International Journal of Food Microbiology 241 (2017) 23–29

Contents lists available at ScienceDirect

International Journal of Food Microbiology

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

Ultrasound improves chemical reduction of natural contaminant microbiota and *Salmonella enterica* subsp. *enterica* on strawberries

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ARTICLE INFO

Article history:
Received 30 June 2016
Received in revised form 3 October 2016
Accepted 9 October 2016
Available online xxxx

Keywords:
Acetic acid
Peracetic acid
Ultrasound
Salmonella enterica
Synergistic effect
Sensory quality

ABSTRACT

New sanitization methods have been evaluated to improve food safety and food quality and to replace chlorine compounds. However, these new methods can lead to physicochemical and sensory changes in fruits and vegetables. The present study evaluated the effects of acetic acid, peracetic acid, and sodium dodecylbenzenesulfonate isolated or combined with 5 min of ultrasound treatment (40 kHz, 500 W) on strawberry quality over 9 days of storage at 8 °C. The strawberry natural contaminant microbiota (molds and yeasts, mesophilic aerobic and lactic acid bacteria), physicochemical quality (pH, total titratable acidity, total soluble solids, vitamin C, and color), sensory quality (triangle test) and inactivation of *Salmonella enterica* subsp. *enterica* intentionally inoculated onto strawberries were analyzed. Ultrasound increased the effect of all chemical compounds in the reduction of aerobic mesophilic, molds and yeasts. The best treatment for those groups of microorganisms was ultrasound combined with peracetic acid (US + PA) that reduced 1.8 and 2.0 log cfu/g during 9 days of storage. Bactericidal effect of peracetic acid was also improved by ultrasound inactivation of *S. enterica*, reaching a decimal reduction of 2.1 log cfu/g. Moreover, synergistic effects were observed in contaminant natural microbiota inactivation for all tested compounds during storage, without any major physicochemical or sensory alteration to the strawberries. Therefore, ultrasound treatment can improve the effect of sanitizers that are substitutes of chlorine compounds without altering the quality of strawberries during storage.
Acetic acid (PubChem CID: 176); Peracetic acid (PubChem CID: 6585); Sodium dodecylbenzenesulfonate (PubChem CID: 18372154).

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1. Introduction

Sanitization is a critical step in the inactivation of pathogenic and spoilage microorganisms, and its use increases food safety. Chlorine compounds are the most used sanitizers in this step (Alvaro et al., 2009). However, some countries in the European Union have already banned the use of these compound in fresh-cut vegetables due to the formation of carcinogenic products like trihalomethanes (Araújo et al., 2015; Fava et al., 2011; Pérez-Gregorio et al., 2011), putting consumers in danger.

Alternative conservation methods such as ultrasound have been recently studied and have proven beneficial effect on food (Ferrario et al., 2015; Kentish and Feng, 2014). Among its diverse applications, ultrasound has the capacity to inactivate pathogenic microorganisms that are critical to the food industry, and ultrasound is also considered a green technology (Awad et al., 2012; Chemat et al., 2011).

Microorganism inactivation by ultrasound occurs due to the generation of localized mechanical, thermal (hot spots) and chemical (free radicals) energy caused by the cavitation process (Gogate and Kabadi, 2009; São José et al., 2014a). Cavitation generates micro-jets that leads to microorganism disaggregation and membrane lesions, as well as releasing free radicals that damage DNA (Chemat et al., 2011; São José et al., 2014a). All of these effects contribute to surface cleanliness (Erriu et al., 2014; Gogate and Kabadi, 2009).

Combination of ultrasound with chemical compounds has generated promising results with the inactivation of pathogenic and spoilage microorganisms (Afari et al., 2016; Chen and Zhu, 2011; São José et al., 2014b). However, long application periods are needed to achieve satisfying results, besides the undesirable physicochemical changes (Fava et al., 2011; Gani et al., 2016; Park et al., 2016; São José and Vanetti, 2015) that may interfere in the consumers buying intention.

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http://dx.doi.org/10.1016/j.ijfoodmicro.2016.10.009
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Among the currently used sanitizers, peracetic acid has stood out because of its efficient inactivation of pathogenic and spoilage microorganisms, and it is not affected by organic matter and proteins. Moreover, peracetic acid has a low environmental impact because acetic acid and hydrogen peroxide are the products of its decomposition (Srey et al., 2013). The Food and Drug Administration (FDA) approved use of peracetic acid at 80 mg/L in fruits and vegetables as described in 21CFR173.315. Other bactericidal compounds have been used in sanitizing food such as organic acids (Mohan and Pohlman, 2016). Among them, acetic acid is a Generally Recognized As Safe (GRAS) compound (FDA, 2015) that is an antimicrobial agent when used at low concentrations, and it usually does not cause secondary effects (EFSA, 2012). Additionally, surfactants can be used for removing contaminants on food surfaces (Sagong et al., 2013; Wang et al., 2013). Sodium dodecylbenzenesulfonate (SDBS) is a surfactant that has great removal capacity. The FDA approved use of SDBS at 20,000 mg/L in foods as described in 21CFR173.315. Overall, all these compounds can be used to retard microbial spoilage and inactivate pathogens involved in foodborne disease outbreaks.

Recently, consumers have preferred fresh and ready-to-eat foods because those foods consumed raw are a potential risk for foodborne illness. The pathogens currently involved in foodborne illness outbreaks are *Listeria monocytogenes*, *Salmonella*, and *Escherichia coli* (Abadias et al., 2008; CDC, 2014; CDC, 2015a). Although eggs, meat and dairy products are more reported in salmonellosis outbreaks, recent salmonellosis outbreaks of disease involving consumption of fruits and vegetables have been reported (CDC, 2015b; Harris et al., 2003). Among the food of vegetable origin that are consumed fresh, strawberries are preferred by consumers and are also an important feedstock for the food industry (Giampieri et al., 2012).

