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Escherichia coli and *Cronobacter sakazakii* in 'Tommy Atkins' minimally processed mangos: Survival, growth and effect of UV-C and electrolyzed water

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ABSTRACT

These studies were aimed at assessing the growing capacity of *Escherichia coli* and *Cronobacter sakazakii* and the effectiveness of Ultraviolet-C (UV-C) radiation, acidic electrolyzed (AEW) and neutral electrolyzed (NEW) waters in the inhibition of these bacteria on minimally processed 'Tommy Atkins' mangoes (MPM). The fruits were contaminated by dip inoculation and kept 10 days at 4, 8, 12 and 20 °C while enumerating bacteria. Contaminated mangoes were disinfected using UV-C (2.5, 5, 7.5 and 10 kJ/m^2), AEW, NEW and sodium hypochlorite (SH) and the microorganisms were monitored. None of the enterobacteria grew at 4, 8 and 12 °C regardless of having persisted during the 10-day period. At 20 °C, *E. coli and C. sakazakii* grew, after adaption phases of 48 h and 24 h, to values of 8.7 and 8.5 log cfu/g at day eight, respectively. *E. coli* showed the highest reduction counts on the MPM washed with NEW and SH (2.2 log cfu/g). UV-C was more effective in reducing *C. sakazakii* (2.4–2.6 log cfu/g), when compared to AEW, NEW and SH (1.2–1.8 log cfu/g). The efficacy of decontamination technologies depends on microorganisms, highlighting the importance of preventing contamination at the primary production and of combining different methods to increase the safety of fresh-cut fruits.

1. Introduction

According to FAO (2016), the worldwide production of the most popular tropical fruit (mango, pineapple, avocado and papaya) is expected to grow by 3.0% annually over the next decade, which will result in about 116 million tons by 2024 compared with the 84 million tons attained in the period of 2012–2014. The total production of mangoes, which is the largest, followed by pineapple, papaya and avocado, is estimated to increase to 58 million tons by 2024, increasing at an annual rate of 2.8% over the next decade. The top ten mango producers will continue to contribute to about 80 percent of the world's supply, with Asia (60%), Africa (14%) and Latin America (25%), being the biggest producers.

The increase in mango production resulted from the consumers' growing demand, driven by the excellent organoleptic characteristic of this fruit, namely the color, texture, succulence and unique flavor as well as its nutritional properties (Siddiq et al., 2013; Oliveira et al., 2016). The fruits are either used in the food industry for the fabrication of canned fruit, jams and concentrated pulps or may be eaten fresh or after minimal processing (fresh-cut fruits). Due to the fact that the production and post-harvest processing steps are different among the producers, and carried out based on various safety principles, the microbial quality of these fruits is a concern (Strawn et al., 2011; Penteado et al., 2004, 2014; Penteado, 2017). Human microbial pathogens can contaminate food of plant origin during the different phases of production: in the fields, during harvesting, processing, distribution, marketing and preparation for consumption. For example, in a multistate outbreak resultant from the consumption of cantaloupe, in the USA, the contamination was attributed to deficient agricultural practices and hygienic conditions (CDC, 2012). Regarding post-harvest procedures, Penteado et al. (2004) showed that 'Tommy Atkins' mangoes, can become contaminated with *Salmonella enterica* in case the washing water used during the hot treatment is contaminated. High temperatures of the washing/rinsing waters may contribute to the internalization of pathogens eventually present in

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the water (Penteado, 2017). Thus, the microbial quality of waters used in the post-harvest phases is important. This procedure is often used by some producers/packers to eliminate flies' larvae. In all the steps, incorrect human handling is a significant source of contamination (Abadias et al., 2008a; Beuchat, 2002; Graça et al., 2015, 2017a).

Salmonellosis is amongst the most common cases of outbreaks in the United States due to fresh produce consumption, of which some have been associated to mango contaminated with *Salmonella* Newport, in 1999 (Sivapalasingam et al., 2004) and *S.* Saintpaul in 2001, in the USA (Beatty et al., 2004), and *Salmonella* Braenderup in 2012 (CDC, 2012) in the USA and Canada. The second most relevant etiologic agent of food borne diseases are the pathogenic strains of *E. coli. E. coli* 0157:H7 was the casual agents of outbreaks resulting from the ingestion of other fruits such as Cantaloupe and pineapple (Sivapalasingam et al., 2004). In 2006 an outbreak caused by *E. coli* 0157:H7 in spinach, in the United States and Canada was also described (Calvin, 2007).

Additionally, other members of the *Enterobacteriaceae* family may occur naturally on plant material, namely the genera *Pantoea*, *Klebsiella, Pectobacterium* (Leff and Fierer, 2013) and *Cronobacter* (Garbowska et al., 2015; Kucerova et al., 2011; Schmid et al., 2009; Vojkovska et al., 2016), which have been isolated from fruits and/or vegetables. Although naturally occurring bacteria could be epiphytic, their presence in fresh produce may be involved in spoilage processes, but also a possible threat for human infections (Kim and Beuchat, 2005). In the case of *C. sakazakii*, an emergent pathogen for neonates and immunocompromised adults, it has been isolated from various plant food origin, though not associated to outbreaks resulting from the consumption of fruits. However, *C. sakazakii* showed a significant growth in fresh-cut 'Royal gala' apple, 'Rocha' pear, and 'Piel de sapo' melon, at 12 and 20 °C (Santo et al., 2016).

