Antimicrobial effect of blueberry (Vaccinium corymbosum L.) extracts against the growth of Listeria monocytogenes and Salmonella Enteritidis

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ABSTRACT

This study examined the antimicrobial effect of blueberry (Vaccinium corymbosum L.) extracts obtained from four cultivars (Elliott, Darrow, Bluecrop, and Duke) on the growth of Listeria monocytogenes and Salmonella Enteritidis. The minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of the extracts against L. monocytogenes and S. Enteritidis in tryptic soy broth were determined. Concentrations of total phenolic compounds and four individual phenolic (chlorogenic acid, ellagic acid, quercetin, and quercetin-3-galactoside) in the extracts were determined using Folin-Ciocalteau method and HPLC analysis, respectively. All four extracts at 112.5–900 mg/mL exhibited a dose-dependent growth-inhibitory effect against L. monocytogenes and S. Enteritidis. L. monocytogenes was significantly more sensitive to the antimicrobial effect of the extracts than S. Enteritidis. Phenolic compounds in the extracts such as chlorogenic acid, quercetin, ellagic acid, and quercetin-3-galactoside were the active antimicrobial compounds in the blueberry extracts. The results of this study suggest that blueberry extract or extract-derived components may be used to control pathogenic microorganisms. More studies on the use of blueberry as a natural antimicrobial in food products are warranted.

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1. Introduction

Chemical preservatives are commonly added to foods to enhance microbiological food safety. However, consumers are increasingly concerned about the potential harmful effects of chemical preservatives and prefer foods containing no chemical preservatives (Marta et al., 2012; Wu, Qiu, Bushway, & Harper, 2008).

Listeria monocytogenes is a foodborne pathogen that can cause listeriosis with symptoms such as nausea, vomiting, and diarrhea. The infection can lead to septicemia, meningitis, encephalitis, and intrauterine or cervical infections in pregnant women, which may result in abortion or still birth. L. monocytogenes infection is a significant risk to pregnant women, newborns, elderly, and immunocompromised individuals (Rocourt, Ben-Embarek, Toyoefuku, & Schlundt, 2003; Vazquez-Boland, Kuhn, Berche, & Chakraborty, 2001). In 1999, an outbreak of listeriosis resulted in 97 cases of illness, 6 miscarriage, and 14 deaths (Song & Yang, 2010). In the U.S., the average annual cases of listeriosis are about 2500 with mortality rates of 20–30% (Scallan et al., 2004). Salmonella Enteritidis is another common and important foodborne pathogen that can cause fever, diarrhea, vomiting, and abdominal cramps. The symptoms are usually mild, however, to small children, weakened, or elderly patients, the symptoms could lead to life-threatening complications. In the U.S., S. Enteritidis is the second most common serovar isolated from humans (Amanda, Wisner, & Wolfgang, 2011; Sikanch & Cherayil, 2007).

Berries are rich in phenolics and have been reported to protect against cancer and cardiovascular diseases (Almeida, Farah, Silva, Nunam, & Glória, 2006; Santos, Almeida, Lopes, & Souza, 2006; Serafini et al., 2009). Studies have been conducted to determine the content and antimicrobial activity of phenolic compounds in berries (Lacombe, Wu, Tyler, & Edwards, 2010; Scalbert, 1991). Wu et al. (2008) reported that cranberry extracts could break the outer
membrane of *L. monocytogenes* cells and lead to the death of cells. Among berries, blueberries (*Vaccinium* spp.) contain relatively high amounts of acids and phenolic compounds that display potential health benefits (Kalt et al., 2008; Szajdek & Borowska, 2008). Many studies reported that blueberries contain health-promoting compounds including phenolic acids and flavonoids, such as chlorogenic acid, ellagic acid, quercetin, and quercetin-3-galactoside (McDougall et al., 2005; Michelle, Anita, Tessa, & Elvira, 2011; Talcott & Lee, 2002). Blueberries have been commercially produced for many years in North America, and nowadays blueberries are cultivated worldwide. In China, about 10 blueberry cultivars are commercially planted and harvested. Favored by Chinese consumers for the fruits' good taste and perceived health benefits, blueberry production in China has increased significantly, and blueberry has become an important economic crop in China. Lacombe, Wu, White, Tedepaill, and Enroe (2012) reported a significant antimicrobial activity of North American lowbush blueberries against the growth of *E. coli* O157:H7 and identified the phenolic compounds in the extract as the main antimicrobial component and the possible mode of action. A thorough literature search showed that no previous study examined the antimicrobial activity of blueberries cultivars grown in China. Accordingly, the objectives of this study were to 1) study the antimicrobial activity of four blueberry cultivars widely grown in China against *L. monocytogenes* and *Salmonella Enteritidis* and 2) determine the contents of polyphenols in each blueberry cultivar. The identification of the antimicrobial activity of the blueberries and responsible active compounds is important for developing the use of blueberries as antimicrobial agents in foods and medicines.

