



## Fruit phenolics as natural antimicrobial agents: Selective antimicrobial activity of catechin, chlorogenic acid and phloridzin

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Received 11 May 2007, accepted 27 July 2007.

### Abstract

Fruit phenolics have attracted a great interest recently as potential natural antimicrobial agents that could be used to extend the shelf life of value-added fruit and vegetable products. The antimicrobial activity of catechin, chlorogenic acid and phloridzin at 1, 5, 10 and 25 mM concentrations was assessed against three marker pathogenic bacteria, one probiotic bacterium, two yeasts and one food spoilage fungus using the turbidity assay. The growth of pathogenic bacteria, *Escherichia coli* O157:H7, *Listeria innocua* and the food spoilage fungus, *Penicillium chrysogenum*, were suppressed by all the phenolics at 25 mM but the growth of food spoilage yeast *Saccharomyces cerevisiae* was inhibited only by chlorogenic acid and phloridzin. Chlorogenic acid exhibited a greater inhibitory effect on opportunistic pathogen, *Candida albicans*, than that of catechin and phloridzin. The growth of probiotic bacterium, *Lactobacillus rhamnosus*, was not affected by the three phenolics at any tested concentrations except by 25 mM phloridzin.

**Key words:** Phenolics, natural antimicrobials, microorganisms, catechin, chlorogenic acid, phloridzin, value-added food.

### Introduction

The demand for value-added fruits and vegetables such as fresh-cut produce is steadily increasing. During storage, the safety and quality of un-pasteurized products of fruits and vegetables becomes questionable due to the growth of disease causing and spoilage microorganisms<sup>1</sup>. Therefore, maintaining the shelf life of value-added fruits and vegetables using preservatives has become significant. However, the use of synthetic chemical preservatives, especially in fruit and vegetable products, is a concern for consumers<sup>2</sup> and hence the identification of consumer acceptable natural antimicrobials has become valuable<sup>3</sup>. Plant derived natural antimicrobials such as polyphenols, terpenoids, essential oils, alkaloids, lectins and polypeptides have played a major role as food preservatives since prehistoric times<sup>4</sup>. Polyphenols are secondary metabolites produced in higher plants for plant defense, odor, flavor and pigments and are well known for their antioxidant and antimicrobial properties<sup>4</sup>. Polyphenols act on the microbes by disrupting their membranes, depriving the substrate or inactivating the enzymes<sup>5</sup>.

Fruit extracts and their phenolics have been shown to inhibit the growth of several Gram-positive and Gram-negative bacteria, i.e., *Aeromonas hydrophila*, *Bacillus subtilis*, *Enterobacter aerogenes*, *Enterococcus faecalis*, *Escherichia coli* O157:H7, *Klebsiella pneumoniae*, *Listeria monocytogenes*, *Salmonella enteritidis*, *Sal. typhimurium*, *Sal. enterica*, *Staphylococcus epidermidis*, *Staph. aureus* and *Vibrio parahaemolyticus*<sup>6-10</sup>. It has been estimated that 4 to 6 million kg of apple by-products (skins and cores) are generated as a result of processing apples for pies in Nova Scotia, Canada and these kinds of bio-resources could be used for extraction of fruit phenolics<sup>11</sup>. Catechin, chlorogenic acid and phloridzin are among the major phenolic

compounds in apples<sup>12</sup>. In general, apple phenolics are suggested to provide many health benefits including reduce risk of lung cancer, cardiovascular disease, asthma and Type II diabetes<sup>12</sup>. However, the antimicrobial properties of major phenolic compounds of apples especially phloridzin is limited. The specific objectives of this study were to (i) establish an appropriate solvent system for dissolving catechin, chlorogenic acid, and phloridzin to study their antimicrobial properties, and (ii) evaluate the antimicrobial activity of catechin, chlorogenic acid, and phloridzin, against selected pathogenic bacteria and yeasts, food spoilage fungi and yeasts, and probiotic bacteria.

