

Antimicrobial Effects of the Combination of Cranberry and Wild Blueberry Extracts on Escherichia coli O157:H7, Listeria Monocytogenes, Salmonella typhimurium, and Staphylococcus aureus

Qiu X¹ and Wu VCH^{2*}

¹College of Biological Engineering, Jimei University, Yindou Road 43, Xiamen, 361021, China

²School of Food and Agriculture, Pathogenic Microbiology Laboratory, The University of Maine, 5395 Hitchner Hall, Orono, ME 04469-5735, USA

Abstract

Antimicrobial effects of the combination of cranberry and wild blueberry extracts on Escherichia coli O157:H7, Listeria monocytogenes, Salmonella typhimurium, and Staphylococcus aureus in Distilled Water (DW) and Brain Heart Infusion (BHI) were investigated. Results showed that pathogens were significantly inhibited. L. monocytogenes was the most sensitive pathogen to the DW treatment. The berry extract had stronger antimicrobial effects on E. coli O157:H7 and S. Typhimurium than on L. monocytogenes and S. aureus. For E. coli O157:H7, about a 7 log CFU/ml difference between the control and the treatments was observed on day 5 at 21°C. S. Typhimurium was reduced to non-detectable level by the powder blend on day 5. Approximately 3 to 4 log CFU/ml reduction by the berry concentrate combination or the powder blend was observed for L. monocytogenes and S. aureus on day 5. The combination of berry extracts may be considered for application in the food industry.

Keywords: Cranberries; Wild blueberries; Antimicrobial effects; Foodborne pathogens

***Corresponding Author:** Wu VCH, School of Food and Agriculture, Pathogenic Microbiology Laboratory, The University of Maine, 5395 Hitchner Hall, Orono, ME 04469-5735, USA, Tel: 1-207-5813101; Fax:1-207-5811636; E-mail: vivian.wu@umit.maine.edu

Introduction

Cranberries and blueberries belong to Vaccinium and are two important native fruits with significant commercial value in North America. They contain various phenolics and organic acids which have antimicrobial activities [1-4]. In addition to the antimicrobial effects, berries have long been known to have positive health effects. Cranberries were used by native New Englanders for treating wounds and blood poisoning, urinary

disorders, diarrhea, and diabetes [5]. Cranberries also can help passengers and the crew on long ocean voyages to relieve scurvy symptoms [5]. The consumption of cranberries may also help to lower incidences of urinary tract infections and inhibit peptic ulcer-associated bacterium, *Helicobacter pylori* [6]. Blueberries have a high antioxidant capacity and thus may have possible protective effects against human degenerative diseases [7]. Recent research found that phenolic compounds from blueberries can inhibit cancer cell proliferation [8,9].

Many chemical preservatives are used in the food industry to enhance food safety. However, consumers today are increasingly concerned about the residue of these chemicals in foods and tend to choose natural, healthful, and safe foods [10]. Natural ingredients made from cranberries or blueberries may be used to control the foodborne pathogens to replace traditional chemical preservatives.

Antibiotic resistance is another issue of concern to people. The mode of action of these naturally occurring compounds could be different from the chemical preservatives [1] thus hypothesized that these natural bioactive compounds may confer a broad spectrum of antimicrobial activity and potentially overcome the issue of antimicrobial resistance. This could be another advantage to use these bioactive berry compounds in the food industry. In addition, the combination of berry ingredients may also provide benefits of both cranberries and blueberries and may be more attractive to consumers.

The major pathogens that often cause diseases in the food industry include *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella*

typhimurium, and *Staphylococcus aureus*. *S. typhimurium* is a Gram negative, rod shaped, and nonsporeforming pathogen. It can cause fever, abdominal cramps, and diarrhea in humans. Poultry and swine are two important food vehicles for this pathogen. *E. coli* O157:H7 is an emerging foodborne pathogen and can cause hemorrhagic colitis and even Hemolytic Uremic Syndrome (HUS). Many kinds of foods may be associated with this pathogen like ground beef, spinach, unpasteurized apple juice, etc. *L. monocytogenes* can cause listeriosis especially for pregnant women, newborns, elderly and immune compromised people. *L. monocytogenes* can grow at as low as 3°C and thus may cause problems for refrigerated foods. Raw vegetables, meats, raw milk and many other foods have been contaminated with this pathogen. *S. aureus* can excrete an exotoxin and cause staphylococcal food poisoning, which is a very common foodborne disease. Food handlers are the main source of this pathogen. Foods that require much handling like salads often cause outbreaks [11].

The combination of berry extracts can offer more benefits to consumers' health than one berry extract only [12,13]. The objective of this study was thus to investigate the antimicrobial effects of the cranberry and wild blueberry concentrate combination as well as the powder blend on these four foodborne pathogens. It will provide important information for future application in the food industry.

Materials and Methods

Wild blueberry concentrate was from Maine Wild Blueberry Company (Machais, ME). Cranberry concentrate was from Ocean Spray cranberries, Inc (Rapids, WI). A cranberry and wild

blueberry powder combination was from Decas Botanical Synergies (Wareham, MA). After receipt, they were stored at 4°C in refrigerator. Tryptic Soy Agar (TSA; Difco) was used for enumerating all four pathogens. MacConkey Sorbitol Agar (MSA; Difco) with a cefixime Tellurite supplement (Dynal Inc. Lake Success, NY) was used for *E. coli* O157:H7. Xylose Lysine Desoxycholate agar (XLD; Difco) was used for *S. typhimurium*. Baird-Parker agar (BP; Difco) was used for *S. aureus*. Modified Oxford medium (MOX; Difco) for *L. monocytogenes*. Thin Agar Layer (TAL) plates were used to enumerate both injured and uninjured cells. TAL was made by overlaying TSA agar twice onto selective agar [14,15].

Microbial strains: Four pathogens used in this experiment were *E. coli* O157:H7 (ATCC 35150), *L. monocytogenes* (ATCC 49594), *S. Typhimurium* (ATCC 14028), and *S. aureus* (ATCC 25923). Cultures were checked for purity by Gram staining and commercial diagnostic kits. *E. coli* O157:H7 was tested using API 20E (bioMerieux, Hazelwood, MO) and RIM Latex agglutination test (Remel, Lenexa, KS). *S. typhimurium* was tested by API 20E. *L. monocytogenes* and *S. aureus* were confirmed by BBL Crystal Gram-Positive ID system (Becton Dickinson and Co. Sparks, MD).

Total phenolics analysis: Total phenolics analysis for wild blueberry concentrate was the same as cranberry concentrate. For the powder blend, approximately 0.2 g was weighed accurately and dissolved in distilled water and diluted to 100 ml. Then this solution was analyzed following the procedure by Waterhouse [16].

Total anthocyanin analysis: Wild blueberry concentrate was diluted 1:10 with distilled water.

Then 0.4 ml of this solution was transferred to cuvettes to analyze total anthocyanin following the previous procedure. Approximately 5 g powder blend was accurately weighed and dissolved in distilled water and diluted to 100 ml. Then it was analyzed by the pH differential method and results were reported in equivalents of cyanidin-3-glucoside [17].

Antimicrobial activity test: Each culture was grown in Brain Heart Infusion (BHI) broth for 18 to 24 hours. After incubation in 100 ml BHI for another 18 to 24 hours, cultures were then centrifuged for 20 min at 10,000 rpm (10080 x g). The supernatant was discarded, and the pellet was washed with 0.1% peptone water (Bacto, Sparks, MD) and suspended with 100 ml 0.1% peptone water. The individual culture was diluted twice with 0.1% peptone water. A cocktail (approximately 6 log CFU/ml) was made by combining four diluted individual cultures.

Sterilized distilled water (100ml) and BHI broth (100ml) were inoculated with the pathogen cocktail to achieve initial inoculum level of ca. 4 log CFU/ml. The inoculated samples were then mixed with cranberry and wild blueberry concentrate; powder blend to get 0% (control), 10% concentrate combination (v/v), and 10% powder blend (w/v). All these samples were kept at 21 °C and 7 °C. Pathogens were enumerated using the Spiral Biotech Plating System (Norwood, MA) after serial 10-fold dilutions with 0.1% peptone water. Plates were incubated at 37 °C for 24 hours. Pathogen counts were made for the DW at 0, 1, 5, 7, and 24 h and for the BHI on day 0, 1, 3, and 5. pH was measured at the starting time of the experiment. All experiments were individually repeated 3 times.

Statistical analysis: Bacterial numbers were reported as log CFU/ml. The experimental design was Randomized Complete Blocks (RCB). Data were analyzed by Analysis Of Variance (ANOVA) using SAS General Linear Models. Statistical Significance was defined as $P < 0.05$. Differences among treatments were examined for the level of significance by Tukey.

