Influence of Heating and Sodium Acidic Polyphosphate on Inhibition of Salmonella Enteritidis and Lactobacillus Rhamnosus in Pomelo Juice

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Abstract. The research focused on the heat resistance of Salmonella Enteritidis (S. Enteritidis) and Lactobacillus rhamnosus (L. rhamnosus) in pomelo juice. Sodium acidic polyphosphate was used to enhance the inhibition of these bacteria in heat treatment of pomelo juice. Temperature increased from 52 to 58°C, D-values of S. Enteritidis decreased from 1.94 to 0.15 min. With L. rhamnosus, D value reduced from 0.15 to 0.72 min when the temperature increased from 60 to 75°C. Z values of L. rhamnosus and S. Enteritidis were 16.31 and 5.37°C, respectively. It means that heat resistance of L. rhamnosus is more than that of S. Enteritidis. Adding 0.1% of sodium acidic polyphosphate significantly enhanced the inhibition of S. Enteritidis and L. rhamnosus in the heating treatment of pomelo juice. The result can be applied for pasteurization of pomelo juice.

1. Introduction

The foodborne outbreaks due to the consumption of contaminated unpasteurized fruit juices have led the Food and Drug Administration (FDA) to enact the HACCP program to ensure the safety of juice products. This program demands manufacturers to subject juice product treatment with a 5-logs reduction for the target pathogen. According to FDA, acidic juices (pH 4.6 or less) contaminated pathogens such as E. coli O157:H7, Salmonella spp, and Cryptosporidium parvum have caused foodborne serious illness outbreaks. In particular, Salmonella spp may be considered as the "pertinent microorganism" for citrus juice products due to the foodborne outbreak in orange juice product of a plant in Arizona [1] and a big outbreak diseases affecting more than 400 people in Australia associated to Salmonella enterica [2]. The symptoms of Salmonella poisoning are diarrhea, abdominal pain, mild fever, and chills [3]. Salmonella enterica is a common pathogen species in humans, causes more than...
one million illnesses and more than 350 deaths annually in the United States [4],[5]. Among them, *S. Enteritidis* is one of the most commonly causing of salmonellosis [6]. They can infect fruit juice products through contaminated water supplies, or inadequate control of pathogens during juice processing, which causes outbreaks. *Salmonella* does not grow but can survive in low pH fruit juices [7]. While, lactic acid bacteria *may not* cause illness; however, they cause spoilage of acid foods, especially, citrus juice [8].

These microorganisms can be easily eliminated through heat, but sensorial and nutritional attributes of fruit juice are extensively damaged. To reduce the thermal effects, sodium acidic polyphosphate has been applied to enhance bacteria inactivation, requiring a shorter time and lower temperature in heating treatment. Several studies have used polyphosphates to inhibit the growth of pathogens and spoilage bacteria successfully [9],[10]. Although polyphosphates are not mentioned as microbial inactivation agents by the FDA, the food industry uses polyphosphates with various functions, such as improving color, emulsifying, binding mixtures, absorbing water, and preventing oxidation [11] so polyphosphates are allowed to be used within a safe limit.

Pomelo juice (*Citrus grandis*) has been also called pommels, pummelo, and Chinese grapefruit juice, a rich bioactive resource inhibits or quench free radical reactions and delays or prevents cellular damage [12]. Beside, pomelo juice contains complex mixtures of aromatic volatiles include esters, aldehydes, ketones [13] and bitter, named naringin [14]. These compounds are easy to change after the traditional heating process causing low commercial pomelo juice value [15]. Thus, it is necessary to enhance the inactivation of bacteria in mild heating treatment to reduce the loss of juice quality.

Thus, the aim of study was to (1) evaluate the heat resistance of *S. Enteritidis* and *L. rhamnosus* in pomelo juice, (2) determine the decimal reduction time (D-value) and the decimal reduction temperature (z-value) of *S. Enteritidis* and *L. rhamnosus* in pomelo juice, (3) investigate inhibition of *S. Enteritidis* and *L. rhamnosus* by adding sodium acidic polyphosphate in heat treatment of pomelo juice.