2. Materials and methods

2.1. Bacteria strains

Two pathogens, *L. monocytogenes* (ATCC 19115) and *S. Enteritidis* (CMCC50041), were used. *L. monocytogenes* and *S. Enteritidis* were initially cultivated on PALCAM and DHL agar (Land Bridge Technology Co., Ltd., Beijing, China), respectively, at 37 °C for 24 h. Colonies were positively identified by specific microbial biochemical identification kit (Land Bridge Technology Co., Ltd.). Colonies were chosen and cultured in tryptic soy broth (TSB, 9 ml, Land Bridge Technology Co., Ltd.) at 37 °C for 24 h, and the cell suspensions were stored at −80 °C in TSB added with 30% glycerin (v/v). Throughout the experiment, the pathogen samples were subcultured every 4 weeks on tryptic soy agar and stored at 4 °C. A single colony was subcultured twice in fresh TSB and incubated at 37 °C for 24 h for use as inocula.

2.2. Preparation of blueberry extract

Four blueberry cultivars, Elliott (*Vaccinium elliottii*), Darrow (*Vaccinium darrowii*), Duke (*Vaccinium duke*), and Bluecrop (*Vaccinium bluecrop*), were purchased from Wolin Agriculture Co., Ltd. (Qingdao, China). The blueberries were kept at −20 °C and used for experiments within 6 months.

The extraction method was adapted from work by Yang, Jia, and Zu (2009). Blueberries of each cultivar were blended for 1 min at room temperature (about 25 °C) in a food processor (Model 8010S, R204B3, Senco Technology Ltd., Shanghai, China). The concentrated solution was added with 5 mL distilled water and the initial concentration was designated as 2 g/mL; 5 mL extract from 20 g blueberries in 10 mL final extract. The samples were kept at −4 °C.

2.3. Determination of antimicrobial activities of four blueberry extracts

The antimicrobial activities of four blueberry extracts were determined according to the methods described by the health industry standards of the People’s Republic China (Department of Health Policy and Regulation, 2005). Elliott was chosen to test the initial antimicrobial activity in the pre-experiment. The initial concentrations of Elliott were adjusted to 2, 1.8, and 1.6 g/mL, respectively. Then, Elliott solution was diluted to 1:2, 1:4, 1:8, and 1:32 in series with TSB. The result showed that these two bacteria could be inhibited at 0.45, 0.9, and 1 mg/mL. In the following study, the concentrations of blueberry extracts were adjusted from 2 g/mL to 1.8 g/mL with fresh TSB. Each blueberry extract was diluted to 1:2, 1:4, 1:8, 1:16 and 1:32 in series with TSB broth. The final concentrations of the extracts were 900, 450, 225, and 112.5 mg/mL, and 2 mL of each extract were placed into sterile test tubes for testing. The inocula of *L. monocytogenes* and *S. Enteritidis* (10 μL) were added into each tube to reach an initial inoculation level of approximately 5 log CFU/mL. The positive control was TSB inoculated with the test pathogens, and the negative control was TSB without inoculation. All extracts were at their native pH. The samples were incubated at 37 °C for 24 h, and the total viable counts (log CFU/mL) were determined at 0 and 24 h by a standard plating method using TSA plates.

