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ORIGINAL ARTICLE

Antimicrobial activity of flavonoids extracted from bergamot (*Citrus bergamia* Risso) peel, a byproduct of the essential oil industry

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Abstract

Aims: To evaluate the antimicrobial properties of flavonoid-rich fractions derived from bergamot peel, a byproduct from the *Citrus* fruit processing industry and the influence of enzymatic deglycosylation on their activity against different bacteria and yeast.

Methods and Results: Bergamot ethanolic fractions were tested against Gram-negative bacteria (*Escherichia coli*, *Pseudomonas putida*, *Salmonella enterica*), Gram-positive bacteria (*Listeria innocua*, *Bacillus subtilis*, *Staphylococcus aureus*, *Lactococcus lactis*) and the yeast *Saccharomyces cerevisiae*. Bergamot fractions were found to be active against all the Gram-negative bacteria tested, and their antimicrobial potency increased after enzymatic deglycosylation. The minimum inhibitory concentrations of the fractions and the pure flavonoids, neohesperidin, hesperetin (aglycone), neoeriocitrin, eriodictyol (aglycone), naringin and naringenin (aglycone), were found to be in the range 200 to 800 μg ml^{-1}. The interactions between three bergamot flavonoids were also evaluated.

Conclusion: The enzyme preparation Pectinase 62L efficiently converted common glycosides into their aglycones from bergamot extracts, and this deglycosylation increased the antimicrobial potency of *Citrus* flavonoids. Pairwise combinations of eriodictyol, naringenin and hesperetin showed both synergistic and indifferent interactions that were dependent on the test indicator organism.

Significance and Impact of the Study: Bergamot peel is a potential source of natural antimicrobials that are active against Gram-negative bacteria.

Introduction

There is increasing epidemiological evidence for the beneficial health effects of regular intake of fruits and vegetables as part of a healthier diet (Dauchet *et al.* 2004). Polyphenols from fruits, vegetables and cereals, herbs and spices have been shown to have beneficial effects on human health, and some extracts of polyphenol-rich plants have been used in functional foods or as supplements. Among polyphenols, flavonoids are secondary metabolites well documented for their biological effects, including anticancer, antiviral, antimutagenic and anti-inflammatory activities (Benavente-Garcia *et al.* 1997; Vuorela *et al.* 2005). There is also evidence suggesting that dietary flavonoids can influence gastrointestinal bacterial populations, and there is considerable *in vitro* data on the direct and indirect (toxin inhibition) activity of polyphenols, such as naringenin and hesperetin, against

Helicobacter pylori (Bae *et al.* 1999; Mabe *et al.* 1999; Puupponen-Pimiä *et al.* 2001, 2005, 2006; Fukai *et al.* 2002; Tombola *et al.* 2003; Funatogawa *et al.* 2004; Isobe *et al.* 2006). The term flavonoid includes the following commonly occurring polyphenols: flavanones, flavones, flavan-3-ols, flavonols and anthocyanins. Flavonoids can function as direct antioxidants and free radical scavengers, and have the capacity to modulate enzymatic activities and inhibit cell proliferation (Duthie and Crozier 2000). In plants, they appear to play a defensive role against invading pathogens, including bacteria, fungi and viruses (Sohn *et al.* 2004). Flavonoids are generally present in glycosylated forms in plants, and the sugar moiety is an important factor determining their bioavailability.

Consumers are increasingly trying to avoid foods with chemical preservatives (Beuchat and Golden 1989; Gould 1996), and this is reflected by the food industries' growing interest in finding high quality products with natural compounds exhibiting antimicrobial activity. In addition, the replacement of synthetic colourants and chemicals with natural plant compounds is also being evaluated (e.g. the use of cactus pear betacyanins and various fruit anthocyanins for producing yellow-red-purple colouration in various foods: Castellar *et al.* 2003; Giusti and Wrolstad 2003). Because of legislations governing the use of current preservatives, there is an increasing demand for natural and minimally processed ingredients that can sufficiently extend the shelf life of food products and guarantee a high degree of safety. A number of aromatic plant oils with antimicrobial activities have found industrial applications as preservatives of raw and processed foods (Lis-Balchim and Deans 1997; Hammer *et al.* 1999).

