ORIGINAL ARTICLE

Antibacterial activity of naringin derivatives against pathogenic strains

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Abstract

Aims: To study the antimicrobial activity of naringin (NAR), a flavonoid extracted from citrus industry waste, and NAR derivatives [naringenin (NGE), prunin and alkyl prunin esters] against pathogenic bacteria such as *L. monocytogenes*, *E. coli* O157:H7 and *S. aureus*. The relationship between the structure of the chemical compounds and their antagonistic effect was also analysed.

Methods and Results: The agar dilution technique and direct contact assaying were applied. NGE, prunin and NAR showed no antimicrobial activity at a concentration of 0·25 mmol l^{-1}. Similarly, fatty acids with a chain length between C2 and C18 showed no antimicrobial activity at the same concentration. However, prunin-6″-O-acyl esters presented high antibacterial activity, mainly against Gram-positive strains. This activity increased with increasing chain length (up to 10–12 carbon atoms). Alkyl prunin esters with 10–12 carbon atoms diminished viability of *L. monocytogenes* by about 3 log orders and *S. aureus* by 6 log orders after 2 h of contact at 37°C and at a concentration of 0·25 mmol l^{-1}. The compounds examined were not effective against any of the Gram-negative strains assayed, even at the highest concentration.

Conclusions: Addition of sugars to the aglycone did not enhance its antimicrobial activity. Attachment of a saturated aliphatic chain with 10–12 carbon atoms to the A ring of the flavonoid (or to sugars attached to this ring), seems to be the most promising modification. In conclusion, alkyl prunin esters with a chain length of C10–C12 have promising features as antimicrobial agents because of their high antilisterial and antistaphylococcal activity.

Significance and Impact of the Study: This study shows that it is possible to obtain NAR derivatives with important antimicrobial activity, especially against Gram-positive pathogenic bacteria. It also provides guidelines on the structural modifications in similar molecules to enhance the antimicrobial activity.

Introduction

Flavonoids from citrus fruit have, and are generating a growing interest as they are natural compounds with proven beneficial properties to human health like prevention of cancer (Le Marchand 2002), *in vivo* anti-inflammatory and anticancer activity (Manthey *et al.* 2001). They also play a role in the treatment of venous insufficiency (Lyseng-Williamson and Perry 2003) and in haemorrhoidal disease (Misra and Imlitemsu 2005).

In addition, they exhibit antimicrobial effects (Cushnie and Lamb 2005; Mandalari *et al.* 2007). Naringin (4′,5,7-trihydroxyflavanone-7-β-D-α-L-rhamnosyl(1 → 2)-β-D-glucoside) is one of the most common citrus flavanone glycosides, which is mainly found in grapefruit and sour oranges (Peterson *et al.* 2006a,b) and can easily be obtained from waste from the citrus industry (Poore 1934; Baier 1947). Although many scientific papers exist, which reports on the beneficial properties of this compound to human health, there are only a few articles

that have specifically studied its antibacterial properties (El-Gammal and Mansour 1986; Han and You 1988; Mandalari *et al.* 2007; Tsui *et al.* 2008).

It is well known that the structural modification of a molecule can change its hydrophilic/lipophilic ratio, and hence the derivatives may present different properties when compared with the original molecule. In particular, when this modification involves incorporation of an aliphatic chain, it has been found that acylated derivatives generally present higher antimicrobial activity, mainly against Gram-positive bacteria (Kabara 1984; Tsuchiya *et al.* 1996; Kitahara *et al.* 2004; Mellou *et al.* 2005; Ávila *et al.* 2008). Among pathogenic Gram-positive bacteria, *Listeria monocytogenes* and *Staphylococcus aureus* deserve special attention. *L. monocytogenes* is a pathogen that causes a severe disease known as listeriosis that especially affects immunocompromised patients. It has been established that food-borne transmission constitutes the main route of acquisition of listeriosis, and it has been reported that strains of this pathogen isolated from foods and clinical environments frequently exhibit antibiotic resistance. (Macgowan *et al.* 1990; Charpentier and Courvalin 1999; Walsh *et al.* 2001). Similarly, staphylococcal food poisoning is recognized as among the most common causes of reported food-borne diseases (Mead *et al.* 1999; WHO, 2002, Le Loir *et al.* 2003), requiring hospital attention by at least 10% of affected individuals. Most strains are capable of producing different heat-stable enterotoxins (Balaban and Rasooly 2001; Ortega *et al.* 2010), which are the principal cause of the gastrointestinal symptoms observed during infections (Tamarapu *et al.* 2001). Consequently, there is a continuous search for new and less contaminating agents for biological control.

