were not active against lactic acid bacteria. The flavone luteolin (two hydroxyl groups in the B ring) showed bacteriostatic effects against lactic acid bacteria, as well as against *Ent. faecalis* and *B. lactis*. However, no such effects were found with more lipophilic flavones. Thus, 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. The phenolic acids (cinnamic acid, 3-coumaric acid, caffeic acid, ferulic acid and chlorogenic acid) showed activity only against Gram-negative bacteria. These variations in sensitivities against pure phenolic compounds may reflect differences in cell surface structures between Gram-positive and Gram-negative bacteria (Puupponen-Pimiä et al. 2001).

In general, in our studies phenolic berry extracts of Nordic berries inhibited the growth of *Salmonella*, *Escherichia* and *Staphylococcus* species but not *Lactobacillus* and *Listeria* species. *Salmonella* and *Staphylococcus* strains were the most sensitive bacteria, and cloudberry and raspberry the most efficient berries. Sea buckthorn berry and black currant showed the least activity against *Salmonella* and *Escherichia* strains (Puupponen-Pimiä et al. 2001, 2004b). Rauha et al. (2000) have also studied antimicrobial effects of some Finnish berry extracts against food poisoning bacteria. They found that the widest bacteriocidal activity was also expressed by berries belonging to the genus *Rubus* (cloudberry and raspberry), which are rich in ellagitannins. Cavanagh et al. (2003) confirmed that several fresh berries (liquefied with a blender), including raspberry and black currant, inhibited the growth of wide range of human pathogenic bacteria, both Gram-negative and Gram-positive. In their experiments mulberries and boysenberries did not inhibit any bacteria.

In our study (Puupponen-Pimiä et al. 2004b) isolated ellagitannin fractions from cloudberry and raspberry were highly efficient against *S. aureus* (Fig. 2a). The antimicrobial properties of tannins present in many plant foods have been well documented (Chung et al. 1998b). According to Beuchat and Heaton (1975) tannins were bacteriostatic and/or bacteriocidal for *Staphylococcus aureus*, *Streptococcus pneumonia*, *Bacillus anthracis*, *Shigella dysenteriae* and *Salmonella* ser. Senftenber.

Human intestinal bacteria such as *Bacteroides fragilis*, *Clostridium perfringens*, *E. coli* and *Salmonella* ser. Typhimurium, but not *Bifidobacterium infantis* and *Lactobacillus acidophilus*, were also inhibited by tannins (Chung et al. 1998a). Many pathogenic bacteria were affected by tannins, such as cariogenic bacteria *S. mutans* and *S. sobrinus* (Hada et al. 1989), and pathogens causing diarrhoea (Toda et al. 1989). Chung et al. (1993) demonstrated that tannic acid, but not gallic acid, was inhibitory to a variety of food born bacteria, such as *E. coli*, *Salmonella* ser. Enteritidis, *Salmonella* ser. Paratyphi and *S. aureus*. They found that inhibitory effects were associated with the ester linkage between gallic acid and polyols. Antibacterial properties of tannins present in other berries than cranberry have rarely been reported.

In our experiments (Puupponen-Pimiä et al. 2004b) growth inhibition of *Salmonella* was only partly caused by the berry phenolics, and most of the inhibition seemed to originate from other compounds, such as organic acids (Fig. 2b). Berry phenolics seem to affect the growth of different bacterial species in different mechanisms, yet it is not well understood. There seem to be complex interactions between pH of the growth media and antimicrobial effects of the berry phenolics varying in different bacterial species and in different phenolic compounds.