### Materials and Methods

**Bacterial strains and other materials:** The microbial strains viz., *Escherichia coli* O157:H7 (ATCC 700728), *Listeria innocua* (ATCC 33090), *Enterobacter aerogenes* (ATCC 13048), *Candida albicans* (ATCC 10231), *Saccharomyces cerevisiae* (ATCC 9763), *Penicillium chrysogenum* (ATCC 9179) and *Lactobacillus rhamnosus* (ATCC 7469) were purchased from Microbiologics Inc. (St. Cloud, MN, USA) as lyophilized microorganisms and were stored at 4°C until the day of culturing them. Catechin, chlorogenic acid, phloridzin, dimethyl sulfoxide (DMSO) and N,N-dimethylformamide (DMF) were purchased from Sigma-Aldrich (Oakville, ON, Canada). Randomly methylated  $\beta$ -cyclodextrin (RMCD) was a product of Cyclodextrin Technologies Development (CTD) Inc. (High Springs, FL, USA).

**Selection of solvents for phenolic compounds:** A study was conducted using four solvents, DMSO, DMF, ethanol and RMCD at seven different concentrations (0.5, 1, 1.5, 2, 4, 8 and 16% v/v)

**Table 1.** Percentage inhibition values of different solvents against the pathogenic, probiotic and spoilage microorganisms<sup>A</sup>.

