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Behavior of *Salmonella heidelberg* in fruit juices

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Abstract: The aim of the study is determination of the behavior of Salmonella heidelberg under acidic conditions and variable temperatures. The growth and survival of Salmonella heidelberg (10 isolates) in fresh fruit juices including; apple (pH, 3.2-1.4), orange (pH, 5.4 –1.5), mango (pH, 5.4-2.8), guava (pH, 5.7-2.4), pineapple (pH, 5.6-2.3), and cocktail (pH, 5.7-2.3) were determined after 0, 3, 6, 9, 12, 15, 18 and 21 days of storage at 10 ºC, 0, 3, 6, 9, 12 and 15 days of storage at 25 ºC, and 0, 3, 6 and 9 days of storage at 37 ºC. Survival in fruit juices depended upon their pH, the type of strain, the type of juices and the incubation temperature. Salmonella heidelberg Survived for up to 18 d in mango, guava, pineapple and cocktail juices, orange juice for up to 15 d and apple juice for up to 12 d stored at 10 ºC. At 20 ºC, Salmonella heidelberg was survived for up to 12 d in guava, pineapple and cocktail juices, 9 d for mango juice, orange juice for up to 9 d and apple juice for up to 6 d. The shortest survival time was observed at 37 ºC for 9 d in mango, guava, pineapple, cocktail juices, 6 d for orange as well as 3 d for apple juices respectively. These findings indicated that, as temperatures of acidify fresh fruit juices (apple, orange, mango, guava, pineapple and cocktail juices, respectively) increase as Salmonella heidelberg population decrease. The author reported that, Acid foods, especially if kept at refrigeration temperatures, support survival of Salmonella heidelberg and may cause Salmonella heidelberg food poisoning.

Keywords: Salmonella Heidelberg, Behavior, Fruit Juices, pH And Temperature

1. Introduction

Salmonella sp. are food-borne pathogens of major public health concern in most countries of the world. Salmonella enterica is an important zoonotic pathogen that causes an estimated 1.4 million illnesses, 16,000 hospitalizations, and between 400 and 600 deaths annually in the United States alone [1, 2]. Salmonella infections have become one of the most important groups of bacterial diseases affecting poultry, and, according to [3] domestic poultry constitutes the largest single reservoir of salmonella organisms existing in nature.

In the United States and Canada, Salmonella serovar Heidelberg is among the most frequently isolated serovars both in clinical cases of salmonellosis and from retail meats and food animals. Salmonella serovar heidelberg ranked first and fourth among serovars from food animals in 2002 and 2003, respectively [50], and ranked fifth and fourth among serovars from human in 2003 and 2004, respectively [4, 5].

Salmonella heidelberg was the most common serovar found in retail meats in both years [6, 7] and was found exclusively in poultry meats. The notion that poultry is the major reservoir of human infections in the United States and Canada is supported by case-control studies implicating table eggs [8, 9] and chicken meat [9] as being the main sources of Salmonella serovar Heidelberg infections. Salmonella serovar heidelberg is a major serovar only in the United States and Canada and is not among the top six serovars in other continents [10]. Epidemic outbreaks of Salmonella serovar heidelberg present a significant public health and economic burden in the region [11]. Salmonella serovar heidelberg has caused large outbreaks of food-borne illness in nursing homes, in hospitals, and within the community [12-15].

Unpasteurized fresh fruit is a traditional product that is produced and consumed in the regions of the world, particularly during the fall harvest season. However, this product has been implicated as the vechle for food-borne diseases. Salmonella species are responsible for the highest number of documents cases of food poisoning in the developed country [16, 17]. A variety of foods, including poultry, eggs, meat, milk, fruits, and vegetables, have been implicated as vehicles of one or more of these pathogens in outbreaks of food-borne illness [18-20]. Salmonella is an enterinvasive bacterium and causes infections that may have one of five different clinical presentations [21, 22]. Gastroenteritis is the most common presentation in industrial countries and is considering as an emergent food-borne pathogen caused disease it's a self-limited illness of brief duration, usually characterized by diarrhea and fever [23]. Salmonella heidelberg is an important human pathogen, which causes diarrhea diseases.

