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Short communication

Behavior of *Salmonella* spp. and natural microbiota on fresh-cut dragon fruits at different storage temperatures

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ABSTRACT

The aim of this study was to determine survival or growth of unadapted, acid-adapted and cold-stressed *Salmonella* spp., and natural microbiota on fresh-cut dragon fruits at different storage temperatures. Dragon fruits were sliced and spot inoculated with five-strain cocktail of *Salmonella* spp. at two inoculum levels (2.5 or 5.5 log CFU/g). Inoculated fruits were stored at 28 °C for 48 h and at 4 °C and 12 °C for 96 h. *Salmonella* population significantly increased by 2.4 to 3.0 log CFU/g at low inoculum level, whereas the numbers increased by 0.4 to 0.7 log CFU/g at the high inoculum level on fruits held at 28 °C for 48 h. Only unadapted and acid-adapted cells grew with 0.7 to 0.9 log increase at the low inoculum level at 12 °C for 96 h. No significant growth was observed at both inoculum levels during storage at 4 °C. Overall, acid, starved and cold adaptation of *Salmonella* spp. did not show significant difference in survival or growth on fresh-cut dragon fruits during storage compared to unadapted control cells. For natural microbiota on the fruit, mesophilic bacterial counts reached to 5-log CFU/g at 28 and 12 °C by 9.9 and 52.9 h. Similar with *Salmonella* spp. there was no growth of natural microbiota at 4 °C. These results showed that *Salmonella* spp. could grow on fresh-cut dragon fruits under inappropriate storage conditions, indicating that fresh-cut dragon fruits could be a potential vehicle for salmonellosis. Thus, this study suggests that fresh-cut dragon fruits should be stored at 4 °C to ensure the safety as well as to extend the shelf life of fresh-cut dragon fruits.

1. Introduction

Salmonella is one of the leading causative agents in Singapore and a total of 1480 laboratory-confirmed cases of nontyphoidal salmonellosis were reported in 2010 (MOH, 2011). In the United States, a total of 49,192 cases of salmonellosis were reported during 2009, according to the National Notifiable Disease Surveillance System (NNDSS) data (CDC, 2011a). There have been several salmonellosis caused by consumption of fresh-cut fruits. In 1996, the likely source of *S.* Weltevreden outbreak came from fruit samples (honeydew, papaya, pineapple, and watermelon) taken from a fruit stall at Jurong shipyard in Singapore (Oio et al., 1997). An outbreak of *S.* Litchfield occurred in Australia in 2006 and was linked to consumption of fresh-cut papaya (Gibbs et al., 2009). For both cases, investigators suspected the use of untreated water to wash the fruits resulted in *Salmonella* outbreak. In 2008, most of the outbreak-related illnesses in USA were caused by *Salmonella* in fruits and nuts (CDC, 2011b).

It is forecasted that the expansion of tropical fruit trade and world productions are to take place over the next 10 years (Yusuf and Salau, 2007) and the dragon fruit market is also facing similar trends. Vietnam, which is one of the largest dragon fruit exporting countries, reported that 90% of its dragon fruit export quantity goes to the Asian market in 2004 (Axis research, 2005). Moreover, in 2011, dragon fruit exports to the United States (US), Japan and South Korea are expected to double (2600 tonnes) as compared to previous year (Vietnam Investment Review, 2011). There are many different species of dragon fruits found in the global market, especially in the tropical and sub-tropical regions, and the present study focused on *Hylocereus undatus*. It is a non-climacteric fruit covered with rosy-red skin studded with green scales and its white flesh contains many small black seeds (Le Bellec et al., 2006). Dragon fruit also has a pH ranging between 4.7 and 5.1 and a Brix value ranging between 11 and 19 °Brix (Gunasena et al., 2007). Although there were no reported outbreaks of salmonellosis in fresh-cut dragon fruits, a previous study revealed that dragon fruits from hawker stalls showed the highest prevalence of *Salmonella* spp. (75%), *S.* Typhi (40%) and *S.* Typhimurium (25%) out of all the fruits examined (Pui et al., 2010), indicating the likelihood of salmonellosis by consumption of contaminated dragon fruit. Moreover, there is a high possibility of bacteria infestation on contaminated fresh-cut dragon fruits which are improperly refrigerated as previous studies have reported *Salmonella* survival and growth on acidic fresh-cut fruits such as papayas, watermelons, honeydews, apples and peaches held at temperatures ranging between 5 and 25 °C (Escartin et al., 1989; Golden et al., 1993; Snyder, 1999; Leverentz et al., 2001; Penteado and Leitao, 2004; Alegre et al., 2010a, 2010b).