Solvent/ Microorganism	Concentration of the test solvents (%)						
	0.5	1	1.5	2	4	8	16
<b>DMSO</b>							
<i>E. coli</i> O157:H7	0.4 <sup>c</sup>	10 <sup>cd</sup>	4 <sup>de</sup>	13 <sup>c</sup>	14 <sup>c</sup>	69 <sup>b</sup>	99 <sup>a</sup>
<i>L. innocua</i>	2 <sup>b</sup>	1 <sup>b</sup>	7 <sup>b</sup>	9 <sup>b</sup>	25 <sup>b</sup>	78 <sup>a</sup>	100 <sup>a</sup>
<i>Ent. aerogenes</i>	0 <sup>c</sup>	0 <sup>c</sup>	0 <sup>c</sup>	0 <sup>c</sup>	0 <sup>c</sup>	7 <sup>b</sup>	99 <sup>a</sup>
<i>Lac. rhamnosus</i>	0 <sup>d</sup>	2 <sup>d</sup>	3 <sup>d</sup>	15 <sup>cd</sup>	21 <sup>c</sup>	61 <sup>b</sup>	97 <sup>a</sup>
<i>C. albicans</i>	23 <sup>d</sup>	24 <sup>d</sup>	22 <sup>d</sup>	25 <sup>d</sup>	37 <sup>c</sup>	72 <sup>b</sup>	100 <sup>a</sup>
<i>Sacc. cerevisiae</i>	9 <sup>b</sup>	9 <sup>b</sup>	7 <sup>b</sup>	3 <sup>b</sup>	4 <sup>b</sup>	96 <sup>a</sup>	100 <sup>a</sup>
<i>P. chrysogenum</i>	0 <sup>d</sup>	0 <sup>d</sup>	0 <sup>d</sup>	0 <sup>d</sup>	8 <sup>c</sup>	41 <sup>b</sup>	100 <sup>a</sup>
<b>DMF</b>							
<i>E. coli</i> O157:H7	1 <sup>b</sup>	2 <sup>b</sup>	5 <sup>b</sup>	5 <sup>b</sup>	6 <sup>b</sup>	100 <sup>a</sup>	100 <sup>a</sup>
<i>L. innocua</i>	6 <sup>d</sup>	4 <sup>d</sup>	7 <sup>d</sup>	25 <sup>c</sup>	40 <sup>b</sup>	100 <sup>a</sup>	100 <sup>a</sup>
<i>Ent. aerogenes</i>	0 <sup>c</sup>	0 <sup>c</sup>	0 <sup>c</sup>	2 <sup>d</sup>	12 <sup>c</sup>	93 <sup>b</sup>	100 <sup>a</sup>
<i>Lac. rhamnosus</i>	7 <sup>d</sup>	10 <sup>d</sup>	18 <sup>cd</sup>	24 <sup>c</sup>	67 <sup>b</sup>	93 <sup>a</sup>	100 <sup>a</sup>
<i>C. albicans</i>	11 <sup>d</sup>	17 <sup>cd</sup>	35 <sup>cd</sup>	41 <sup>c</sup>	72 <sup>b</sup>	100 <sup>a</sup>	100 <sup>a</sup>
<i>Sacc. cerevisiae</i>	4 <sup>b</sup>	5 <sup>b</sup>	0 <sup>b</sup>	2 <sup>b</sup>	96 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>
<i>P. chrysogenum</i>	17 <sup>cd</sup>	15 <sup>cd</sup>	20 <sup>cd</sup>	25 <sup>c</sup>	50 <sup>b</sup>	100 <sup>a</sup>	100 <sup>a</sup>
<b>Ethanol</b>							
<i>E. coli</i> O157:H7	0 <sup>c</sup>	0 <sup>c</sup>	0 <sup>c</sup>	0 <sup>c</sup>	11 <sup>b</sup>	99 <sup>a</sup>	99 <sup>a</sup>
<i>L. innocua</i>	3 <sup>c</sup>	7 <sup>c</sup>	8 <sup>c</sup>	17 <sup>c</sup>	69 <sup>b</sup>	100 <sup>a</sup>	100 <sup>a</sup>
<i>Ent. aerogenes</i>	0 <sup>c</sup>	0 <sup>c</sup>	0 <sup>c</sup>	0 <sup>c</sup>	23 <sup>b</sup>	99 <sup>a</sup>	99 <sup>a</sup>
<i>Lac. rhamnosus</i>	2 <sup>d</sup>	0.3 <sup>d</sup>	0 <sup>c</sup>	4 <sup>d</sup>	38 <sup>c</sup>	89 <sup>b</sup>	100 <sup>a</sup>
<i>C. albicans</i>	2 <sup>c</sup>	2 <sup>c</sup>	1 <sup>c</sup>	6 <sup>c</sup>	33 <sup>b</sup>	100 <sup>a</sup>	100 <sup>a</sup>
<i>Sacc. cerevisiae</i>	13 <sup>c</sup>	8 <sup>cd</sup>	1 <sup>cd</sup>	6 <sup>cd</sup>	1 <sup>d</sup>	65 <sup>b</sup>	100 <sup>a</sup>
<i>P. chrysogenum</i>	0 <sup>c</sup>	0 <sup>c</sup>	2 <sup>c</sup>	2 <sup>c</sup>	66 <sup>b</sup>	100 <sup>a</sup>	100 <sup>a</sup>
<b>RMCD</b>							
<i>E. coli</i> O157:H7	24 <sup>c</sup>	37 <sup>d</sup>	39 <sup>cd</sup>	40 <sup>cd</sup>	48 <sup>c</sup>	54 <sup>b</sup>	74 <sup>a</sup>
<i>L. innocua</i>	1 <sup>d</sup>	18 <sup>c</sup>	40 <sup>b</sup>	60 <sup>a</sup>	66 <sup>a</sup>	67 <sup>a</sup>	73 <sup>a</sup>
<i>Ent. aerogenes</i>	7 <sup>bc</sup>	6 <sup>bc</sup>	6 <sup>bc</sup>	3 <sup>c</sup>	3 <sup>bc</sup>	8 <sup>b</sup>	48 <sup>a</sup>
<i>Lac. rhamnosus</i>	0 <sup>c</sup>	0 <sup>c</sup>	0 <sup>c</sup>	67 <sup>b</sup>	82 <sup>ab</sup>	72 <sup>ab</sup>	95 <sup>a</sup>
<i>C. albicans</i>	0 <sup>c</sup>	0 <sup>c</sup>	5 <sup>ab</sup>	10 <sup>ab</sup>	11 <sup>ab</sup>	4 <sup>ab</sup>	27 <sup>a</sup>
<i>Sacc. cerevisiae</i>	13 <sup>b</sup>	19 <sup>ab</sup>	11 <sup>b</sup>	9 <sup>b</sup>	18 <sup>ab</sup>	35 <sup>a</sup>	24 <sup>ab</sup>
<i>P. chrysogenum</i>	29 <sup>bc</sup>	17 <sup>d</sup>	26 <sup>cd</sup>	30 <sup>bc</sup>	34 <sup>bc</sup>	36 <sup>b</sup>	89 <sup>a</sup>

<sup>A</sup>Mean values followed by different letters along the row are significantly different ( $\alpha = 0.05$ ).