Processed berries

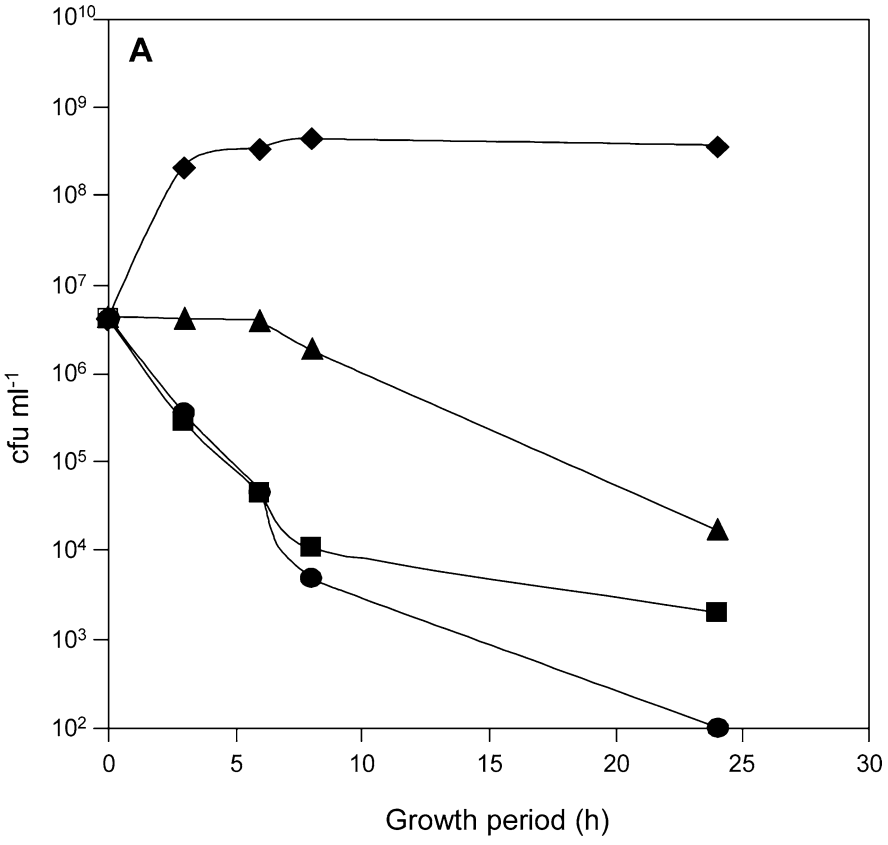
Due to the short harvesting time of berry fruits, most of the crop is processed for further use. Berry juices and cordials are the main products. Raspberry and black currant cordials (100% fruit) display good antibacterial activity against various bacteria, such as *Enterococcus*, *Escherichia*, *Mycobacterium*, *Salmonella* and *Staphylococcus* species, whereas *Mycobacteria phlei* appeared to be susceptible to all the products (Cavanagh et al. 2003). In addition, *M. phlei* showed varying susceptibilities to different raspberry cordials. The variation in activity may be a result of variations in the preparations of the cordials, or alternatively differences in the raspberry varieties used in the production. Ryan et al. (2001) compared antibacterial properties of raspberry juice cordial, raspberry juice, raspberry leaf extract and a commercial brand of raspberry tea against human pathogenic bacteria. Raspberry cordial and juice significantly reduced the growth of several bacterial species, including *Salmonella*, *Shigella* and *E. coli*. However, no antibacterial activity was detected in the leaf extract or tea. Cavanagh et al. (2003) hypothesised that although the origin of antimicrobial activity of raspberry and other berry products was unknown, they may have potential for example as a means of purifying drinking water at areas where drinking water resources may have contaminated with harmful bacteria (Cavanagh et al. 2003).

Transportation and storage of berry juice concentrates at low temperatures prior to final packaging is a common practice in the juice industry and introduces a potential risk for postconcentration contamination with pathogenic bacteria. However, Nogueira and co-workers (2003) have evaluated the likelihood of *E. coli* O157:H7, *L. monocytogenes* and *Salmonella* to survive in cranberry juice concentrates at or above temperatures used for transportation or storage. Cranberry juice possessed intrinsic antimicrobial activity that will eliminate these bacterial pathogens in the events of postconcentration recontamination. At least a 5-log reduction of all these bacteria was demonstrated.