Bergamot (*Citrus bergamia* Risso) is a typical fruit of the Reggio Calabria province in southern Italy, where it is mainly used for its essential oil extracted from the peel. Bergamot essential oil is widely used in the pharmaceutical industry because of its antibacterial and antiseptic activity (Verzera *et al.* 2003). Bergamot peel represents about 60% of the processed fruits and is regarded as primary waste; if not processed further, it may cause environmental problems because of its fermentability. However, bergamot peel contains very useful compounds, such as pectins and flavonoids (Mandalari *et al.* 2006a). The peel contains the characteristic *Citrus* species flavanone rutinosides and neo-hesperosides derived from naringenin, eriodictyol and hesperetin. Moreover, a small amount of flavone *O*- and *C*-glycosides, not previously found in orange and lemon peels, have been identified (Mandalari *et al.* 2006a). It is well documented that the free radical scavenger activity of flavonoids mainly depends on the arrangement of the substituents within its structure. However, the correlation between antioxidant activity and chemical structure of flavonoids is still unclear. Polyphenol glycosides are relatively hydrophilic and do not diffuse across biological membranes. While simple flavonoid glucosides can be taken up into cells, and aglycones are absorbed by passive diffusion, the small intestine is unable to absorb the rutinoside forms. Therefore, a full or partial deglycosylation step is critical for the absorption of flavonoids. We have previously shown that commercial enzyme preparations, Pectinase 62L and Pectinase 690L, can efficiently deglycosylate bergamot flavonoids, potentially improving their uptake and increasing the beneficial effects through greater bioavailability (Mandalari *et al.* 2006b). A bergamot pectic oligosaccharide fraction obtained by treatment with Pectinase 62L has also shown potential prebiotic effect in an *in vitro* fermentation system (Mandalari *et al.* 2007). It has been demonstrated that enzyme treatments of monosaccharidic and disaccharidic flavonoids producing lipophilic derivatives increased both their antimicrobial and antioxidant activities (Mellou *et al.* 2005). The aim of the present study was to evaluate the antimicrobial properties of bergamot fractions rich in flavonoids. In addition, the influence of enzymatic deglycosylation on their antibacterial activity against Gram-negative bacteria, Gram-positive bacteria and yeast was investigated.

Material and methods

Materials

Bergamot peel was obtained from a bergamot processing factory (Consorzio del Bergamotto) in southern Italy, which consisted of a mix of the three major cultivars *Fantastico* (90%), *Femminello* (5%) and *Castagnaro* (5%). An alcohol insoluble residue (AIR) was prepared as previously described (Mandalari *et al.* 2006a), and the four liquid fractions created for each sequential ethanolic fractionation of the peel with two 70% v/v followed by two 100% v/v EtOH extractions were termed 70 E1, 70 E2, 100 E1 and 100 E2. The composition of the alcohol extracts, in terms of sugars, uronic acid and phenolics (simple phenolics, *Citrus* flavonoids and psoralens), has previously been reported by Mandalari *et al.* (2006a). Pectinase 62L (Endogalacturonase 1060 U ml^{-1}) was obtained from Biocatalysts Ltd (Cefn Coed, Wales, UK). Polygalacturonic acid was purchased from Sigma Chemical Co (Dorset, UK). All flavone and flavanone glycosides and aglycones were obtained from Extrasynthese (Genay, France).

Microbial strains and culture conditions

The following strains were used as indicators for antimicrobial testing and were obtained from the in-house

culture collection of Institute of Food Research (IFR, Norwich, UK): *Escherichia coli* K-12 MG1655, *Salmonella enterica* var. Typhimurium LT2, *Pseudomonas putida* ATCC 795, *Bacillus subtilis* ATCC 6633, *Listeria innocua* ATCC 33090, *Lactococcus lactis* MG1614, *Staphylococcus aureus* FI10139 (food isolate supplied by Unilever R&D, Bedford, UK) and *Saccharomyces cerevisiae* NCYC 505. *Escherichia coli*, *Salm. enterica* and *B. subtilis* cultures were grown at 37°C with shaking (200 rev min^{-1}) in L-broth containing (l^{-1}): 10 g bacteriological peptone (Becton Dickinson, Oxford, UK), 5 g yeast extract (Becton Dickinson), 5 g NaCl and 1 g glucose. *Pseudomonas putida* cultures were grown in L-broth at 25°C with shaking (200 rev min^{-1}). *Lactococcus lactis* cultures were grown statically at 30°C in M17 broth (Oxoid, Basingstoke, UK) supplemented with 0·5% (w/v) glucose. *Staphylococcus aureus* and *L. innocua* cultures were grown in BHI broth (Oxoid) at 37°C with shaking (200 rev min^{-1}) and without shaking, respectively. *Saccharomyces cerevisiae* cultures were grown statically at 25°C in YM broth (Difco). For solid media, 1·5% (w/v) agar (Difco) was added.