Results

Total phenolics content, total anthocyanin content, and Ph: Total phenolics content of wild blueberry concentrate was 38.6 g/L (gallic acid equivalents). Total phenolics content of the powder blend was 165.7 g/kg (gallic acid equivalents). Total anthocyanin content of wild blueberry concentrate was 346 mg/L (Cyanidin-3 Glucoside equivalents). Total anthocyanin content of the powder blend was 96 mg/kg (Cyanidin-3 Glucoside equivalents). The pH values of the samples were showed in (Table 1)

Antimicrobial effects in distilled water: E. coli O157:H7 did not decline significantly in general in distilled water compared to the control during 24 h storage (Figure 1). L. monocytogenes was the most sensitive among all these four pathogens in water. It reduced quickly and could not be detected from 5 h at both 21°C and 7°C (Figure 2). S. typhimurium and S. aureus were also significantly reduced by treatments. At both 21°C and 7°C, S. Typhimurium was significantly reduced compared with the control (Figure 3). At 21°C, no S. aureus was detected from both the powder blend and the concentrate combination after 24 h. The powder blend showed a less effective antimicrobial effect on S. aureus at 7°C than at 21°C (Figure 4). TSA agar was used to recover all possible pathogens that survived (Figure 5). The total

number did not drop significantly because of the survival of E. coli O157:H7.

Antimicrobial Effects in BHI: Both the powder blend and the concentrate combination showed significant antimicrobial effects on these four pathogens at 21°C and 7°C. E. coli O157:H7 grew to 9 log CFU/ml in the control sample at 21°C but it was significantly reduced in the treatment samples. At 7°C, E. coli O157:H7 was reduced to a non detectable level on day 5 (Figure 1). L. monocytogenes was significantly reduced by either concentrate combination or powder blend. L. monocytogenes grew from 3 log CFU/ml to 7.7 log CFU/ml in the control sample during 5 days storage at 7°C. In general, an approximately 3 to 4 log CFU/ml difference between the control and the treatments was observed on day 5 (Figure 2). Cranberry and wild blueberry concentrate combination and powder blend showed strong killing effects on S. typhimurium. S. typhimurium was not detected in the treatment samples. In the control samples, S. typhimurium grew to 8.9 log CFU/ml at 21°C on day 5. At 7°C, S. typhimurium was also reduced to a non-detectable level in the sample treated with the powder blend on day 5 (Figure 3). For S. aureus, the concentrate combination and the powder blend showed an inhibition effect at both 21°C and 7°C. S. aureus growth was inhibited during 5 days storage in the treatment samples while it increased 3.4 log CFU/ml approximately in the control samples (Figure 4). TSA was used to recover the total number of pathogens. At 21°C, pathogens grew from 5.1 log CFU/ml to 9.3 log CFU/ml during 5 days storage in the control samples. At 7°C, pathogens grew from 5.2 log CFU/ml to 7.9 log CUF/ml from day 0 to day 5 in the control samples. During the same period, both the concentrate

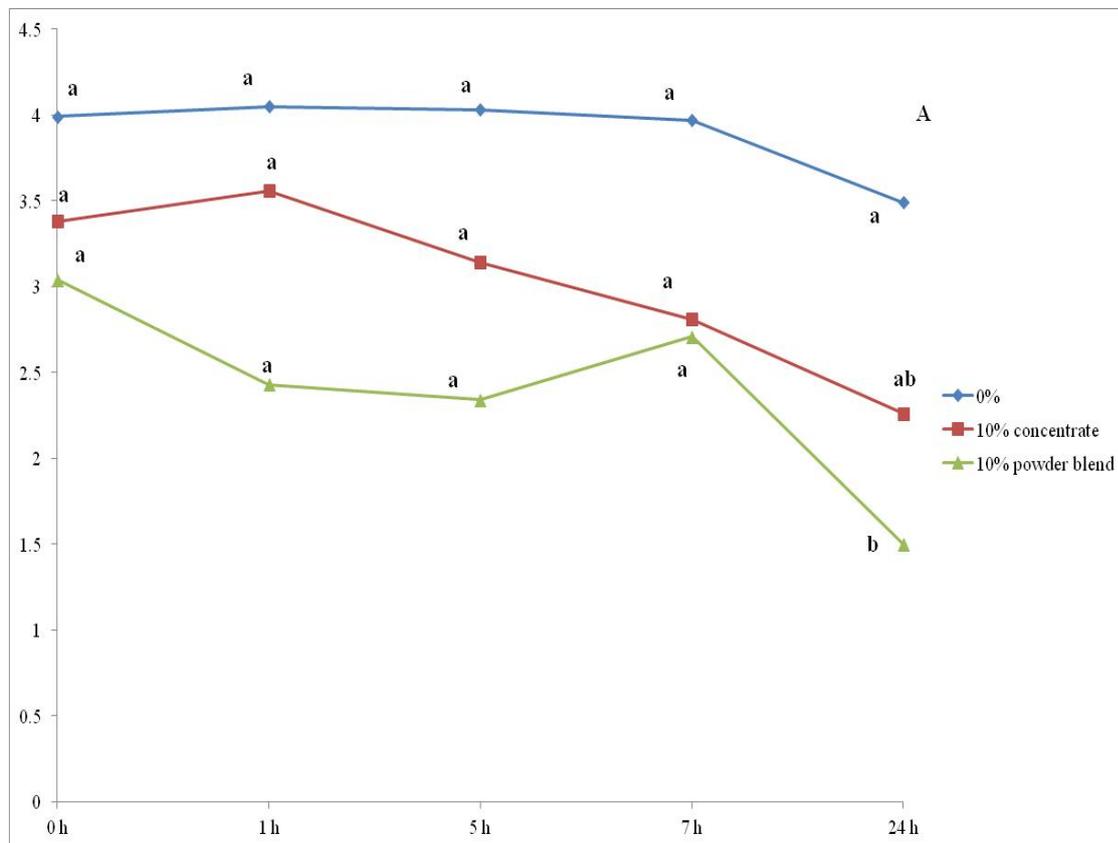
combination and the powder blend prevented pathogen growth and caused a significant reduction at both 21°C and 7°C (Figure 5).

Table 1: pH of the media with the berry concentrate and powder blend treatments

Concentrations of the powder or concentrate in the media (v/v)
Medium 0% 10% powder blend 10% concentrate combination
DW 5.9*a 4.0b 3.1c
BHI 7.4a 4.4b 3.8c

*Values were means of three replicates and measured at the starting time of the experiment. Means with different letters (a, b, c) in the same row indicate significant differences.