2.4. Determination of the minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) of blueberry extracts

The MICs and MBCs of the blueberry extracts for *L. monocytogenes* and *S. Enteritidis* were determined by an agar dilution method as described by Alsterholm, Karami, and Faergemann (2010) with a modification of using broth instead of agar. MIC was the lowest concentration of extract that inhibited the visible growth of a microorganism after 24 h incubation, whereas MBCs was the lowest concentration that at least 99.9% of the cells in the initial inoculation were killed. Blueberry extracts were adjusted to 1.8, 1.5, 1.4, 1.2, and 1.1 g/mL with TSB. These dilutions of blueberries were further diluted to 1:2 and 1:4 in series with TSB. The final concentrations of the extracts were 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 1100, 1200, 1400, 1500, and 1800 mg/mL. *L. monocytogenes* or *S. Enteritidis* inoculum (10 μL) was added into the dilutions of blueberry extract (2 mL), respectively, to reach an initial inoculation level of approximately 5 log CFU/mL. All extracts were at their native pH. The inoculated tubes were incubated with shaking (180 rpm, ZWFR-21L shaker, Hengsheng Co., Ltd., Shanghai, China) at 37 °C for 24 h. The samples were 10-fold serially diluted with 0.85% saline, and the viable counts were counted on TSA to determine the MIC and MBC.

2.5. Determination of pH effect of four blueberry extracts against microorganisms

The native pH of four blueberry extracts at 450 and 900 mg/mL was determined using a pH meter (S20 SevenEasy, Mettler Co., Ltd., Shanghai, China). Among the four blueberry extracts, Elliott cultivar had the lowest pH value. Therefore, to study the pH effect, TSB solutions were added with citric acid (Sinopharm, Shanghai, China) to adjust the pHs to those of the Elliott extracts. The pHs of TSB solutions were adjusted to 3.4 (450 mg/mL extract) and 4.2 (900 mg/mL extract). The acidified TSB solutions were filtered and stored at 4 °C. *L. monocytogenes* or *S. Enteritidis* inoculum (10 μL)
was inoculated in the TSB to reach an initial inoculation level of approximately 5 log CFU/mL. The samples were kept at 37 °C. The counts of *L. monocytogenes* or *S. Enteritidis* were determined at 0 and 24 h using TSA plates.

2.6. Determination of total phenolic acids

The total phenolics of all four blueberries were measured by Folin–Ciocalteau method (Singleton & Rossi, 1965). Absorbance was observed at 765 nm using a UV-spectrophotometer (Model 722, Jingke Meter Company, Shanghai, China). Results were expressed as milligrams of gallic acid equivalents (GAE) per gram of fresh weight using a standard curve with gallic acid at concentrations of 1.25–25 mg/L.

2.7. Contents of four individual phenolic compounds by HPLC analysis

The contents of chlorogenic acid, ellagic acid, quercetin, and quercetin-3-galactoside in the blueberry extracts were analyzed by a Waters 2695 series HPLC system equipped with a diode array detector (Meadows Instrumentations, Bristol, WI, USA) (Rossi et al., 2003). The column, Zorbax-SB-C18 (Agilent Technologies Ltd., 4.6 × 250 mm i.d., particle size 5 μm) was held at 40 °C with a flow rate of 1.0 mL/min. The mobile phases were methanol (solvent A) and water: acetic acid at a 98:2 (v/v) ratio (solvent B). All reagents were HPLC grade. The gradient for separation was as follows: 0 min, 0–20% A and 0–80% B; 0–25 min, 20–70% A and 80–30% B; 25–30 min, 70% A and 30% B; 30–30.5 min, 70–20% A and 30–80% B; 30.5–35 min, 20% A and 80% B. The wavelength was set at 325 nm to detect phenolic compounds.

2.8. Determination of antimicrobial activity of four individual phenolic compounds

The results of HPLC analysis showed the blueberries were rich in chlorogenic acid and also contained ellagic acid, quercetin, and quercetin-3-galactoside. Four pure commercial-available chlorogenic acid, ellagic acid, quercetin, and quercetin-3-galactoside (Sigma Aldrich, Shanghai, China) were used to study their antimicrobial activities. The phenolic compounds at their initial concentration of 1 mg/mL were adjusted in 2 mL TSB to 420 μg/mL, 200 μg/mL, 44 μg/mL, and 8 μg/mL, which were within the concentration range of blueberry fruits. *L. monocytogenes* or *S. Enteritidis* inoculum (10 μL) was added into the TSB to reach an inoculation level at approximately 5 log CFU/mL. The samples were incubated at 37 °C for 24 h, and the cell counts were determined by plating method using TSA plates.