**Table 2.** Solubility of the phenolic compounds in different solvents<sup>A</sup>.

Compound \ Solvent	Water	DMSO	DMF	Ethanol	RMCD
	Catechin	---	+++	+++	+++
Chlorogenic acid	+++	NA	NA	NA	NA
Phloridzin	---	+++	-	-	---

<sup>A</sup>Each compound was dissolved at 25 mM concentration in 4% (v/v) solvent in water. +++, highly soluble; ++, moderately soluble; +, slightly soluble; -, poorly soluble; ---, not soluble; NA, not applicable.

to identify the concentration of each solvent that does not suppress the growth of the tested microorganisms (Table 1). The solubility of each test compound in the solvents was also studied (Table 2). For all the tests against different microorganisms, chlorogenic acid was dissolved in water and then the respective

sterile warm growth medium was added (Table 3). Catechin and phloridzin were dissolved in selected concentrations of DMSO or ethanol as presented in Table 3. The medium was dispensed into 3 ml aliquots in sterile glass tubes and each concentration was maintained in triplicates.

**Inoculation and incubation:** The lyophilized microorganisms were purchased as Kwik-stik™ and the inocula were prepared according to the manufacturer's instructions (Microbiologics Inc., St. Cloud, MN, USA). Briefly, on the day of processing the culture, the cap of the Kwik-stik™ was pinched to allow the hydrating fluid to reach the lyophilized pellet. Then the pellet was crushed completely and the hydrated suspension was swabbed immediately as a small circle on an agar plate. Streaks were made starting from the circle using a sterile inoculation needle and then perpendicular streaks were made on the agar plate to facilitate isolation of colonies. The plates were incubated at 32-37°C for duration of 24 h for the aerobic bacterial and yeast cultures. *P. chrysogenum* was incubated for 5-7 days at 25°C. *Lac. rhamnosus* is anaerobic and hence the required conditions were created with the help of the anaerogen sachet (Oxoid, Basingstoke, Hampshire, England) placed inside an anaerobic glass jar (2.5 l) and then the jar was incubated at 37°C for 48-72 h. An anaerobic indicator (Oxoid, Basingstoke, Hampshire, England) was placed inside the jar along with the anaerogen to ensure that the anaerogen had increased the levels of carbon dioxide. The anaerobic indicator stays white under anaerobic conditions and turns pink under aerobic conditions. The isolated colonies were transferred to the respective slant cultures of growth medium and incubated at 25-37°C for 1-7 days. Then these were stored as stock cultures at 4°C. Nutrient agar and sheep blood agar (Becton Dickinson C., Le Pont de Claix, France) were used for culturing *E. coli* O157:H7 and *L. innocua*, respectively, whereas de Mane, Rogosa and Sharpe (MRS, Oxoid, Basingstoke, Hampshire, England) agar was used to culture *Lac. rhamnosus*. Tryptic soy agar (Becton Dickinson C., Le Pont de Claix, France) was used as the primary growth medium for the remaining cultures. Malt extract broth (MEB, Becton Dickinson C., Le Pont de Claix, France) was used as the growth medium for the two yeast strains, MRS broth for growing *Lac. rhamnosus*, and tryptic soy broth (TSB) was used for the remaining strains (Table 3).