Figure 1



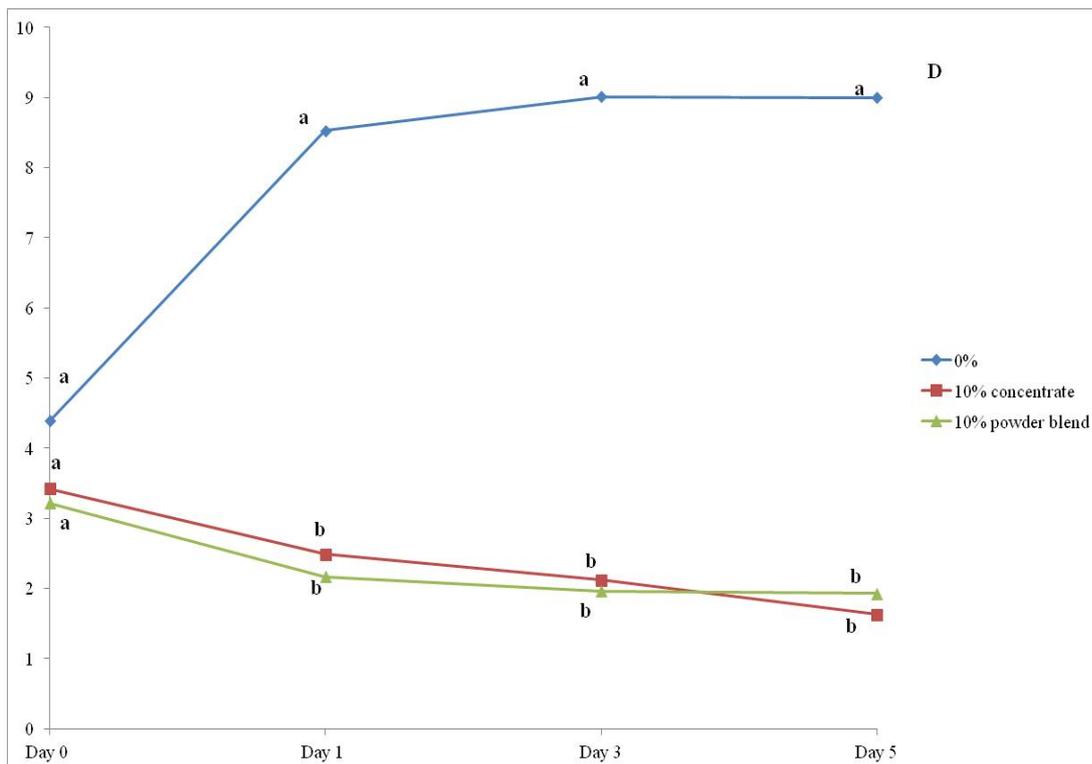
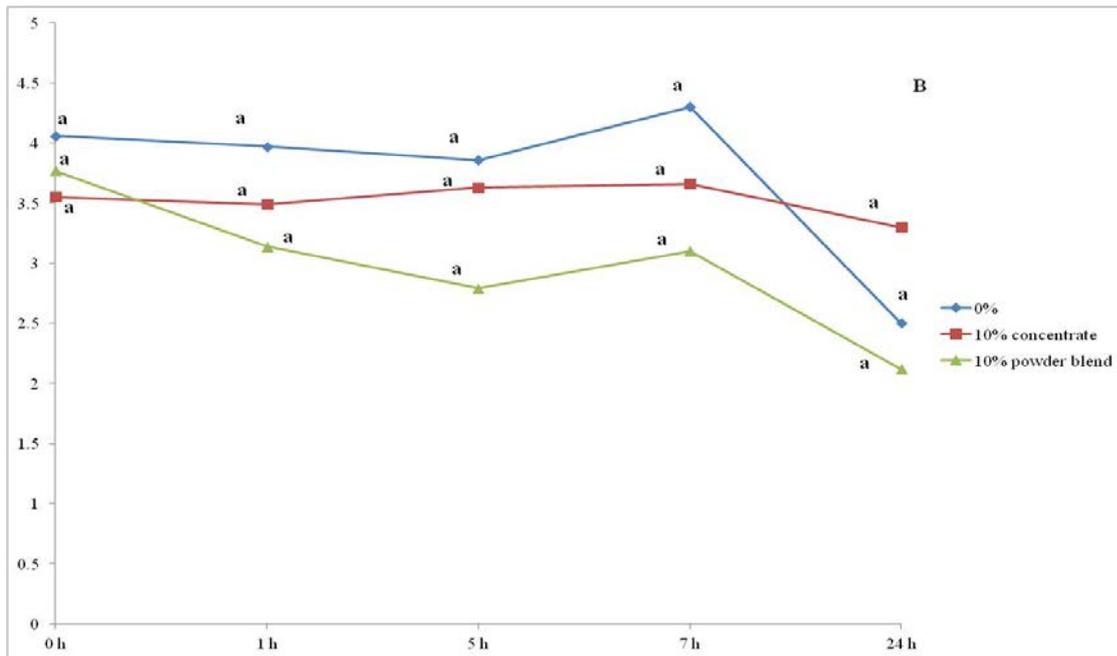
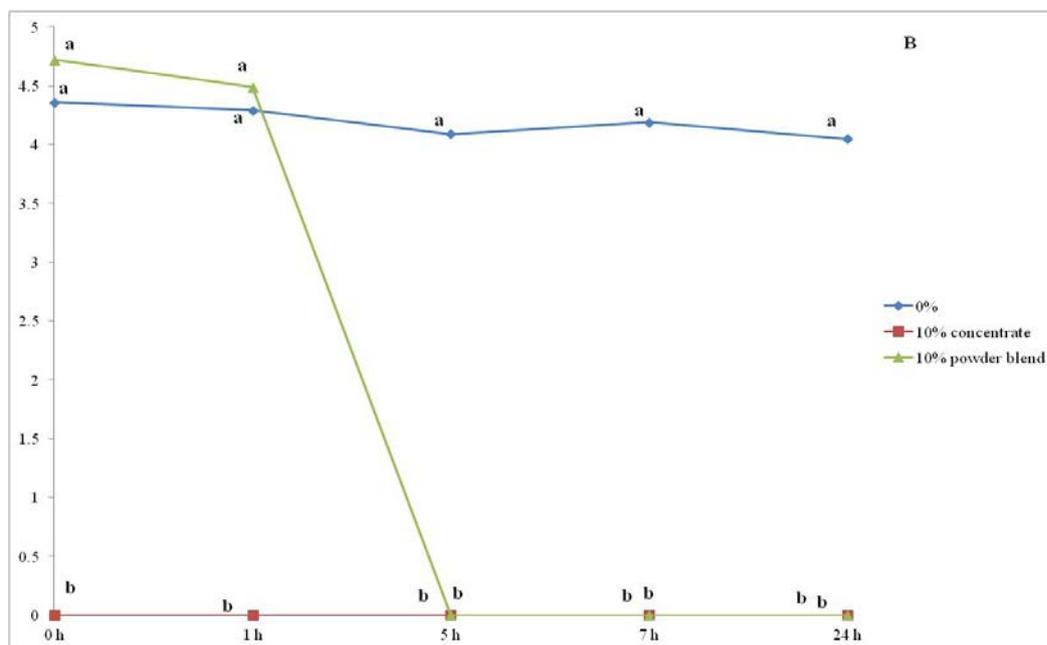
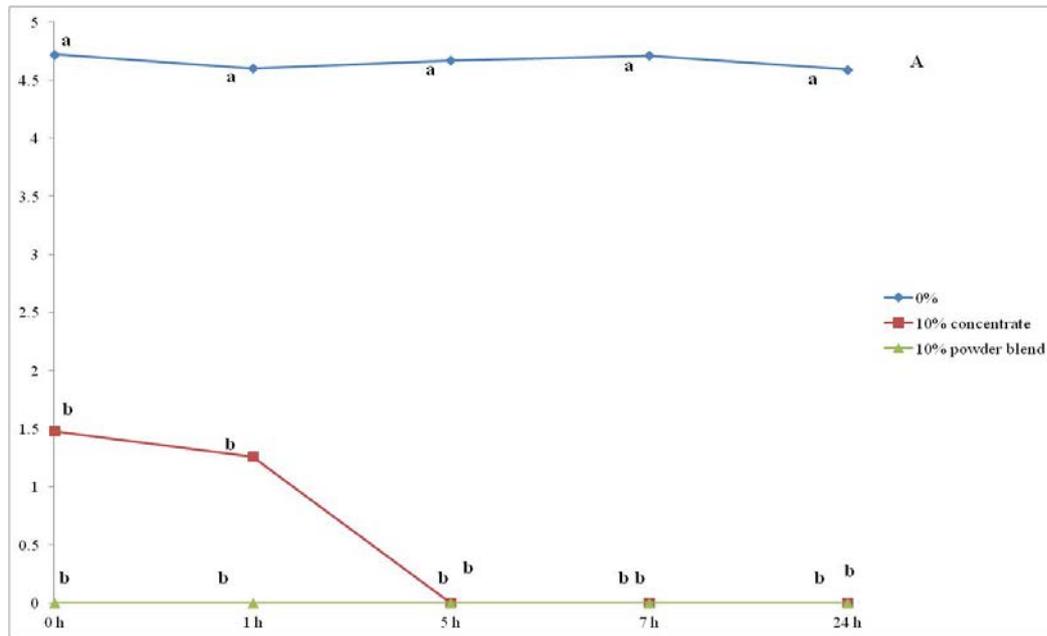


Figure 1: Antimicrobial effects of cranberry and wild blueberry concentrate combination and powder blend on Escherichia coli O157:H7 in sterilized distilled water at 7°C (1A) and 21°C (1B) for 0, 1, 5, 7, and 24 hours and in BHI at 7°C (1C) and 21°C (1D) on day 0, 1, 3, and 5. Means with different letters were significantly different ($P < 0.05$) for the same experimental sampling time. Y-axis is viable cell counts log CFU/ml and X-axis is treatment time.