The objective of this study was to examine antimicrobial activity of the flavonoids, naringin (NAR), prunin and NGE, as well as alkyl prunin esters obtained through enzymatic catalysis, against pathogenic bacteria. Furthermore, the relationship between the chemical structure of the compounds and their antagonistic effect was analysed especially against *L. monocytogenes* and *S. aureus*.

Materials and methods

Flavonoids

The following flavonoids were analysed: NAR, NGE and prunin (P). Naringin was recovered from residues from citrus industries according to Geronazzo *et al.* (2000) Prunin (P) was obtained by enzymatic hydrolysis of a supersaturated solution of NAR according to the methodology described by Ellenrieder *et al.* (1998). NGE was obtained through acid-catalysed hydrolysis of NAR according to the method described by Robin *et al.* (2007).

Prunin esters

The following prunin esters were assayed: prunin 6″-O-acetate (PA), prunin 6″-O-butyrate (PB), prunin 6″-O-hexanoate (PH), prunin 6″-O-octanoate (PO), prunin 6″-O-decanoate (PD), prunin 6″-O-laurate (PL) and prunin 6″-O-stearate (PS). All these compounds were synthesized from prunin (glucosyl-7-O-naringenin) according to a previous work (Céliz and Daz 2011) by enzymatic catalysis in an organic medium using lipase B, a commercial enzyme from *Candida antarctica*, immobilized in an acrylic resin, Novozym® 435 (donated by Novozymes Latin America Ltda., Paraná, Brazil) with different fatty acids or alkyl vinyl esters, which provided the acyl group (Sigma and Fluka, USA). Figure 1 shows the chemical structure of the compounds assayed and a synthesis diagram.

Bacterial strains

The following bacteria were used: *Listeria monocytogenes* 01/155 and 99/287 (Instituto de Microbiología 'Dr. Carlos Malbrán', Buenos Aires, Argentina), *L. monocytogenes* 99/287RB6, a bacteriocin-resistant clone of *L. monocytogenes* 99/287 (Ibarguren *et al.* 2010), *Salmonella* Enteritidis, *Pseudomonas aeruginosa* ATCC27853, *Staphylococcus aureus* ATCC29213, *Enterococcus faecium* CRL1385, *Escherichia coli* O157:H7 and *Bacillus subtilis* C4 (Sabaté *et al.* 2009). *B. cereus* C1 was supplied by Dr. Morea (ISPA, Bari, Italy). The strains were routinely propagated in brain-heart infusion (BHI) broth at 37°C for 12 h. Stock cultures were stored at −20°C in BHI broth containing 10% (w/v) glycerol.

Culture media

Brain-heart infusion (BHI; Britania, Buenos Aires, Argentina) and Mueller-Hinton broth (Britania) were used for antimicrobial assaying. When a solid medium was needed, 1·5% w/v agar (Britania) was added.

Minimum inhibitory concentrations (MICs)

MIC values of NAR, NGE, prunin and the alkyl prunin esters mentioned earlier were determined according to the microdilution method published by the Clinical and Laboratory Standards Institute (CLSI, 2003). Several compound concentrations were assayed on Mueller-Hinton agar at 37°C for 24 h against different strains.

Simultaneously, with the analysis of antimicrobial activity of NAR and its derivatives, fatty acids ranging from 2 to 18 carbon atoms were assayed to determine whether the hydrocarbon chain per se would produce a certain effect on the viability of the strains examined.

The compounds were dissolved in dimethyl sulfoxide (DMSO) at the desired concentration, and 100 μl of each solution was added to 10 ml of Mueller-Hinton agar broth. Control of the culture medium was performed to verify the viability of the strains, and control of 100 μl DMSO (1% v/v) was performed to confirm that this solvent at the maximum concentration assayed would not produce inhibition of the micro-organisms under study. Each experiment was carried out in triplicate.

