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Growth inhibition effect of fruit juices and pomace extracts on the enteric pathogens Campylobacter jejuni and Salmonella ser. Typhimurium

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Citation

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Abstract

The antibacterial effect of fruit juices and pomace extracts from 13 wild and cultivated fruits (Prunus avium, P. cerasus, P. armeniaca, Crataegus monogyna, Morus alba, M. nigra, Ribes nigrum, R. rubrum, R. uva-crispa, R. nidigrolaria, Rubus idaeus, R. fruticosus) against two foodborne enteric pathogens (Salmonella ser. Typhimurium, Campylobacter jejuni) was evaluated by broth microdilution assays. Juices and/or extracts of sour cherry, apricot, raspberry, blackcurrant, redcurrant, gooseberry and jostaberry efficiently inhibited the growth of both bacteria (growth ≤ 25%). Juices and extracts from cherry (red and yellow cultivars), hawthorn, blackberry and pomace extracts from black and white mulberry had a similar strong inhibitory effect on the growth of C. jejuni, but had weak or no effect on S. ser. Typhimurium. Sour cherry, jostaberry and raspberry pomace extracts revealed a substantial antibacterial effect at both acidic and neutral pH.

INTRODUCTION

Bacteria of the genus Campylobacter and Salmonella are important human pathogens causing foodborne infections worldwide. Campylobacter jejuni infects the intestinal tract, with occasional spread to the blood, and causes stomach pain, fever, mild to severe or bloody diarrhoea, nausea, and sometimes vomiting (Kotula and Stern 1983). Salmonella species multiply in the small intestine and invade the gut lining. In the last 30 years, several reports have been published on outbreaks of Salmonella gastroenteritis in hospitalized patients caused by multiple antibiotic resistant Salmonella strains (Lamb et al. 1984). Salmonella ser. Typhimurium is one of the most important serovariants of the genus in almost all countries (Kobilinsky et al. 2007).

In spite of the use of traditional and modern methods in food safety techniques (e.g. irradiation, Lacriox and Ouattara 2000; application of essential oils, Burt 2004) as many as 30% of the people in industrialized countries suffer from a food borne disease each year (Burt 2004). Therefore, the need for new methods of reducing or eliminating food born pathogens remains (Leistner 1978).

Plants have a natural defense mechanism against microbial infections. Antimicrobial peptides, lectins, phenolic compounds, terpenoids, essential oils and various other compounds are likely to be involved in this phenomenon (Cowan 1999). Raw and processed fruits, as well as waste products remaining after processing (peel, seeds, stems, flesh) are good sources of these ingredients. In previous in vitro experiments, juices and extracts from berries, guava, and citrus fruits revealed antibacterial activities against Gram-negative and -positive bacteria (Ryan et al. 2001; Cavanagh et al. 2003; Lee et al. 2003; Vattem et al. 2005; Sagdic et al. 2006; Mahfuzul Hoque et al. 2007). In most of the cases, whole fruits were extracted and little attention was paid to the by-products of juice making.

In the present study, the in vitro biological activity of juices, water and methanol extracts of pomace (peels, seeds, flesh) remaining after juice pressing, of 13 fruits were investigated on the human pathogen bacteria Salmonella ser. Typhimurium and Campylobacter jejuni by broth dilution method.

MATERIALS AND METHODS

BACTERIAL ISOLATES AND THEIR MAINTENANCE

Clinical isolates of Salmonella ser. Typhimurium and Campylobacter jejuni were investigated. S. ser.

Typhimurium was maintained on T1 medium (10g glucose, 4g beef extract, 4g peptone, 1g yeast extract, 1l H_2O) and C. jejuni, on Campylobacter blood-free selective agar medium (CCDA; Charcoal cefoperazone deoxycholate agar, Merck).

FRUITS AND EXTRACTION METHODS

Fruits investigated were cherry (Prunus avium – two cultivars), sour cherry (P. cerasus), apricot (P. armeniaca), black and white mulberry (Morus nigra, M. alba), blackcurrant (Ribes nigrum), redcurrant (R. rubrum), gooseberry (R. uva-crispa), jostaberry (R. nidigrolaria), blackberry (Rubus fruticosus), raspberry (R. idaeus) and hawthorn (Crataegus monogyna). Fresh fruits were purchased on a local market (Szeged) or were collected in the neighbourhood of Szeged. Fruit juices were freshly pressed, filtered through a cheese cloth, centrifuged (10,000 rpm, 10 min., Sorvall RC-5B) and stored at -20oC. The remaining pomace was dried overnight at 60oC in an oven and then grounded to powder. One gram of each powdered pomace was extracted 3 times with 10 ml of distilled water or methanol per cycle. After each cycle the extracts were centrifuged (8000 rpm, 10 min, Sorvall RC-5B), and the supernatants were combined and evaporated to dryness at 100oC 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.

