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Behavior of *Salmonella* spp. on fresh-cut tropical fruits

Chen Ma*, Jianguo Li, Qun Zhang

Hainan Provincial Key Laboratory of Quality and Safety for Tropical Fruits and Vegetables, Laboratory of Quality & Safety Risk Assessment for Tropical Products (Haikou) Ministry of Agriculture, Analysis and Testing Center, Chinese Academy of Tropical Agriculture Sciences, Haikou 571101, PR China

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ABSTRACT

The behavior of *Salmonella* spp. on fresh-cut dragon fruit, banana, starfruit, mango, pineapple, guava and wax apple at 28 °C and 4 °C was studied. Growth of *Salmonella* on the fresh-cut starfruit was studied at 7, 10, 15, 20, 25 or 30 °C. Tropical fruits were cut into cubes and spot inoculated with three-strain cocktail of *Salmonella* spp. at four inoculum levels (0.1, 1.0, 2.0 and 3.0 log CFU/g). Results indicated that *Salmonella* grew well at 28 °C on all tested fruits at different inoculum levels [growth potential (δ) = 2.57–4.95] except for mango and pineapple, with the maximum populations ranging from 2.67 to 6.95 log CFU/g. Starfruit was the most suitable matrix for *Salmonella* growth. *Salmonella* exhibited a poor growth on mango and no growth on pineapple. At 4 °C for 6 days, no obvious *Salmonella* growth was observed on all the tested fruits. In addition, *Salmonella* growth was inhibited by different ratios of mango or pineapple ethanol extracts to Luria–Bertani (LB) broth. The inhibition percent of pure mango or pineapple ethanol extracts on *Salmonella* growth was 96.3% or 108.9%, respectively. Predicted growth parameters fitted by the Baranyi and Robert model indicated that on fresh-cut starfruit, *Salmonella* spp. grew immediately at 25 and 30 °C with no lag time and grew slowly at 7–20 °C with lag times of 74.74, 34.19, 9.32, 2.61 h. The predicted growth rate (μ_{max}, log CFU/g/h) for *Salmonella* on starfruit was 0.0048 ± 0.00022, 0.011 ± 0.00067, 0.039 ± 0.0027, 0.088 ± 0.0022, 0.19 ± 0.0061, 0.29 ± 0.023 at 7, 10, 15, 20, 25 and 30 °C, respectively. At higher storage temperatures, the growth rate and maximum population of *Salmonella* were higher. This study suggests that *Salmonella* may grow and reach high populations on fresh-cut tropical fruits depending on storage conditions except for mango and pineapple and should be stored at low temperatures (<4 °C) to ensure the safety and extend the shelf life of fresh-cut tropical fruits.

1. Introduction

Approximately 140 million tons of over 3000 types of tropical fruits are produced annually worldwide (Strawn et al., 2011). It is forecasted that the world production and expansion of the tropical fruit trade will take place over the next 10 years (Yusuf and Salau, 2007). China is one of the largest tropical fruit producing countries, and is also the largest importer in the world (FAO, 2009). Vietnam, Philippines, Thailand and Malaysia are the main exporters to China. Banana, mango, pineapple, papaya and dragon fruit are the favorite tropical fruits, often consumed in whole forms, freshly cut and as juice in markets in China. In the majority of regions producing tropical fruits, no uniform food safety standards exist in production and postharvest practices, and outbreak surveillance systems and pathogen detection capabilities do not exist or are very poor, thus the safety of these products remains a concern (Strawn et al., 2011).

Outbreaks of foodborne disease have occurred because of the consumption of various tropical fruits, including mango, papaya, pineapple, coconut, banana, dragon fruit, avocado and mamey (CDC, 2003, 2005; Pui et al., 2010; Strawn et al., 2011). *Salmonella* is the leading bacterial pathogen (Strawn et al., 2011). Multiple serovars of *Salmonella* have caused foodborne illness associated with tropical fruits, such as Saintpaul, Heidelberg, Litchfield, Typhi, Newport, Paratyphi, Senftenburg (Beatty et al., 2004; CDC, 2004; Gibbs et al., 2009; Kzta et al., 2002; Sivaapalasingam et al., 2003; Teoh et al., 1997; Wilson and Mackenzie, 1955). In China, salmonellae are responsible as the top bacterial pathogen for reported foodborne disease outbreaks (NHFPC, 2014). Tropical fruits contain ample sugars and high water activity, providing conditions for microbial growth and survival (Bassett and McClure, 2008). Previous studies have reported that *Salmonella*, *Escherichia coli* O157:H7, *Listeria monocytogenes* and other pathogens can survive or grow on

* Corresponding author.
E-mail address: mc19860112@163.com (C. Ma).