Quantification of the antagonistic effect against *S. aureus* ATCC29213

The effects of the prunin 6″-O-laurate (PL) were assayed against *S. aureus* ATCC29213. A bacterial suspension in peptone water (0·1% w/v meat peptone) was used, and each cell suspension was supplemented with the tested compound dissolved in DMSO to obtain concentrations of 0·025, 0·10 and 0·25 mmol l^{-1} (1% DMSO). Samples were incubated at 37°C, and bacterial viability was assayed by cell counts on Mueller-Hinton plates after 30, 60 and 120 min of contact. All assays were carried out in duplicate.

Structure–activity relationship of the compounds against bacteriocin-resistant *L. monocytogenes* and *S. aureus* ATCC29213

The effects of NAR derivatives were analysed against *S. aureus* ATCC29213 and bacteriocin-resistant *L. monocytogenes* 99/287RB6 viability. Each 10 ml water peptone bacterial suspension (0·1% w/v meat peptone) was supplemented with 50 μl (50 mmol l^{-1}) of the compound

to be studied to obtain a final concentration of 0·25 mmol l^{-1} (0·5% DMSO) in each tube. Samples were incubated at 37°C for 2 h, and bacterial viability was determined by cell counts on Mueller-Hinton plates. All assays were carried out in duplicate.

Statistical analysis

Statistical analysis was carried out according to Tukey's test, and results were considered significant when $P < 0.05$.

Results

Minimum inhibitory concentrations (MICs)

Antibacterial activity of NAR, NGE, prunin and alkyl prunin esters was assessed against seven Gram-positive and three Gram-negative strains using the agar dilution method. Table 1 shows the antibacterial activity results.

DMSO (1% v/v) or any of the fatty acids at a concentration of 0·25 mmol l^{-1} in DMSO (maximum 1% v/v) did not show any inhibitory effect against the strains assayed. Naringin, prunin and the aglycone NGE did not present any inhibitory effect at the concentrations assayed (max. 0·25 mmol l^{-1}) nor did PA below 0·25 mmol l^{-1}. Prunin butyrate (PB) inhibited *P. aeruginosa*, *S. aureus*, *B. cereus* and the three listeria strains only at a concentration of 0·25 mmol l^{-1}. Prunin hexanoate (PH) showed a similar behaviour to that observed for PB, but the MICs for *L. monocytogenes* 99/287 S and R reached 0·10 mmol l^{-1}. Prunin octanoate (PO) and prunin decanoate
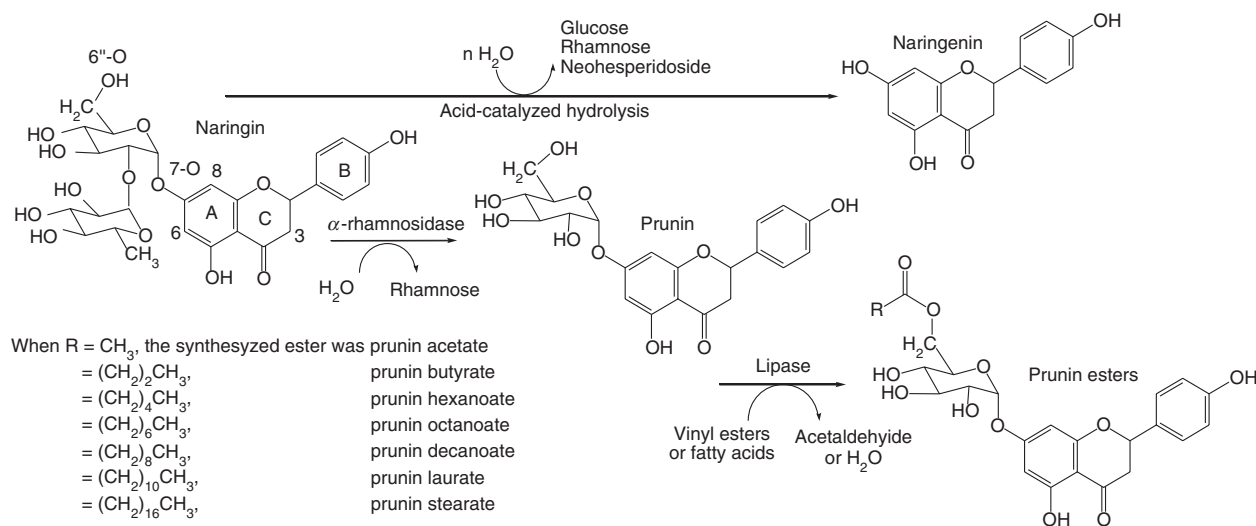

Figure 1 Scheme of reactions carried out with naringin. Flavonoid rings and some important atoms are specified.