DETERMINATION OF ANTIBACTERIAL EFFECT BY BROTH MICRODILUTION METHOD

In vitro antibacterial activities were evaluated by microdilution plate assay. Absorbance of the bacterial cultures was measured at 492/620 nm in the presence of the fivefold diluted (with T1 medium) juice or extract, without pH modification (unbuffered method). The most efficient juices and extracts (growth inhibition ≥ 75%) were buffered to pH 7 with 0.165 M morpholino-propane sulfonic acid, and their inhibitory effect was tested in the concentration range of ten- to eighty-fold dilutions (buffered method). In each well, 100 μl of diluted and sterile-filtered (0.45um, Millipore) juice or extract was mixed with 100 μl cell suspension (105 cells/ml). Each test plate contained an uninoculated control, a positive growth control, a medium-free control and a drug sterile control. The uninoculated control was used as background for the spectrophotometric calibration for the determination of the antibacterial acitivity of fruit juices and extracts. The samples were tested by triplicate and the results were recorded after 48 h. C. jejuni was growing under CO_2 atmosphere.

RESULTS AND DISCUSSION

The tested strains were sensitive to most juices or extracts (Table 1). Mainly the anthocyanin-rich and acidic fruits had strong antibacterial activity. The dark coloured Ribes and Rubus berries had excellent antibacterial action. Raspberry, blackcurrant, redcurrant and jostaberry juice (pH 2.8 - 3.8) totally inhibited the growth of both bacteria, while the growth of C. jejuni was reduced but not stopped by gooseberry juice. Among the water extracts of berries, only blackberry pomace showed total inhibitory effect on both of bacteria while the other extracts exhibited medium or strong inhibitory effect while C. jejuni showed higher sensitivity than S. ser. Typhimurium. Methanol extracts had similar effect as water extracts.

Figure 1

Table 1: Antibacterial activity of juices, water extracts, and methanol extracts

	Bacteria					
	Campylobacter jejuni			*Salmonella* ser. Typhimurium		
Fruit (concentration, mg/ml)	juice	Water extract	MetOH extract	juice	Water extract	MetOH extract
	Growth					
Crataegus monogyna (n.d., 8.5, 6.8)	n.d.	1	1	n.d.	3	3
Morus alba (9.25, 4.0, 2.5)	4	2	1	4	4	4
Morus nigra (13.96, 7.1, 6.3)	4	2	1	4	2	4
Prunus armeniaca (5.3, 7.2, 4.2)	1	1	1	1	2	1
Prunus avium (14.2, 10.3, 6.6)	1	2	2	3	2	3
Prunus avium var. *Gold* (20.8, 4.9, 6.3)	1	1	2	4	4	4
Prunus cerasus (9.8, 8.4, 14)	1	1	1	1	2	1
Ribes x *nidigrolaria* (15.7, 8.1, 3.1)	0	2	2	0	1	1
Ribes nigrum (6.5, 6.3, 7.3)	0	0	4	0	0	1
Ribes rubrum (8.3, 4.9, 7.6)	0	1	1	0	1	1
Ribes uva-crispa (3.8, 7.2, 4.2)	1	2	1	0	1	1
Rubus fruticosus (3.2, 4.3, 5.6)	2	2	1	2	4	3
Rubus idaeus (9.1, 2.1, 2.8)	0	1	1	0	3	3

(0: no growth; 1: growth < 25%; 2: growth < 50%, 3: growth < 75%; 4: growth > 75%; untreated control is taken as 100%, n.d.=no data)

Antibacterial activity of berry juices and extracts from whole fruits has been intensively studied in the recent years but little attention was paid to the by-products of juice making. Inhibitory effect of raspberry juice was demonstrated against Escherichia coli, S. ser. Typhimurium and Staphylococcus epidermidis (Ryan et al. 2001; Lee et al. 2003). In the study of Cavanagh et al. (2003), blackberry juice had no growth inhibitory effect on Salmonella species (S. california, S. enteritidis, S. ser. Typhimurium) but strongly inhibited

Klebsiella pneumoniae. Our results show weak inhibition against S. ser. Typhimurium and stronger effect against C. jejuni. Puupponen-Pimiä and co-workers (2001) found that blackcurrant juices and extracts were more efficient against Gram-positive bacteria than against Gram-negative ones, which is in contrast to our results where both berry juice and aqueous extract of pomace caused total growth inhibition on both investigated Gram-negative species.