Berry pomace is a by-product of the juice-pressing industry. Due to its low pH value it may posses significant ecological and environmental problem. Berry pomaces containing the berry skins are, however, very rich sources of phenolic compounds. Vattem et al. (2003, 2004) and Vattem and Shetty (2003) have recently shown that solid-state bioprocessing of cranberry pomace, using food-grade fungus *Rhizopus oligosporus* or *Lentinus edode*, resulted in enrichment of total soluble phenolics and of ellagic acid. They also found that bioprocessing improved the antimicrobial activities of the extracts against important food borne pathogens *L. monocytogenes*, *Vibrio parahaemolyticus* and *E. coli* O157:H7. Tested microorganisms showed different sensitivities to various functional properties of the extracts, which may indicate different mechanisms of action in the antimicrobial activity. Antimicrobial activity against *L. monocytogenes* by the extracts correlated well with the increase of soluble phenolics, antioxidant activity and enrichment of ellagic acid during bioprocessing. Inhibition of *V. parahaemolyticus* and *E. coli* O157:H7 correlated with highest ellagic acid concentration and/or antioxidant activity. The two suggested mechanisms of action were disruption of the plasma membrane by localised hyper-acidification, and disruption of membrane-transport and/or electron transport. They hypothesised that the profile of phytochemicals that are present in natural sources confer a broad spectrum antimicrobial capacity and potentially limit the development of antimicrobial resistance because of their possible differences in modes of action. Thus, bioprocessing of cranberry and also other berries may offer an innovative approach to produce broad spectrum antimicrobials against important pathogens.

Conclusion

In recent years knowledge about bioactive berry compounds, especially the phenolic compounds, has increased a lot. Several studies show that berry compounds inhibit the growth of human pathogenic bacteria, such as *Salmonella*, *staphylococcus*, *Helicobacter* and *E. coli* O157:H7. Utilisation of antimicrobial activity of berry phenolic compounds as natural antimicrobial agents may offer many new applications for food industry and medicine. Functional foods targeted for gut well-being and balanced gut microflora form a very important group of novel products. Natural food preservatives targeted to foods which are easily contaminated by bacteria, such as *Salmonella* and *Staphylococcus*, are highly desired. A