Antimicrobial testing

The minimum inhibitory concentrations (MICs) of the bergamot fractions (70 E1, 70 E2, 100 E1 and 100 E2) and the pure flavonoid compounds [neohesperidin, hesperetin (aglycone), neoeriocitrin, eriodictyol (aglycone), naringin and naringenin (aglycone)] were determined using a Bioscreen C (Labsystems, Helsinki, Finland). The test organisms were grown for 16 h in appropriate media and the optical density at 600 nm (OD_{600}) was adjusted to 0·1 by dilution in fresh media. All assays were performed in duplicate and growth in the presence of ethanol (maximum 1% v/v) acted as controls. In the combination assays, the 'checkerboard' procedure described by White *et al.* (1996) was followed. This method allows varying the concentrations of each antimicrobial along different axes, thus ensuring that each well of the Bioscreen assay plate contained a different combination. The combination assays were performed in Bioscreen honeycomb 100-well plates containing appropriate media with test compounds represented by flavonoid aglycones (naringenin, eriodictyol and hesperetin). Diluted cell cultures were then added and the bacterial growth was monitored using Bioscreen C for 16 h. The OD_{600} was measured at 10-min intervals. Controls grown with equivalent levels of ethanol (maximum 1% v/v) were included in all assays.

The MIC of each bergamot fraction or flavonoid compound, alone or in combination, was considered as the lowest concentration which completely inhibited bacterial growth (OD_{600} cutoff point ≤0·1 = no growth) after 16 h.

The MIC data of each flavonoid aglycone tested were converted into fractional inhibitory concentration (FIC), defined as the ratio of the concentration of the antimicrobial in an inhibitory concentration with a second compound to the concentration of the antimicrobial by itself (Olasupo *et al.* 2004).

$$FIC_A = \text{MIC of A with B/MIC of A}$$

The FIC index was then calculated as follows:

$$\text{FIC index} = FIC_A + FIC_B$$

The interaction of the antimicrobial combinations was determined with an isobologram as previously described (Davidson and Parish 1989; Hwang *et al.* 2004; Olasupo *et al.* 2004).

Enzymatic treatment of bergamot 70 E1 fraction

In order to potentially improve the antimicrobial activity, the 70 E1 fraction (0·5 g) was incubated with 10 U Polygalacturonase (PGase)-equivalent activity of Pectinase 62L in 50 mmol l^{-1} Na-acetate buffer, pH 5·0, for 2 h in a shaking incubator (37°C, 100 rev min^{-1}) in a final volume of 50 ml. PGase activity was determined against 1% (w/v) orange peel pectin (Mandalari *et al.* 2006b). One unit of activity was defined as the amount of enzyme required to release 1 μmol galacturonic acid per minute at 37°C, pH 5·0.

Flavonoid glycoside and aglycone analysis

The flavonoid glycosides and aglycones released in the fraction after enzyme treatment were analysed using a Phenomenex Luna C_{18} (2) reverse phase column (250 × 4·6 mm, 5 μm; Phenomenex, Macclesfield, UK) in combination with an Agilent HP1100 HPLC instrument (Agilent Ltd, West Lothion, UK) with diode-array detector as previously described (Mandalari *et al.* 2006a).