Many of the studies describing the survival of infectious pathogens in foods carried out in recent years have focused on or included Salmonella heidelberg as a target organism. Many of the studies have been done to determine the effect of environmental conditions on the survival and growth of pathogenic bacteria. There are a number of considerations to make when carrying out survival studies. Strain to strain variability has been shown to be important for some conditions. The pH-dependent and pH-independent stationary-phase acid tolerant phenotypes. In addition to that the preadaptation of cells is important for survival studies with acid foods [24].

The recovery method and medium may underestimate the numbers of survivors present if those organisms are stressed. If selective media are used to recover injured or stressed cells, modification to the recovery protocol may be required as described by [25]. In addition to the recovery medium, the composition of diluent used for serial dilutions may be also important [26]. Other factors shown to be important for survival include pH and acid type [27] and storage temperature or for inadequately short incubation periods [28]. The present work aimed at providing data on behaviour of Salmonella heidelberg in fruit juices during storage for variable time and temperature.

2. Materials and Methods

2.1. Inoculum Preparation

Three strains of *Salmonella heidelberg* were studied. Cultures were maintained on tryptic soya agar (TSA; 7.0; Difco, Laboratories, Detroit, MI) slants at 5 °C with monthly transfers to maintain viable cells and propagated in tryptic soy broth (TSB, Difco) for 24 h at 37 °C to provide approximately 106 CFU/ml-1. Cultures of the three isolates were combined in equal volumes to serve as a mixture to be used as inocula for the experiments.

2.2. Fruit Juices

Commercial, pasteurized clarified six different fruit juices including, apple, orange, mango, guava, pineapple, and cocktail, were purchased from the local supermarkets. No preservatives and artificial color was added (i.e. did not contain any of microbial inhibitors).

2.3. Inoculation of Fruit Juices

For inoculating fruit juices samples populations of Salmonella heidelberg were applied as mentioned by [29]. A population suspension (106 CFU ml-1) of Salmonella heidelberg was prepared by adding 10 ml-1 of the undiluted 3-isolates mixture to 10 liters of 0.1 % peptone water. On the other hand, each fruit juices sample included control, which was un-inoculated. The population suspensions were prepared to obtain a final concentration on the fruit juices samples (106 CFU/ml-1).

2.4. Storage Conditions

Fruit juices samples (2000 ml) of each juice were placed into separate sterilized conical flasks. Fruit juices samples were stored for time intervals of 0, 3, 6, 9, 12, 15, 18 and 21days at 10 ºC. Subsequently, 0, 3, 6, 9, 12 and 15 days at 20 ºC as well as 0, 3, 6 and 9 days at 37 ºC. before microbiological analyses respectively. For microbiological analysis samples were taken after 3 days.

2.5. pH Measurement

The pH of fruit juices samples were measured after time intervals of 10 to 20 min at each time of microbiological analysis using a model Jenway 3020 pH meter.

2.6. Microbiological Analyses

At each sampling time, duplicates of 50 ml fruit juices from each sample were analyzed. Samples were combined with 225 ml sterile 0.1 % peptone water (Difco; pH 7.0) in sterilized conical flasks and mixed. The resulting fruit juices slurries were serially diluted in 0.1 % peptone water. Aliquots of 0.1 ml were plated in duplicates onto nutrient agar (Oxoid) and modified brilliant green agar (MBGA) (Oxoid) and xylose-lysine-desoxycholate agar (XLDA) (Oxoid) to facilitate detection of Salmonella heidelberg populations inoculated onto fruit juices and for differentiating test isolates used from other organisms which might present in fruit juices samples. Plates were incubated at 37 °C for 18 to 24 h before Salmonella heidelberg colonies were counted. Samples yielding colonies eventually confirmed by biochemical and serological tests to Salmonella heidelberg were recorded positive.

