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Removal of *Salmonella enterica* Enteritidis and *Escherichia coli* from green peppers and melons by ultrasound and organic acids

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ABSTRACT

The aim of this study was to evaluate the effectiveness of ultrasound treatment combined with organic acids in the decontamination step for green peppers and melons. The influence of the surface roughness of the peppers and melons on bacterial adhesion was evaluated, as measured using a profilometer. The adhesion of *Salmonella enterica* serovar Enteritidis and *Escherichia coli* to the green pepper and melon surfaces was also evaluated by measuring the hydrophobicity of the microorganisms and the surfaces. The bacteria that adhered to the surface of green peppers and melons was quantified by plate count and visualized by scanning electron microscopy. In addition, the efficiency of ultrasound and organic acids to remove bacteria from the pepper and melon surfaces was examined. The average roughness (R_a) of the green peppers (13.0 ± 2.7 nm) was significantly different ($p > 0.05$) from the melons (33.5 ± 7.9 nm). Adherence of *S.* Enteritidis and *E. coli* are thermodynamically unfavorable for both surfaces studied ($\Delta G_{adhesion} > 0$). Despite these data, good adhesion occurred on both surfaces. The number of bacteria on green pepper slices was 7.3 and 7.0 log CFU/cm^2 for *E. coli* and *S. enterica* Enteritidis, respectively. For melon surfaces, the number of bacteria was 7.0 and 6.9 log CFU/cm^2 for *E. coli* and *S.* Enteritidis, respectively. The greater adherence of both bacteria on the green peppers can be explained by its hydrophobic surface; the hydrophilic surfaces of melons resulted in lower adherence. These results suggest that the adhesion observed in this experiment is a multifactorial process. Among the treatments evaluated for green peppers, a higher removal of pathogens was observed after use of a combination of ultrasound and 1% lactic acid; this treatment reduced *E. coli* and *Salmonella* by 2.9 and 2.8 log CFU/cm^2, respectively. For melons, the combination of ultrasound and lactic acid showed a reduction of 2.5 and 3.1 log CFU/cm^2 for *E. coli* and *S.* Enteritidis, respectively. These results indicate that it is possible to replace the chlorinated compounds that are commonly used to sanitize fruits and vegetables. These results confirm that ultrasound, an emerging technology for food processing applications, could enhance the microbial safety of fresh produce.

1. Introduction

Fresh vegetables and minimally processed fruit and vegetable consumption have increased in recent years as modern society seeks multiple benefits including greater convenience and healthier lifestyles (Forghani and Oh, 2013). Produce can become contaminated with foodborne pathogens while growing in fields or orchards, or during harvesting, postharvest handling, processing, and distribution (Beuchat, 1998). To minimize the risk of food containing microorganisms, the sources of contamination in the production environment should be identified and specific preventive measures should be implemented (São José and Vanetti, 2012). The surface properties of plants can be described by surface hydrophobicity, constitutive characteristics, and topography (Bastos et al., 2005; Wang et al., 2009). The adhesion of bacteria to plant surfaces has been a problem for food safety and has become a challenge for the food industry.

A washing and sanitizing step is commonly applied in the production process to reduce the numbers of pathogens and spoilage organisms. Chlorine compounds are typically used to sanitize these foods (Allende et al., 2008; Artés and Allende, 2005; Ruíz-Cruz et al., 2007). These compounds, however, have also been the focus of environmental concern, and some environmental groups have recommended ending their use worldwide (Rico et al., 2007; Ruíz-Cruz et al., 2007; Selma et al., 2008).

The adherence of pathogenic micro-organisms on the surfaces of some fruits is a food safety problem and has been a challenge for processors (Ukuku and Fett, 2002). Researchers are interested in finding new options to reduce pathogens and, at the same time, ensure the safety of minimally processed fruits and vegetables (Sagong et al., 2011; São José and Vanetti, 2012). These strategies should consider alternatives

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that do not use or result in toxic residues that can endanger human health and the environment, and according Sagong et al. (2011), consumers increasingly demand that food industries reduce their use of chemical additives.

One option is organic acids (lactic, acetic, citric and ascorbic acid). These substances are GRAS (generally recognized as safe) and are recognized to have the ability to inactivate bacterial foodborne pathogens (Akbas and Olmez, 2007) due to environmental pH reduction, disturbance of membrane transport and/or permeability, anion accumulation, or a decrease in internal cellular pH (Parish et al., 2003; Ramos et al., 2013).

Ultrasound is widely applied in the areas of science and engineering as a non-thermal method with many capabilities that makes it appropriate for different applications, including the food industry (Golmohamadi et al., 2013). This method was adopted by the electronics industry to decontaminate surfaces, and its use has recently been recommended as an alternative sanitization step in the food industry (Adekunte et al., 2010; Cao et al., 2010; Sagong et al., 2011; São José and Vanetti, 2012; Forghani and Oh, 2013). Ultrasonic waves promote cavitation, i.e., the formation, growth and collapse of air bubbles. These bubbles generate localized mechanical and chemical energies that are capable of inactivating microorganisms (Adekunte et al., 2010; Gogate and Kabadi, 2009; Patil et al., 2009; Piyasena et al., 2003).

This approach can contribute to the processing of minimally processed fruits and vegetables and help in adapting to new market trends. The aim of this work was to evaluate how the adhesion process occurs and to apply ultrasound in combination or not with organic acids to remove *Escherichia coli* and *Salmonella enterica* Enteritidis adhering to the surface of green peppers and yellow melons.