Results

Antimicrobial activity of bergamot fractions

The MIC values of bergamot fractions, determined before and after treatment with Pectinase 62L, against all the bacteria tested and the yeast *S. cerevisiae* are presented in Table 1. The results of negative controls containing ethanol (maximum 1% v/v) indicate the complete absence of inhibition of all the strains tested (data not shown). Before treatment with Pectinase 62L, all bergamot fractions, with the exception of 100 E2, showed activity against the Gram-negative bacteria, but were not active against any of the Gram-positive bacteria or the yeast

Table 1 Minimum inhibitory concentration (MIC) of bergamot fractions against Gram-positive bacteria, Gram-negative bacteria and *Saccharomyces cerevisiae*

Strain	Fraction	MIC
Escherichia coli K-12	70 E1	600
MG1655	**70 E1 post 62L**	**400**
	70 E2	300
	100 E1	200
	100 E2	No effect
Salmonella enterica ser.	70 E1	1000
Typhimurium LT2	**70 E1 post 62L**	**800**
	70 E2	400
	100 E1	400
	100 E2	No effect
Pseudomonas putida	70 E1	1000
ATCC 795	**70 E1 post 62L**	**800**
	70 E2	500
	100 E1	500
	100 E2	No effect
Bacillus subtilis ATCC 6633	70 E1	No effect
	70 E1 post 62L	**1000**
	70 E2	No effect
	100 E1	No effect
	100 E2	No effect
Listeria innocua ATCC 33090	No effect	
Lactococcus lactis MG1614		
Staphylococcus aureus FI10139		
Saccharomyces cerevisiae		
NCYC 505		

Values are expressed as μg ml^{-1}.

Table 2 Flavonoid profile of 70 E1 before and after treatment with Pectinase 62L

Compound ID	70 E1	70 E1 post 62L treatment
Apigenin 6,8-Di-C-glucoside	68·97 ± 1·25	71·05 ± 0·47
Diosmetin6,8-Di-C-glucoside	44·79 ± 0·58	39·38 ± 0·69
Eriocitrin	74·35 ± 0·79	0·00
Neoeriocitrin	1393·59 ± 2·47	70·82 ± 1·25
Luteolin-Glc/Rha isomer 1	71·97 ± 1·29	23·72 ± 0·47
Diosmetin mono-Glc isomer 1	84·63 ± 1·47	10·48 ± 0·58
Diosmetin mono-Rha	22·91 ± 0·41	21·07 ± 0·25
Narirutin	115·64 ± 1·52	95·53 ± 0·69
Naringin	1721·32 ± 3·58	84·02 ± 0·56
Apigenin-Glc/Rha	281·79 ± 0·47	0·00
Eriodictyol-Rha	165·25 ± 0·58	0·00
Diosmetin mono-Glc isomer 2	54·93 ± 0·74	0·00
Neohesperidin	1143·42 ± 1·47	199·76 ± 0·25
Narigenin mono-Rha	385·16 ± 1·25	63·41 ± 0·69
Hesperetin mono-Rha	716·71 ± 2·48	401·10 ± 0·58
Bergapten	0·00	6·10 ± 0·02
Bergamottin	0·00	0·00
Dehydro-neohesperidin	0·00	204·81 ± 1·58
Eriodictyol aglycone	0·00	289·62 ± 0·69
Apigenin aglycone	0·00	44·99 ± 0·74
Naringenin aglycone	0·00	169·41 ± 0·58
Diosmetin aglycone	0·00	19·66 ± 0·47
Hesperetin aglycone	0·00	94·50 ± 0·58

Glc, glucose; Rha, rhamnose.
Values are expressed as μg ml^{-1}.

tested in this study. Among the different fractions, 100 E1 was found to be the most effective, followed by 70 E2 and 70 E1. The amount of flavonoid present in 70 E1 was higher than that in both 70 E2 and 100 E1, suggesting that the latter fractions contained nonflavonoid lipophilic compounds that are antimicrobial, such as terpenes and psoralens (bergapten and bergamottin). *Escherichia coli* was the most sensitive strain (complete inhibition achieved with a concentration of 200 μg ml^{-1} 100 E1), followed by *Salm. enterica* (400 μg ml^{-1} 100 E1) and *Ps. putida* (500 μg ml^{-1} 100 E1). As expected, the antimicrobial properties of 70 E1 increased after treatment with Pectinase 62L because of the conversion of flavonoid glycosides into their more lipophilic and biologically active aglycones (Table 2). The fraction 70 E1 showed activity against the Gram-positive bacterium *B. subtilis*, but only after treatment with Pectinase 62L.

