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ARTICLE

Effect of fruit juices and pomace extracts on the growth of Gram-positive and Gram-negative bacteria

Judit Krisch[1]*, László Galgóczy[2], Mónika Tölgyesi[1], Tamás Papp[2] and Csaba Vágvölgyi[2]

[1]Institute of Food Engineering, Faculty of Engineering, University of Szeged, Szeged, Hungary, [2]Department of Microbiology, Faculty of Science and Informatics, University of Szeged, Szeged, Hungary

ABSTRACT Extracts and juices of cultivated and wild fruits belonging to the families Rosaceae, Grossulariaceae, Moraceae, Berberidaceae, Polygonaceae, Caprifoliaceae and Cornaceae were examined for their growth reducing activity on four bacteria (*Bacillus subtilis*, *B. cereus* var. *mycoides*, *Escherichia coli* and *Serratia marcescens*). In vitro antibacterial activities were evaluated by microdilution plate assays. Black currant (*Ribes nigrum)*, cornelian cherry (*Cornus mas)* and European rowan (*Sorbus aucuparia)* had the highest growth inhibition capacity.
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KEY WORDS
fruit
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Fruits are rich sources of vitamins, minerals and fibres, therefore, their consumption have positive role in the maintenance of human health. Phenolic compounds present in plants (phenolic acids, flavonoids, stilbenes, lignans and complex phenolic polymers) have antioxidant activity due to their redox properties (Kahkönen et al. 1999). They can also act as metal chelators and can have antimicrobial properties. The main target of antibacterial action is usually the cell membrane where destabilization and/or permeabilisation can occur. Phenolics can also inhibit extracellular enzymes or the multidrug resistance pumps of certain bacteria. Some berry extracts exhibit bacterial antiadhesion activity, so that bacteria cannot adhere to mucosal surfaces, which is an important prerequisite for colonisation and infection (Puupponen-Pimia et al. 2004).

There has been a growing interest in plant-derived active compounds among food technologists and pharmacologists. Food industry is searching for natural preservatives and antioxidants; and medicine, for new antimicrobial agents without rapidly developing resistance. In this study, the antibacterial activity of 21 wild and cultivated fruits was investigated.

Materials and Methods

Bacteria and culture conditions

Bacillus subtilis ssp. *subtilis* BD 170, *B. cereus* var. *mycoides* ATCC 9634, *Escherichia coli* SZMC 0582, and *Serratia marcescens* SZMC 0567 were grown on T1 medium (10g glucose, 4g beef extract, 4g peptone, 1g yeast extract, 1L H_2O).

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*Corresponding author. E-mail: krisch@mk.u-szeged.hu.

Fruits and extraction methods

The fruits tested are listed in Table 1. Fresh fruits were purchased on a local market (Szeged) or were harvested in the neighbourhood of Szeged or in the mountains of North-East Hungary. Fruit juices were freshly pressed and stored at -20°C. The remaining pomace was dried at 60°C in an oven for 12 h and then ground to powder. One gram of each powdered pomace was extracted 3 times with 10 ml of distilled water or methanol per cycle. The extracts were combined and evaporated to dryness at 100°C in an oven (water extracts) or at 35-40°C in a water bath (methanol extracts). The dry material was redissolved in 4 ml distilled water (water extracts) or 10% methanol-water solution (methanol extracts), and frozen in 1 ml aliquots. One sample from each extracts was dried again and weighed for dry matter content calculation. Juices and extracts were diluted in the appropriate media for the tests.

Broth microdilution method

Inocula from each bacterium (10^5 cells/ml) were prepared in LB medium (10g triptone, 10g NaCl, 2g yeast extract, 1L H_2O). 100 µl of fivefold diluted and sterile filtered juice was mixed with 100 µl cell suspension in triplicates, incubated at 37°C for 48 h, and then absorbance was measured at 620 nm. For determination of growth curves absorbance was measured repeatedly in a time span of 48-72 hours.

Results

In our tests black currant (*Ribes nigrum)*, cornelian cherry (*Cornus mas)* and European rowan (*Sorbus aucuparia)* had the best inhibition capacity (Table 1). Sweet cherry cultivars, hawthorn (*Crataegus monogyna)* and elderberry (*Sambucus*

Table 1. Growth inhibition effect of fruit juices. 0 - no growth; 1 - growth ≤ 25%; 2 - growth ≤ 50%; 3 - growth ≤ 75%; 4 - growth > 75 %; 100 % is taken as the growth control. J -juice; W - water extract; M - methanol extract

