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Influence of electro-activated solutions of weak organic acid salts on microbial quality and overall appearance of blueberries during storage

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ABSTRACT

The aim of this work was to study the potential of diluted electro-activated solutions of weak organic acid salts (potassium acetate, potassium citrate and calcium lactate) to extend the shelf life of blueberries during post-harvest storage. The sanitizing capacity of these solutions was studied against pathogenic bacteria *Listeria monocytogenes* and *E. coli* O157:H7 as well as phytopathogenic fungi *A. alternata*, *F. oxysporum* and *B. cinerea*. The results showed that a 5-min treatment of inoculated blueberries with electro-activated solutions resulted in a 4 log CFU/g reduction in *Listeria monocytogenes* for all solutions. For *E. coli* O157:H7, the electro-activated potassium acetate and potassium citrate solutions achieved a decrease of 3.5 log CFU/g after 5 min of berry washing. The most important fungus reduction was found when blueberries were washed with an electro-activated solution of potassium acetate and a NaOCl solution. After 5 min of blueberry washing with an electro-activated potassium acetate solution, a very high reduction effect was observed for *A. alternata*, *F. oxysporum* and *B. cinerea*, which showed survival levels of only 2.2 ± 0.16, 0.34 ± 0.15 and 0.21 ± 0.16 log CFU/g, respectively. Regarding the effect of the washing on the organoleptic quality of blueberries, the obtained results showed no negative effect on the product color or textural profile. Finally, this work suggests that washing with electro-activated solutions of weak organic acid salts can be used to enhance the shelf-life of blueberries during post-harvest storage.

1. Introduction

According to the World Health Organization (World Health Organization, 1998), fruits and vegetables and their processed products contain high levels of vitamins, phenols and minerals and have become an essential part of the human diet (Goodburn and Wallace, 2013). This tendency was primarily induced by epidemiological studies of diets rich in fruit (El-Ramady et al., 2015). The Codex Alimentarius Commission (Codex Alimentarius Commission, 2010) has shown various health benefits for diets rich in fruit and vegetables, which are associated with protective effects against cancer and chronic diseases such as coronary heart disease (Goodburn and Wallace, 2013). Due to their biochemical composition and nutritional and health benefits, blueberries are fruits with one of the highest antioxidant activities. They contain high levels of vitamins (C and E), anthocyanins and polyphenolics (Chun et al., 2013; Kim et al., 2000). Diets containing blueberries have demonstrated potential to limit the development and severity of certain cancers and vascular diseases, including atherosclerosis, ischemic stroke, and the neurodegenerative diseases of aging (Neto, 2007; Schmidt et al., 2006).

Considering the growing consumer demand for convenient, fresh, high-quality fruit that is nutritious, flavorful and stable, the marketing interest for blueberries has increased (Rolle, 2006). Since 2012, the production of blueberries has increased in USA and Canada by 6.07 and 5.65%, respectively (Lambert and Criner, 2014). However, increased production of blueberries places higher importance on ensuring microbiological safety and maintaining nutritional quality during post-harvest storage and selling. Moreover, blueberries are minimally processed or consumed raw and are not washed before packaging (Kim et al., 2000). Thus, contamination risks must be balanced with minimizing shelf-life, competing outcomes that are both economically undesirable. Many methods of disinfection have been used, including chemical washing and spraying procedures, irradiative treatments and natural (biological)

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methods (El-Ramady et al., 2015; Rolle, 2006). Industry has focused on chemical techniques despite the potential storage risks for these reagents in concentrated form (Yoo et al., 2010) and health concerns. For example, to reduce microorganism populations, blueberries are usually washed or sprayed with chlorinated water containing 50–200 mg kg^{-1} of active chlorine (Wu and Kim, 2007). Although other chemical methods were assessed for microbial reduction (bromine, iodine, trisodium phosphate, quaternary ammonium compounds, acids, hydrogen peroxide, ozone) the efficacy of these methods varied greatly and scientific data were lacking, making it difficult to draw firm conclusions concerning their efficacy (El-Ramady et al., 2015; Rolle, 2006). In fact, 95% of post-harvest blueberries exhibit a broad range of fungal contamination (Gabler et al., 2004; Tournas and Katsoudas, 2005). Several outbreaks of foodborne illnesses have also been associated with berry consumption and have been attributed to possible fecal contamination during growing, harvesting and handling (Goodburn and Wallace, 2013; Kim et al., 2000; Kim and Hung, 2012; Li and Wu, 2013; Wu and Kim, 2007; Zhang et al., 2015). As the choice and the efficacy of decontamination methods are significantly reflected in the reduction of microbes, more effective and secure methods for microbial safety of blueberries are needed. The use of "hurdle" technologies as a preservation strategy appear to be most effective, as they involve combinations of different preservation techniques (Rico et al., 2007).