2. Materials and methods

2.1. Measurement of the contact angle

2.1.1. Surfaces

For the different surfaces, the contact angles between the surface and water (Milli-Q), formamide (LGC Bio, São Paulo, Brazil) and α-bromonaphthalene (Merck, Brazil) were measured on a DSA 100 goniometer (Kruss, Hamburg, Germany). Measurements of the contact angle of one 2.0 μL drop were taken each second for 30 s for all liquids and surfaces.

2.1.2. Microorganisms

Measuring the contact angle on the surfaces of *S. enterica* Enteritidis cells was performed on a layer of vegetative cells using the method described by Busscher et al. (1984). First, *S. enterica* Enteritidis cells were grown twice in brain heart infusion (BHI) to obtain a suspension of active cultures with approximately 1.0×10^7 CFU/mL Later, the suspension was centrifuged at 4000 *g* (4 °C) for 10 min and then washed three times in 0.1 M phosphate-buffered saline (PBS). The cell mass was resuspended in the buffer and deposited on a cellulose acetate membrane filter (0.45 μm pore size and 47 mm diameter) by filtration using negative pressure. During the filtration, 30 mL of pure water (Milli-Q) was added.

To standardize the moisture content, the filters were transferred into Petri dishes containing 1% agar (w/v) and 10% glycerol (v/v). The membranes were cut into three pieces to determine the angle of contact with the three liquids of different polarities.

2.1.3. Determination of the total interfacial tension (γ_s^{tot})

The total interfacial tension was determined by the sum of the apolar and polar components of the respective surfaces (Eq. (1)):

$$\gamma_l^{TOT}(1+\cos\theta) = 2\sqrt{\gamma_s^{LW}\gamma_l^{LW}} + 2\sqrt{\gamma_s^-\gamma_l^+} + 2\sqrt{\gamma_s^+\gamma_l^-} \quad (1)$$

where γ_l tot is the total interfacial tension of the liquid; γ^{LW} is the interfacial tension of the interactions of the Lifshitz–van der Waals forces; γ^+ is the interfacial tension of the electron acceptor component of the acid–base component; γ^- is the interfacial tension of the electron donor component of the acid–base component, θ is the contact angle, and *s* and *l* indicate surface and liquid, respectively (van Oss and Giese, 1995).

The three components of the interfacial tension of the surfaces were determined from the contact angles obtained from three liquids with different polarities, whose interfacial tensions are known, as shown in Table 1.

The interfacial tension is the result of the sum of the two components (γ_s^{LW} and γ_s^{AB}):

$$\gamma_s^{LW} = 11.1(1+\cos\theta_B)^2 \quad (2)$$

$$\gamma_s^{AB} = 2\sqrt{\gamma_s^+\gamma_s^-} \quad (3)$$

$$\gamma_s^{tot} = \gamma_s^{LW} + \gamma_s^{AB} \quad (4)$$

where γ_s^{LW} is the interfacial tension of the interactions of the Lifshitz–van der Waals forces; θ_B is the contact angle obtained with α-bromonaphthalene; γ_s^{AB} is the polar component of the Lewis acid–base interaction; γ_s^+ is the interfacial tension of the electron acceptor component of the acid–base component; γ_s^- is the interfacial tension of the electron donor component of the acid–base component; and γ_s^{tot} is the total interfacial tension of the surface.

2.1.4. Free energy of the hydrophobic interaction (ΔG_{sws}^{TOT})

The total free energy of interaction among molecules of the surface (*s*) immersed in water (*w*) was determined by the sum of the apolar and polar free energies of interaction, ΔG_{sws}^{LW} and ΔG_{sws}^{AB}, respectively.

$$\Delta G_{sws}^{tot} = \Delta G_{sws}^{LW} + \Delta G_{sws}^{AB} \quad (5)$$

$$\Delta G_{sws}^{LW} = -2.\sqrt{\gamma_s^{LW}-\gamma_w^{LW}} \quad (6)$$

$$\Delta G_{sws}^{AB} = -4\left(\sqrt{\gamma_s^+\gamma_s^-} + \sqrt{\gamma_w^+\gamma_w^-} - \sqrt{\gamma_s^+\gamma_w^-} - \sqrt{\gamma_w^+\gamma_s^-}\right) \quad (7)$$

2.1.5. Determination of the total free energy of adhesion ($\Delta G_{adhesion}$)

Using the values of the components of the interfacial tensions, it is possible to determine the $\Delta G_{adhesion}$ between two surfaces (microbial cells (*b*) and food surfaces (*s*)):

$$\gamma_{bs} = \gamma_{bs}^{LW} + \gamma_{bs}^{AB} \quad (8)$$

$$\gamma_{bs}^{LW} = \gamma_b^{LW} + \gamma_s^{LW} - 2\sqrt{\gamma_b^{LW}\gamma_s^{LW}} \quad (9)$$

Table 1
Components of the interfacial tensions of the substances at 25 °C.

Substances	Interfacial tension (mJ/m^{-2})			
	γ_l^{TOT}	γ_l^{LW}	γ_l^+	γ_l^-
α-Bromonaphthalene	44.4	44.4	0.0	0.0
Water	72.8	21.8	25.5	25.5
Formamide	58.0	39.0	2.28	39.6