Table 1 Minimum inhibitory concentration (MIC) in mmol l^{-1} of naringin (NAR), prunin (P), naringenin (NGE), prunin 6″-O-acetate (PA), prunin 6″-O-butyrate (PB), prunin 6″-O-hexanoate (PH), prunin 6″-O-octanoate (PO), prunin 6″-O-decanoate (PD), prunin 6″-O-laurate (PL) and prunin 6″-O-stearate (PS) against the Gram-positive and Gram-negative strains assayed. 'N.I.' means no inhibition observed at 0·25 mmol l^{-1}

Bacterium/compound	NAR	P	NGE	PA	PB	PH	PO	PD	PL	PS
Gram-positive bacteria										
Bacillus cereus	N.I.	N.I.	N.I.	N.I.	0·250	0·250	0·050	0·050	0·250	N.I.
Bacillus subtilis C4	N.I.	N.I.	N.I.	N.I.	N.I.	0·250	0·100	0·100	0·250	N.I.
Listeria monocytogenes 99/287 S	N.I.	N.I.	N.I.	N.I.	0·250	0·100	0·050	0·010	0·010	0·250
Listeria monocytogenes 99/287R	N.I.	N.I.	N.I.	N.I.	0·250	0·100	0·025	0·025	0·025	N.I.
Listeria monocytogenes 01/155	N.I.	N.I.	N.I.	N.I.	0·250	0·250	0·025	0·050	0·050	0·250
Enterococcus faecium CRL1385	N.I.	N.I.	N.I.	N.I.	N.I.	0·250	N.I.	0·100	0·100	0·100
Staphylococcus aureus ATCC29213	N.I.	N.I.	N.I.	N.I.	0·250	0·250	0·100	0·100	0·100	0·025
Gram-negative bacteria										
Pseudomonas aeruginosa	N.I.	N.I.	N.I.	N.I.	0·250	0·250	0·250	0·250	0·250	N.I.
Salmonella enteritidis	N.I.	N.I.	N.I.	N.I.	N.I.	N.I.	N.I.	N.I.	0·250	N.I.
Escherichia coli	N.I.	N.I.	N.I.	N.I.	N.I.	N.I.	N.I.	N.I.	0·250	N.I.

(PD) inhibited *P. aeruginosa* at a concentration of 0·25 mmol l^{-1}, and they showed the highest inhibition of *B. cereus* and *B. subtilis* C4 with MICs of 0·05 and 0·10 mmol l^{-1}, respectively. Against listeria strains, PO and PD presented MICs between 0·01 and 0·05 mmol l^{-1} and against *S. aureus* a MIC of 0·10 mmol l^{-1}. Prunin laurate (PL) inhibited *P. aeruginosa* and the *Bacillus* strains at a concentration of 0·25 mmol l^{-1}. Furthermore, it inhibited the *Listeria* strains at MICs between 0·01 and 0·05 mmol l^{-1}. Prunin stearate (PS) showed the lowest MIC against *S. aureus*: 0·025 mmol l^{-1}. PD, PL and PS markedly affected *E. faecium* at a MIC of 0·10 mmol l^{-1}. *E. coli* and *S. enteritidis* were only inhibited by PD and PL at a concentration of 0·25 mmol l^{-1}.

Quantification of the antagonistic effect of PL against *S. aureus* ATCC29213

The effect of PL against *S. aureus* was tested at 0·025, 0·10 and 0·25 mmol l^{-1}. To carry out this experiment, direct contact was established between the compound and the micro-organism.