Electro-activated aqueous solutions offer a uniquely strong potential to achieve the aforementioned objective of ensured microbial safety for fresh blueberries. Electro-activation is a principle based on applied electrochemistry. The properties of aqueous solutions can be modified through surface reactions at the electrode/solution interface when an external electric current is applied to an electro-activation reactor filled with the appropriate aqueous solutions. The oxidation-reduction processes involved at the solution/anode interface yield aqueous solutions with acidic pH, oxidative (positive) redox potential up to +1100 mV and saturation with active oxygen. These solutions are highly reactive and have strong antibacterial and antifungal activities. Simultaneous reactions at the cathode/solution interface enable the production of solutions with reductive properties that can also be used as washing solutions (Liato et al., 2015a,b). Thus, electro-activation solutions (EAS) provide a triple combination of oxidation-reduction (Redox) potential (ORP), pH and oxidizing species (Aider et al., 2012). Several studies showed successful application of EAS as effective disinfectants for killing bacteria, spores, virus and fungus (Aider et al., 2012; Buck et al., 2002; Huang et al., 2008). Moreover, EAS exhibited a high sanitizing effect against microbial contamination of fresh or minimally processed fruit and vegetables (Goodburn and Wallace, 2013; Park et al., 2001; Rico et al., 2007). For example, Pangloli and Hung (2013) studied the microbiological safety risk for blueberries and demonstrated important reductions, from 4.98 ± 0.2 to 0.54 ± 0.34 log CFU/g, of *E. coli* O157:H7 (Pangloli and Hung, 2013). Treatment with chlorine-containing disinfectants showed significant pathogen reduction for a variety of foods, but the possible association of chlorine may lead to the eventual formation of carcinogenic chlorinated compounds (trihalomethanes), calling into question the use of chlorinated water in food processing (Chun et al., 2013; Huang et al., 2008; Rico et al., 2007). This is also related to electrolyzed NaCl solutions, which contain high levels of active chlorine. However, electro-activated solutions (EAS) can be successfully generated without producing toxic chlorine. In our previous work, electro-activated solutions of weak organic acid salts were obtained. Indeed, solutions of potassium acetate, calcium lactate and potassium citrate were acidified through electro-activation and successfully used to study their destructive effects against *S. enterica*, *L. monocytogenes* and *S. aureus*. We found higher destruction kinetics for these foodborne pathogens after treatment with EAS of weak organic acid salts compared to conjugated commercial acids. Thus, it has been suggested that generation of EAS without chlorinated compounds could be an effective disinfectant method for post-harvest preservation of vegetable products and extension of post-harvest shelf-life (Liato et al., 2015a,b).

This work aimed to study the effect of electro-activated organic solutions on pathogen and fungal reduction and sensory qualities during post-harvest storage.

2. Material and methods

2.1. Blueberries

Canadian blueberries (*Vacinnium corymbosum*) were purchased from local grocery stores (Quebec, QC, Canada) on the day of experimentation and left at room temperature (21 ± 1 °C) for 1 h prior to analysis. Fresh, uniform (0.012–0.014 m in diameter and height) and visibly non-damaged blueberries with intact surfaces were selected for sensorial quality and microbiological assessment.

2.2. Microbial and fungal cultures preparation

Cultures of *Listeria monocytogenes* (ATCC Scott A3), and *Escherichia coli* O157:H7 (ATCC 35150) were kept frozen as glycerol stocks at −80 °C in cryovials. The strains were prepared by inoculating 10 mL of tryptic soy broth medium (211823 BD, Becton-Dickinson & Company, Franklin Lakes, NJ, USA) supplemented with 0.5% yeast extract and incubated 24 h at 37 °C in aerobic conditions. A loopful of each culture was inoculated into 0.5 mL of fresh TSB and incubated under the same conditions. The grown cultures were washed twice in sterile saline solution (0.85%) by centrifuging at 5000 g for 5 min. The final pellet was re-suspended in sterile saline solution to a concentration calculated to yield approximately 1×10^9 CFU/mL. The bacterial population in each culture was confirmed by serial 10-fold dilution in sterile 0.1% peptone water (211677 BD, Becton-Dickinson & Company, Franklin Lakes, NJ, USA) by plating 0.1 mL portions of diluted culture onto the appropriate agar mediums: PALCAM (263620 BD, Difco) and MacConkey (220100 BD, Difco) for *Listeria monocytogenes* and *E. coli* O157:H7, respectively. The inoculated plates were aerobically incubated at 37 °C for 24 h.

Fungal strains of *Alternaria alternata* (LMA-369, cheese isolate), *Fusarium oxysporum* (LMA-590, cheese isolate) and *Botrytis cinerea* (raspberry isolate) used in this study were obtained from the Food Sciences Culture Collection of Université Laval (Quebec, QC, Canada). A suspension of sporulating fungal material was stored at −80 °C as a stock culture and subsequently cultivated on potato dextrose agar (PDA, 213400 BD, Difco, Becton-Dickinson & Company, Franklin Lakes, NJ, USA). After seven days of incubation at 21 °C in the dark, the cultures were subcultured by transferring mycelium fragments to fresh PDA medium. On the fourteenth day of incubation, the conidia from each culture were harvested by flooding the surface of the plate with 10 mL of a 0.05% detergent solution (Tween-80, Sigma-Aldrich, MO, USA) and scraped using a glass rod. The suspension was filtered through several layers of sterile gauze to remove large mycelium fragments and centrifuged at 3000 g for 15 min (Narayanasamy, 2011). The pellet was re-suspended and the centrifugation cycle was repeated three times to replace the detergent with sterile saline solution (0.85%). The spore concentration in the final volume was determined microscopically using a counting chamber (Levy Nº 3301, Thomas Scientific, Swedesboro, NJ, USA) to a spore concentration of 1×10^7 CFU/mL.