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Thermal death times of acid-habituated *Escherichia coli* and *Salmonella enterica* in selected fruit beverages

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A B S T R A C T

The study established the decimal reduction times at 60 and 73 °C (D_{60} and D_{73}) of each of acid-adapted cocktails of *Escherichia coli* (NRRL 3704, ATCC 8739, ATCC 92522) and *Salmonella enterica* serovars Typhimurium (NRRL B-4420), Typhi (NRRL B-573), and Enteritidis (Biotech 1963) in some fruit beverages. Tested beverages included apple and orange juices, which are most commonly reported vectors of diseases; and tropical fruit beverages such as mango, guava, and soursop nectars, which are not frequently used as suspending media in thermal inactivation studies. The fruit beverages had pH of 3.30–4.73, titratable acidities of 0.15–0.64% organic acid, and soluble solids of 11.13–14.33 °Brix. At 60 °C, *E. coli* and *Salmonella* were 1.26–3.13 and 1.21–2.33 folds, respectively, more resistant to heating than at 73 °C. At 60 °C, *E. coli* and *S. enterica* had D_{60} value ranges of 5.90 s (orange juice) to 12.42 s (guava nectar) and 7.50 s (orange juice) to 11.46 s (soursop nectar), respectively. At 73 °C, *E. coli* had D_{73} values ranging from 3.56 s (apple juice) to 5.82 s (soursop nectar), while those of *S. enterica* ranged from 3.59 s (guava nectar) to 9.74 s (soursop nectar). The variations in the observed heat resistance in both heating temperatures were attributed to the differences in the physicochemical properties of the suspending fruit beverages. The results obtained in this work contribute to further understanding the behaviors of these pertinent pathogens in heat-treated fruit beverages. These data also provide baseline information for the establishment of heat pasteurization process schedules for better control of fruit juice product safety.

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1. Introduction

The consumption of fruit juices is considered a convenient means of complying with the 5-serving recommended daily intake of fruits and vegetable for health and well-being (FNRI 1994; USDHHS and USDA 1998). However, unpasteurized fruit juice consumption has also been associated with a number of occurrences of outbreaks of infections. Pathogenic *Escherichia coli* and *Salmonella enterica* serovars have been demonstrated to cause infections due to contaminated apple and orange juice products, respectively (Harris et al., 2003). Hence, the United States Food and Drug Administration (USFDA) ratified the Fruit Juice Hazard Analysis Critical Control Point, mandating processors to subject their products to kill steps that will inactivate a pertinent target population by 5-log (99.999%) reduction (Federal Register, 2001).

The application of heat or thermal processing is a traditional yet very effective means of decontaminating food of pathogenic microorganisms (Buchanan & Edelson, 1999). In developing economies where a significant number of industry stake holders come from micro- and small scale processors, heating is an appropriate food processing technique as the technology is considered cheap and readily available (Mak, Ingham, & Ingham, 2001). The efficacy of heat treatment in inactivating microorganisms is however, dependent on intrinsic factors of food commodities such as pH, acidity, and soluble solids; and extrinsic processing parameters such as processing time and temperature (Gabriel, Barrios, & Azanza, 2008; Li Wan Po et al., 2002). Furthermore, implicit microbial characteristics have also been demonstrated to affect their susceptibility towards heat. Several studies have demonstrated the ability of *E. coli* and *S. enterica* to develop heterologous adaptive

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mechanism towards heat treatment when previously subjected to sublethal acid stresses (Mak et al., 2001; Mazzota, 2001; Sharma, Adler, Harrison, & Beuchat, 2005). Such dependence of efficacy therefore underscores the importance of establishing product-, process- and reference microorganism-specific thermal process schedules to ensure safety.

This study was conducted to establish the decimal reduction times of acid-habituated *E. coli* and *S. enterica* in a number of fruit beverages. Each of the tested organisms was composed of a cocktail of three different isolates/serovars to account for inter-isolate/serovar variation in heat susceptibility. Some of the fruit beverages used as suspending media included mango, guava, and soursop juices, which are tropical fruits not commonly used in microbiological challenge studies. The established results contribute to existing knowledge pool to further understanding how physiological state affects selected pathogen behavior in heated juices, and provide baseline information that may guide processors in designing appropriate thermal process schedules for specific fruit beverage types.

2. Materials & methods

2.1. Physicochemical analyses of the test juices

Five different brands of commercially available fruit beverages (with no declared added preservatives) were purchased from a local supermarket. To make sure of uniformity of physicochemical characteristics of the test beverages, the study took samples from the same production batches. The test fruit beverages included commercially available apple and orange juices; and mango, soursop, and guava nectars. The commercial designations of the tested beverages were according to label declarations, and further confirmed using the descriptions enumerated by Bates, Morris, and Crandall (2001). According to Bates et al. (2001) products declared as 'juice' are composed of pure fruit extracts that may or may have not been subjected to concentration and reconstitution. On the other hand, nectars usually contain 20–50% juice, sweetened and acidified, and may be clear or pulpy. All the nectar samples used in this study contained visible amounts of pulps that made the samples noticeable more viscous than the tested juices.