To evaluate the absence of viable Salmonella heidelberg populations in the negative samples, aliquots of 25 ml of the enrichments were combined with 225 ml of modified brilliant green agar (MBGA) (Oxoid) and xylose- lysine- desoxycholate agar (XLDA) (Oxoid. Enriched cultures (100 µl) were plated on MBGA and XLDA and incubated for 18 to 20 h at 37 °C.

2.7. Statistical Analyses

Data were subjected to the statistical analysis system (SAS Institute, Cary, N.C.) for analysis of variance and Duncan's multiple range testes. Each value presented the mean of eight values (duplicate values from duplicate samples analyzed from two independent trials).

3. Results and Discussion

Fruit juices are an important part of the modern diet in many countries. The bacteriological survey of commercially sold, pasteurized, shelf-stable fruit juices from retail markets. The pH of the tested juices was 2.4-4.8. Bacteria were isolated from 51 samples (42.5%) and fungi from 78 samples (65%). Escherichia coli O157:H7 was detected in four of the analyzed samples (3.34%), and Staphylococcus aureus was detected in four different samples (3.34%). In 11 samples (9.1%), the total number of microorganisms detected was as high as 125 colony forming units (CFU). Acidophilic microorganisms were isolated from 26 samples (21.7%) and Blastomyces was detected in 46 samples (38.3%). Many of the microorganisms detected may cause disease in humans; thus, a number of the tested samples did not meet the Greek guidelines for the microbiological quality of juices. Use of a Hazard Analysis Critical Control Point (HACCP) system should be generally introduced into the juice industry sector to improve the quality of fruit juices, as well as other manufactured foods [30].

The Food and Drug Administration (FDA) has recently concluded that there is a risk of serious illness from consuming juice products that have not been processed in a manner designed to destroy target pathogens, including E. coli O157:H7 and Salmonella spp. Thus, in 1998 the FDA published regulations requiring a warning statement on packaged juices not processed in a manner to produce at least a 5-log10- unit reduction in the pertinent target microorganism for a period of at least as long as the shelf life of the product when stored under normal and moderate abuse conditions [31].

In addition to that, In total, 33 cases were reported, and a matched case-control study (23 cases/24 controls) identified consumption of fresh (unpasteurized) fruit juice purchased from a large retailer (X) as the only significant risk factor for illness (matched odds ratio: 7.4, 95% confidence interval: 1.5-37.2). Though the bacterium could not be isolated from fruit juice, the minimal pH value for growth of the causative strain of the outbreak (3.4) was compatible with survival in fruit juice from X. The outbreak strain showed acid resistance and adaptive properties that may explain how it could have caused infection through fresh orange juice [32]. To our knowledge, this is the first study related to survival and growth of Salmonella heidelberg in fruit juices. Survival of Salmonella heidelberg during storage in different fresh fruit juices including; apple, orange, mango, guava, pineapple, and cocktail at 10, 20 and 37 ºC, were observed.

A limited number of studies have evaluated the ability of Salmonella hiedelberg to survive and grow in acidifies fruit juices. Survival in fruit juices depended upon their pH, the type of strain, the type of juice and the incubation temperature [33].

At 10 °C, the results of the present study indicated that, Salmonella hiedelberg were survived on fresh fruit juices throughout 21 days of storage (Fig. 1), death was more rapid as the acidity of juices increased and reached to zero level (undetectable) of Salmonella hiedelberg population. Salmonella hiedelberg survived up to 18 days in mango (pH, 4.8-3), guava (pH, 5.21-3.14), pineapple (pH, 5.22-3.16), and cocktail (pH, 5-3) juices, while, in orange (pH, 3.23 –2.03) juice survived for up to 15 days and apple (pH, 3.08-1.58) juice for up to 12 days (Fig. 1). Control samples, untreated with Salmonella hiedelberg were observed.