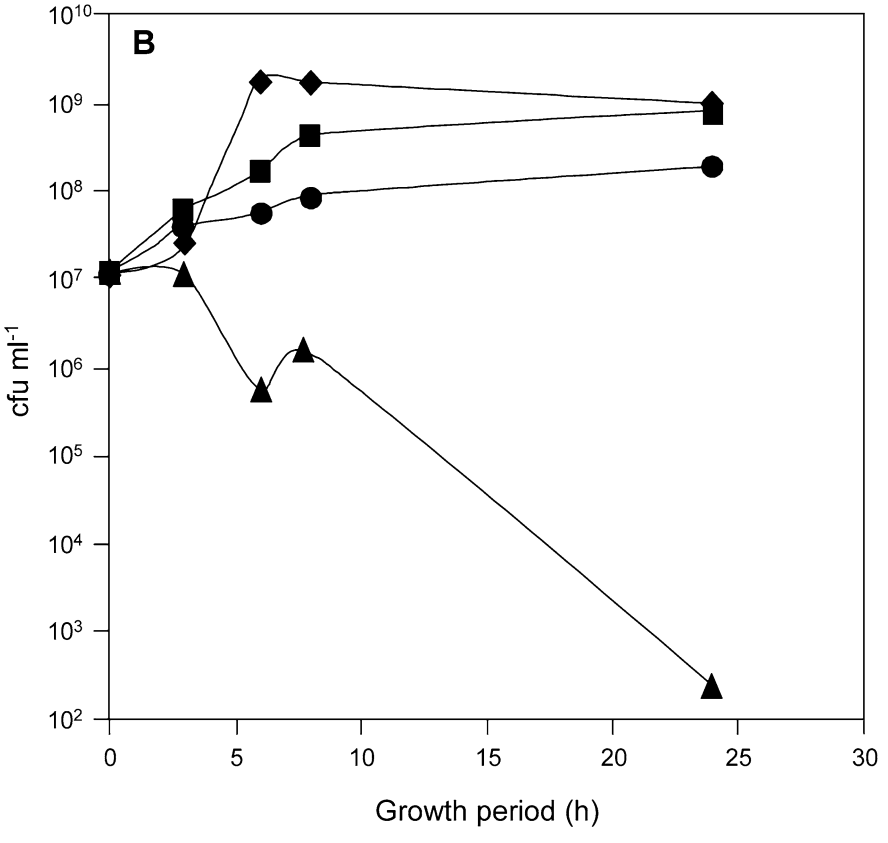


Fig. 2 Comparison of antimicrobial activity of berries, extracts and fractions of cloudberry on bacterial growth. **a***Staphylococcus aureus* VTT E-70045: (◆) control growth curve, (▲) freeze-dried cloudberries 10 mg ml^{-1}, (●) cloudberry extract 1 mg ml^{-1}, (■) cloudberry ellagitannin fraction 1 mg ml^{-1}. **b***Salmonella enterica* sv. Typhimurium VTT E-981151: (◆) control growth curve, (▲) freeze-dried cloudberries 10 mg ml^{-1}, (●) cloudberry extract 1 mg ml^{-1}, (■) cloudberry ellagitannin fraction 1 mg ml^{-1}

concrete example in the food industry is utilisation of berry phenolics, dried berry powder or concentrated berry juice in chicken, meat and seafood marinades and dressings or in food packages. Capsules containing berry powder or berry concentrate that balance gut microflora or even prevent diarrhoea are examples in health care area. In medicine, antiadhesion therapy by the berry compounds to prevent, relieve and even cure microbial diseases seems to be a very promising approach. Development of alternative regimen using berry compounds for the prevention and control of infections caused by bacteria resistant to antibiotics will also be very important issue in the future. Recent findings reinforce the suggestions that berry-derived antimicrobials might act on a broad spectrum of bacteria and could be included as an effective addition to traditional antimicrobial compounds and treatments. However, further studies concerning safety, toxicology, combined use with traditional medicines and legislation are needed.

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References

Akiyama H, Fujii K, Yamasaki O, Oono T, Iwatsuki K (2001) Antibacterial action of several tannins against *Staphylococcus aureus*. J Antimicrob Chemother 48:487–491

Alakomi H-L, Skyttä E, Saarela M, Mattila-Sandholm T, Latva-Kala K, Helander IM (2000) Lactic acid permeabilizes gram-negative bacteria by disrupting the outer membrane. Appl Environ Microbiol 66:2001–2005

Alakomi H-L, Saarela M, Helander I (2003) Effect of EDTA on *Salmonella enterica* serovar Typhimurium involves a component not assignable to lipopolysaccharide release. Microbiology 149:2015–2021

Alberto MR, Farias ME, Manca de Nadra MC (2001) Effect of gallic acid and cathechin on *Lactobacillus hilgardii* 5w growth and metabolism of organic compounds. J Agric Food Chem 49:4359–4363

Aruoma OI, Murcia A, Butler J, Halliwell B (1993) Evaluation of the antioxidant and prooxidant actions of gallic acid and its derivatives. J Agric Food Chem 41:1880–1885

Audia JP, Webb CC, Foster JW (2001) Breaking through the acid barrier: an orchestrated response to proton stress by enteric bacteria. Int J Med Microbiol 291:97–106

Barber MS, McConnell VS, DeCaux B (2000) Antimicrobial intermediates of the general phenylpropanoid and lignin specific pathways. Phytochemistry 54:53–56

Bate-Smith EC (1973) Haemanalysis of tannins: the concept of relative astringency. Phytochemistry 12:907–912

Belofsky G, Percivill D, Lewis K, Tegos GP, Ekart J (2004) Phenolic metabolites of *Dalea versicolor* that enhance antibiotic activity against model pathogenic bacteria. J Nat Prod 67:481–484

Beuchat LR, Heaton EK (1975) *Salmonella* survival on pecan as influenced by processing and storage conditions. Appl Microbiol 29:795–801

Brul S, Coote P (1999) Preservative agents in foods. Mode of action and microbial resistance mechanisms. Int J Food Microbiol 50:1–17