Figure 2



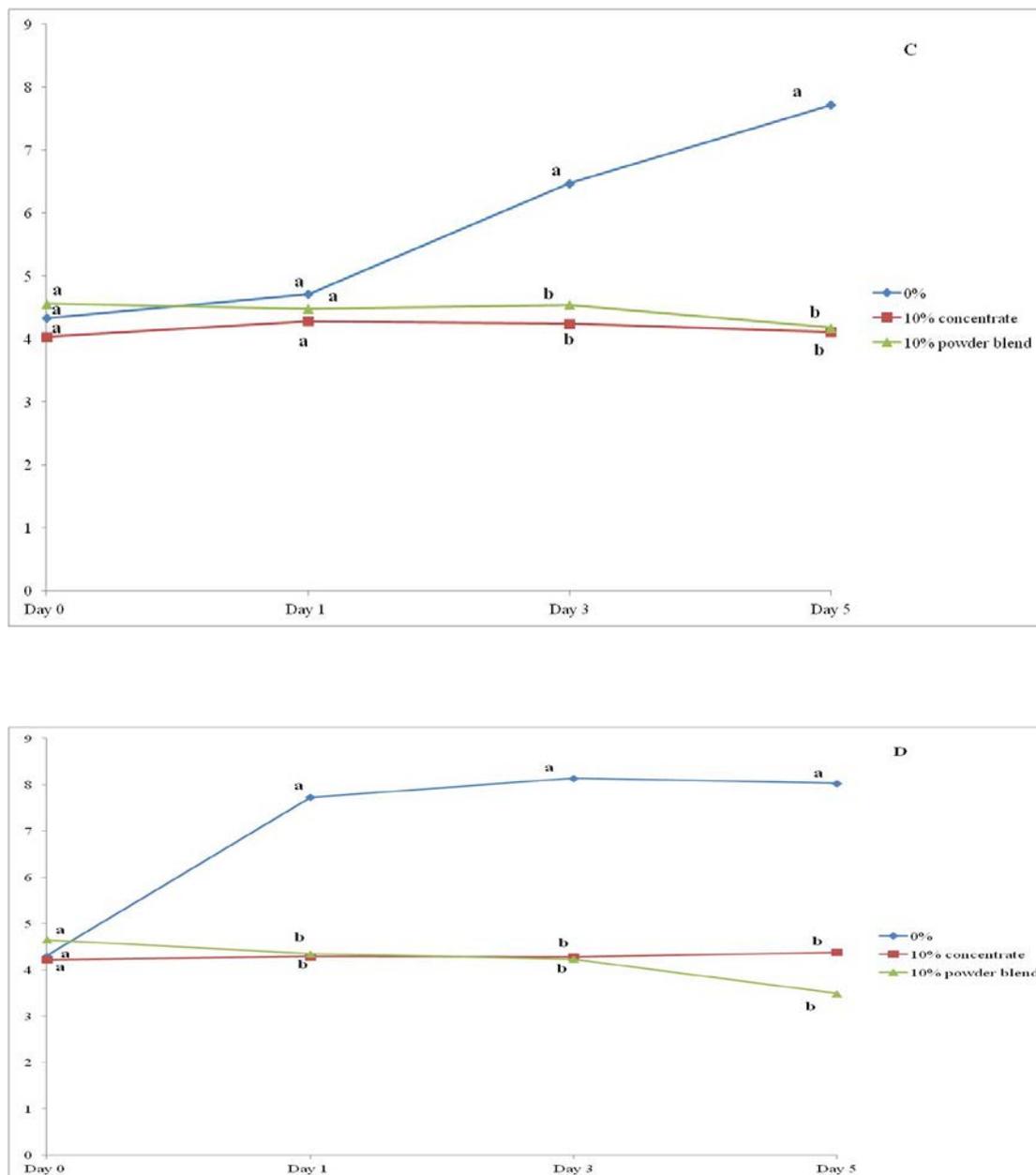
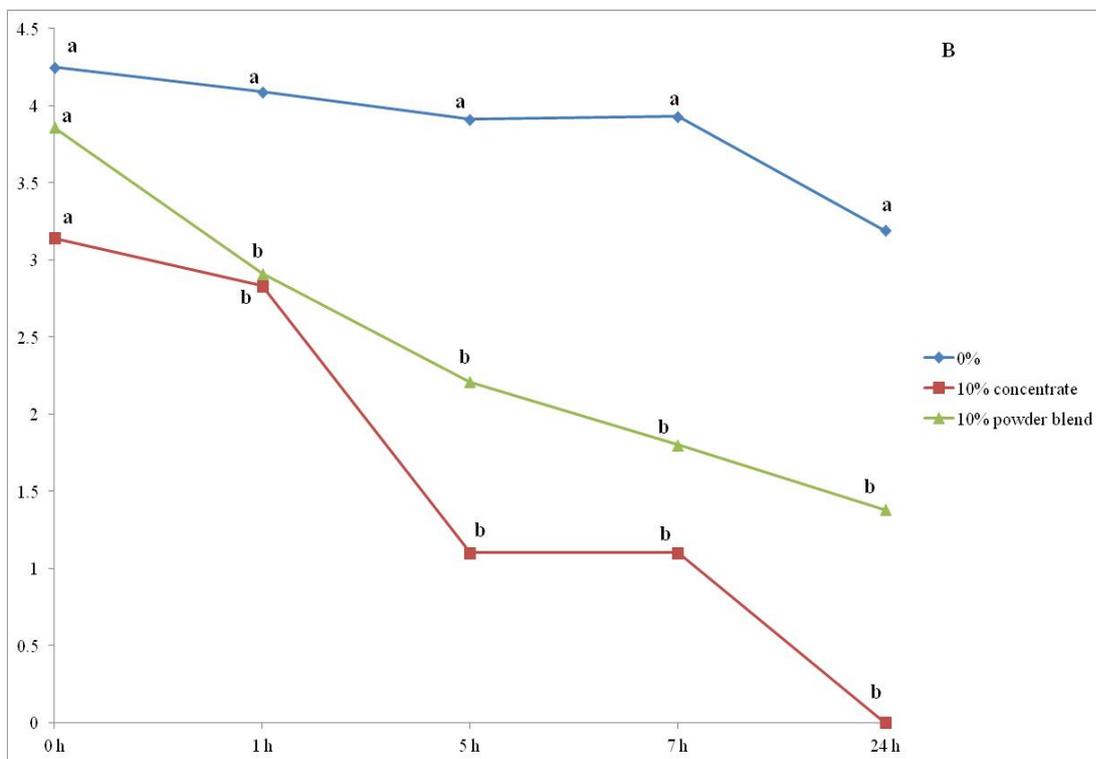
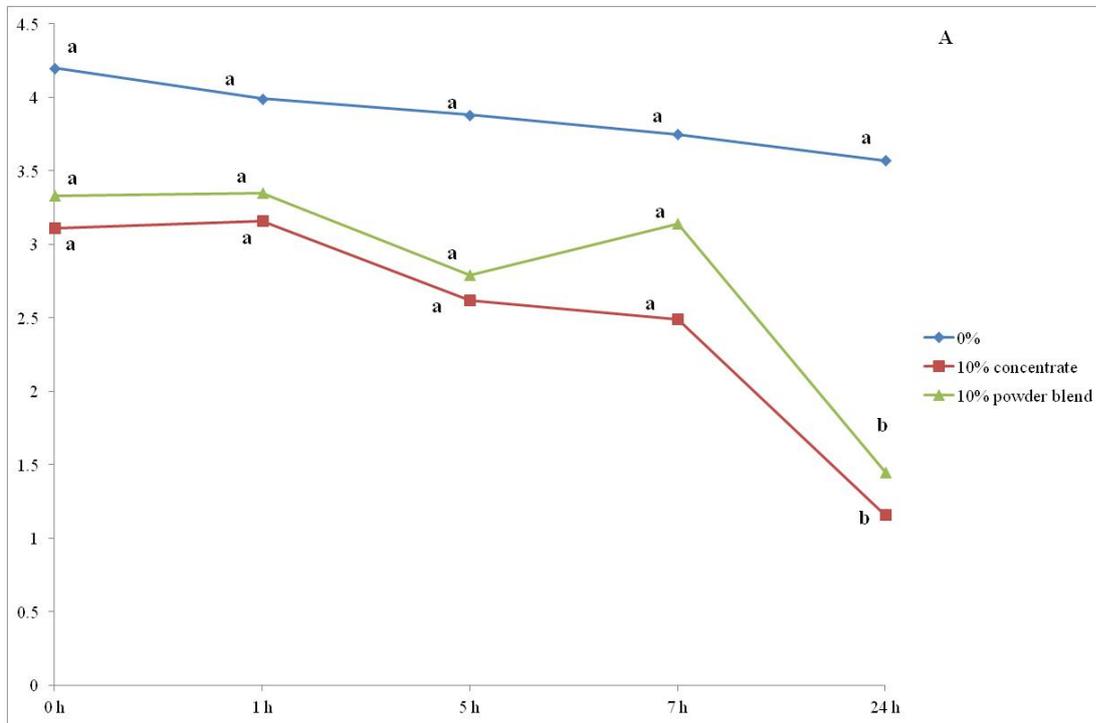


Figure 2: Antimicrobial effects of cranberry and wild blueberry concentrate combination and powder blend on *Listeria monocytogenes* in sterilized distilled water at 7°C (2A) and 21°C (2B) for 0, 1, 5, 7, and 24 hours and in BHI at 7°C (2C) and 21°C (2D) on day 0, 1, 3, and 5. Means with different letters were significantly different ($P < 0.05$) for the same experimental sampling time. Y-axis is viable cell counts log CFU/ml and X-axis is treatment time.