Previous studies have reported that pathogenic enterobacteria can survive and grow in mango. Strawn and Danyluk (2010) describe that five different serovars of *S. enterica* were able to grow on 'Tommy Atkins' mangoes at 23 °C and 12 °C, as opposed to *E. coli* O157:H7 that could not grow. Ma et al. (2016), presented results showing that the sevorars of *S. enterica* tested on the Palmer cultivar were not able to grow at 28 °C and 4 °C. However, *S. enterica* Enteritidis was able to grow in pasteurized Palmer mango pulp, at 25 °C, after an adaptation phase of 19 days (Penteado et al., 2014).

In general, data on the behavior of human pathogens on tropical fruits remain limited, though they are necessary to increase the consumers' confidence and satisfaction and evaluate the potential microbial risks associated to them. Additionally, in the flowchart of minimally processed fruit production, a phase of disinfection is mandatory, and washing with solutions of sodium hypochlorite is the most common method. However, this method has been attributed to various drawbacks, namely its safety and the consequences of its application for man and environment (Castro-Ibáñez et al., 2016; Pezzuto et al., 2016; Meireles et al., 2016; Ramos et al., 2013).

Electrolyzed water (acid electrolyzed water and neutral electrolyzed water) and short wave Ultraviolet-C (UV-C) illumination (Bintsis et al., 2000; Huang et al., 2008; Ramos et al., 2013) are decontamination technologies that can be applied to fresh vegetables and fruits as alternatives to sodium hypochlorite solutions. The efficacy of electrolyzed water has been demonstrated on utensils and surfaces (Rahman et al., 2016) and various food products of plant origin, such as fresh-cut apple (Graça et al., 2011), blueberries (Kim and Hung, 2012), fresh-cut pear (Graça et al., 2017b), broccoli (Martínez-Hernández et al., 2015), cilantro (Hao et al., 2015), lettuce, carrot and endive (Abadias et al., 2008b) and tomatoes and lettuce (Pangloli and Hung, 2011). The efficacy of electrolyzed water was also observed in animal origin food products such as pork (Rahman et al., 2013), chicken, and beef as well as fish (Al-Holy and Rasco, 2015). On the other hand, UV-C was also used as a substitution for sodium hypochlorite solutions in order to decrease microbial populations and/or lengthen shelf-life of apples (Graça et al., 2013), apricots (Yun et al., 2013), kiwifruit (Beirão-da-Costa et al., 2014), mango and pineapple (George et al., 2015), melon (Manzocco et al., 2011), pear (Graça et al., 2017b) and watermelon (Artés-Hernández et al., 2010).

Therefore, the objective of the present work was to study the survival and growth of *Escherichia coli* and *Cronobacter sakazakii* on fresh-cut mango of the cultivar 'Tommy Atkins' at different temperatures. Additionally, the effect of the UV-C irradiation, acidic electrolyzed (AEW) and neutral electrolyzed (NEW) waters on the populations of the two *Enterobacteriaceae* species on contaminated fresh-cut mango was studied.

2. Methods

2.1. Mango preparation

Imported 'Tommy Atkins' (cv) mangoes (*Mangifera indica*) were bought in local supermarkets and prepared on the day the study began. Fruits were washed in running tap water and disinfected by immersion and scrubbing with a sodium hypochlorite solution (0.5%) during 30 s. After drying at room temperature, mangoes were aseptically peeled and, with a sterile cork borer, cut in pieces of 1 g each (1 cm long and radius 0.6 cm) without the kernel and skin and used in the microbial growth experiments. Using a disinfected knife, 10 g of these unpeeled pieces were prepared to test the physical and chemical decontaminations.

2.2. Enterobacteria and inocula preparation

The microorganisms used in this study were *Escherichia coli* (the non-toxicogenic strain of *E. coli* O157:H7 NCTC 12900, *E. coli* ATCC 25922 and *E. coli* ATCC 10536) and *Cronobacter sakazakii* [a strain isolated from a baby, ATCC BAA 894 and a strain isolated from soil, 4E (Universidade do Algarve) (Santo et al., 2016)], prepared in a mixture (cocktail). The enterobacteria were stored at −80 °C and maintained on Tryptone Soy Agar (TSA) (Oxoid, Hampshire, UK) at 4 ± 1 °C. To contaminate the fruit, the bacteria were grown on TSA at 37 ± 1 °C for 24 ± 2 h, following a growth in 50 mL of Tryptic Soy Broth (TSB) (Biokar Diagnostics, Allonne, France) at 37 ± 1 °C in an orbital shaker (VWR, Incubating Mini Shaker, USA) at 150 rpm, during 24 h. The bacterial cells were recovered by centrifugation (15 min at 9016 g) (Heraeus, Multifuge 1 L-R, Germany) originating a pellet that was resuspended in 50 mL of sterile saline peptone [8.5 g/L NaCl (Panreac, Barcelona, Spain) and 1 g/L peptone (Biokar)] to prepare suspensions with 10^7 cfu/mL which were used as fresh-cut mango inocula. The adjustments of the bacterial counts were made using a standard curve, measuring the transmittance at 420 nm in a spectrophotometer (Spectrophotometer UV–Vis, 175 Shimadzu-UV160, USA). The bacterial concentration of the inocula were checked using the colony count method described by Miles and Misra (1938), inoculating droplets (20 μL) of ten-fold dilutions on the surface of the TSA medium, in triplicate, following an incubation of the plates during 24 ± 2 h at 37 ± 1 °C.

2.3. Growth of E. coli and C. sakazakii on fresh-cut mangoes

The mango portions (1 g) were contaminated by "dip inoculation" through immersion in 10^7 cfu/mL suspensions of *E. coli* and *C. sakazakii* separately during 3 min at 150 rpm in an orbital shaker. Next, the mango pieces were dried in a laminar flow hood (Bioquell,