Burger O, Weiss E, Sharon N, Tabak M, Neeman I, Ofek I (2002) Inhibition of *Helicobacter pylori* adhesion to human gastric mucus by a high-molecular-weight constituent of cranberry juice. Crit Rev Food Sci Nutr 42 [Suppl]:279–284

Burt S (2004) Essential oils: their antibacterial properties and potential applications in foods—a review. Int J Food Microbiol 94:223–253

Campos FM, Couto JA, Hogg TA (2003) Influence of phenolic acids on growth and inactivation of *Oenococcus oeni* and *Lactobacillus hilgardii*. J Appl Microbiol 94:167–174

Caturla N, Vera-Samper E, Villalain J, Mateo CR, Micol V (2003) The relationship between the antioxidant and antibacterial properties of galloylated catechins and the structure of phospholipid model membranes. Free Radical Biol Med 34:648–662

Cavanagh HM, Hipwell M, Wilkinson JM (2003) Antibacterial activity of berry fruits used for culinary purposes. J Med Food 1:57–61

Chung K-T, Stevens SE, Jr, Lin W-F, Wei CI (1993) Growth inhibition of selected food-borne bacteria by tannic acid, propyl gallate and related compounds. Lett Appl Microbiol 17:29–32

Chung K-T, Lu Z, Chou MW (1998a) Mechanism of inhibition of tannic acid and related compounds on the growth of intestinal bacteria. Food Chem Toxicol 36:1053–1060

Chung K-T, Wei C-I, Johnson MG (1998b) Are tannins a double-edged sword in biology and health? Trends Food Sci Technol 9:168–175

Cotter PD, Hill C (2003) Surviving the acid test: responses of Gram-positive bacteria to low pH. Microbiol Mol Biol Rev 67:429–453

Doores S (1993) Organic acids. In: Davidson PM, Branen AL (eds) Antimicrobials in food, 2nd edn. Marcel Dekker, New York, pp 95–136

Friedman M, Jürgens HS (2000) Effect of pH on the stability of plant phenolic compounds. J Agric Food Chem 48:2101–2110

Hada N, Kakiuchi N, Hattori M, Namba T (1989) Identification of antibacterial principles against *Streptococcus mutans* inhibitory principles against glucosyltransferase from the seed of *Areca catechu* L. Phytother Res 3:140–144

Haddock EA, Gupta RK, Al-Shafi SMK, Layden K, Haslam E, Magnolato D (1982) The metabolism of gallic acid and hexahydroxydiphenic acids in plants: Biogenetic and molecular taxonomic considerations. Phytochemistry 5:1049–1062

Harmand MF, Blanquet P (1978) The fate of total flavonolic oligomers (OFT) extracted from *Vitis vinifera* L. in the rat. Eur J Drug Metab Pharmacokinet 1:15–30

Haslam E (1989) Plant polyphenols: vegetable tannins revisited. Cambridge University Press, Cambridge

Hatano T, Kusuda M, Hori M, Shiota S, Tsuchiya T, Yoshida T (2003) Theasinensin A, a tea polyphenol formed from (-)-epigallocatechin gallate, suppresses antibiotic resistance of methicillin-resistant *Staphylococcus aureus*. Planta Med 69:984–989

Helander I, Alakomi H-L, Latva-Kala K, Mattila-Sandholm T, Pol I, Smid E, Gorris L, Wright von A (1998) Characterization of the action of selected essential oil components on gram-negative bacteria. J Agric Food Chem 46:3590–3595

Helander IM, Mattila-Sandholm T (2000) Fluorometric assessment of Gram-negative bacterial permeabilization. J Appl Microbiol 88:213–219

Herald PJ, Davidson PM (1983) Antibacterial activity of selected hydroxycinnamic acids. J Food Sci 48:1378–1379

Herrmann K (1989) Occurence and content of hydroxycinnamic and hydroxybenzoic acid compounds in foods. Crit Rev Food Sci Nutr 28:315–347