Figure 3



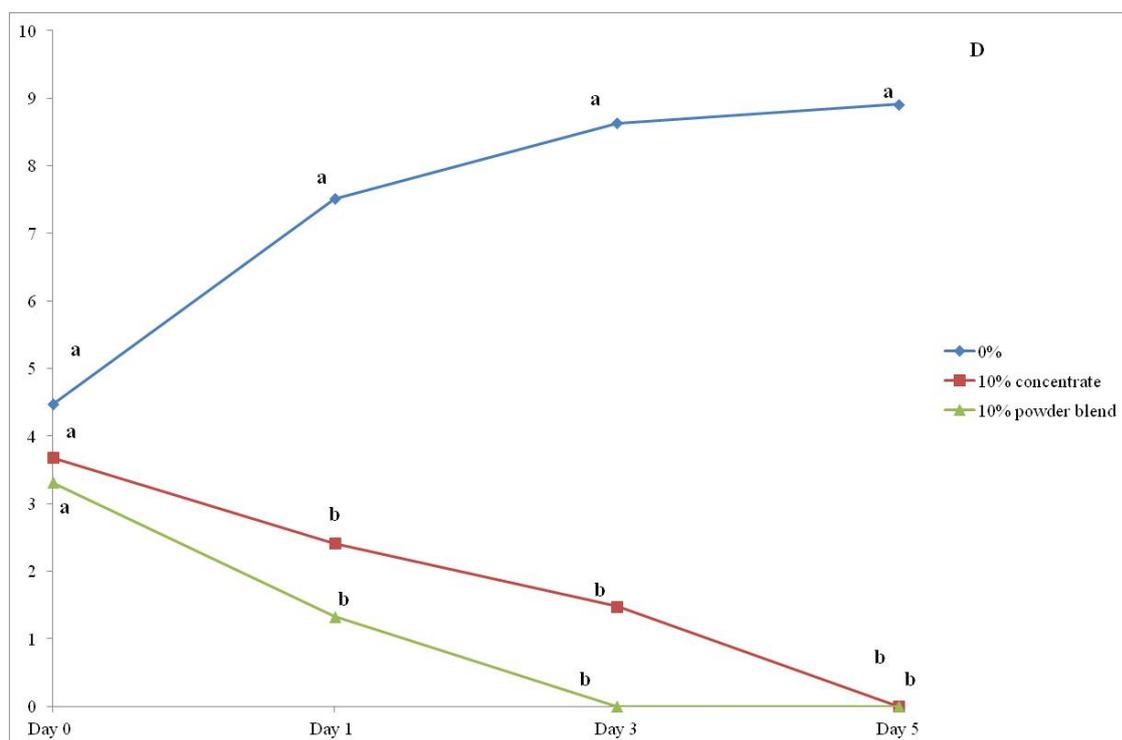
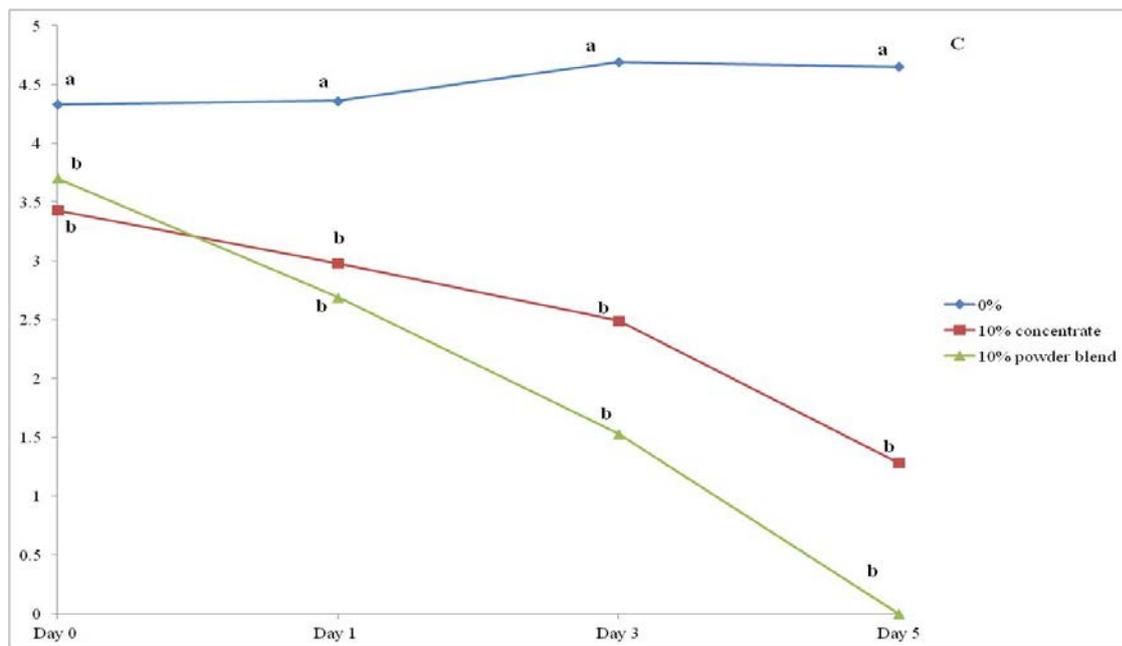
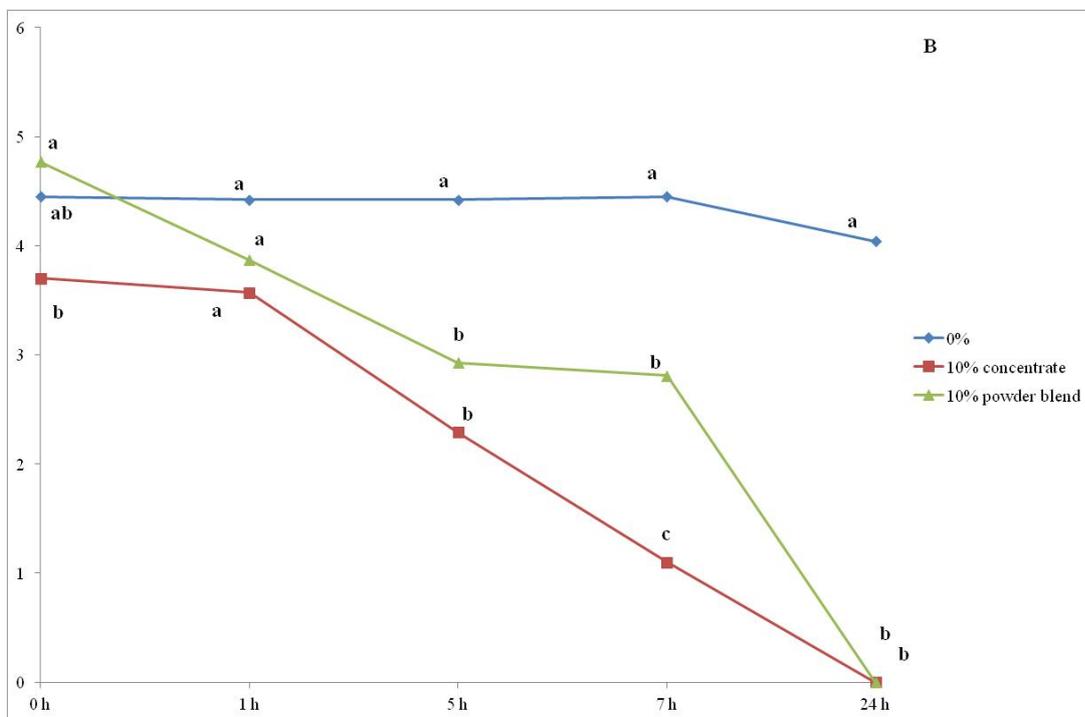
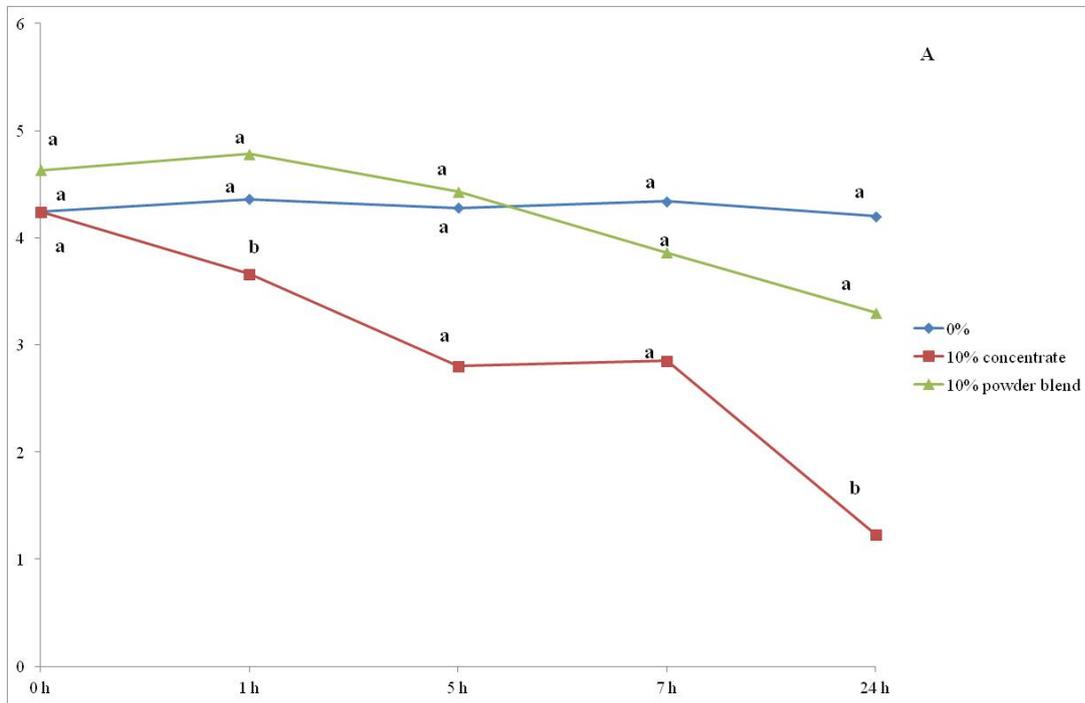