**Antimicrobial assay:** For the turbidity assay, a loopful (~20 ml) of bacteria or yeasts was inoculated into 10 ml of sterile growth medium and incubated overnight at 32-37°C (Table 3). After incubation the bacterial or yeast inoculum was prepared to a concentration of approximately 8 log cfu/ml using McFarland's

**Table 3.** Growth conditions for the test microorganisms.

Microorganism	Nature of the microorganism	Incubation temperature °C	Growth medium <sup>A</sup>	Solvent used to dissolve		
				Catechin	Chlorogenic acid	Phloridzin
<b>Bacteria</b>						
<i>E. coli</i> O157:H7	pathogen	35	TSB	2% DMSO	water	4% DMSO
<i>L. innocua</i>	pathogen	32	TSB	2% DMSO	water	4% DMSO
<i>Ent. aerogenes</i>	pathogen	35	TSB	4% DMSO	water	4% DMSO
<i>Lac. rhamnosus</i>	probiotic	32	TSB	1.5% DMSO	water	2% DMSO
<b>Yeast</b>						
<i>C. albicans</i>	pathogen	32	MEB	2% ethanol	water	2% DMSO
<i>Sacc. cerevisiae</i>	spoilage	32	MEB	4% DMSO	water	4% DMSO
<b>Fungi</b>						
<i>P. chrysogenum</i>	spoilage	25	TSB	2% ethanol	water	4% DMSO

standard (Med-Ox Diagnostics Inc., Ottawa, ON, Canada), and 50 ml of the inoculum was used to inoculate each of the tubes containing the respective growth medium with four different concentrations (1, 5, 10 and 25 mM) of each test compound. The control tubes contained the corresponding solvent (in appropriate concentration) of the growth medium for all the test compounds (Table 3). The inoculated tubes with the aerobic bacteria were incubated overnight at 32-37°C with a gentle shaking at 180 rpm using a shaker-oven (Model Apollo HP 50, CLP Inc, San Diego, CA, USA) and the tubes with anaerobic bacteria were incubated anaerobically for 48-72 h at 37°C at 180 rpm.

The fungus, *P. chrysogenum*, was grown on Petri plates and when there was enough conidiogenesis, 0.1% (v/v) peptone water was poured over the thallus and scrapped gently with a sterile inoculation needle. It was allowed to stand for 10-15 min so that the mycelial bits stay at the bottom and the conidia alone were transferred to the tube. The conidial suspension was adjusted to McFarlands standard (8 log cfu/ml) and 60 µl of the inoculum was used to inoculate each of the tubes containing the TSB growth medium with four different concentrations of each test compound. The control tubes for each compound contained the equivalent amount of solvent in the growth medium. The tubes were incubated at 25°C for 36-48 h. The mycelium in the liquid culture medium was homogenized using a polytron homogenizer (Model PT 10/35, Brinkmann, Estbury, NY, USA) for 30 s and allowed to settle for 2-3 min. Then the absorbance was read at 600 nm using a spectrophotometer (Model DU®70, Beckman Instruments Inc., Fullerton, CA, USA) after adjusting the absorbance to the blank with the respective growth medium. The results are expressed as percentage inhibition of the microorganism with compared the respective control. The experiment with each microorganism was repeated independently two times.

**Statistical analysis:** One-way ANOVA was performed to the percentage inhibition values using SAS general linear model<sup>13</sup>. To obtain normality, square root or arcsine transformations were performed but the original data are presented in the tables. When there were significant differences at  $p < 0.05$ , the means were compared using Tukey's Honestly Significant Difference (HSD) test at  $\alpha = 0.05$ .

## Results

**Influence of solvents on microbial growth:** Seven different concentrations (0.5, 1, 1.5, 2, 4, 8 and 16%) of the solvents, DMSO, DMF, ethanol and RMCD, were evaluated for their influence on the growth of the test microorganisms *in vitro* (Table 1). A concentration dependent response was observed for the inhibition of the growth of all microorganisms by all four tested solvents. For example, 16% of DMSO, DMF and ethanol were completely inhibitory to all the tested microorganisms whereas the inhibitory effect of the same solvents at 1.5% ranged 0-24%. Inhibitory effect of DMF was relatively greater than that of DMSO and ethanol at corresponding higher concentrations (8 and 16%). In contrast to the above three solvents, RMCD did not inhibit any microorganism completely at any of the tested concentrations but its inhibitory effect on the growth of each microorganism was more pronounced at low concentrations of the solvent (0.5-2%) when compared with that of DMSO, DMF and ethanol. Present results showed that 2-4% of DMSO or ethanol as the most

appropriate concentrations of the respective two solvents did not inhibit the growth of the microorganisms considerably (Table 1) and facilitated the solubility of catechin and phloridzin completely (Table 2). Chlorogenic acid was dissolved in water. Based on the above results, appropriate solvent and concentration was assigned for dissolving the three phenolic compounds for assessing their antimicrobial activity against seven different microorganisms (Table 3).