2.9. Statistical analysis

All experiments were repeated three times. Bacterial populations were reported as log CFU/mL. Analysis of variance (ANOVA) was performed on cell counts using the General Linear Models (GLM) procedure of SPSS software version 17.0. Differences among treatments were compared using Fisher’s least-significant-difference test. The level of significance for comparisons was set at *p* = 0.05.

3. Results

3.1. Determination of antimicrobial activities of blueberry extracts

Figs. 1 and 2 show the results of screening antimicrobial activity of the four blueberry extracts. In general, the extracts at 900 mg/mL showed significant antimicrobial activity against *L. monocytogenes* (Fig. 1). At 450 mg/mL, Elliott produced 100% reduction and Darrow produced 50% reduction in *L. monocytogenes* population after 24 h at 37 °C, however, Bluecrop and Duke showed no effect (Fig. 1). Fig. 2 shows the antimicrobial effect of the extracts on *S. Enteritidis*. Elliott or Darrow at 900 mg/mL reduced *S. Enteritidis* population to <1 CFU/mL and Elliott at 450 mg/mL reduced *S. Enteritidis* by 2.54 logs. Overall, the reduction of *L. monocytogenes* and *S. Enteritidis* made by the Elliott extract was the most significant (Figs. 1 and 2).

Table 1 shows the MICs and MBCs of the blueberry extracts for *L. monocytogenes* and *S. Enteritidis*. The MICs and MBCs of Elliott, Darrow, Bluecrop, and Duke extracts were lower for *L. monocytogenes* than for *S. Enteritidis*. At 300 mg/mL of Elliott, 4.68 log reduction was observed when compared with control at 24 h. Darrow at 350 mg/mL inactivated 5.1 log *L. monocytogenes*, and Bluecrop at 550 mg/mL and Duke at 750 mg/mL inactivated 5.44 log and 5.1 log *L. monocytogenes*, respectively (Fig. 3). At MICs, Elliott, Darrow, and Bluecrop showed significant log reductions of *S. Enteritidis* when compared with the control at 24 h. However, Duke was ineffective against *S. Enteritidis*, and no MIC was observed (Fig. 2d, Table 1).

3.2. The effect of pH on antimicrobial activity of blueberry extracts

Table 2 shows the pH values of TSB containing four blueberry extracts at 450 mg/mL and 900 mg/mL. Significant differences (*p* < 0.05) were observed in the pH values of TSB containing different concentrations of blueberry extracts (Table 2). The pH values of Elliott were the lowest among all extracts at concentrations of 450 and 900 mg/mL. Table 3 shows the survival counts of *L. monocytogenes* and *S. Enteritidis* treated with acidic solutions at pH levels similar to Elliott extracts at 450 mg/mL and 900 mg/mL. Elliott at 450 mg/mL reduced *L. monocytogenes* to below detection limit (1 log CFU/mL), but the acidic solution (pH 4.2) reduced only 2.4 log *L. monocytogenes* when compared with the control (Table 3). The antimicrobial effect of Elliott at 900 mg/mL was significantly better than that of acidic solution at the same pH level (pH 3.4). Elliott at 900 mg/mL inactivated *S. Enteritidis* below detection limit, but only 2.5 log *S. Enteritidis* were inactivated at concentration of 450 mg/mL. Acidic solution at pH 4.2 was ineffective in inhibiting the growth of *S. Enteritidis*. However, acidic solution at pH 3.4 showed growth-inhibitory effects toward *S. Enteritidis*. Elliott concentrations at 450 mg/mL and 900 mg/mL showed greater antimicrobial activities (*p* < 0.05) than those of acidic solutions at pH 4.2 and 3.4.