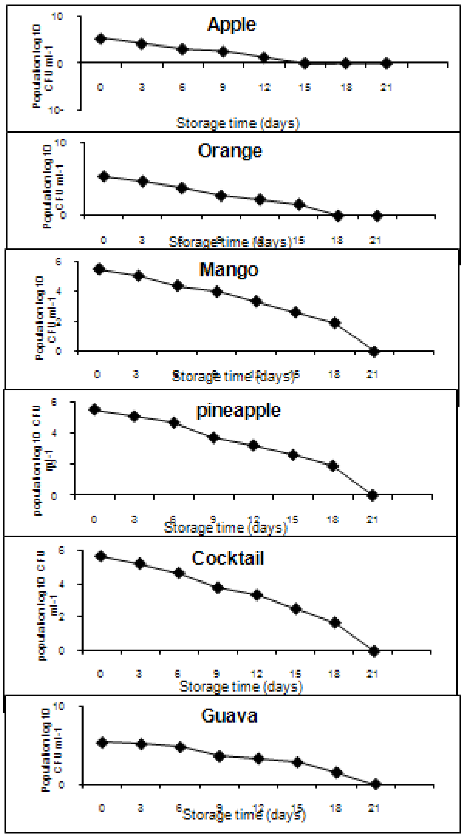

Fig.. 1. *Showing changes in population of Salmonella heidelberg in fruit juices during storage at 10 ºC for up to 21 days.*

Significant changes in pH values of fresh fruit juices were observed during storage treated with Salmonella hiedelberg (Fig. 4, A). The same results were reported by El-Safey [34] showing that, the use of higher acids concentration should result in a higher reduction of the bacterial load at all. Similarly, bacterial reduction was optimal at higher concentrations of acids, combination of acids, if the acid temperature was elevated, or if bacteria were attached to adipose tissue [35-37]. In addition to that, bacterial reductions were maximal with higher concentrations of acids [35-37].

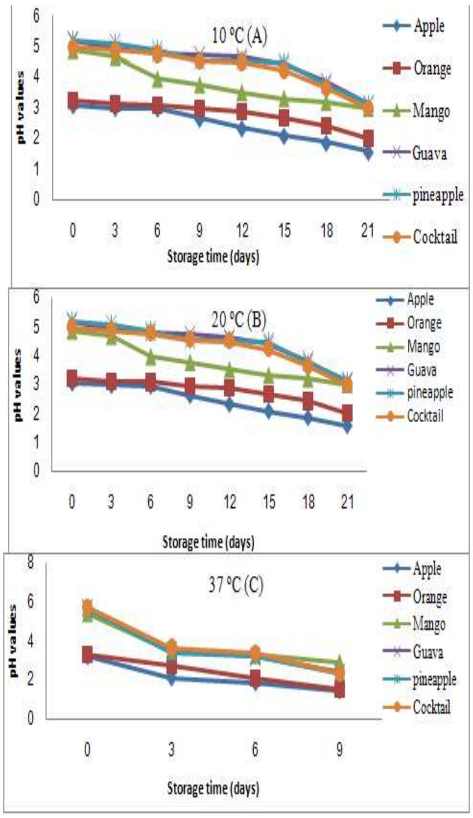

Fig. 4. *Showing the changes in pH values of fruit juices inoculated with Salmonella heidelberg stored at 10 ℃ for up to 21 days (A), 20 ℃ for up to 18 days (B) and 37 ℃ for up to 9 days (C), respectively.*

On the other hand, several foodborne human pathogens, when exposed to harsh conditions, enter viable but noncul-turable (VBNC) state; however, still open is the question whether VBNC pathogens could be a risk for public health, because, potentially, they can resuscitate. Moreover, cultural methods for food safety control were not able to detect VBNC forms of foodborne bacteria. Particularly, it has not been established whether food chemophysical characteris-tics can induce VBNC state in contaminating pathogen bacterial populations, especially in food, such as salads and fresh fruit juices, not subjected to any decontamination treatment. Salmonella Typhimurium and S. flexneri, de-pending on inoculum size, lost culturability but maintained viability and were able to resuscitate; moreover, S. flexneri was still able to form colonies after 48 h at high inoculum size. However, the entry into VBNC state differs on the species, depending, in turn, on inoculum size and time of incubation [38].