The inhibitory effect of bergamot fractions against all the strains tested was bacteriostatic rather than bactericidal. This was indicated by colony formation on agar plates inoculated with cells from cultures exposed to MIC levels of the samples under investigation (data not shown).

Antimicrobial activity of bergamot flavonoids

The MICs of three bergamot flavonoid glycosides and their corresponding aglycones against Gram-negative bacteria, Gram-positive bacteria and *S. cerevisiae* are reported in Table 3. The pure organic compounds showed varying degrees of activity against the strains tested. As demonstrated with the bergamot fractions, the Gram-negative bacteria were the most sensitive to the pure compounds. Eriodictyol aglycone showed the greatest activity with MICs in the range of 250 and 800 μg ml^{-1}. Naringenin was the next most effective compound. Except for the activity of neoeriocitrin against *E. coli*, no inhibition was evident with any of the flavonoid glycosides (neohesperedin, neoeriocitrin and naringin).

The modes of interaction of the flavonoid aglycones eriodictyol, hesperetin and naringenin are presented as FIC isobolograms in Figs 1–3. As mentioned in the earlier studies (Davidson and Parish 1989; Olasupo *et al.* 2004), the shape of the isobologram, curve convex, linear and concave, represents the synergistic, additive (or indifference) and antagonistic interactions, respectively. The interpretation of the FIC indices depends on which of the several definitions described in the literature are used (Te Dorsthorst *et al.* 2002). In this study, we have

Table 3 Minimum inhibitory concentration of bergamot flavonoids against Gram-positive bacteria, Gram-negative bacteria and *Saccharomyces cerevisiae*

Compound	*Escherichia coli*	*Salmonella enterica*	*Pseudomonas putida*	*Bacillus subtilis*	*Listeria innocua*	*Lactococcus lactis*	*Staphylococcus aureus*	*Saccharomyces cerevisiae*
Neohesperedin	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000
Hesperetin	1000	1000	1000	>1000	>1000	>1000	>1000	>1000
Neoeriocitrin	800	>1000	>1000	>1000	>1000	>1000	>1000	>1000
Eriodictyol	250	800	800	250	800	800	800	800
Naringin	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000
Naringenin	800	1000	1000	1000	>1000	250	>1000	>1000

Values are expressed as μg ml^{-1}.

interpreted as synergistic if the FIC index is ≤0·5, additive or indifferent if it is >0·5 but ≤4, and antagonistic if it is >4 (Visalli *et al.* 1998). Indifference to synergism was observed between eriodictyol and hesperetin against *E. coli* and *Salm. enterica* (Figs 1a and 2a) but not against *Ps. putida* where an indifference tending towards antagonistic effect between the two compounds was observed (Fig. 3a). The combination of eriodictyol and naringenin showed an indifference to synergistic effect against *Salm. enterica* (Fig. 2b) and *Ps. putida* (Fig. 3b), whereas a mainly indifferent interaction was observed against *E. coli* (Fig. 1b). Indifference tending to antagonism was evident in the combination of hesperetin and naringenin against *E. coli* and *Salm. enterica*, but indifference tending to synergism was observed against *Ps. putida* (Figs 1c, 2c and 3c).

Discussion

The present study has demonstrated that bergamot peel, a byproduct of the *Citrus* fruit processing and essential oil industries, is a potential source of natural antimicrobials. Many natural compounds, including plant phenolics and terpenoids, have been widely used because of their strong antimicrobial properties against food-borne pathogens, and therefore they can be applied as novel preservatives in the food industry (Friedman *et al.* 2002). There are numerous reports on the antimicrobial activity of crude plant extracts (Puupponen-Pimiä *et al.* 2006). Bergamot essential oil is effective against both Gram-positive and Gram-negative bacteria (Fisher and Philips 2006). The antimicrobial activity of purified flavonoids has also been described (Taguri *et al.* 2004). Various flavanones (hesperetin and naringenin) have been shown to be active against *Helicobacter pylori* (Bae *et al.* 1999). The focus of this study was to establish the biological activities of flavonoid-rich fractions from bergamot peel; we compared their antimicrobial properties with pure natural organic compounds alone and in combinations. Native bergamot peel fractions were inhibitory to Gram-negative bacteria only. *Bacillus subtilis* was the only Gram-positive organism that was inhibited but only at high concentration (1000 μg ml^{-1}) of enzyme treated bergamot fraction. We have shown that the fractions containing low levels of flavonoids (100 E1) is more active against the Gram-negative bacteria as compared with the more flavonoid-rich fraction (70 E1). This may be because of the presence of as yet uncharacterized nonflavonoid lipophilic antimicrobial compounds such as residual terpenes, sterols and psoralens (bergapten and bergamottin).