As shown in Fig. 2, there was a relationship between the PL concentration and the antimicrobial effect. After 30 min of contact, a reduction of 2 log orders in the number of viable cells was observed at a concentration of 0·025 mmol l^{-1}, compared with about 4 log orders at 0·25 mmol l^{-1}. The three concentrations assayed showed a similar pattern: rapid loss of viability that increased with prolonged contact time. The most important antagonistic effect was obtained with a PL concentration of 0·25 mmol l^{-1} after 2 h. A reduction of more than 6 log orders was registered in the number of colony-forming units per ml. A similar time-concentration
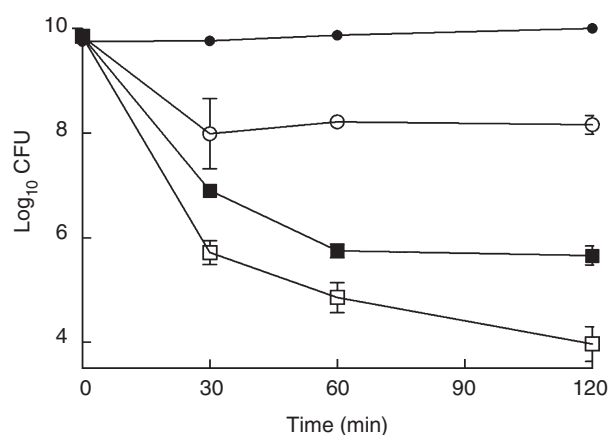

Figure 2 Quantification of the antagonistic effect of prunin 6″-O-laurate (PL) against *S. aureus* ATCC29213 at 0.025 mmol l^{-1} (○), 0.10 mmol l^{-1} (■) and 0.25 mmol l^{-1} (□). ● Control. The assay was carried out in duplicate. Data are expressed as the mean ± standard error.

behaviour was previously found for PL against *L. monocytogenes* 99/287, a bacteriocin-sensitive strain and the parent strain of *L. monocytogenes* 99/287RB6 (Céliz *et al.* 2010).

Structure–activity relationship of the compounds against *S. aureus* ATCC29213 and the bacteriocin-resistant *L. monocytogenes* 99/287RB6 clone

To find a relationship between antimicrobial activity and the chemical structure of the compounds, a fixed concentration of 0·25 mmol l^{-1} (0·5% DMSO) was used. Because of their low solubility in water, the compounds were first dissolved in DMSO. The addition of 100 μl of the

mentioned dissolutions in 10 ml of Mueller-Hinton broth produced a crystalline solution in the case of NAR, NGE, P, PA, acetic acid and butyric acid and a liquid–liquid suspension in the cases of PB, PH, PO, PD, PL, PS and fatty acids with six or more carbon atoms. Particularly, in the case of stearic acid and PS, fast precipitation from the dispersion could be observed. Samples were incubated at 37°C for 2 h, and bacterial viability was determined by cell counts. The results for each bacterium are given later.

Listeria monocytogenes 99/287RB6
The flavonoids and the alkyl prunin esters with six or less carbon atoms presented only small differences compared with controls (Fig. 3). The prunin esters with eight or more carbon atoms showed the highest inhibitory effect. After 2 h of contact, a decrease of about 3 log orders was observed in the number of the bacteriocin-resistant *L. monocytogenes* viable cells.

Staphylococcus aureus ATCC29213
When the antagonistic effect of the compounds was tested against *S. aureus*, a similar behaviour was observed to that of *L. monocytogenes*. The aglycone NGE and its glycosylated derivatives did not show any activity. The prunin esters with chains up to four carbon atoms did not demonstrate significant inhibition, whereas esters with 6–12 carbon atoms presented an inhibitory effect that increased with increasing chain length. The most effective compounds were those with a chain length of 10–12 carbon atoms, obtaining a diminution in viability of 6 log orders. The ester with 18 carbon atoms (PS) showed a decrease in viability of about 1 log order (Fig. 3).
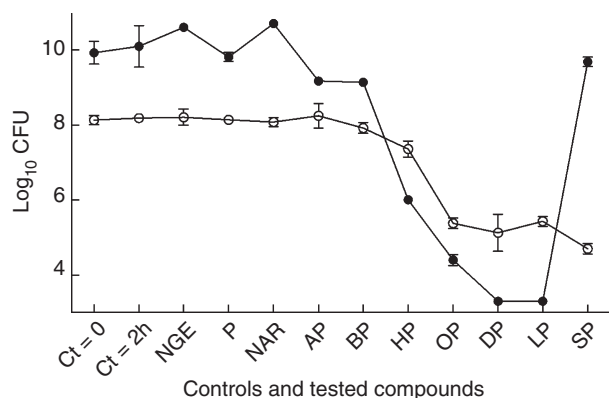

