LWT - Food Science and Technology 77 (2017) 517–524

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Effects of pulsed electric field processing on microbial survival, quality change and nutritional characteristics of blueberries☆

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ARTICLE INFO

Article history:
Received 5 October 2016
Received in revised form
29 November 2016
Accepted 6 December 2016
Available online 8 December 2016

Keywords:
Pulsed electric fields
Blueberries
Microbial
Quality
Nutrition
Sanitizer

Chemical compounds studied in this article:
Peroxyacetic acid (PubChem CID: 195769)
Hydrogen peroxide (PubChem CID: 784)
Sodium chloride (PubChem CID: 5234)

ABSTRACT

Whole fresh blueberries were treated using a parallel pulsed electric field (PEF) treatment chamber and a sanitizer solution (60 ppm peracetic acid [PAA]) as PEF treatment medium with square wave bipolar pulses at 2 kV/cm electric field strength, 1μs pulse width, and 100 pulses per second for 2, 4, and 6 min. The effects of PEF on native microbiota and artificially-inoculated *Escherichia coli* K12 and *Listeria innocua* populations on blueberries were determined. Color, texture, anthocyanins and total phenolic compound concentrations were also evaluated. The combination of PEF and PAA was able to achieve up to 3 log reduction of *E. coli* and *Listeria* as well as 2 log/g reduction of native microbiota. PEF treatments did not cause any changes in color and appearance of the blueberries. The treatments did, however, cause the blueberries to soften in texture. Anthocyanins and phenolic compounds in blueberries increased by 10 and 25%, respectively, after PEF treatments. The results demonstrate the potential of PEF applications to enhance the safety and improve the quality and nutritional value of fruits and their derived products.

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1. Introduction

Small fruits such as raspberries and blueberries are high value commodities for their well-reported health benefits. In terms of U.S. fruit consumption, blueberries rank only second to strawberries in popularity of berries. Blueberries are not only popular, but also repeatedly ranked in the U.S. diet as having one of the highest antioxidant capacities among all fruits, vegetables, spices and seasonings. Antioxidants are essential to optimizing health by helping to combat the free radicals that can damage cellular structures as well as DNA (Whfoods, 2016).

Berry fruits are usually grown in open fields and are constantly exposed to sources of potential pre-harvest contamination such as soils, insects, birds, and irrigation water as well as human contact during harvesting. Therefore, they are susceptible to contamination caused by pathogenic bacteria. In recent years, an increasing number of foodborne illness outbreaks has been associated with small fruits such as raspberries and blueberries (Calder et al., 2003; Luna, Mody, Griffin, 2010; Miller, Rigdon, Robinson, Hedberg, & Smith, 2013; Sarvikivi et al., 2012). Two outbreaks and 52 illnesses were related to the consumption of blueberries in 2014 (CDC, 2015). In addition, the warm temperature during the harvest season prompts fruit spoilage. Wu and Kim (2007) observed that visible signs of decay can develop on warm, wet blueberries in less than 12 h. Therefore, proper intervention controls are needed and should be applied as soon as the fruits are harvested. The intervention processes currently being used may not be sufficient for controlling the biological hazards in these foods, creating urgency for the development of new intervention technologies.

Fruit washing with sanitizers has been used by farms, processors, and distributors to reduce initial microbial loads, but the effectiveness has been limited. Chlorinated water sprays (100 ppm) have demonstrated reductions of less than 1 log CFU/g against APC

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http://dx.doi.org/10.1016/j.lwt.2016.12.009
0023-6438/Published by Elsevier Ltd.

on the surface of low bush blueberries, when compared to unwashed berries (Crowe, Bushway, & Bushway, 2005). Alexandre, Brandão, and Silva (2012) reduced total aerobic mesophilic bacteria on strawberries by 1.5 and 2.2 log CFU with 1% and 5% hydrogen peroxide, respectively. Hung, Tilly, and Kim (2010) reduced *E. coli* O157:H7 populations artificially inoculated onto strawberries by 1.17 log CFU/g when treated with electrolyzed oxidizing water at 4 °C for 1 min, and by 1.58 log CFU/g when the treatment time was increased to 5 min. When ultrasonication was added, *E. coli* O157:H7 populations on strawberries were further reduced by 1.49 and 1.89 log CFU/g, respectively. The efficacy of electrostatic sprays of electrolyzed oxidizing water, UV light, ozone, and a combination of ozone and UV light in inactivating *E. coli* O157:H7 artificially inoculated onto blueberries was studied and a synergistic effect for the combination was observed (Kim & Hung, 2012). Tsunami® 100 is an Environmental Protection Agency (EPA) registered antimicrobial water additive for pathogen reduction in fruit and vegetable processing water, composed of acetic acid, peracetic acid and hydrogen peroxide. Tsunami 100 is recommended for use in the process waters of post harvest, fresh cut, and processed fruits and vegetables in both batch and continuous operations. EPA (1998) authorizes the use of peroxyacetic acid-based additives for fruits and vegetables and washed water up to 80 ppm. Alvaro et al. (2009) used the peracetic acid sanitizer (Tsunami®100, Ecolab) for sweet pepper, tomato and cucumber (500 mg/L for 2 min) and stated that the perorganic acid compound is better for washing fruit and improving postharvest shelf life as it is environmentally friendly, breaking down into acetic acid and oxygen after use, does not have any adverse effects on human health, and does not affect the taste characteristics of fruits and vegetables.