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It is also common for bacteria to undergo various stress conditions (Abee and Wouters, 1999) from pre- to post-harvesting stages where subsequent handling and processing of foods are required. For instance, acidic soil and fruits can induce acid stress on *Salmonella* (Gunasena et al., 2007; Patil et al., 2009) while pathogens which are attached to equipment surfaces or any abiotic surfaces with low or no available nutrients can experience starvation stress (Lou and Yousef, 1996). Cold storage of fruits during transportation also induces cold stress on *Salmonella* and other microorganisms (Axis research, 2005; Rodriguez-Romo and Yousef, 2005). These stress conditions may influence bacterial growth on fresh-cut fruits.

The objective of this study was to determine the behavior of unadapted, acid-adapted, starved and cold-stressed *Salmonella* spp. on fresh-cut dragon fruits at ambient and refrigeration temperatures in order to investigate the effects of temperature abuse and stressed conditions on growth of *Salmonella*. Survival and growth of pathogens at two different inoculum sizes were also examined to determine the effect of inoculation level on the ability of the bacteria to overcome environmental conditions and grow. In addition, growth of inherent microbiota on the fruit was observed under different storage temperatures and modeled to predict the microbiological safety of fresh-cut dragon fruit.

2. Materials and methods

2.1. Measurement of pH, titratable acidity and Brix

Dragon fruits (*H. undatus*) were purchased from local fruit stores in Singapore. The fruits used in this study were ripe with no visible microbial growth and were free from visible physical defects. The fruits were homogenized and the pH of the sample was measured using a calibrated pH meter (model 744, Metrohm, Riverview, FL, USA). Brix was determined using a refractometer (model RX-5000CX, ATAGO, Honcho, Itabashi-ku, Tokyo). Titratable acidity was determined by titrating dragon fruit juice with 0.01 N NaOH (Sigma-Aldrich, St. Louis, MO, USA) to an end point at pH 8.1 and expressed as percent citric acid (Lum and Norazira, 2011). The sugar–acid ratio of the fruit was calculated as soluble solids (°Brix) divided by titratable acidity (%).

2.2. Preparation of inoculum

The five *Salmonella* serovars used in this study were *S.* Newport (ATCC 6962; tomato outbreak), *S.* Agona (ATCC BAA-707; alfalfa sprout outbreak), *S.* Saintpaul (ATCC 9712; cantaloupe outbreak), *S.* Montevideo (ATCC BAA-710; tomato outbreak) and *S.* Gaminara (ATCC BAA-711; orange juice outbreak) and were purchased from American Type Culture Collection (ATCC; Manassas, VA, USA). Frozen cultures were activated in 10-ml tryptic soy broth (TSB; Oxoid, Basingstoke, Hampshire, England). All cultures were adapted to 150 μg/ml nalidixic acid (Sigma-Aldrich) by stepwise increment of nalidixic acid after each transfer of the respective culture. All media used in this study were supplemented with 150 μg/ml nalidixic acid so that *Salmonella* cells isolated from inoculated dragon fruits were relatively free from other background bacterial contaminants (Beuchat and Mann, 2008; Strawn and Danyluk, 2010a, 2010b). Nalidixic acid resistant *Salmonella* serovars were transferred weekly to maintain viability. Prior to inoculation, each nalidixic acid resistant serovar was incubated in 10 ml of TSB supplemented with 150 μg/ml nalidixic acid (TSBN) at 37 °C for 24 h with two consecutive transfers. The cultures were centrifuged at 3000 *g* for 10 min at 4 °C and washed twice by removing the supernatant and suspending the cell pellet in 1 ml of 0.1% peptone (Oxoid). Equal aliquot (1 ml) of each *Salmonella* serovar was aseptically combined to produce a cocktail of five serovars with the final inoculum concentrations of approximately 8.0 log CFU/ml.