Howell AB (2002) Cranberry proanthocyanidins and the maintenance of urinary tract health. Crit Rev Food Sci Nutr 42 [Suppl]:273–278

Howell AB, Vorsa N, Marderosian AD, Foo LY (1998) Inhibition of the adherence of P-fimbriated *Escherichia coli* to uro-epithelial surfaces by proanthycyanidin extracts from cranberries. NE J Med 339:1085–1086

Howell AB, Leahy M, Kurowska E, Guthrie N (2001) In vivo evidence that cranberry proanthocyanidins inhibit adherence of P-fimbriated *E. coli* bacteria to uroepithelial cells. FASEB J 15 (4):A284

Hu Z-Q, Zhao W-H, Hara Y, Shimamura T (2001) Epigallocatechin gallate synergy with ampicillin/sulbactam against 28 clinical isolates of methicillin-resistant *Staphylococcus aureus*. J Antimicrob Chemother 48:361–364

Häkkinen S, Kärenlampi S, Mykkänen H, Heinonen M, Törrönen R (2000) Ellagic acid-content in berries: influence of domestic processing and storage. Eur Food Res Technol 212:75–80

Häkkinen SH, Kärenlampi SO, Heinonen MI, Mykkänen HM, Törrönen RA (1999) Content of the flavonols quercetin, myricetin, and kaempferol in 25 edible berries. J Agric Food Chem 47:2274–2279

Ikigai H, Nakae T, Hara Y, Shimamura T (1993) Bactericidal catechins damage the lipid bilayer. Biochim Biophys Acta 1147:132–136

Kolodzeij H, Kayser O, Latte KP (2003) Evaluation of the antimicrobial potency of tannins and related compounds using the microdilution broth method. Planta Med 65:444–446

Kontiokari T, Laitinen J, Järvi L, Pokka T, Sundqvist K, Uhari M (2003) Dietary factors protecting women from urinary tract infection. Am J Clin Nutr 77:600–604

Kubo I, Xiao P, Fujita K (2002) Anti-MRSA activity of alkyl gallates. Bioorg Med Chem Lett 12:113–116

Kubo I, Fujita K, Nihei K, Masuoka N (2003) Non-antibiotic antibacterial activity of dodecyl gallate. Bioorg Med Chem 11:573–580

Levy SB (2002) Active efflux, a common mechanisms for biocide and antibiotic resistance. J Appl Microbiol Symp Suppl 92:65S–71S

Maillard JY (2002) Bacterial target sites for biocide action. J Appl Microbiol Symp [Suppl] 90:16S–27S

Mazur WM, Uehara M, Wähälä K, Adlercreutz H (2000) Phyto-oestrogen content of berries, and plasma concentrations and urinary excretion of enterolactone after a single strawberry-meal in human subjects. Brit J Nutr 83:381–387

Mullen W, Stewart AJ, Lean MEJ, Gardner P, Duthie GG, Crozier A (2002) Effect of freezing and storage on the phenolics, ellagitannins, flavonoids, and antioxidant capacity of red raspberries. J Agric Food Chem 50:5197–5201

Määttä K, Kamal-Eldin A, Törrönen R (2001) Phenolic compounds in berries of black, red, green, and white currants (*Ribes* sp.). Antioxid Redox Sign 3:981–993

Nanayakkara NP, Burandt CL Jr, Jacob MR (2002) Flavonoids with activity against methicillin-resistant *Staphylococcus aureus* from *Dalea scandens* var. *paucifolia*. Planta Med 68:519–522

Nikaido H (2003) Molecular basics of bacterial outer membrane permeability revisited. Microbiol Mol Biol Rev 64:593–656

Nogueira MCL, Oyarzábal OA, Gombas DE (2003) Inactivation of *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* in cranberry, lemon, and lime juice concentrates. J Food Protect 66:1637–1641

Ofek I, Goldhar J, Zafriri D, Lis H, Adar R, Sharon N (1991) Anti-*Escherichia coli* adhesin activity of cranberry and blueberry juices. NE J Med 324:1599