PEF treatments have been studied for killing foodborne pathogenic microorganisms, such as *E. coli* O157:H7, *Salmonella* spp. and *L. monocytogenes* as well as spoilage bacteria, molds and yeasts (Jin, Zhang, Hermawan, & Dantzer, 2009a, 2009b, 2014, 2015; Guo et al., 2014; Gurtler, Rivera, Zhang, & Geveke, 2010, 2011; Min, Jin, Min, Yeom, & Zhang, 2003; Reina, Jin, Zhang, & Youself, 1998). PEF treatments have exhibited the potential of maintaining the physico-chemical quality of liquid food products, without substantially impacting the sensory properties and health-related compound makeup (Guo et al., 2014; Jin & Zhang, 1999). Most of these studies have been focused on liquid foods, such juices and beverages. There is an increasing interest in PEF pretreatment of fresh produce products, such as potato and apple (Angersbach, Heinz, & Knorr, 2000; Bazhal, Lebovka, & Vorobiev, 2001; Lebovka, Praporscic, & Vorobiev, 2004). However, there is no information available for inactivation of microbial contaminants on fresh whole fruits, particularly combined with the use of a sanitizing solution as a PEF treatment medium, and their impact on microbial reduction, product quality and nutritional value of whole berry fruits. Fresh berries are, traditionally, a seasonal food, and their consumption is highest during the summer months. Frozen berries, berry jams, juices/nectars, and dehydrated berries are all typically available at other times of the year.

The central idea behind this current study was to evaluate PEF processing for its potential use as a pretreatment that would extend the shelf-life, enhance the safety and improve nutritional and other characteristics of whole fresh fruits and their derived constituent products (i.e., juice, puree, jam, pastes, raisin and other dehydrated products). The specific objectives of this first study were to: 1) investigate the antimicrobial efficacy of PEF treatment in combination with sanitizing solution for blueberries; 2) evaluate the effects of these treatments on the quality and nutritional attributes of fresh fruits, and 3) assess the potential of these treatments to manufacture derived products.

2. Materials and methods

2.1. Blueberry preparation

Fresh blueberries were obtained from local supermarkets, stored in a refrigerator at 4 °C until the time of the experiment and used within *ca*. 1 week of purchasing. Blueberries with a diameter of about 12–14 mm and average weight of 2 g/piece were selected for the experiments.

2.2. Inoculation of blueberries

E. coli K12 and *Listeria innocua* were used as surrogates of pathogenic *E. coli* O157:H7 and *Listeria monocytogenes*, respectively. *E. coli* K12 (ATCC 23716) and *Listeria innocua* (ATCC 33090) were obtained from the culture collection of the U.S. Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center (Wyndmoor, PA, USA). Frozen stock cultures of each strain were cultured independently in 9 mL Tryptic Soy Broth (TSB, BBL/Difco Laboratories, Sparks, MD, USA) in sterile 150 mm glass tubes at 37 °C for 18 h.

For studies involving the evaluation of quality and nutritional properties or natural microbiota, blueberries were only washed with tap water. For studies involving the artificial inoculation of *Listeria* or *E. coli*, blueberries were further sprayed with 70% ethanol to remove background microorganisms and dried for 2 h in biohood prior to inoculation. Blueberries were immersed into 100 mL of the inoculum for 1 min and then placed on a sterile tray in a laminar flow hood and dried under continuous circulating laminar flow for 2 h at 22 ± 2 °C to facilitate microbial attachment on the fruit surface.

2.3. Sanitizing solution and PEF treatment

Tsunami 100 (EcoLab, St. Paul, MN) (15.2% peroxyacetic acid, 11.2% hydrogen peroxide) was used in this study. One liter of sterile distilled water was conditioned at 22 °C overnight, and 250 μl or 500 μl Tsunami 100 (T100) was added to the sterile distilled water just before each experiment. The concentration of peroxyacetic acid in each sanitizing solution was determined using a test kit acceding to the manufacturer's instruction. The concentration of peroxyacetic acid corresponding to 0.25% and 0.5% solutions were 30 and 60 ppm, respectively.

A bench-scale PEF processing system (OSU–4H Model) located at the Eastern Regional Research Center, Agricultural Research Service, USDA (Wyndmoor, PA, USA) was used for this study (Fig. 1). The system provided bipolar square waveform pulses with a maximum peak voltage of ±11 kV. The high voltage pulse generator operated at a maximum repetition rate of 2000 pulses per second and a pulse width of 1–10 μs Pulses were monitored with a high voltage probe (VD-60; Northstar, Albuquerque, NM, USA), current monitors (Model 110; Pearson, Palo Alto, CA, USA) and oscilloscopes (TDS-210; Tektronix, Beaverton, OR, USA). A specially designed parallel-electrode (stainless steel) PEF treatment chamber with 2.5 cm gap distance and an area of 56 cm^2 (7 × 8 cm) was used to treat blueberry samples (Fig. 1).

Before PEF treatment, fresh blueberries (30 pieces, ca. 60 g) were placed in the treatment chamber, and then the chamber was filled with the salt solution (0.025% w/v; 500 μS/cm electrical conductivity) with or without sanitizer. The salt solution was used to improve electrical contact between electrodes and have a more homogeneous electric field distribution. The PEF treatment conditions in this study were 2 kV/cm field strength, 1 μs pulse width, and 100 pulses per second. The treatment times were 2, 4, and