At 20 °C, Salmonella heidelberg succeeded to survive for up to 12 days in guava, pineapple and cocktail juices. In mango and orange juice the pathogen survived for up to 9 days. Moreover, Salmonella hiedelberg in apple juices sur-vived for up to 6 days (Fig. 2). The significant decrease in Salmonella hiedelberg populations occurred in apple, orange, mango, guava, pineapple and cocktail it might refer to the acidity of juices. Significant changes in pH values of fruit juices may contribute to the major effect on Salmonella heidelberg during storage at 20 °C (Fig. 4, B). Growth oc-curred in one brand of apple cider (pH 3.98) incubated at 20 ºC. Regardless of test parameters, there was no indication that cell types differed in tolerance to the acidic environment in apple cider or orange juice. The shortest survival time was observed at 37 ºC for 9 d in guava, pineapple, cocktail juices, 6 d for orange as well as 3 d for apple juices respectively.

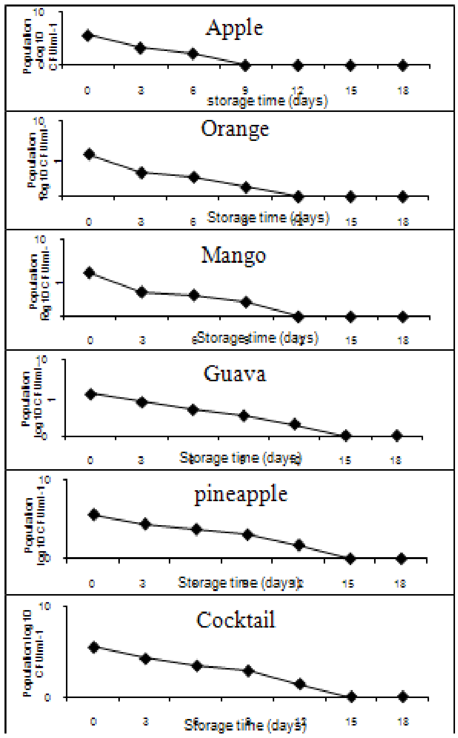

Fig. 2. *Showing the changes in survival and growth of Salmonella heidel-berg in fruit juices storage at 20 ℃ for up to 18 days.*

Other investigators study the effect of alpha- cyclodex-trin-cinnamic acid inclusion complexes on populations of Escherichia coli O157:H7 and Salmonella enterica in fruit juices at two different incubation temperatures (4 and 26 ºC) and reported that, populations of E. coli O157:H7 in apple cider were significantly reduced ($P <$ or $= 0.05$) during the 7-day sampling period in all solutions regardless of tem-perature. Compared with the controls, populations were significantly reduced by the addition of 400 and 1,000 mg/liter inclusion complex, but reductions were not signif-icantly different ($P \geq 0.05$) between the two treatment groups (400 and 1,000 mg/liter). Salmonella was significantly re-duced in all solutions regardless of temperature. There were significant differences between the control and each inclu-sion complex concentration at 4 and 26 degrees Celsius. Coupled with additional processing steps, al-pha-cyclodextrin-CA inclusion complexes may provide an alter native to traditional heat processes [39].

At 37 ºC, significant decreases in population of Salmo-nella heidelberg and shortest survival time was observed at 37 ºC in fruit juices under investigation (Fig. 3). The result indicated that, Salmonella heidelberg survival and grew for 9 d in guava, pineapple, cocktail juices, 6 d for orange as well as 3 d for apple juices respectively. The presence of

Salmonella hiedelberg in fresh fruit juices, significant decrease was detected, where the initial mean log10 population of Salmonella hiedelberg was 5.49 CFU ml-1 and after 3 days storage decreased to 2.66 CFU ml-1 (<2.83 CFU ml-1) in apple juice, however, the Salmonella hiedelberg able to survive for up to 3 days. In orange juice Salmonella hiedelberg was survived for up to 6 days storage at 35 ºC. where the initial mean log10 population was 5.22 CFUml-1 decreased to 2.18 CFUml-1 (<3.04 CFU ml-1). The Salmonella hiedelberg survival in mango, guava, pineapple, and cocktail at 3 7 ºC for up to 9 days were observed. Death was more rapid as the temperature increased and reached to zero level of Salmonella hiedelberg population. The pH values changes during storage of fruit juices including apple, orange, mango, guava, pineapple, and cocktail from 5.72 to 1.48 are shown in Fig.ure (4, C).