We have also demonstrated that Pectinase 62L is able to efficiently convert the bergamot flavonoid glycosides into their aglycones, and this treatment resulted in increased antimicrobial activity of the flavonoids. About 800 μg ml^{-1} was present as free aglycones in the 70 E1 fraction after Pectinase 62L treatment, and this amount was comparable with the MIC values found for pure aglycone compounds. We tested three pure flavonoid conjugates and their aglycones against the test organisms. As was expected, most of the flavonoid conjugates were inactive. Of the aglycones tested, eriodictyol was the most active and inhibited all the bacteria and the yeast *S. cerevisiae* with MIC values that ranged between 250 μg ml^{-1} and 800 μg ml^{-1}. In the enzyme-treated bergamot fraction, this aglycone was detected at 289 μg ml^{-1}. Many of the deleterious effects of flavonoids on bacterial cells could specifically occur in the presence of aglycones, which are known to be readily transported into and across cell membranes by diffusion.

In applying bioactive phenolic compounds as food preservatives, it is important to consider the dietary intake of these compounds on the complex gut microflora. Flavonoids are commonly present in plants as glycoside conjugates, and the sugar moiety is the major determinant of their absorption in the human GI tract. It has been shown that flavonoid rhamnosides and complex glycosides, such as rutinosides and neohesperosides, are poorly absorbed as compared with their aglycones and simple glucosides (Hollman *et al.* 1999). There is considerable variation in the average daily dietary intake of flavonoids;

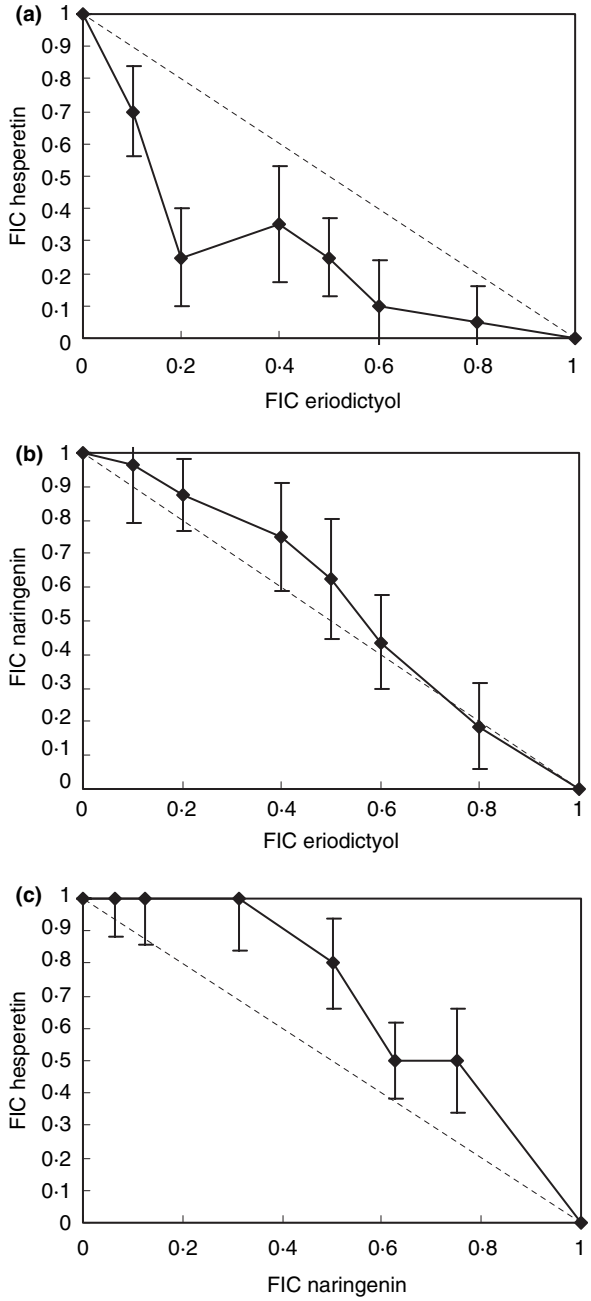

Figure 1 FIC isobolograms for combinations of eriodictyol with hesperetin (a) or naringenin (b) and hesperetin with naringenin (c) against *Escherichia coli*. The dotted line indicates the theoretical additive line.