**Antimicrobial activity of phenolic compounds:** The phenolic compounds, catechin, chlorogenic acid and phloridzin were tested for their antimicrobial activity against seven microorganisms using four different concentrations, 1, 5, 10 and 25 mM (Table 4). In general, a dose-dependent but selective response of the compounds was observed on the inhibition of growth of specific microorganism. It is interesting to note that none of the three phenolic compounds (1, 5, 10 or 25 mM) inhibited the growth of *Ent. aerogenes*. Catechin at 25 mM demonstrated a moderate inhibition of *E. coli* O157:H7 and *L. innocua* (39% and 65%, respectively) and did not inhibit *Ent. aerogenes*, *Lac. rhamnosus* and *Sacc. cerevisiae* at any of the tested concentrations. In comparison to catechin, chlorogenic acid at 25 mM concentration inhibited the growth of *E. coli* O157:H7, *L. innocua* and *Sacc. cerevisiae* by >90% with comparison to the control. However, chlorogenic acid did not inhibit *Ent. aerogenes* and *Lac. rhamnosus* at any of the tested concentrations (1, 5, 10 or 25 mM). Similar to catechin, the inhibitory effect of phloridzin on *E. coli* O157:H7 and *L. innocua* was moderate. Phloridzin at 5 mM and above concentrations inhibited *Sacc. cerevisiae* completely. Phloridzin was inhibitory to *Lac. rhamnosus* only at the 25 mM concentration. However, phloridzin did not have any inhibitory effect on *Ent. aerogenes* up to 25 mM concentration and its effect on other microorganisms was moderate (Table 4).

## Discussion

One of the key important factors in assessment of antimicrobial compounds *in vivo*, is the selection of the appropriate concentration of the specific solvent that is not inhibitory to the growth of the testing microorganisms. The literature shows that the reported antimicrobial activity of the same compound differs when different solvents were used to dissolve the same hydrophobic phenolic compound<sup>14,15</sup>. Among the most commonly used solvents to dissolve phenolic compounds are DMSO, ethanol<sup>14</sup>, methanol<sup>8,9</sup> and propylene glycol<sup>16</sup>. The solvent could be synergistic or antagonistic to the antimicrobial compounds and therefore use of appropriate controls equivalent to the concentration of the solvent is also important. In this study, we evaluated four selected solvents, DMSO, DMF, ethanol and RMCD, for their influence on the growth of seven microorganisms that were intended to use for screening three phenolic compounds. Based on this experiment, we were able to identify the appropriate concentration(s) of the most efficient solvent for dissolving the test compounds that also had a minimum inhibitory effect to the microorganisms. In general, 2-4% of DMSO or ethanol were the most appropriate for 1-25 mM catechin and phloridzin. RMCD is a recently introduced solubility enhancer of lipophilic compounds in aqueous solution<sup>17</sup> but has never been studied for the application in antimicrobial activity screening. Results indicate that RMCD is a better candidate than other solvents when a greater

**Table 4.** Percentage inhibition values of catechin, chlorogenic acid and phloridzin against the pathogenic, probiotic and spoilage microorganisms<sup>A</sup>.