Fruits from the family Rosaceae (the two cherry cultivars, sour cherry, apricot and hawthorn) had also a strong inhibitory effect on C. jejuni but, except for sour cherry and apricot, had a week or no effect on S. ser. Typhimurium. Other authors also found good inhibition results with cherry (Lee et al. 2003), and apricot (Rashid et al. 2007).

Mulberry juices showed no effect on the growth of the bacteria but the pomace extracts inhibited the growth of C. jejuni. Water and ethanol extracts, or dark and white mulberry, had no difference in the inhibitory effect.

The inhibitory potential of fruit juices and pomace extracts seen at acidic pH was in most cases lost at pH 7. Water extracts from sour cherry and raspberry as well as methanol extracts from jostaberry, however, revealed a good inhibition effect with MIC values of 8.95, 1.82 and 7.37 mg/ml, respectively (Table 2). These inhibitions are thus independent of the low pH and could be attributed e.g. to anthocyanins and ellagitanins, as demonstrated in case of some other coloured fruits and vegetables (Harborne and Williams 2000; Lee et al. 2003).

Figure 2

Table 2: Minimal Inhibitory Concentration (MIC, mg/ml) of pomace water and methanol extracts effective at pH 7 (48 h incubation)

Fruit extract (pH 7.0)	Bacteria	
	C. jejuni	*S.* ser. Typhimurium
Cerasus vulgaris water extract	8.95	-
Ribes x *nidigrolaria* MetOH extract	-	7.37
Rubus idaeus water extract	1.82	-

The low pH of fruit juices is caused by weak organic and phenolic acids. In their undissociated forms (mainly at pH 3 to 5) they can interact with the cell membrane and penetrate into the cell causing the acidification of the cytoplasm. The effect of acidity can also be mediated by the pH-dependent dissociation of other antibacterial molecules and compounds (Burt 2004). The juices and extracts in our tests had a pH range 2.8-5.5 (Table 3), accompanied with the highest antibacterial activities. However, as shown by Table 2, some extracts had strong inhibitory effect on pH 7, too.

Figure 3

Table 3: pH of fruit juices and pomace extracts

Fruit	pH		
	juice	Water extract	MetOH extract
Crataegus monogyna	4.9	5.2	5.5
Morus alba	4.7	4.8	4.8
Morus nigra	5.5	5.6	5.6
Prunus armeniaca	3.3	4.1	3.8
Prunus avium	3.6	3.8	3.6
Prunus avium var. *Gold*	3.6	3.6	3.2
Prunus cerasus	3.3	3.8	4.1
Ribes x *nidigrolaria*	2.8	3.2	3.1
Ribes nigrum	2.8	3.0	3.0
Ribes rubrum	2.8	3.8	3.8
Ribes uva-crispa	3.1	3.2	3.3
Rubus fruticosus	3.3	3.3	3.8
Rubus idaeus	3.3	3.6	3.8

A number of factors can influence the antimicrobial activity of plant extracts, including growth conditions, seasonal variations and extraction methodology. The components dissolved in aqueous and alcoholic extraction are partly dissimilar. Water extract contains the majority of anthocyanins, tannins, starches, saponins, polypeptides and lectins present, while methanol extracts, in addition, polyphenols, lactones, flavones, and phenons (Cowan 1999). Ryan et al. (2001) described the different antibacterial activity of raspberry leaf aqueous and ethanol extracts. We observed similar phenomenon with water and methanol extracts of the pomace.

Gram-negative and Gram-positive organisms show different sensitivity to antibacterial agents because the former possess an outer membrane surrounding the cell wall (Ratledge and Wilkinson 1988) restricting the diffusion of hydrophobic compounds. 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. Extracts of common Finnish berries (including raspberry) were, on the contrary, more effective against Gram-negative compared to Gram-positive bacteria, except blackcurrant which was a poor inhibitor of Gram-negative bacteria in that work (Puupponen-Pimiä et al. 2001). Yet another study (Cavanagh et al. 2003) revealed no correlation between Gram-positive or Gram-negative bacterial status and susceptibility to the berries.

The fruits and berries investigated in our study were good growth inhibitors of both Gram-negative bacteria used in our study, but C. jejuni was more sensitive and was affected by more juices and extracts than S. ser. Typhimurium. It seems that the difference of inhibitory potential of fruit juices and their extracts on Gram-negative and -positive organisms is not unambiguous so that further investigations are necessary.

All the same, the observed inhibitory potential of the investigated fruit juices and pomace extracts on bacterial growth may be utilized in the development of functional foods and natural food preservatives. The by-products of juice industry may represent an economically interesting source for the extraction of active compounds.

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