Chemical compounds in industry are used to reduce contamination of fruits and vegetables, and this effect can be increased when combined with ultrasound. Therefore, we evaluated the isolated and combined effects of using chemicals with ultrasound in fruits and vegetables, microbial inactivation, and physicochemical, nutritional and sensory changes. Specifically, this paper evaluated effects of ultrasound treatment combined with acetic acid, peracetic acid, and sodium dodecylbenzenesulfonate in the reduction of natural contaminant microbiota, physicochemical, nutritional and sensory quality of strawberries.

2. Material and methods

Strawberries (*Fragaria* × *ananassa* Duch.) cultivar Oso Grande were obtained in local commerce during the period of June to November of 2015 and transported in isothermal boxes. The fruits were selected, and the peduncle and calyx were removed. After the application of each sanitization procedure on 800 g of strawberries, the treated fruits were placed in boxes of polyethylene terephthalate and stored at 8 ± 1 °C under atmospheric air.

2.1. Sanitization procedures

The applied procedures were sterilized water (SW), acetic acid (AA) at 800 mg/L (Toscano®, São Paulo, Brazil), sodium dodecylbenzenesulfonate (SDBS) at 1200 mg/L (Sigma-Aldrich®, Missouri, USA), peracetic acid (PA) at 40 mg/L (Proxitane®, Paraná, Brazil), and ultrasound bath (US) at 40 kHz and 500 W (Model Soniclean 15, Sanders Medical®, Minas Gerais, Brazil). Using the same concentrations, the three chemical compounds described previously were combined simultaneously with ultrasound (US + AA, US + SDBS, and US + PA). All treatments were applied for 5 min (Sagong et al., 2011), and 1 L of solution at 7 ± 1 °C was also used. The control treatment was the strawberries washed in potable water without any sanitization (NS, no sanitizer).

2.2. Microbiological analysis

2.2.1. Natural contaminant microbiota

The procedures on this step were performed following the methodology from the American Public Health Association described in the Compendium of Methods for the Microbiological Examination of Foods (Downes and Ito, 2001). First, 25 g of strawberries were homogenized in 225 mL of 0.1% peptone water at a dilution 10^{-1}. Homogenization was performed in a stomacher (Marconi®, Piracicaba, São Paulo) for 1 min. Appropriate dilutions were made in 0.1% peptone water and transferred to the culture medium specific for each microbial group. Mesophilic aerobic bacteria were plated on Plate Count Agar (PCA) (HiMedia®, Mumbai, India) and incubated at 35 °C for 48 h. Molds and yeasts were plated on Potato Dextrose Agar (PDA) (HiMedia®, Mumbai, India) and incubated at 25 °C for 7 days. Lactic acid bacteria were plated on Man, Rogosa and Sharpe Agar (MRS) (Merck®, Darmstadt, Germany) and incubated at 35 °C for 72 h. All results were expressed in colony-form units per gram (cfu/g).

2.2.2. Intentionally inoculated Salmonella enterica subsp. enterica in strawberries

2.2.2.1. Inoculum preparation. Salmonella enterica subsp. *enterica* was isolated from vegetables commercialized in the city of Alegre, Espírito Santo, Brazil, and identified by the sequencing of the 16S rRNA gene in a previous work (data not published). *Salmonella enterica* cells were stored at −80 °C in nutrient broth and glycerol (80:20) and activated before using. The activation was performed by two consecutive transfers to Brain Heart Infusion (BHI) broth (HiMedia®, Mumbai, India). BHI broth was incubated at 35 °C for 24 h until population reaches between 10^8 and 10^9 cfu/mL, confirmed by plate count on Hektoen Enteric Agar (HiMedia®, Mumbai, India).

2.2.2.2. Strawberries inoculation. First, the strawberries were pre-selected and washed in potable water, and then 200 g of fruit were transferred to sterilized polyethylene bags. Next, 500 mL of 0.1% peptone water and 10 mL of the inoculum were added to the bags, obtaining a solution with approximately 10^6 cfu/mL cells of *S. enterica*. The strawberries were maintained in the solution for 60 min at 25 ± 1 °C (São José, 2013). The suspension was drained, and the strawberries were transferred to sterilized polyethylene bags and incubated at 25 °C for 24 h to allow better bacterial adhesion (São José, 2013). After, 500 mL of each sanitizing solution described in item 2.1 were applied for 5 min at 7 ± 1 °C to 200 g of strawberries.

2.2.2.3. S. enterica quantification. After each sanitization treatment, 25 g of strawberries were homogenized in 225 mL of a 0.1% peptone water at a dilution 10^{-1}. Homogenization was held in a stomacher (Marconi®, Piracicaba, São Paulo) for 1 min. Appropriate dilutions were made in 0.1% peptone water and plated on Hektoen Enteric Agar (HiMedia®, Mumbai, India) by the spread plate technique, and the *S. enterica* colonies were counted after 18 to 24 h of incubation at 37 °C. The results were expressed in cfu/g (Downes and Ito, 2001). After the sanitization, the sanitizing solution was also plated, and the cells were counted using the same technique and media as the strawberry samples to verify if the cells were inactivated or just washed off of the fruits. The results were expressed in cfu/mL.

2.3. Physicochemical analysis

2.3.1. Total titratable acidity, total soluble solids, and ratio

Total titratable acidity analysis (TTA) was performed using the titrimetric technique, described in modified AOAC (AOAC, 2005). The results were expressed in mg of citric acid per 100 mg of strawberry pulp. Total soluble solids (TSS) was determined by refractometry, using direct read of the strawberry pulp with a bench refractometer

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