Name of the compound/ Microorganism	Concentration of the test compounds (mM)			
	1	5	10	25
<b>Catechin</b>				
<i>E. coli</i> O157:H7	2 <sup>c</sup>	3 <sup>c</sup>	10 <sup>b</sup>	39 <sup>a</sup>
<i>L. innocua</i>	7 <sup>b</sup>	2 <sup>b</sup>	4 <sup>b</sup>	65 <sup>a</sup>
<i>Ent. aerogenes</i>	No inhibition	No inhibition	No inhibition	No inhibition
<i>Lac. rhamnosus</i>	No inhibition	No inhibition	No inhibition	No inhibition
<i>C. albicans</i>	3 <sup>a</sup>	4 <sup>a</sup>	6 <sup>a</sup>	5 <sup>a</sup>
<i>Sacc. cerevisiae</i>	No inhibition	No inhibition	No inhibition	No inhibition
<i>P. chrysogenum</i>	0 <sup>c</sup>	10 <sup>b</sup>	49 <sup>a</sup>	53 <sup>a</sup>
<b>Chlorogenic acid</b>				
<i>E. coli</i> O157:H7	3 <sup>c</sup>	10 <sup>b</sup>	94 <sup>a</sup>	90 <sup>a</sup>
<i>L. innocua</i>	1 <sup>c</sup>	63 <sup>b</sup>	88 <sup>a</sup>	95 <sup>a</sup>
<i>Ent. aerogenes</i>	No inhibition	No inhibition	No inhibition	No inhibition
<i>Lac. rhamnosus</i>	No inhibition	No inhibition	No inhibition	No inhibition
<i>C. albicans</i>	8 <sup>c</sup>	19 <sup>bc</sup>	27 <sup>b</sup>	41 <sup>a</sup>
<i>Sacc. cerevisiae</i>	1 <sup>c</sup>	2 <sup>c</sup>	43 <sup>b</sup>	92 <sup>a</sup>
<i>P. chrysogenum</i>	20 <sup>b</sup>	11 <sup>b</sup>	5 <sup>b</sup>	73 <sup>a</sup>
<b>Phloridzin</b>				
<i>E. coli</i> O157:H7	8 <sup>b</sup>	9 <sup>b</sup>	20 <sup>b</sup>	61 <sup>a</sup>
<i>L. innocua</i>	12 <sup>b</sup>	16 <sup>b</sup>	14 <sup>b</sup>	47 <sup>a</sup>
<i>Ent. aerogenes</i>	No inhibition	No inhibition	No inhibition	No inhibition
<i>Lac. rhamnosus</i>	No inhibition	No inhibition	No inhibition	52
<i>C. albicans</i>	0 <sup>c</sup>	0 <sup>c</sup>	2 <sup>b</sup>	13 <sup>a</sup>
<i>Sacc. cerevisiae</i>	12 <sup>b</sup>	99 <sup>a</sup>	99 <sup>a</sup>	99 <sup>a</sup>
<i>P. chrysogenum</i>	45 <sup>c</sup>	57 <sup>bc</sup>	62 <sup>ab</sup>	71 <sup>a</sup>

<sup>A</sup> Mean values followed by different letters along the row are significantly different ( $\alpha = 0.05$ ).

solvent concentration (8-16%) required for dissolving test compounds.

In this study the antimicrobial activity of selected apple phenolics was tested against seven different microbes including one probiotic bacterium. All three tested phenolic compounds, catechin, chlorogenic acid and phloridzin, suppressed the growth of *E. coli* O157:H7, a Gram-negative bacterium and *L. innocua*, a Gram-positive bacterium, the two pathogenic bacteria that are commonly associated with the food-borne disease outbreaks of fresh and minimally processed fresh produce<sup>18</sup>. In this study, *L. innocua* was considered as a substitute for *L. monocytogenes*<sup>19</sup> due to the concern of the possible risk of handling *L. monocytogenes*. However, none of the three compounds (up to 25 mM) has any inhibitory effect on the growth of *Ent. aerogenes*, another Gram-negative bacterium. Puupponen-Pimiä *et al.*<sup>8</sup> found that chlorogenic acid hindered the growth of *E. coli* CM 871. In contrast, Wen *et al.*<sup>15</sup> reported that chlorogenic acid (1%) did not reduce the growth of *L. monocytogenes*. In another study, (+)-catechin suppressed *E. coli* growth<sup>9</sup>.