Figure 3: Antimicrobial effects of cranberry and wild blueberry concentrate combination and powder blend on Salmonella Typhimurium in sterilized distilled water at 7°C (3A) and 21°C (3B) for 0, 1, 5, 7, and 24 hours and in BHI at 7°C (3C) and 21°C (3D) on day 0, 1, 3, and 5. Means with different letters were significantly different (P < 0.05) for the same experimental sampling time. Y-axis is viable cell counts log CFU/ml and X-axis is treatment time.

Figure 4



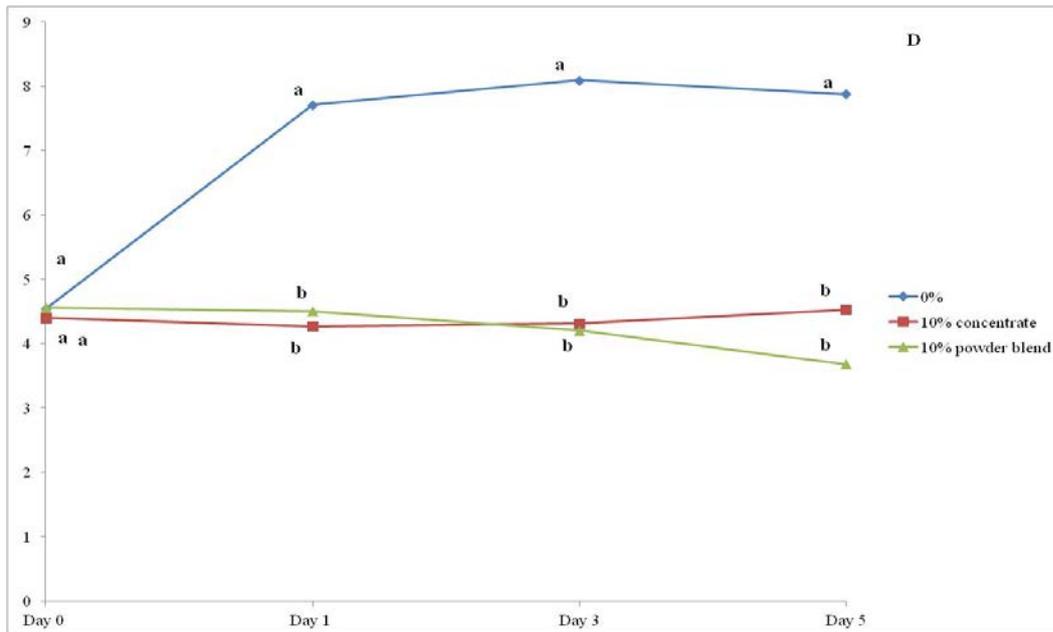
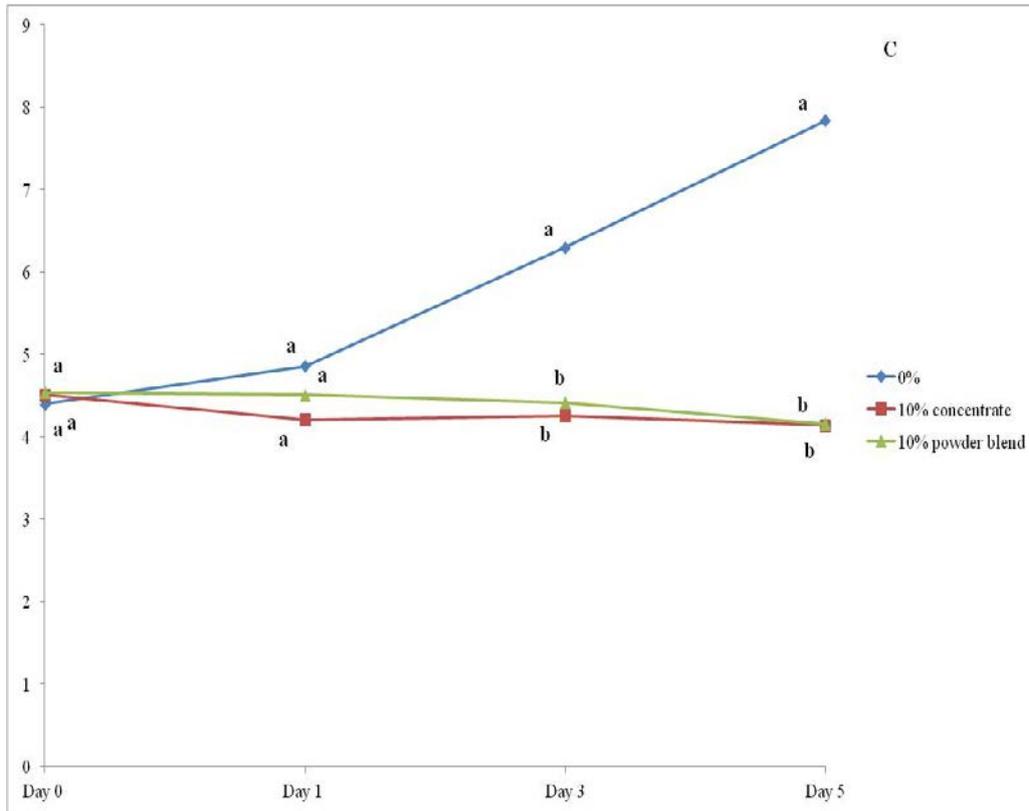
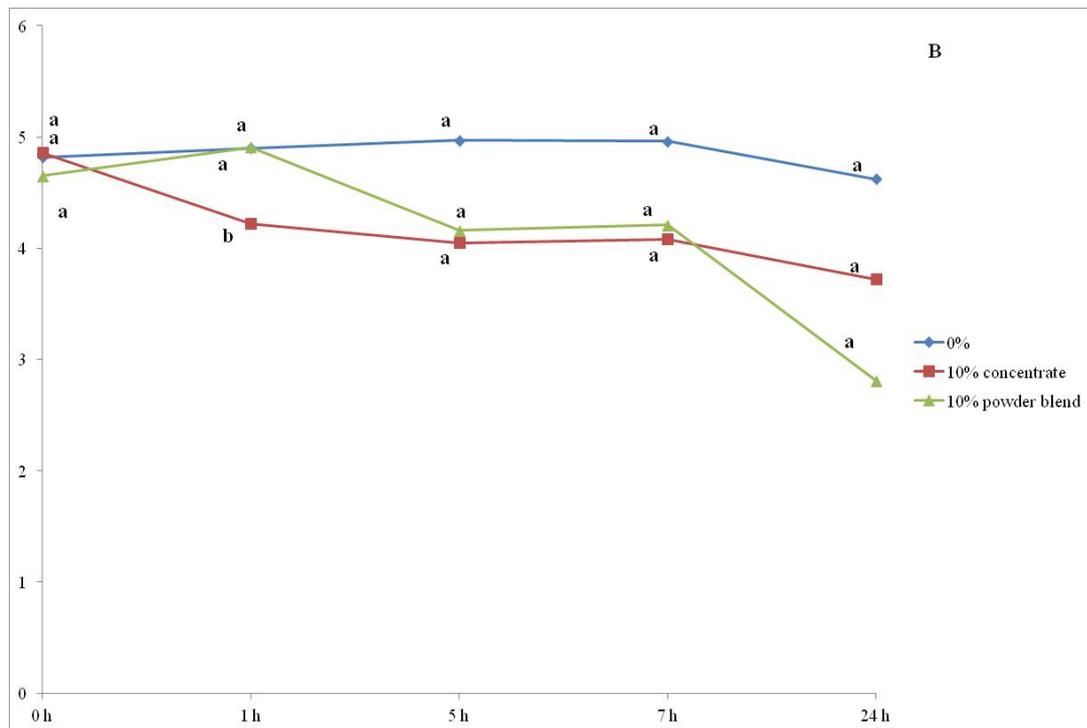
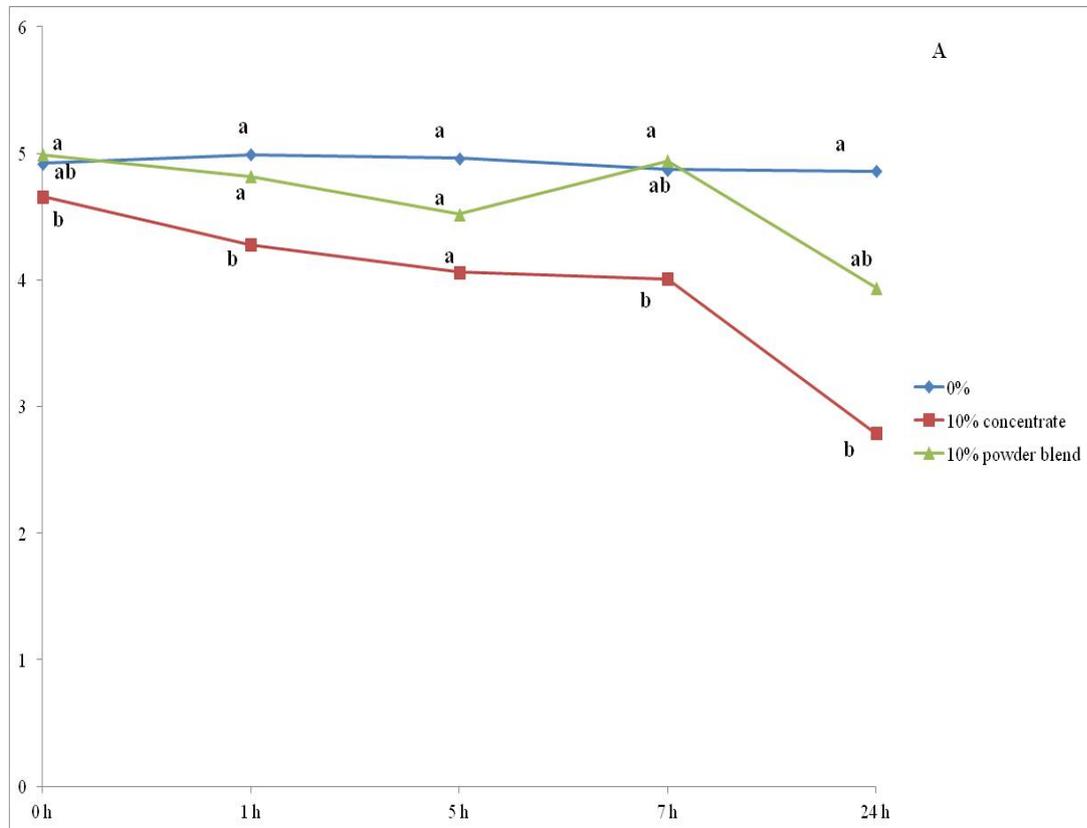