	Gram positive bacteria						Gram negative bacteria					
	Bacillus subtilis			*Bacillus cereus*			*Escherichia coli*			*Serratia marcescens*		
	J	W	M	J	W	M	J	W	M	J	W	M
Rosaceae												
Prunus avium	4	4	1	4	4	4	4	4	2	4	4	4
Prunus avium Gold	4	4	4	4	3	4	4	4	2	4	4	4
Prunus cerasus	4	4	4	3	2	4	2	3	1	4	4	1
Prunus armeniaca	4	4	4	3	1	3	2	2	2	4	4	4
Crataegus monogyna	4	4	4	3	4	4	4	4	4	4	4	4
Rubus idaeus	4	4	4	1	3	4	1	4	2	0	4	1
Rubus fruticosus	4	1	1	1	1	0	1	4	1	4	4	2
Fragaria ananassa	4	4	1	1	4	1	3	4	3	4	4	4
Sorbus aucuparia	1	1	2	2	1	4	1	1	1	4	4	1
Grossulariaceae												
Ribes nigrum	1	1	1	0	4	0	0	1	1	2	1	4
Ribes rubrum	1	4	4	4	4	4	1	4	1	1	4	1
Ribes uva-crispa	1	1	4	4	1	4	1	1	1	4	1	1
Ribes x nidigrolaria	1	4	4	0	2	4	0	3	1	0	4	4
Moraceae												
Morus alba	4	4	2	4	4	0	4	4	3	4	4	4
Morus nigra	4	4	1	4	4	0	3	4	4	4	4	4
Berberidaceae												
Berberis thunbergii	4	4	1	2	4	1	1	4	2	1	4	4
Mahonia aquifolium	1	4	2	2	2	4	1	4	1	1	4	4
Caprifoliaceae												
Sambucus nigra	4	4	1	4	4	0	4	4	4	4	4	4
Sambucus alba	4	4	4	4	3	4	4	4	3	4	4	4
Polygonacea												
Rheum rhabarbarum	4	0	4	4	0	4	2	4	1	4	4	1
Cornaceae												
Cornus mas	1	1	1	2	1	0	2	3	1	4	4	0

nigra) had no or weak inhibitory effect on the tested bacteria; in some cases they even facilitated bacterial growth. Members of the *Ribes* and *Rubus* genus were generally efficient inhibitors while other members of the Rosaceae family showed a poor growth reducing effect. When inhibition was observed, best results were obtained with methanolic extracts of the pomace, followed by the juices and finally by the water extracts. In general, Gram-positive bacteria showed moderate sensitivity. The Gram-negative *E. coli* was the most sensitive strain, especially to the juices and methanolic extracts. The other Gram-negative bacterium, *S. marcescens*, turned out to be the most insensitive one (for details, see Table 1).

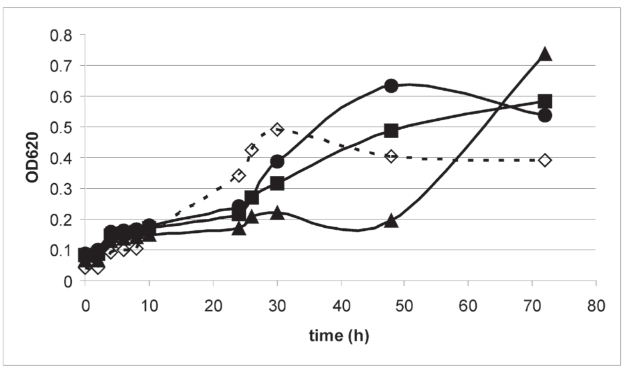

Figure 1. Effect of raspberry juice, water and methanol extract on the growth of *B. subtilis*. Empty diamond: control; filled triangle: juice; filled square: water extract of pomace, filled circle: methanolic extract of pomace.

To have a more detailed picture of the effect of fruit juices and extracts on bacterial growth, growth curves were obtain with *B. subtilis* and *E. coli* using raspberry. As seen in Figure 2, raspberry juice totally inhibited the growth of *E. coli* while the extracts had a moderate inhibitory effect. *B. subtilis* treated with raspberry juice reached, however, after a 48 hours adaptation period, a higher cell number than the untreated control. With the extracts, the adaptation period was shorter, only 24 hours, and the cell number was also higher than in the control (Fig. 1).

Raspberry juice had a two-face action on the growth of *B. subtilis* as shown by Figure 3. In ten- and twenty-fold dilutions, there was an adaptation phase of 48 and 24 hours, respectively, but then the maximal cell number considerably surpassed that of the control. With the higher dilutions, there was no adaptation phase but the second facilitatory effect was

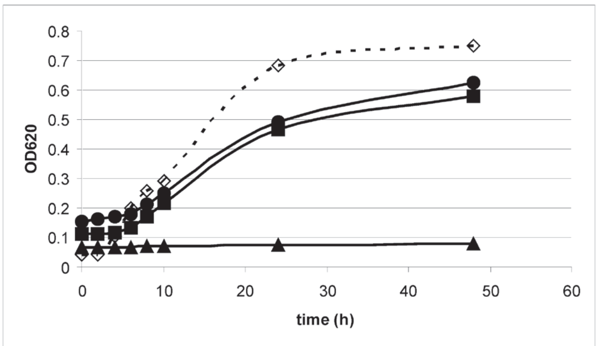

Figure 2. Effect of raspberry juice, water and methanol extract on the growth of *E. coli*. Empty diamond: control; filled triangle: juice; filled square: water extract of pomace, filled circle: methanolic extract of pomace.