3.3. Content of total phenolics and four phenolic compounds of blueberry extracts

The phenolic contents of the extracts of the four blueberry cultivars are shown in Table 4. The phenolic contents among the four extracts were significantly different (*p* < 0.05). Elliott had the highest amount of total phenolic compounds with an average of 505 mg gallic acid equivalent (GAE) in 100 g fresh berry fruits. At 450 mg/mL, Elliott extract displayed the greatest growth inhibition on *L. monocytogenes* and *S. Enteritidis*. Phenolic acids in 100 g Darrow fresh berries were approximately 476 mg GAE. The inhibitory effect of Darrow on both pathogens was less than that of Elliott at same concentrations. Blueberry extracts demonstrated a significant dose-dependent inhibition upon the test pathogens. Duke had a higher content of total phenolic compounds than Bluecrop, however, its antimicrobial activity was weaker than Bluecrop (Table 4 and Figs. 1 and 2).

Previous studies showed that cultivars of blueberries contain several health-promoting compounds including phenolic acids and flavonoids, such as chlorogenic acid, ellagic acid, quercetin and quercetin-3-galactoside, (Johnson, Lucius, Meyer, & Gonzalez de Mejia, 2011; McDougall et al., 2005; Talcott & Lee, 2002).
Fig. 1. Screening the antimicrobial effect of four blueberry extracts against the growth of *L. monocytogenes* at 37 °C. a) Elliott, b) Darrow, c) Bluecrop, and d) Duke. Results represent average of three repeats. Data were expressed as means ± standard deviation. *: Represents a significant (*p* < 0.05) difference from the control at 24 h.

Fig. 2. Screening the antimicrobial effect of four blueberry extracts against the growth of *S. Enteritidis* at 37 °C. a) Elliott, b) Darrow, c) Bluecrop, and d) Duke. Results represent average of three repeats. Data were expressed as means ± standard deviation. *: Represents a significant (*p* < 0.05) difference from the control at 24 h.
Among the compounds, ellagic acid was most effective in inhibiting the growth of L. monocytogenes and S. Enteritidis. Though the total phenolic content in Bluecrop was higher in Bluecrop than that in Duke, the quantity of these four phenolic compounds in blueberries contributing to the antimicrobial activity were quercetin, ellagic acid, and quercetin-3-galactoside. The antimicrobial activities of the four commercial phenolic compounds are shown in Fig. 4. Chlorogenic acid at 200 μg/mL demonstrated no antimicrobial effect on L. monocytogenes and S. Enteritidis, however, it showed antimicrobial effect at 500 μg/mL (data not shown). Quercetin was ineffective at 8 μg/mL, but ellagic acid, quercetin, and quercetin-3-galactoside at 200 μg/mL showed significant (p < 0.05) inhibitory effect when compared to the control. Among the compounds, ellagic acid was most effective in inhibiting the growth of L. monocytogenes and S. Enteritidis at concentrations as low as 44 μg/mL (data not shown). The data confirmed that the tested phenolic compounds in blueberry contributing to the antimicrobial activity were quercetin, ellagic acid, and quercetin-3-galactoside.

### Table 1
Assessment of MICs and MBCs for four blueberries extracts. Results represent the average of three repeats.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Blueberry cultivar</th>
<th>MIC (mg/mL)</th>
<th>MBC (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. monocytogenes</td>
<td>Elliott</td>
<td>300</td>
<td>450</td>
</tr>
<tr>
<td></td>
<td>Darrow</td>
<td>350</td>
<td>500</td>
</tr>
<tr>
<td></td>
<td>Bluecrop</td>
<td>550</td>
<td>750</td>
</tr>
<tr>
<td></td>
<td>Duke</td>
<td>750</td>
<td>900</td>
</tr>
<tr>
<td>S. Enteritidis</td>
<td>Elliott</td>
<td>450</td>
<td>600</td>
</tr>
<tr>
<td></td>
<td>Darrow</td>
<td>600</td>
<td>800</td>
</tr>
<tr>
<td></td>
<td>Bluecrop</td>
<td>1200</td>
<td>1800</td>
</tr>
<tr>
<td></td>
<td>Duke</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

*Detection limit is <1 log CFU/mL.