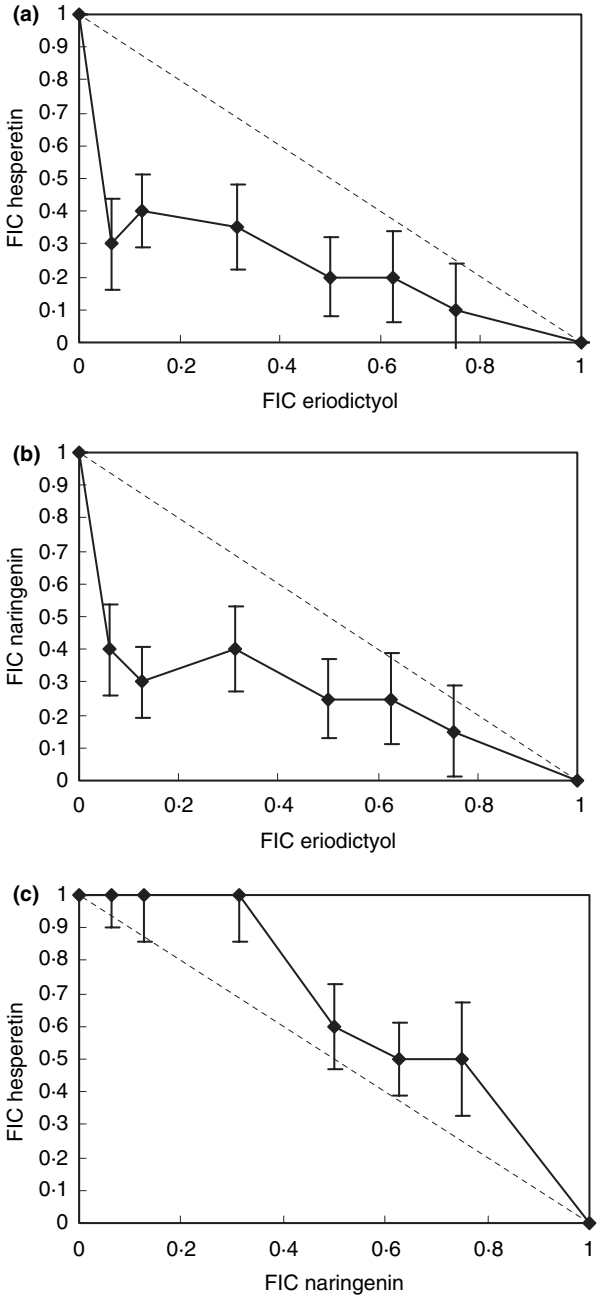

Figure 2 FIC isobolograms for combinations of eriodictyol and hesperetin (a) or naringenin (b) and hesperetin with naringenin (c) against *Salmonella enterica*. The dotted line indicates the theoretical additive line.

the values have been reported from 1 g per day as glycosides (or 650 mg per day aglycones) to 117·1 mg gallic acid equivalents per day in the American diet, and clearly the type of food consumed influences exposure and uptake (Manach *et al.* 2004; Chun *et al.* 2005). From the gut, those phenolic conjugates not absorbed will reach the large intestine where they may be converted to aglycones by the activity of gastrointestinal bacteria known to produce glycosidases capable of converting flavonoid rutinosides, neohesperosides, rhamnosides and glucosides into their aglycones, and further to simple phenolic acids (Rechner *et al.* 2004; Jenner *et al.* 2005; Simons *et al.* 2005).