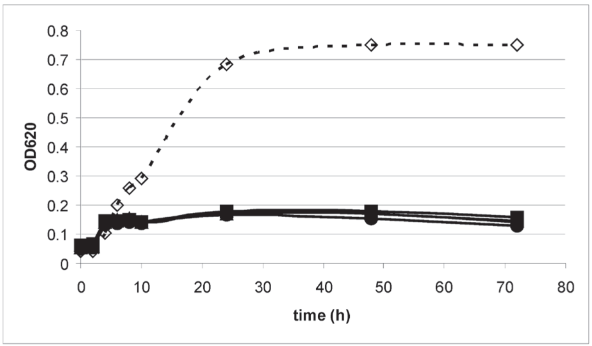

Figure 4. Effect of raspberry juice dilutions on the growth of *E. coli*. Empty diamond: control; filled square:10x; filled triangle:20x;, asterisk: 40x; filled circle: 80x dilution.

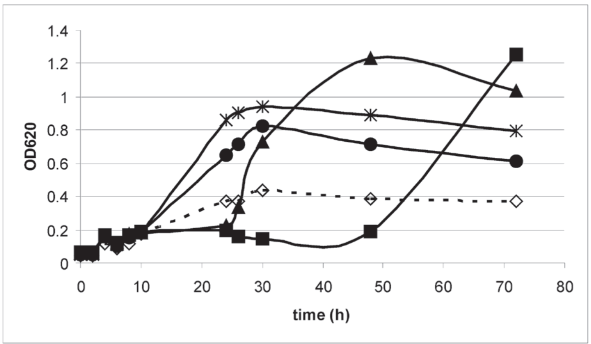

Figure 3. Effect of raspberry juice dilutions on the growth of *B. subtilis*. Empty diamond: control; filled square:10x; filled triangle:20x;, asterisk: 40x; filled circle: 80x dilution.

also less intense. In case of *E. coli*, all dilutions had more or less the same inhibitory effect (Fig. 4).

Discussion

Each juice and extract had an acidic pH ranging from 2.8 (*Ribes* spp.) to 5.5 (*Morus nigra*). The low pH of fruit juices is caused by weak organic and phenolic acids: in their undissociated forms (mainly on pH 3-5) these can interact with cell membranes and penetrate into the cells causing acidification of the cytoplasm. However, in our experiments there was only a weak correlation between acidity of the samples and their antibacterial effect.

Consumption of various berries has an important role in human health maintenance. Raspberry (*R. idaeus*) has a long tradition of use in curing diarrhoea (Ryan et al. 2001). Antibacterial activity of raspberry juice was demonstrated against *E. coli*, *S. typhimurium* and *S. epidermidis* (Ryan et al. 2001; Lee et al. 2003). In the study of Cavanagh et al. (2003), blackberry (*R. fruticosus*) juice had no growth inhibitory effect on *Salmonella* species but strongly inhibited *Klebsiella pneumoniae.* In our experiments raspberry and blackberry juice decreased the growth of *B. cereus* and *E. coli.*

In European folk medicine, *R. nigrum* (blackcurrant) fruits have been used to support the immune and digestive systems. In previous studies (Puupponen-Pimiä et al. 2001; Cavanagh et al. 2003), blackcurrant juices and extracts were more efficient against Gram-positive bacteria than against Gram-negative ones. In our study, the blackcurrant juice caused growth inhibition on all the investigated species except of *S. marcescens*. Other investigated *Ribes* species (*R.* x *nidigrolaria*, *R. uva-crispa* and *R. rubrum*) were also among the most efficient inhibitors in our study.

Gram-negative and Gram-positive organisms show different sensitivity to several antibacterial agents because the former possess an outer membrane as part of their cell wall (Ratledge and Wilkinson 1988) restricting the diffusion of hydrophobic compounds, e.g.: essential oils (Burt 2004), and other oily substances such as guava and neem extracts (Mahfuzul Hoque et al. 2007) showed higher antimicrobial activity against Gram-positive than Gram-negative bacteria. At the same time, certain small hydrophobic compounds are known to penetrate easily to the cells generating pores in the outer membranes. The efficiency of methanolic extracts (and juices) on *E. coli* in our study suggest the presence of such non-polar molecules. Previous studies demonstrated that purple and red fruits and vegetables, with high content of anthocyanins, had substantial antibacterial effect (Harborne and Williams 2000; Lee et al. 2003). Our results with closely related coloured and non-coloured fruit species and varieties (*Sambucus nigra* and *S. alba*, *Morus nigra* and *M. alba*, *Prunus avium* Germersdorfi and *P. avium* Gold) showed, however, no real difference in their inhibitory effect. On the other hand, the dark coloured *Ribes* and *Rubus* species had excellent antibacterial action. The role of anthocyans in the growth inhibition of bacteria needs further investigation.

Acknowledgements

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