2.3. Preparation of acid-adapted cells

Acid-adapted cells were prepared by transferring 0.1 ml of respective nalidixic acid resistant *Salmonella* strains into 9.9 ml of TSBN supplemented with 1% glucose (Sinopharm chemical reagent, Shanghai, China) and incubated at 37 °C for 24 h with two consecutive transfers prior to use (Beuchat and Mann, 2008). Before inoculation on the fresh-cut dragon fruit, acid-adapted cells were washed and prepared as a cocktail as described above.

2.4. Preparation of cold-stressed and starved cells

To prepare cold stressed cells, cells grown in TSBN were centrifuged at 3000 *g* for 10 min at 4 °C. The cells were washed as described above followed by resuspension of pelleted cells in 1 ml fresh TSBN. The cocktail of five serovars was prepared and subsequently stored at 5 °C for 5 days (Bang and Drake, 2002). For starved cells, the cells were washed as described above followed by resuspension of pelleted cells in 1 ml phosphate buffered saline (PBS; 1st Base Pte Ltd, Singapore) (Wesche et al., 2005). The cocktail of five serovars was prepared and subsequently stored at 37 °C for 2 days (Bang and Drake, 2002).

2.5. Inoculation on dragon fruits

The dragon fruits were peeled and cut into 10 g wedge slices (3 cm length, 3.5 cm width and 0.5 cm height) using a flame-sterilized knife. 10-μl of inoculum was spot inoculated on the cut surface of the fruit at two inoculum levels (2.5- or 5.5-log CFU/g). The inoculated fruits were air-dried in a biosafety cabinet for 30 min at room temperature and were placed into sterile stomacher bags. Each bag was folded over once and stored in an incubator at 4 or 12 °C for 0, 1, 2, 3 and 4 days, or 28 °C for 0, 6, 24, 30 and 48 h.

2.6. Growth of natural microbiota

Dragon fruits without inoculation were also stored at 4 or 12 °C for 0, 8, 24, 32, 48, 52, 56, 72, 80 and 96 h, or 28 °C for 0, 2, 4, 6, 8, 10, 24, 30, 32 and 48 h to observe growth of aerobic mesophilic and psychrotrophic microbiota during storage at each temperature. Growth curves of natural microbiota at 12 and 28 °C were fitted to the modified Gompertz model using the following equation (Zwietering et al., 1990);

$$\log x = A + C\exp\left(-\left(\left(2.718\left(\frac{R_g}{C}\right)\right)(\lambda - t) + 1\right)\right)$$

where $A = \log x_0$(CFU/g), x_0 = the initial cell number, C = the asymptotic increase in population density (log CFU/g), Rg = the growth rate (log CFU/h), λ = the lag-phase duration (h), and t = time (h).

2.7. Enumeration

To enumerate the bacterial population on the fruits during storage, 90-ml of 0.1% peptone water was added into a stomacher bag containing the 10-g fruit and homogenized for 1 min using stomacher. After stomaching, serial dilutions were made with 0.1% peptone water and pour-plated onto trypticase soy agar (TSA; Oxoid) supplemented with 150 μg/ml nalidixic acid (TSAN) for *Salmonella* spp. and onto TSA for natural microbiota. Plates were incubated at 37 °C for 24 to 48 h for *Salmonella* spp. and aerobic mesophilic microbiota or for 10 days at 7 °C for psychrotrophic microbiota (Cousin et al., 2001), followed by manual counting of colonies. The populations of bacterial cells were expressed in log CFU/g of fruit flesh.