Figure 3 Structure–activity relationship of the compounds assayed against *S. aureus* ATCC29213 (○) and the bacteriocin-resistant *L. monocytogenes* clone (●). Abbreviations: Ct = 0: control at *T* = 0, Ct = 2 h: control after 2 h, NAR: naringin, P: prunin, NGE: naringenin, PA: prunin 6″-O-acetate, PB: prunin 6″-O-butyrate, PH: prunin 6″-O-hexanoate, PO: prunin 6″-O-octanoate, PD: prunin 6″-O-decanoate, PL: prunin 6″-O-laurate and PS: prunin 6″-O-stearate. Data are expressed as the mean ± standard error of two assays.

Discussion

Acylated flavonoids are relatively new compounds and therefore little scientific information exists about their biological properties. It has been reported that some biological, biochemical and biophysical properties of a flavonoid may vary significantly because of acylation (Gatto *et al.* 2002; Céliz *et al.* 2010; Salem *et al.* 2010; Céliz and Daz 2011).

In general, researchers agree that an improvement exists in microbial activity of a large variety of compounds when lipophilic groups are added. In fact, fatty acids that can be used as donators of hydrophobic groups possess inhibitory properties per se against different bacteria (Nieman 1954).

It is known that the cytoplasmic membrane is the site where fatty acids must interact first to produce inhibition, regardless of the mechanism (Desbois and Smith 2010). Whereas the external membrane of Gram-negative bacteria behaves as a fatty acid barrier, the cell wall of Gram-positive bacteria allows circulation of fatty acids towards the internal membrane (Galbraith and Miller 1973; Sheu and Freese 1973). Therefore, antimicrobial activity of free fatty acids is usually more important in Gram-positive than in Gram-negative bacteria (Nieman 1954; Sheu and Freese 1973; Desbois and Smith 2010).

While carrying out the agar diffusion technique at a concentration of 0·25 mmol l^{-1} in this study, none of the saturated fatty acids (from 2 to 18 carbon atoms) showed any inhibitory effect against the 10 strains assayed. This result agrees with that reported by Kitahara *et al.* (2004), who studied the antimicrobial activity of saturated fatty acids and fatty amines against methicillin-resistant *S. aureus* (MRSA). The authors found that lauric acid and decanoic acid only showed anti-MRSA activity against six MRSA strains at concentrations above 400 μg ml^{-1} (2·0 mmol l^{-1}) and 800 μg ml^{-1} (4·65 mmol l^{-1}), respectively. Stearic and octanoic acid showed no activity even at a concentration of 1600 μg ml^{-1} (5·6 and 11·1 mmol l^{-1}, respectively). Furthermore, they informed that MICs of fatty amines depended on the hydrophobic chain length, and the highest activity was found for myristylamine, a saturated C14 amine.

When specifically considering flavonoid activity in a previous study, we observed that prunin did not present antimicrobial activity against different strains, whereas its 6″-O-lauroyl ester did (Céliz *et al.* 2010). Tsuchiya *et al.* (1996) studied the antibacterial activity of phytochemical flavanones against 18 methicillin-resistant *S. aureus* strains and found that NGE had antimicrobial activity at concentrations between 0·75 and 1·5 mmol l^{-1}. In addition, aliphatic groups at the 6- or 8-position (A flavonoid ring) enhanced the flavanone antibacterial activity. Moreover, substitution with a lavandulyl group instead of a prenyl

group upon the 8th position enhanced the activity of sophoraflavanone G more than leachianone G, indicating that a substituent with a relatively long chain is more active (Tsuchiya *et al.* 1996). Alcaráz *et al.* (2000) studied the structure–activity relationship of flavanones, flavones and hydroxy or methoxy chalcone substituents against *S. aureus*. They found that flavanones were the least active, and the activity was observed between 0·5 and 6·0 mmol l^{-1}, which would agree with the range found by Tsuchiya *et al.* (1996) for similar compounds. Mellou *et al.* (2005) found that from two totally inactive glycosyl flavones isolated from Greek endemic plants one became active against *S. aureus* at a concentration of 1000 μg ml^{-1} (1·3 mmol l^{-1}), after esterification with a lauroyl chain in the 6″-O position, where the sugar is linked to the 7-O-flavone position of the A ring. On the contrary, Gatto *et al.* (2002) worked with quercetin and its 3-O-acyl esters (with chain lengths between 2 and 16 carbon atoms) against yeasts, Gram-negative and Gram-positive strains. They found that at a concentration of 100 μg ml^{-1} (0·21 mmol l^{-1} for quercetin-3-O-laurate), none of the compounds showed any inhibitory effect against the micro-organisms assayed. Although the authors believe that this lack of activity was because of the concentration used, it may also have been the result of the fact that acylation of the C ring (3-position) did not allow adequate interaction between the molecule and the target cell at membrane level.