Figure 4: Antimicrobial effects of cranberry and wild blueberry concentrate combination and powder blend on *Staphylococcus aureus* in sterilized distilled water at 7°C (4A) and 21°C (4B) for 0, 1, 5, 7, and 24 hours and in BHI at 7°C (4C) and 21°C (4D) on day 0, 1, 3, and 5. Means with different letters were significantly different ($P < 0.05$) for the same experimental sampling time. Y-axis is viable cell counts log CFU/ml and X-axis is treatment time.

Figure 5



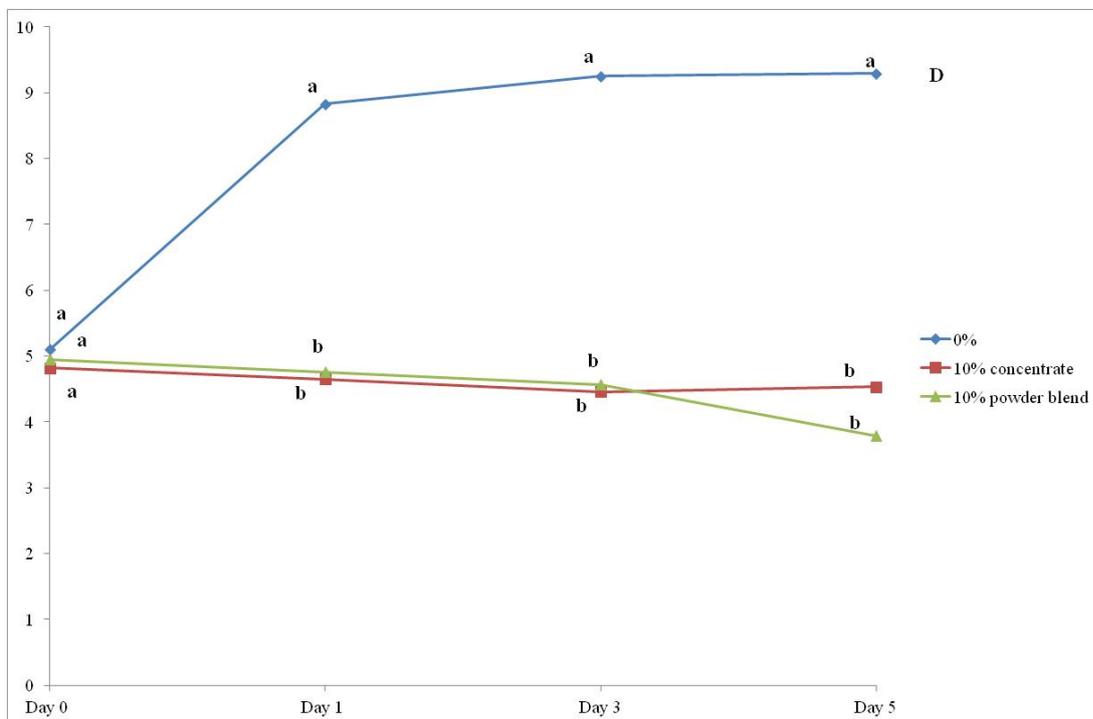
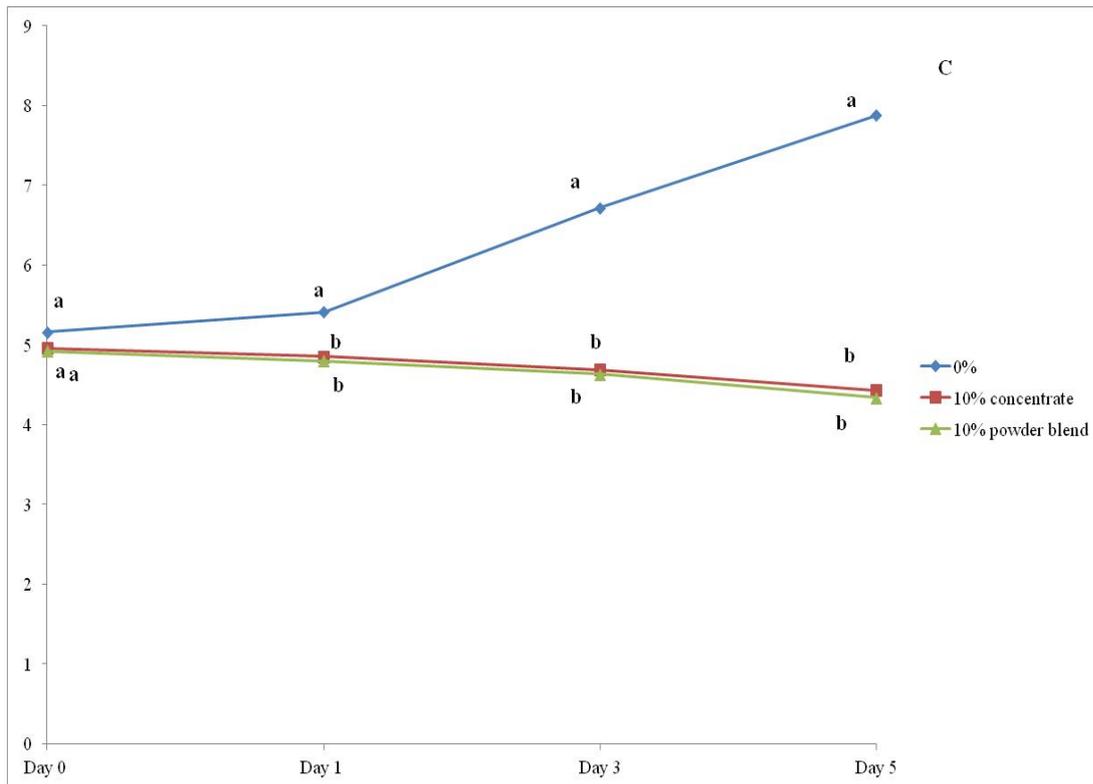


Figure 5: Antimicrobial effects of cranberry and wild blueberry concentrate combination and powder blend on total aerobic bacteria in sterilized distilled water at 7°C (5A) and 21°C (5B) for 0, 1, 5, 7, and 24 hours and in BHI at 7°C (5C) and 21°C (5D) on day 0, 1, 3, and 5. Means with different letters were significantly different (P < 0.05) for the same experimental sampling time. Y-axis is viable cell counts log CFU/ml and X-axis is treatment time.