2. Materials and methods

*Preparation of juice*

Pomelo fruits (*Citrus grandis*) were purchased from Binh Minh farms (Vinh Long, Viet Nam). The average weight of pomelo fruits was 1.0±0.2 kg. The age of fruits was 8 months from flowering. The fruit was peeled, followed by pressing with a squeezer (Fujiyama -FJ-400, sieve opening: 1 mm). Then, the juice was poured into the dark cylindrical tubes (2 cmx10 cm) and sterilized by autoclave at 121°C in 15 min. Then, it was cooled in ice water (0°C) until the temperature of juice reached 25°C and used for experiments. Brix and pH values of juice were 11.0 and 4.0, respectively.

*Sodium Acidic Polyphosphate (commercially called: Sporix)*

Sporix was manufactured by SDBNI Co., Ltd, Korea (INS No. 452 (i). EEC No. E450. CAS No. 68915-31-1) with P2O5 of 72 %.

*Microbiological procedures*

Freeze-dried culture of *S. Enteritidis* (ATCC 13076) was obtained from the American Type Culture Collection (USA). The treated procedures were prepared using the method of Park and Kang [16] with minor modifications. *S. Enteritidis* was plated on TSA and incubated at 37°C for 18 h. A culture was prepared by transferring a single colony of *S. Enteritidis* into 5 mL of TSB and incubated at 37°C for 18 h.

*L. rhamnosus* isolated from fermenting pomelo juice was used in this work. The culture of the *L. rhamnosus* strain was prepared as described previously [17] with minor modifications. A single colony of *L. rhamnosus* on deMan, Rogosa, Sharpe (MRS) agar was grown into 5 mL of MRS broth and incubated at 37°C for 24 h.
The pellets were collected by centrifugation at 4,000 g for 20 min at 4°C, washed three times with 0.2 % peptone water (PW), and then resuspended in 5 mL of PW. The cultures stored at 4 °C for use within 8 h, corresponding to a density of approximately 10^8-10^9 CFU/mL.

**Heat treatment with sodium acidic polyphosphate**

Each of the glass tubes with dimension 20 mmx100 mm (diameter x length) containing 9 mL of sterilized pomelo juice was heated in a water bath (WNB14, Memmert, Germany). The temperature in sample tubes was determined by a thermometer immersed in the control tube. When the juice reached the investigated temperature, 1 mL of culture was added into the juice to obtain approximately 10^7-10^8 CFU/mL. To investigating the influence of sodium acidic polyphosphate, sporix was added into the juice (0.1 % (w/v)). When the mixture (sporix-juice) reached a temperature of investigation, 1.0 mL culture was added into the mixture of juice. The samples were heated to investigated temperatures (52-60°C for *S. Enteritidis* and 60-75°C for *L. rhamnosus*) for 0 to 50 s, cooled immediately in an ice-water bath. One mL of the sample was pipetted for enumeration.

**Enumeration of *S. Enteritidis* and *L. rhamnosus***

The population of *S. Enteritidis* and *L. rhamnosus* was enumerated colony-forming units (CFU) using a surface spread-plate technique. The sample was serially diluted 10-fold by 0.2 % PW. Then, 0.1 mL of the diluted sample was spread onto selective media Xylose Lysine Deoxycholate (XLD) Agar for *S. Enteritidis* and De Man, Rogosa and Sharpe (MRS) agar for *L. rhamnosus*. In case that the bacterial cells survived at a low density, 1 mL of the undiluted sample was immediately spread on four Petri plates (about 250 μL per plate) with a diameter of 90 mm. Then, all plates were incubated at 37°C for 24-48 h before counting colonies. Colonies were counted from plates of appropriate dilutions (only use plates containing 30 to 300 colonies), the microbial populations were computed.

**Determination of D, z values**

Logarithm survivors of *S. Enteritidis* and *L. rhamnosus* were plotted against heating times at each temperature (1).

\[ \log N = \log N_0 - \frac{kt}{2303} = \log N_0 + s \cdot t \] (1)

where \( N_0 \) is the number of survival cells (CFU/mL) at time \( t = 0 \), \( N \) is the number of surviving cells (CFU/mL) after heat treatment, and \( t \) is treatment time (s) at a specified temperature. \( s \) is a slope of the survival curve. The decimal reduction time (D), the decimal reduction temperature (z) were calculated as described previously [18]. D-values were determined at each temperature by taking the negative inverse of the relevant s-value as in Figure 1 (a). The z values were determined by taking the negative inverse slope of the plot of log (D) versus temperature as in Figure 1 (b).