Ow YY, Stupans I (2003) Gallic acid and gallic acid derivatives: effects on drug metabolizing enzymes. Curr Drug Metab 4:241–248

Paulus W (1993) Microbicides for the protection of materials—a handbook. Chapman & Hall, London, p 496

Poole K (2004) Efflux-mediated multiresistance in Gram-negative bacteria. Clin Microbiol Infect 10:12–26

Puupponen-Pimiä R, Nohynek L, Meier C, Kähkönen M, Heinonen M, Hopia A, Oksman-Caldentey K-M (2001) Antimicrobial properties of phenolic compounds from berries. J Appl Microbiol 90:494–507

Puupponen-Pimiä R, Aura A-M, Karppinen S, Oksman-Caldentey K-M, Poutanen K (2004a) Interactions between plant bioactive food ingredients and intestinal flora—effects on human health. Biosci Microflora 23:67–80

Puupponen-Pimiä R, Nohynek L, Schmidlin S, Kähkönen M, Heinonen M, Määttä-Riihinen K, Oksman-Caldentey K-M (2004b) Berry phenolics selectively inhibit the growth of intestinal pathogens. J Appl Microbiol (in press)

Rauha J-P, Remes S, Heinonen M, Hopia A, Kähkönen M, Kujala T, Pihlaja K, Vuorela H, Vuorela P (2000) Antimicrobial effects of Finnish plant extracts containing flavonoids and other phenolic compounds. Int J Food Microbiol 56:3–12

Reed J, Howell A, Cunningham D, Krueger C (2003) Differences in structure and bacterial anti-adhesion activity of cranberry proanthocyanidins compared to proanthocyanidins from other foods. Proceedings of the 1st International conference on polyphenols and health. 18–21 November 2003, Vichy, France. Poster abstract P28

Reid G, Hsieh J, Potter P, Mighton J, Lam D, Warren D, Stephenson J (2001) Cranberry juice consumption may reduce biofilms on uroepithelial cells: Pilot study in spinal cord injured patients. Spinal Cord 39:26–30

Roccaro AS, Blanco AR, Giuliano F, Rusciano D, Enea V (2004) Epigallocatechin-gallate enhances the activity of tetracycline in staphylococci by inhibiting its efflux from bacterial cells. Antimicrob Agents Chemother 48:1968–1973

Ryan T, Wilkinson JM, Cavanagh HMA (2001) Antibacterial activity of raspberry cordial in vitro. Veter Sci 71:155–159

Scalbert A (1991) Antimicrobial properties of tannins. Phytochemistry 30:3875–3883

Sobota AE (1984) Inhibition of bacterial adherence by cranberry juice: potential use for the treatment of urinary tract infections. J Urol 131:1013–1016

Stapleton PD, Shah S, Anderson JC, Hara Y, Hamilton-Miller JMT, Taylor PW (2004) Modulation of β-lactam resistance in *Staphylococcus aureus* by catechins and gallates. Int J Antimicrob Agents 23:462–467

Tanaka T, Tachibana H, Nonaka B, Nishioka I, Hsu F-L, Kohda H, Tanaka O (1993) Tannins and related compounds CXXII. New dimeric, trimeric and tetrameric ellagitannins, lambertianins A-D, from *Rubus lambertianus* SERINGE. Chem Pharm Bull 41:1214–1220

Tegos G, Stermitz FR, Lomovskaya O, Lewis K (2002) Multidrug pump inhibitors uncover remarkable activity of plant antimicrobials. Antimicrob Agents Chemother 46:3133–3141

Toda M, Okubo S, Hiyoshi R, Shimamura T (1989) The bactericidal activity of tea and coffee. Lett Appl Microbiol 8:123–125

Törrönen R, Häkkinen S, Kärenlampi S, Mykkänen H (1997) Flavonoids and phenolic acids in selected berries. Cancer Lett 114:191–192

Ulltveit G (1998) (Wild berries) Ville baer. Technologisk forlag, 2nd edn. NW Damm, Oslo, Norway, pp 1–166