Therefore, in the present study, these active compounds in blueberries potentially responsible for the antimicrobial activity against L. monocytogenes and S. Enteritidis were examined and their quantities were determined. The polyphenolic compositions in the four blueberry cultivars were determined by HPLC analysis (Table 4). The contents of phenolics were statistically different (p < 0.05) among the cultivars. Chlorogenic acid was higher in Elliott (0.42 mg/g) than in others, whereas Darrow had the highest amount of ellagic acid (0.044 mg/g). Quercetin-3-galactoside in Darrow was also the highest (0.2 mg/g). Elliott, Darrow, and Bluecrop showed growth inhibition toward L. monocytogenes and S. Enteritidis. Though the total phenolic content in Bluecrop was lower than that in Duke, the quantity of these four phenolic compounds was higher in Bluecrop.

3.4. The antimicrobial activities of four pure individual phenolic products

The antimicrobial activities of the four commercial phenolic compounds are shown in Fig. 4. Chlorogenic acid at 200 μg/mL demonstrated no antimicrobial effect on L. monocytogenes and S. Enteritidis, however, it showed antimicrobial effect at 500 μg/mL (data not shown). Quercetin was ineffective at 8 μg/mL, but ellagic acid, quercetin, and quercetin-3-galactoside at 200 μg/mL showed significant (p < 0.05) inhibitory effect when compared to the control. Among the compounds, ellagic acid was most effective in inhibiting the growth of L. monocytogenes and S. Enteritidis at concentrations as low as 44 μg/mL (data not shown). The data confirmed that the tested phenolic compounds in blueberry contributing to the antimicrobial activity were quercetin, ellagic acid, and quercetin-3-galactoside.

![Fig. 3](image-url) Viable cell counts (VCC) of L. monocytogenes and S. Enteritidis at MIC (mg/mL) at 0 and 24 h. Data were expressed as means ± standard deviation. #: Represents a significant (p < 0.05) difference from the control at 24 h.

### Table 2
Assessment of pH values of four blueberry extract in TSP. Results are the average of three trials measured at the start of the experiment. Means in the same column with different letters are significantly different (p < 0.05).

<table>
<thead>
<tr>
<th>Blueberry cultivar</th>
<th>Extract concentration (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>450</td>
</tr>
<tr>
<td>Elliott</td>
<td>4.20 ± 0.02a</td>
</tr>
<tr>
<td>Darrow</td>
<td>4.51 ± 0.1b</td>
</tr>
<tr>
<td>Bluecrop</td>
<td>5.02 ± 0.03c</td>
</tr>
<tr>
<td>Duke</td>
<td>5.82 ± 0.02d</td>
</tr>
</tbody>
</table>

4. Discussion

In this study, the antimicrobial capacity of four blueberry cultivars against Gram-negative S. Enteritidis and Gram-positive L. monocytogenes was determined. Both pathogens were inhibited by Elliott and Darrow extracts to non-detectable levels at 900 mg/mL in TSB. At the same concentration, Bluecrop and Duke extracts were effective in inhibiting growth of L. monocytogenes, but had weak effect against S. Enteritidis. TSB containing blueberry extract could be an adverse environment for foodborne pathogens due to the low pH and the presence of phenolic compounds. The results of acidic solution study indicated that at the same pH level, the antimicrobial effect of acidic solution was less than that of blueberry extracts. In addition, there were significant pathogen reductions at higher blueberry concentrations. The results implied that the phenolic compounds in blueberries might contribute to the antimicrobial effect. Many studies have focused on the contents of phenols in berries and their potential therapeutic effect and health promoting actions, such as reversal age-related declines in neuronal signal transduction as well as cognitive and motor deficits and the improvement of learning activity and memory (Andres-Lacueva et al., 2005; Lau, Shukitt-Hale, & Joseph, 2005; Molan et al., 2008). In addition, the phenolic compounds contained hydroxyl groups that showed phenolic toxicity, which was thought to be the main factor inhibiting the enzymatic activity in microbial cells (Mason & Wasserman, 1987). Phenolic acids, such as gallic and caffeic acids, displayed potential as metabolic inhibitors of proline in L. monocytogenes (Apostolidis, Kwon, & Shetty, 2008). Blueberries have one of the highest antioxidant activities of all fruits and vegetables and are excellent sources of phytochemicals (Lau, Joseph, McDonald, & Kalt, 2009; Stoner, 2009). Recent reports highlight the antimicrobial effects of phenolic compounds in blueberries (Lacombe et al., 2012; Puupponen-Pimiä et al., 2001). Proanthocyanidins of wild blueberries have been recognized for their anti-oxidation properties with particular emphasis on the prevention of microbial adhesion to gut epithelial tissue (Schmidt et al., 1998).