Puupponen-Pimiä *et al.*<sup>8</sup> proposed that phenolics were inhibitory to Gram-positive bacteria and not to Gram-negative bacteria. Present results do not agree with this generalized statement because the tested phenolics in this study have inhibited *E. coli* O157:H7, a Gram-negative bacterium. The differences in the sensitivity of the bacteria against different phenolics could be attributed to the differences in their cell wall structure. Gram-negative bacteria have an outer membrane (OM), which acts as a molecular sieve and facilitates the passage of hydrophilic compounds and certain hydrophobic compounds through porins (proteins in the OM), whereas, Gram-positive bacteria lack the

outer membrane. The passage of the hydrophobic compounds through porins is also limited by the size of the penetrating solute<sup>20</sup>. Literature suggests that *Ent. aerogenes* is resistant to  $\beta$ -lactam antibiotics because its OM lacks porins or it possesses efflux mechanisms whereas the OmpF porin of *E. coli* translocates  $\beta$ -lactam antibiotics<sup>21-23</sup>. Hence, it could be proposed that OM of *E. coli* O157:H7 is permeable to the tested phenolics whereas the OM of *Ent. aerogenes* is not. Besides, phenolics exhibit their antimicrobial activity on Gram-positive bacteria by disrupting the ATP synthesis or by disintegrating the cell wall<sup>10</sup>.

Interestingly, the results from our findings revealed that catechin, chlorogenic acid or phloridzin did not inhibit the growth of the probiotic bacteria, *Lac. rhamnosus*, except 25 mM phloridzin inhibited the bacteria by 52%. Similarly, previous findings suggest that 17 phenolic compounds were not inhibitory to the probiotic strain, lactic acid bacteria<sup>8</sup>. *Lactobacillus* species are commonly used in probiotic products to achieve balanced intestinal microflora<sup>24,25</sup>. Given their protective nature on beneficial gut microorganism, the survival of these probiotics is encouraging to further studies toward the potential use of

these fruit phenolics as natural antimicrobial agents in value-added food.

The findings of this study indicated an inhibitory effect of the three phenolics on the pathogenic yeast strain, *C. albicans*, very low to marginal at the tested concentrations. However, chlorogenic acid and phloridzin suppressed the spoilage yeast, *Sacc. cerevisiae*, completely at 25 and 5 mM, respectively, but catechin did not inhibit its growth at any of the tested concentrations. It has been shown in another study that (+)-catechin did not inhibit the yeasts, *Sacc. cerevisiae* and *C. albicans*<sup>9</sup>. It is interesting to note from the present results that the spoilage fungus, *P. chrysogenum*, was inhibited by all the tested phenolics. Therefore, the results from our study are in agreement with others<sup>8,9,15</sup>, which suggest that different microorganism have selective sensitivity to different phenolic compounds at different concentrations.

## Conclusions

In conclusion, catechin, chlorogenic acid and phloridzin, three phenolic compounds that are abundant in apple processing by-products, exhibited varying degree of inhibitory action toward the growth of tested food pathogenic and spoilage bacteria, fungi and yeasts. However, it is important to note that these phenolics (except 25 mM phloridzin) did not inhibit the probiotic bacterium *Lac. rhamnosus* suggesting no or minimal threat to the beneficial colon microflora, if the phenolics are used as food additives at the desirable concentrations. Therefore, catechin, chlorogenic acid and phloridzin have a potential to be included as natural antimicrobial agents in value-added foods such as minimally processed fruits and vegetables, juices, jellies, jams, etc., provided

the compounds are tested for the organoleptic properties and safe levels of consumption. Future research should focus on the assessment of the combinations of these phenolics for their additive and synergistic antimicrobial activity and on testing different extracts from apple processing by-products for their antimicrobial properties.

### Acknowledgement

This research was funded by the Technology Development Program of the Nova Scotia Department of Agriculture, the Agri-Futures Nova Scotia Program of the Agriculture and Agri-Food Canada (AAFC), and Scotian Gold Cooperative Limited, Coldbrook, Nova Scotia.

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