Ultee A, Bennik MHJ, Moezelaar R (2002) The phenolic hydroxyl group of carvacrol is essential for action against the food-borne pathogen *Bacillus cereus*. Appl Environ Microbiol 68:1561–1568

Vattem DA, Lin Y-T, Labbe RG, Shetty K (2003) Antimicrobial activity against select food-borne pathogens by phenolic antioxidants enriched in cranberry pomace by solid-state bioprocessing using the food grade fungus *Rhizopus oligosporus*. Process Biochem 39:1939–1946

Vattem DA, Shetty K (2003) Ellagic acid production and phenolic antioxidant activity in cranberry pomace (*Vaccinium macrocarpon*) mediated by *Lentinus edodes* using a solid-state system. Process Biochem 39:367–379

Vattem DA, Lin Y-T, Labbe RG, Shetty K (2004) Phenolic antioxidant mobilization in cranberry pomace by solid-state bioprocessing using food grade fungus *Lentinus edodes* and effect on antimicrobial activity against select food borne pathogens. Innovat Food Sci Emerg Technol 5:81–91

Viberg U, Sjöholm I (1996) Blåbär och lingon—bär med tradition och framtid (in Swedish). Livsmedesteknik 38:38–39

Viljakainen S (2003) Reduction of acidity in northern region berry juices. PhD Thesis, Helsinki University of Technology

Viljakainen S, Visti A, Laakso S (2002) Concentrations of organic acids and soluble sugars in juices from Nordic berries. Acta Agric Scand 52:101–109

Vivas N, Lonvaud-Funel A, Glories Y (1997) Effects of phenolic acids and anthocyanins on growth, viability and malolactic activity of a lactic acid bacterium. Food Microbiol 14:291–300

Vuorinen H, Määttä K, Törrönen R (2000) Content of the flavonols myricetin, quercetin, and kaempferol in Finnish berry wines. J Agric Food Chem 48:2675–2680

Walsh SE, Maillard JY, Russell AD, Catrenich CE, Charbonneau DL, Bartolo RG (2003) Activity and mechanisms of action of selected biocidal agents on Gram-positive and -negative bacteria. J Appl Microbiol 94:240–247

Weiss EI, Lev-Dor R, Sharon N, Ofek I (2002) Inhibitory effect of a high-molecular-weight constituent of cranberry on adhesion of oral bacteria. Crit Rev Food Sci Nutr 42 [Suppl]:285–292

Wen A, Delaquis P, Stanich K, Toivonen P (2003) Antilisterial activity of selected phenolic acids. Food Microbiol 20:305–311

Zafriri D, Ofek I, Pocino AR, Sharon N (1989) Inhibitory activity of cranberry juice on adherence of type 1 and P fimbriated *Escherichia coli* to eucariotic cells. Antimicrob Agents Chemother 33:92–98

Zhao W-H, Hu Z-Q, Hara Y, Shimamura T (2001a) Inhibition by epigallocatechin gallate (EGCg) of conjugative R plasmid transfer in *Escherichia coli*. J Infect Chemother 7:195–197

Zhao W-H, Hu Z-Q, Okubo S, Hara Y, Shimamura T (2001b) Mechanism of synergy between epigallacatechin gallate and β-lactams against methicillin-resistant *Staphylococcus aureus*. Antimicrob Agents Chemother 45:1737–1742

Zhao W-H, Asano N, Hu Z-Q, Shimamura T (2003) Restoration of antibacterial activity of β-lactams by epigallocatechin gallate against β-lactamase-producing species depending on location of β-lactamase. J Pharm Pharmacol 55:735–740

Zheng Z, Shetty K (1998) Cranberry processing waste for solid-state fungal inoculant production. Process Biochem 33:323–329

Zheng Z, Shetty K (2000) Solid-state bioconversion of phenolics from cranberry pomace and role of *Letinus ododes* beta-glucosidase. J Agric Food Chem 48:895–900

Yoda Y, Hu Z-Q, Zhao W-H (2004) Different susceptibilities of *Staphylococcus* and Gram-negative rods to epigallocatechin gallate. J Infect Chemother 10:55–58