**Data analysis**

All experiments were carried out in triplicated. The result was stated as the mean +/- standard deviation. The significant difference of mean values was assessed with a one-way analysis of variance (ANOVA) followed by Fisher’s test using Excel software ver.2010 at a significance level of (P < 0.05).
3. Results and discussion

Heat resistant of *S*. *Enteritidis* and *L*. *rhamnosus* in pomelo juice

The survival of *S*. *Enteritidis* and *L*. *rhamnosus* in pomelo juice in heating treatment is showed in Figure 2. The population of *S*. *Enteritidis*, and *L*. *rhamnosus* decreased with an increase in treatment temperatures. At 60°C for 20 s, the level of log-reductions for *S*. *Enteritidis*, and *L*. *rhamnosus* was 5.2, and 0.9 logs, respectively. *S*. *Enteritidis* fell below the detection limit (1 log CFU/mL) at 60°C for 30 s, while *L*. *rhamnosus* was 75°C for 30 s.

![Figure 2. Survival curve in heat treatment of pomelo juice: (a) for *S*. *Enteritidis* (● 52°C, ■ 55°C, ▲ 58°C, ♦ 60°C) and (b) for *L*. *rhamnosus* (ο 60°C, □ 65°C, ▼ 70°C, ◊ 75°C)](image)

The D and z-values for *S*. *Enteritidis* and *L*. *rhamnosus* in the pomelo juice are showed in Table 1. Comparing to Mazzotta’s results, the D value of *S*. *Enteritidis* in pomelo juice in this study was lower than that in apple, orange, and white grape juice [19]. This author reported that the D-values of *Salmonella* (composite of serotypes *Enteritidis* and others) at 56 to 60°C were 1.21 and 0.23 min in apple juice, 2.52, and 0.45 min for orange, and 1.38 and 0.38 min in white grape juice. On the contrary, Gabriel and Nakano [20] reported that D values of *S*. *Enteritidis* in apple juice was 0.61 min at 55°C, lower than that in pomelo juice (0.98 min). z value in pomelo juice was 5.4°C, similar to that in orange juice (5.4°C), apple juice (5.7°C), and white grape juice (5.3°C) [19].

Results in Table 1 also indicate that D-values of *L*. *rhamnosus* in pomelo juice were re higher than that of *L*. *plantarum* in apple juice [17]. The authors reported that the D value of *L*. *plantarum* is 0.37, 0.14 min at 60, 70°C in apple juice, respectively.

| Temp (°C) | *S*. *Enteritidis* | *L*. *rhamnosus* |
|----------|-------------------|-----------------|
|          | D (min)           | z (°C)         | D (min) | z (°C)         |
| 52       | 3.60 ± 0.41       | 5.37           | 60      | 0.73 ± 0.10    |
| 55       | 0.98 ± 0.17       | 5.37           | 65      | 0.24 ± 0.04    | 16.31    |
| 58       | 0.15 ± 0.01       | 5.37           | 70      | 0.15 ± 0.01    |

The results also showed that spoilage bacteria, *L*. *rhamnosus* was more resistant against the lethal effect of temperature in comparison with the pathogenic bacteria, *S*. *Enteritidis*. These results are the same as other studies. For instance, Park & Kang showed *Salmonella* Typhimurium (*S*. Typhimurium) was below detection threshold (log (N) <1) in apple juice (pH 3.5, 11.8 °Brix) at 60°C for 30 s from initial density of 10⁷ CFU/mL [16]. Ananta & Knorr studied the heat resistance of *L*. *rhamnosus* in BPS at 75°C in 30 s and showed reductions approximately 7 logs [24].

3.1. Influence of sodium acidic polyphosphate on inactivation of *S*. *Enteritidis* and *L*. *rhamnosus* in heat treatment of pomelo juice.