### Table 3
Viable cell counts of L. monocytogenes and S. Enteritidis in TSB of different treatments. Means (n = 3) in the same column with different letters are significantly different (p < 0.05).

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Viable cell counts (log CFU/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L. monocytogenes</td>
</tr>
<tr>
<td>0 h</td>
<td>8.6 ± 0.1a</td>
</tr>
<tr>
<td>24 h</td>
<td>8.6 ± 0.1a</td>
</tr>
<tr>
<td>0 mg/mL</td>
<td>0.1 ± 0.1</td>
</tr>
<tr>
<td>PH 4.2 acidic solution a</td>
<td>6.2 ± 0.1</td>
</tr>
<tr>
<td>450 mg/mL Elliott extract c</td>
<td>4.7 ± 0.1</td>
</tr>
<tr>
<td>PH 3.4 acidic solution a</td>
<td>2.3 ± 0.0d</td>
</tr>
</tbody>
</table>

*Acidic solutions of pH 3.4 and 4.2 in TSB have the same pH value as blueberry concentrate (highbush, Vaccinium elliottii) of 900 and 450 mg/mL in TSB, respectively.

b Detection limit is <1 log CFU/mL.
cultivars were inhibitory to the growth of L. monocytogenes and S. Enteritidis. Among the four cultivars, Elliott was most effective against L. monocytogenes and S. Enteritidis. The total phenolic content in Elliott was the highest among the four cultivars and was more than other Elliott cultivars planted in other countries (Dragovic-Uzelac, Savic, Brala, & Levaj, 2010; Michelle et al., 2011). Elliott also has higher content of quercetin and ellagic acid (Table 4). The result showed that ellagic acid, quecerstin, and quecerstin-3-galactose at 200 \( \mu \)g/mL were effective against pathogens. Partic-

ularly, ellagic acid showed excellent inhibition toward L. monocytogenes and S. Enteritidis. Among the four cultivars, Elliott was most effective against L. monocytogenes and S. Enteritidis. This study demonstrated that extracts from the four blueberry cultivars were inhibitory to the growth of L. monocytogenes and S. Enteritidis. Among the four cultivars, Elliott was most effective against L. monocytogenes and S. Enteritidis. The total phenolic content in Elliott was the highest among the four cultivars and was more than other Elliott cultivars planted in other countries (Dragovic-Uzelac, Savic, Brala, & Levaj, 2010; Michelle et al., 2011). Elliott also has higher content of quercetin and ellagic acid (Table 4). The antimicrobial activity of commercially sourced pure compounds was evaluated in order to further investigate the compounds which might contribute to the antimicrobial activity of blueberry. The result showed that ellagic acid, quecerstin, and quecerstin-3-galactose at 200 \( \mu \)g/mL were effective against pathogens. Partic-