Discussion

Pathogens in the media combined with the berry extract may be sublethally injured. These injured cells may resuscitate and become functionally normal in a favorable environment. Selective medium contains some selective compounds which can inhibit the recovery of injured cells. In this study, TAL was used in order to recover both injured and uninjured cells [15].

Pathogens survived better in BHI than in distilled water. Water with concentrate or powder is a very harsh environment for these bacteria, since it lacks nutrients which can support the metabolic activities of these four pathogens. Bacteria died easily and quickly in water at both 21°C and 7°C except for *E. coli* O157:H7. In distilled water, the pH was 0 (powder blend) and 3.1 (concentrate combination), respectively (Table 1). *E. coli* O157:H7 was known to be resistant to low pH. It did not decline as fast as other pathogens in water (Table 2). Other studies showed that *E. coli* O157:H7 can survive in apple cider (pH 3.5 to 1) and orange juice (pH 3.5 to 0) [18]. Miller et al. [19] reported that *E. coli* O157:H7 withstood pH 2 (pH was adjusted with HCl) with no significant drop in CFU after 24 h. All these studies showed that *E. coli* O157:H7 can tolerate an acid environment in a way.

Nogueira et al. [20] reported that cranberry juice possesses intrinsic antimicrobial properties that will eliminate *L. monocytogenes*, *S. typhimurium* and *E. coli* O157:H7. A 5 log reduction of all these pathogens was demonstrated in their study. Puuponen-Pimiä et al. [21] reported that cranberry and wild blueberry extracts rich in anthocyanins generally inhibited Gram negative but not Gram positive bacteria. In the present study, the berry powder and concentrate had antimicrobial

effects on all these four foodborne pathogens. Although low pH played a significant role, it is not the only factor to inhibit bacteria according to our previous studies [3, 4]. Cranberries and blueberries contain organic acids, anthocyanin and phenolics, which all can have antimicrobial activity. The antimicrobial activity of phenolics from cranberry extract was independent of low pH, while the anthocyanin may lose the antimicrobial activity partially at neutral pH [4]. Results from BHI showed that *E. coli* O157:H7 and *S. typhimurium* (Gram negative) were more sensitive than *L. monocytogenes* and *S. aureus* (Gram positive). This suggested that this berry extract may possibly contain some compounds which have better inhibitory effects on Gram negative than Gram positive bacteria. Since Gram positive bacteria contain much more peptidoglycan than that of Gram-negative cells and have a more rigid cell structure, the antimicrobial effect may be related to the difference of the cell surface structure of these pathogens [3].

In summary, the cranberry and wild blueberry powder blend and concentrate combination showed strong antimicrobial activity. The advantage of using both cranberry and wild blueberry concentrate and the powder blend from cranberries and blueberries is to offer more health benefits than either cranberry or blueberry alone. Based on various health benefits of berries and the antimicrobial effects, it is worthwhile to do further research to apply them in foods to enhance food safety.

Acknowledgments

This study was supported by USDA CSREES Special Grant Blueberry Research, Maine Agriculture Center, and the Maine Agricultural and

Forest Experiment Station at the University of Maine with external publication number 3351 and the Science Foundation of Jimei University, China. This work is based upon research supported in part by Hatch Grant number ME08562-10 from the USDA National Institute of Food and Agriculture.

References

1. Vattem DA, Lin YT, Labbe RG, Shetty K (2004) Phenolic antioxidant mobilization in cranberry pomace by solid-state bioprocessing using food grade fungus *Lentinusedodes* and effect on antimicrobial activity against select food borne pathogens. *Innovative Food SciEmergTechnol* 5: 81-91.
2. Puupponen-Pimiä R, Nohynek L, Alakomi HL, Oksman-Cadentey KM (2005) Bioactive berry compounds—novel tools against human pathogens. *Appl Microbiol Biotechnol* 67: 8-18.
3. Wu VCH, Qiu X, Bushway A, Happers, L (2008) Antibacterial effects of American cranberry (*Vaccinium macrocarpon*) concentrate on foodborne pathogens. *LWT-Food SciTechnol* 41: 1834-1841.
4. Lacombe A, Wu VCH, Tyler S, Edwards K (2010) Antimicrobial action of the American cranberry constituents, phenolics, anthocyanins, and organic acids, against *Escherichia coli* O157:H7. *Int J Food Microbiol* 139: 102-107.
5. Cunningham DG, Vannozzi SA, Turk R, Roderick R, O'Shea E, et al. (2004) Cranberry phytochemicals and their beneficial health benefits. *Nutraceutical beverages chemistry, nutrition, and health effects*. ACS symposium series 871: pp. 35-51. Washington, DC.
6. Vattem DA, Ghaedian R, Shetty K (2005) Enhancing health benefits of berries through phenolic antioxidant enrichment: focus on cranberry. *Asia Pac J Clin Nutr* 14: 120-130.
7. Madhujith T, Shahidi F (2004) Antioxidant activity of blueberry and other *Vaccinium* species. In: Shahidi F, Weerasinghe DW (edn), *Nutraceutical beverages chemistry, nutrition, and health effects*, 871: 149-160. ACS, Washington, DC.
8. Yi WG, Fischer J, Krewer G, Akoh CC (2005) Phenolic compounds from blueberries can inhibit colon cancer cell proliferation and induce apoptosis. *J Agric Food Chem* 53: 7320-7329.
9. Kraft TFB, Schmidt BM, Yousef GG, Knight CTG, Cuendet M, et al. (2005) Chemopreventive potential of wild lowbush blueberry fruits in multiple stages of carcinogenesis. *J Food Sci* 70: 159-166.
10. Gould GW (1996) Industry perspectives on the use of natural antimicrobials and inhibitors for food applications. *J Food Prot Supplement*: 82-86.
11. Wong ACL, Bergdoll MS (2002) Staphylococcal food poisoning. In: Cliver, DO, Riemann HP (edn), *Foodborne diseases*, pp. 231-248. Academic Press, CA.
12. Vattem DA, Lin YT, Ghaedian R, Shetty K (2005) Cranberry synergies for dietary management of *Helicobacter pylori* infections. *Process Biochem* 40: 1583–1592.
13. Liu RH (2004) Potential synergy of phytochemicals in cancer prevention: mechanism of action. *J Nutr* 134: 3479S–3485S.

14. Wu VCH, Fung DYC (2001) Evaluation of thin agar layer method for recovery of heat-injured foodborne pathogens. *J Food Sci* 66: 580–583.
15. Wu VCH, Fung DYC, Kang DH, Thompson LK (2001) Evaluation of thin agar layer method for recovery of acid-injured foodborne pathogens. *J Food Prot* 64: 1067–1071.
16. Waterhouse AL (2005) Unit 11.1. Determination of total phenolics. In: Wrolstad RE, Acree TE, Decker EA, Penner MH, Reid DS, Schwartz SJ, Shoemaker CF, Smith DM, Sporns P (edn), *Handbook of Food Analytical Chemistry*, pp. 463-470. John Wiley & Sons, NJ.
17. Lee J, Finn CE, Wrolstad RE (2004) Comparison of anthocyanin pigment and other phenolic compounds of *Vaccinium membranaceum* and *Vaccinium ovatum* native to the Pacific northwest of North America. *J Agric Food Chem* 52: 7039-7044.
18. Han Y, Linton RH (2004) Fate of Escherichia coli O157:H7 and Listeria monocytogenes in strawberry juice and acidified media at different pH values and temperatures. *J Food Prot* 67: 2443-2449.
19. Miller LG, Kaspar CW (1994) Escherichia coli O157:H7 acid tolerance and survival in apple cider. *J Food Prot* 57: 460-464.
20. Nogueira MCL, Oyarzábal OA, Gombas DE (2003) Inactivation of Escherichia coli O157:H7, Listeria monocytogenes, and Salmonella in cranberry, lemon, and lime juice concentrates. *J Food Prot* 66: 1637-1641.
21. Puupponen Pimiä R, Nohynek L, Meier C, Kahkonen M, Heinonen M, et al. (2001) Antimicrobial properties of phenolic compounds from berries. *J Appl Microbiol* 90:494-507.