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**Table 1. D and z values of *S*. *Enteritidis* and *L*. *plantarum* in heating**
Inactivation of *S. Enteritidis* and *L. rhamnosus* by adding sodium acidic polyphosphate (commercially called: Sporix) in heat treatment of pomelo juice is shown in Figure 3. The survival of *S. Enteritidis*, and *L. rhamnosus* towards treatment with acid sporix at 0.1 % (w/v) significantly more reduced compared to that without sporix. Heating with sporix added at 58°C, 20 s, the log-survivals of *S. Enteritidis* was 3.31, compared to 5.10 (CFU/mL) without adding sporix. In case of *L. rhamnosus*, heating with sporix added at 65°C, 20 s, the log-survivals (CFU/mL) were 4.31, compared to 5.41 without adding sporix. These results indicate that, sodium acidic polyphosphate significantly contributed into inactivation of *S. Enteritidis* and *L. rhamnosus* in heat treatment of pomelo juice.

Obritsch, Ryu *et. al.* also showed long-chain polyphosphates (P$_2$O$_5$ content of 67-69%) reduced the survival of gram-positive bacteria, *L. plantarum* at concentrations above 750 ppm, and gram-negative bacteria, *S. Typhimurium* at concentrations above 500 ppm [10]. Greater inhibition of bacterial growth is at higher polyphosphate concentrations until 5,000 ppm. The antimicrobial activity of a polyphosphate may be affected by the number of phosphorus units, with a higher inhibitory effect related to long-chain polyphosphates [9]. Sporix with P$_2$O$_5$ approximately 74 %, belongs to long-chain polyphosphates so it is suitable to inhibit microorganism. Influence of sodium acidic polyphosphate on inactivation of *S. Enteritidis* and *L. rhamnosus* could be explained by the formation of complexes between polyphosphate groups with metal ions [25].

![Figure 3](image-url)

**Figure 3.** Population of bacteria in heat treatment of pomelo juice with adding 0.1 % of sporix: (a) *S. Enteritidis* at 58°C, 20 s and (b) *L. rhamnosus* at 65°C, 20s

The cell walls of bacteria contain a layer of peptidoglycan, teichoic acid, which are linked to metals such as Ca$^{2+}$, Mg$^{2+}$, Mn$^{2+}$, Fe$^{3+}$ [26]. The main function of the anionic polymers in the cell wall is to maintain the concentration of divalent cations in the membrane area. Polyphosphates have a higher affinity for metal cations than both peptidoglycan and teichoic acid [27]. Thus, it is expected that the removal of essential cations from unique cation-binding sites inhibits bacteria development.

![Figure 4](image-url)

**Figure. 4** Survival curves by heating (●) and heating with sporix (■): for (a) *S. Enteritidis* at 58°C, and heating (○) and (b) heating with sporix (□) for *L. rhamnosus* at 65°C
The *S.* Enteritidis and *L.* rhamnosus had lower resistance with adding of sodium acidic polyphosphate in the heat treatment of pomelo juice (Figure 4). The D value of *S.* Enteritidis with sporix added was 0.08 min, compared to 0.15 min without adding sporix at 58°C. The D value of *L.* rhamnosus with sporix added was 0.11 min, compared to 0.24 min without adding sporix at 65°C. D-values of these organisms with sporix and temperature treatments reduced approximately 40 % compared to that only temperature treatments. Besides, *L.* rhamnosus should be considered as the target organism in pomelo juices, because the heat resistance of *L.* rhamnosus at the tested temperatures was higher than that of *S.* Enteritidis.

4. Conclusions

Heat resistance of *S.* Enteritidis and *L.* rhamnosus in pomelo juice was determined. Obtained D- and z-values indicate that heat resistant of *L.* rhamnosus was higher than that of *S.* Enteritidis. Adding of sodium acidic polyphosphate enhanced the inactivation of *S.* Enteritidis and *L.* rhamnosus in heating treatment of pomelo juice. With 0.1 % w/w of sodium acidic polyphosphate in the heating treatment, microbial density reduced approximately 10 folds. The adding of sodium acidic polyphosphate can be applied to improved quality of pomelo juice in pasteurization.

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