ularly, ellagic acid showed excellent inhibition toward L. monocytogenes and S. Enteritidis. 99.9% were killed at 0.044 mg/mL (data not shown). Ellagic acid, quecerstin, and quecerstin-3-galactose in blueberries also contributed to the inhibitory effect against the pathogens. Many studies have reported the antimicrobial activity of ellagic acid, quecerstin-3-galactose, and quecerstin. Ellagic acid has been used to treat diseases in many countries, particularly those in Asia. Ellagic acid in Quercus infectoria Oliv. extract could destroy cell membrane and reduce the tolerance of bacteria to osmotic pressure that lead to cell death. Quecerstin had an anti-VacA effect and was demonstrated to have strong iron binding capabilities at pH 5 and pH 7.4 in phosphate buffer and can outcompete known Fe\(^{2+}\) chelators such as ferrizone that lead to cell breakage (Guo et al., 2007; Pastene et al., 2010). Rauha et al. (2000) reported that quecerstin from Finnish plant extracts could inhibit the growth of microorganisms. In addition, quecerstin-3-galactoside displayed growth inhibition toward Staphylococcus aureus and Escherichia coli (Yow, Tang, Chu, & Huang, 2012). While it was abundant in blueberries (Table 4), chlorogenic acid has little antimicrobial effect at 200 mg/mL. However, chlorogenic acid could inhibit pathogens at 500 \( \mu \)g/mL (data not shown). Studies have reported chlorogenic acid at higher concentrations could inhibit the growth of bacteria by interfering with the biofilm formation of bacteria (Fiamegos et al., 2011). In general, phenolic compounds in low pH condition could complex with proteins on the outer membrane of microorganisms through nonspecific forces, such as hydrogen bonding and hydrophobic effects, covalent bond formation (Haslam, 1996; Stern, Hagerman, Steinberg, & Mason, 1996), and changed Na\(^+\)/H\(^+\) antiporter systems to reduce the tolerance of bacteria to low osmotic environments. These interactions resulted in death of microorgan-isms. Gram-positive bacteria have thicker peptidoglycan cell walls, which serve to protect cells against hostile environments. In addition, Gram-negative bacteria like S. Enteritidis have efflux pumps, which are the first defense of bacteria, allowing them to selectively extrude specific phenolic compounds to protect them-

selves from the toxicity of phenolic compounds (Yow et al., 2012). However, L. monocytogenes was observed to be the most suscepti-

ble to blueberry extracts in the present study. It implied that blueberry phenolics could overcome membrane protection system and disrupt metabolism via changing in the fatty acid profile of L. monocytogenes as reported by Kleerebezem et al. (2010). Although the efflux pump of S. Enteritidis is an effective barrier helping it to adapt to and survive the harsh environment, blue-

berries still could significantly weaken it.

In conclusion, the present study is the first to show that extracts of four blueberry cultivars widely planted in China have significant antibacterial effects against L. monocytogenes and S. Enteritidis. Further studies are warranted to examine the applications of blueberries or their extracts in food products and medicines for food safety and health purposes.

Acknowledgment

This research was supported by the Maine Agricultural and Forest Experiment Station at the University of Maine with external

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Content ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elliott</td>
<td>5.05 ± 0.03a</td>
</tr>
<tr>
<td>Darrow</td>
<td>4.76 ± 0.01b</td>
</tr>
<tr>
<td>Bluecrop</td>
<td>3.10 ± 0.03c</td>
</tr>
<tr>
<td>Duke</td>
<td>4.30 ± 0.05d</td>
</tr>
</tbody>
</table>

Values in the same row with different letters are significantly different (\( p < 0.05 \)).

Table 4
Contents of total phenolic compounds and four polyphenolic compounds of four blueberry extracts. The experiments were repeated three times, and data are expressed as mean ± standard deviation. Means in the same row with different letters are significantly different (\( p < 0.05 \)).

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Content ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total phenolic content(^a)</td>
<td>5.05 ± 0.03a</td>
</tr>
<tr>
<td>Chlorogenic acid(^b)</td>
<td>0.42 ± 0.27a</td>
</tr>
<tr>
<td>Ellagic acid(^b)</td>
<td>0.037 ± 0.018a</td>
</tr>
<tr>
<td>Quercetin(^b)</td>
<td>0.007 ± 0.000a</td>
</tr>
<tr>
<td>Quercetin-3-galactoside(^b)</td>
<td>0.11 ± 0.014a</td>
</tr>
</tbody>
</table>

\(^a\) Total phenolic content in blueberries were determined with the Folin–Ciocalteau method (mg GAE/g fresh fruit weight).

\(^b\) Four polyphenolic compounds content in blueberries were determined with HPLC analysis (mg/g fresh fruit weight).

Fig. 4. Antimicrobial effect of four pure phenolic compounds (EA: ellagic acid, Q-3-G: quercetin-3-galactoside, CA: chlorogenic acid, and Q: quercetin) against L. monocytogenes and S. Enteritidis at 24 h. Data were expressed as means ± standard deviation. \( p < 0.05 \) compared with the control (24 h).
References