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the pulp, juice and freshly cut surfaces of tropical fruits such as mango, papaya, coconut, guava, dragon fruit, pineapple and avocado held at temperatures between 4 and 37 °C or at a frozen temperature (−20 °C) (EI-Safey, 2013; Mutaku et al., 2005; Penteado et al., 2014; Strawn and Danyluk, 2010a; Sim et al., 2013; Walter et al., 2009). However, data on the behavior of human pathogens on or in tropical fruits and their associated products remain limited. Additionally, several tropical fruits in different parts (pulp, peel, seed, wastes, etc.) also exhibited antimicrobial activity against human pathogens to varying degrees (Das, 2012; Joshi et al., 2012; Mohamed et al., 1994). Anti-microbial effects of pineapple peel, mango peel and seed kernels have also been documented (Chanda et al., 2010; Engels et al., 2011; Gupta et al., 2012; Kabuki et al., 2007; Tewtrakul et al., 2008). Natural bioactive compounds in tropical fruits may be of use as a good source of antimicrobial agents against human pathogens.

In many Asian countries, fresh-cut fruits are often displayed and sold without any refrigeration and proper packaging (James and Ngarmsak, 2010). In warm and humid climates in tropical countries or during the summer season, there may be higher chances of *Salmonella* outbreaks (Lee and Lam, 1996) as the bacteria could survive or grow on contaminated fresh-cut fruits because of the temperature fluctuation during refrigeration. The purpose of this research was to determine the behavior of *Salmonella* spp. on seven types of tropical fruits [dragon fruit (*Hylocereus undatus*), banana (*Musa nana Lour*), starfruit (*Averrhoa carambola* Dah Pon), mango (*Mangifera indica* Palmer), pineapple (*Ananas sativus* Comte de Paris), guava (*Psidium guajava* Pearl) and wax apple (*Syzygium samarangense* Black Diamond)] at ambient and refrigeration temperatures. The negative effect of selected fruit ethanol extracts on the growth of *Salmonella* spp. was also investigated. In addition, the growth of *Salmonella* spp. on fresh-cut starfruit as a function of temperature (7–30 °C) was modeled separately using both primary and secondary models. The results obtained from this research will be helpful for regulatory agencies to conduct a risk analysis of tropical fruits contaminated with *Salmonella*.

2. Materials and methods

2.1. Microorganisms and inoculum preparation

A cocktail of three serotypes of *Salmonella* (two isolated from produce outbreaks) were used in this study: *S.* Newport (ATCC 6962; tomato outbreak), *S.* Saintpaul (ATCC 9712; cantaloupe outbreak) and *S.* Enteritidis (ATCC 13076). Prior to each experiment, frozen stock cultures (−20 °C) of each strain were streaked onto tryptic soy agar (TSA) and incubated at 37 °C for 24 h. A single colony from each strain was transferred to 5 ml of tryptic soy broth (TSB) and incubated at 37 °C for 24 h. Then, 1 ml aliquots were transferred to 50 ml fresh tryptic soy broth and incubated at 37 °C for 18–20 h. The culture was centrifuged at 4 °C for 10 min at 4000 × *g*. The cells were washed twice by removing the supernatant and suspending the cell pellet in sterile 0.1% peptone water. Equal aliquots (10 ml) of each *Salmonella* serovar were aseptically combined to produce a cocktail of three serovars with a final cell concentration of approximately 8.0 log CFU/ml.