The fruit beverages were subjected to determination of pH using a digital pH meter (Philips PW9420, England) with an electrode previously calibrated with pH 4 and 7 buffers. Titratable acidity (TA, as % citric acid) of each sample was also determined potentiometrically by titration with standardized 0.1 N NaOH until pH 8.2. Finally, soluble solids (SS, °Brix) in the samples were determined using a handheld refractometer (Atago, Japan). All physicochemical analyses were conducted in triplicate.

2.2. Microbial isolates

The study utilized separate composites of three *E. coli* strains and three *S. enterica* serovars as test organisms. For the tested *E. coli*, nutrient agar (NA) slants containing strains 3704 of the Northern Regional Research Laboratory (NRRL 3704) and 8739 of the American Type Culture Collection (ATCC 8739) were obtained from the Philippine National Collection of Microorganisms, National Institute of Molecular Biology and Biotechnology of the University of the Philippines, Los Baños (NIMBB-UPLB). On the other hand, NA slant of the ATCC 25922 strain was obtained from the Natural Science Research Institute, University of the Philippines Diliman, Quezon City (NSRI-UPD). The NA slants of the tested *S. enterica* serovars, namely Typhimurium (NRRL B-4420), Typhi (NRRL B-573), and Enteritidis (Biotech 1963) were all obtained from the NIMBB-UPLB.

Working stock cultures were first prepared by obtaining cells from each of the slants, and separately transferring each strain or serovar into 1 ml sterile nutrient broth (NB, HiMedia, Mumbai India) before incubating at 37 °C for 24 h. Cells from the NB cultures were then transferred into sterile NA slants and incubated for another 24 h before storing at 4 °C. Weekly subculture of all organisms were conducted following previously described protocols.

2.3. Acid stress exposures and composite inoculum preparation

All reference strains were individually propagated and exposed to acid stress following previously described protocols by Parish, Goodrich, and Miller (2004) and Mak et al. (2001). Briefly, loop inoculum was obtained from each of the refrigerated culture slants, transferred into 3 ml NB, and subsequently incubated at 37 °C for 24 h. Acid stress exposure was then conducted by transferring the 3 ml cultures into 12 ml NB with pH adjusted to 5.0 using 0.25% w/v citric acid solution. The acidified cultures were then incubated for another 24 h at 37 °C. To prepare the composite inoculum, 10 ml of acid-stressed cell cultures were obtained from each of the 3 *E. coli* strains or 3 *S. enterica* serovars and combined in a sterile centrifuge tube. The composite acid-stressed cells were then harvested by subjecting the culture mix to centrifugation (REMI, Bombay, India) at 3500 *g* for 10 min. The supernate was immediately decanted and replaced with 5 ml test fruit beverage to come up with a suspension of 7.0 log acid-stressed *E. coli* or *S. enterica* cells/ml juice or nectar. The cells were allowed to acclimatize in the juice suspension for not longer than 15 min.

2.4. Thermal inactivation and inactivation kinetics characterizations

Screw capped test tubes containing 5 ml test fruit beverages were heated in a water bath (Citenco Co. Ltd., Somerset, UK). A separate control tube was inserted with a probe digital thermometer (Cooper DFP450W, China) through its cold point for temperature monitoring. When the fruit beverage temperature reached 60 or 73 °C, 0.5 ml aliquot of the acid-stress cell suspension was introduced into each tube and heated at different time intervals. Heated tubes were immediately withdrawn from the water bath and immediately placed into an ice bath. The suspensions were kept in ice for not longer than 15 min until subjected to survivor enumerations.

Survivor enumerations were conducted by subjecting the heated fruit juice beverage suspensions to serial ten-fold dilution with 0.1% peptone (BBL, Gelysate, Pancreatic Digest of Gelatin, Cockeysville, MD, USA) prior to surface-plating onto pre-solidified NA (HiMedia, Mumbai, India) and incubating at 37 °C fro 24 h. The populations of surviving *E. coli* and *S. enterica* were enumerated and reported as log CFU/ml. The inactivation kinetics of each challenge organism per heating temperature per suspending fruit beverage was determined by plotting the survivor population against heating time. The inactivation curves were traced from the plots by determining the best-fitted regression line. The inactivation kinetics was expressed in terms of the decimal reduction times (D values), which was equivalent to the negative inverse of the slope of the regressed line. In this study, D values were only obtained from inactivation curves that traversed at least 1 log cycle and with a coefficient of determination (R^2) $\geq$ 0.96.

2.5. Statistical analyses

The test fruit beverage physicochemical attributes and microbial D values were subjected to single-factor analyses of variance using the general linear model procedure of the SAS statistical software

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