This study determined that although prunin and the free fatty acids did not present inhibitory activity, the alkyl prunin esters turned out to be the strong antimicrobial agents at a concentration of 0·25 mmol l^{-1}, especially against *L. monocytogenes* and *S. aureus*. Also, it was found that antimicrobial activity of the prunin-derived esters was important against Gram-positive bacteria but not against Gram-negative bacteria. This activity was directly related to the hydrophobicity of the substituent, with an optimum of between 10 and 12 carbon atoms. The chain would provide more hydrophobicity to the glucosyl aglycone, which certainly would allow better interaction at the membrane level.

It should be noted that the antistaphylococcal activity of PS differed significantly according to the method employed. A reason could be the low solubility of PS in aqueous matrices. Whereas PS remains uniformly dispersed, and in continuous contact with the organism during the agar dilution technique, when the direct contact assay was performed, PS precipitated while the organism stayed in suspension. Consequently, its effective concentration diminished drastically. Other authors have observed significant differences in the determination of antimicrobial activities in accordance with the properties of flavonoids and the techniques used (Cushnie and

Lamb 2005). This situation was not observed against *L. monocytogenes*, which is almost certainly because of the higher inhibition rate (Céliz *et al.* 2010). The examination of the antimicrobial property was independent of the detection technique for all other compounds assayed.

Based on the current results and those found in literature, several general observations can be made regarding the structure–activity relationship of the compounds analysed.

Glycoside substituents

Low concentrations of the aglycone NGE (0·25 mmol l^{-1}) did not inhibit Gram-positive bacteria. Moreover, the presence of glycosides (glucose in the case of prunin and di-glycoside neohesperidose in NAR), did not enhance antimicrobial activity. Other authors have compared the antibacterial activity of aglycones and their glycosides. Mandalari *et al.* (2007) compared antimicrobial activity of NGE, hesperetin and eriodictyol and their NAR, neohesperidin and neoeriocitrin di-glycosides against *Listeria innocua* and *S. aureus* FI10139, among the other strains. Their results suggest that if there exists a difference between aglycones and di-glycosides, aglycones would be more active. Han and You (1988) found that the aglycone NGE was more effective than NAR at inhibiting Gram-positive bacteria. This would indicate that the attachment of hydrophilic substituents to the 7-O-aglycone would diminish the interaction between the aglycone and the target strain. This is almost certainly true because of the lack of affinity for the phospholipid bi-layer or specific receptors on the cell membrane.

Alkyl substituents

Regarding their lipophilic character, it is obvious that the incorporation of hydrophobic groups is not enough to turn an inactive flavonoid (or with very low activity) into an active compound against Gram-positive bacteria. However, attachment of a lipophilic group with an approximate length of 10–12 carbon atoms (saturated aliphatic chains) to the A ring of the flavonoid (or to sugars attached to this ring) seems to be the most promising modification. These conclusions would agree with those reported by other authors who, working with essential oils, showed that hydrophobicity and steric properties play an important role in their antibacterial activity (Shapiro and Guggenheim 1998a,b).

Conclusions

In this paper, we observed that 7-O-glycosyl moieties did not enhance the inhibitory activity of the aglycone NGE

against seven Gram-positive and three Gram-negative strains. Moreover, alkyl prunin esters showed important antimicrobial activity against Gram-positive strains, and the inhibitory activity gradually increased with the increasing chain length; maximum inhibition was observed with 10–12 carbon atoms. Concentrations of 0.25 mmol l^{-1} of alkyl prunin esters with these chain lengths diminished viable *L. monocytogenes* with about 3 log orders and *S. aureus* with about 6 log orders after 2 h. These results show that it is possible to obtain NAR derivatives with important antimicrobial activity, especially against Gram-positive pathogenic bacteria.

Acknowledgements

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