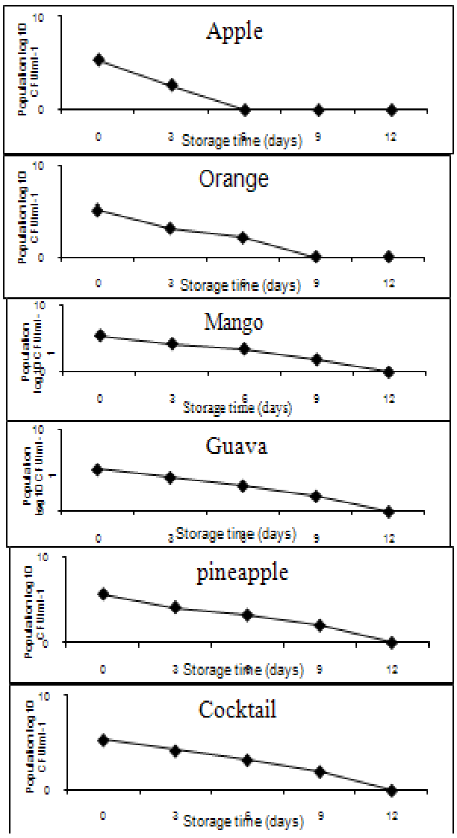

Fig. 3. *Showing the changes in survival and growth of Salmonella heidelberg in fruit juices storage at 37 ºC for up to 12 days.*

Several investigators examined the behavior of pathogenic bacteria in fruit juices, unpasteurized fruit and vegetable juices [40], fruit juices [41], orange juice [42], fresh fruit juices and melon and watermelon juices [43, 44], orange and apple juices [45], strawberry juice [46], and orange juice [32], Moreover, the influence of preadaptation strains to an acidic environment by subculturing individual strains of a 15-strain cocktail at pH 5.5 prior to mixing and inoculation into high salt (13.5 % w/v), reduced pH (4.9), TSB stored at 20 °C. Under these storage conditions, there is no advantage conferred to cells that were acid-adapted [47]. In addition to that, acid-adapted cells retain higher viability than unadapted cells in only two of nine foods tested [27].

On the other hand, there are many methods used to safe the fruit juices, [48], for instance, Osmosonication represents a potential processing alternative for producing safe and high-quality concentrated fruit juice without applying thermal treatments. Findings reported in this article can also be applied by industries when concentrating juices by classical means at relatively low temperature. It provides industries with a mathematical model specific for blackberry juice, from which different combinations of sonication time and storage time of concentrate can be chosen to achieve safety and quality goals. Moreover, the effect of continuous ohmic heating to inactivate Escherichia coli O157:H7, Salmonella Typhimurium and Listeria monocytogenes in orange juice and tomato juice and reported that, continuous ohmic heating can be effective in killing foodborne pathogens on orange juice and tomato juice with lower degradation of quality than conventional heating [49].

4. Conclusion

Survival in fruit juices depended upon their pH, the type of strain, the type of juices and the incubation temperature. Salmonella heidelberg Survived for up to 18 d in mango, guava, pineapple and cocktail juices, orange juice for up to 15 d and apple juice for up to 12 d stored at 10 ºC. At 20 ºC, Salmonella heidelberg was survived for up to 12 d in guava, pineapple and cocktail juices, 9 d for mango juice, orange juice for up to 9 d and apple juice for up to 6 d. The shortest survival time was observed at 37 ºC for 9 d in mango, guava, pineapple, cocktail juices, 6 d for orange as well as 3 d for apple juices respectively. These findings indicated that, as temperatures of acidify fresh fruit juices (apple, orange, mango, guava, pineapple and cocktail juices, respectively) increases as Salmonella heidelberg population decrease.

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