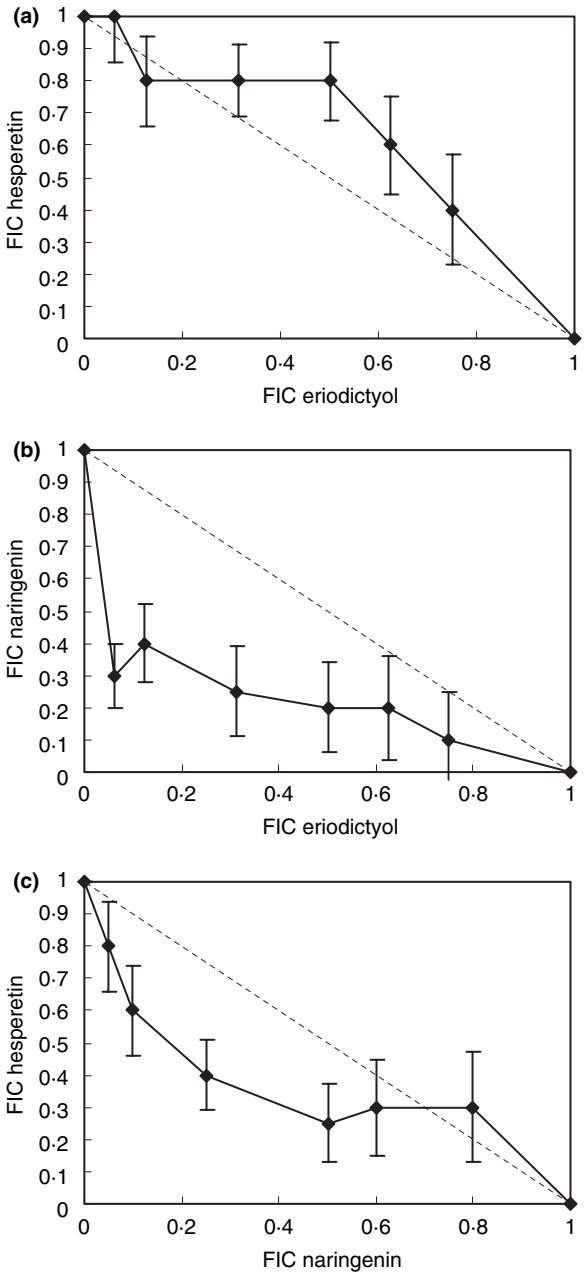

Figure 3 FIC isobolograms for combinations of eriodictyol and hesperetin (a) or naringenin (b) and hesperetin with naringenin (c) against *Pseudomonas putida*. The dotted line indicates the theoretical additive line.

Very little is known about the structure–function relationships of natural antimicrobials, but it seems that different substituent groups within the compounds have a great influence on their biophysical and biological properties. Structural features such as the presence of an aromatic ring or the numbers of hydroxyl and methoxyl groups can significantly change membrane permeability and subsequent affinity to external and internal binding sites in the bacteria, thus influencing the compound's antimicrobial properties (Fitzgerald *et al.* 2004). It has also been demonstrated that hydrophobicity and steric properties play important roles in the antibacterial activities of essential oils (Shapiro and Guggenheim 1998).

Most studies on the antimicrobial properties of flavonoids have focussed on the inhibitory activity of individual components, while information on the effects of these natural compounds in combination against food borne microorganisms is limited. We have shown that the interactions between different aglycones can alter the antimicrobial effectiveness of the bergamot flavonoids against food borne bacteria. The synergism observed between eriodictyol and hesperetin against *E. coli* and *Salm. enterica*, and between eriodictyol and naringenin against *Salm. enterica* and *Ps. Putida*, could be because of their combined reaction with the cell membrane as a possible primary target site but with different mode of inhibitory action (Sikkema *et al.* 1994). A slight antagonistic interaction was observed for naringenin and hesperetin against *E. coli* and *Salm. enterica*, and for eriodictyol and hesperetin against *Ps. putida.* This response may be the result of a number of mechanisms, such as competition for specific target sites or inhibition of uptake by the bacterial cells. Alternatively, direct interaction between the two compounds may lead to changes in structural conformation, thus resulting in the reduction of inhibitory activity. However, further studies need to be performed to understand the precise mechanisms responsible for these interactions. The overall bacteriostatic effects of the bergamot flavonoids may be because of a combination of these biophysical properties and subsequent biochemical effects, and also the ability of the different bacteria to transform flavonoids to less active/inactive metabolites.

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