2.2. Tropical fruit preparation, inoculation with pathogens and storage temperature

Seven types of common tropical fruits were purchased from a supermarket in the city of Haikou, China. All the tropical fruits were selected based on their production and consumption in China, including dragon fruit (*H. undatus*), banana (*M. nana Lour*), starfruit (*A. carambola* Dah Pon), mango (*M. indica* Palmer), pineapple (*A. sativus* Comte de Paris), guava (*P. guajava* Pearl) and wax apple (*S. samarangense* Black Diamond). All fruits were individually washed in sterile distilled water and surface-sterilised with 70% ethanol prior to the peeling and cutting process. Banana, dragon fruit and pineapple were peeled using a flame sterilized knife. Then the flesh of the three fruits and other whole fruit were cut into cubes (ca. 2.0 cm by 2.0 cm by 2.0 cm) using a sterile knife on a sterilized cutting board. The cubes of each fruit were disinfected by immersion in a solution of 200 ppm chlorine for 15 min, and rinsed using sterile distilled water four times and then allowed to dry in the biosafety cabinet for 1 h (Danyluk et al., 2014; SantAna et al., 2012). Human pathogens test indicated that no human pathogens were observed on both untreated and treated fruits.

Portions of 25 g of each fruit (cubes) were packaged in sterilized plastic bags (Stomacher) individually and spot inoculated with 1.0 ml cell suspension in each plastic bags to reach the inoculum levels of 0.1, 1.0, 2.0 and 3.0 log CFU/g, respectively. The cell suspension was obtained from serial dilutions of *Salmonella* inocula (8.0 log CFU/ml) in 0.1% peptone (9.0 ml) to ca. 3.0×10^4, 10^3, 10^2, 10 CFU/ml (equal to ca. 4.47, 3.47, 2.47 and 1.47 log CFU/ml). The population was enumerated on Xylose lysine deoxycholate (XLD) agar and CHROMagar chromogenic agar. Samples inside of the open stomacher bags were held in a biosafety cabinet to dry at ambient temperature for 1 h. After drying, the bags were folded over once and placed in incubator. All the samples were stored at 28 ± 2 °C (2 days) or 4 °C (6 days). To study *Salmonella* growth on the starfruit, 25 g starfruit cubes packged in sterilized plastic bags were prepared as above and spot inocubated with 1.0 ml 4.47 log CFU/ml cell suspension and then were stored at one of six temperatures: 7, 10, 15, 20, 25 or 30 °C. Control samples were prepared by repeating the same procedure above in non-inoculated sterile distilled water.

2.3. Enumeration of pathogens

Samples were collected at different time intervals and were analyzed for *Salmonella*. The time intervals varied depending on the storage temperature and ended once the microorganisms reached the stationary phase. To study the behavior of *Salmonella* on seven tropical fruits, bacterial populations were enumerated after 2 days at 28 ± 2 °C and 6 days at 4 °C. For the samples of starfruit held at 7, 10, 15, 20 °C, bacterial populations were enumerated every 12 h for the first 3 days and every 24 h for the last 3 days. For the samples of starfruit held at 25 or 30 °C, bacterial populations were enumerated every 40 min within fist 4 h, and every 4 h on the first day and every 24 h for the last 5 days. At each sample point, 25 g samples were combined with 225 ml 0.1% peptone water, and stomached for 2 min in a stomacher (Model BagMixer, Interscience Co., France). Serial dilutions of each sample were plated onto Xylose lysine deoxycholate (XLD) agar and CHROMagar chromogenic agar, respectively for *Salmonella* sp. and onto TSA for natural microbiota, and incubated at 37 °C for 24–48 h. The bacterial colonies were counted and converted to the logarithm of base 10, recorded as log CFU/g (converted to the logarithm of the natural base, recorded as Ln CFU/g when used in Ratkowsky square-root model).

2.4. Negative effect of selected fruit ethanol extracts on the Salmonella growth

To determine the negative effect of selected fruit ethanol extracts on *Salmonella* growth, the culture medium was a mixture of 0, 1/1, 5/1, 10/1 and 100% fruit ethanol extracts to Luria–Bertani (LB) broth. The samples were stored at 28 ± 2 °C with the inoculum level of 2.5–2.8 log CFU/ml. Fruit ethanol extracts were obtained as the following procedure (Abdullah et al., 2012): 50 g fruit homogenates was extracted with 100 ml ethanol at 60 °C for 4 h, and then filtered