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The behavior of *Salmonella* Typhimurium, *Salmonella* Typhi, *Salmonella* Agona and *Salmonella* Montevideo in raw carrot and in fresh unpasteurized carrot juice

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The behavior of *Salmonella* Typhimurium, *Salmonella* Typhi, *Salmonella* Agona and *Salmonella* Montevideo in raw carrot at 30 ± 1 and 3 ± 1°C and in unpasteurized carrot juice at 3 ± 1, 12 ± 1, 20 ± 1, 30 ± 1 and 37 ± 1°C was determined. Fresh juice was obtained from carrots in the laboratory. Raw carrots were collected from a market in Pachuca city, Hidalgo, Mexico. On whole carrots stored at 20 ± 1 or 3 ± 1°C, no growth was observed for *Salmonella* strains. After 15 days at 30 ± 2°C, *Salmonella* serotypes decreased from an initial inoculum level of approximately 6 log CFU to 3.4 log in all the carrots; and at 3 ± 1°C, they decreased from approximately 2.6 log CFU to 1.7 log. All *Salmonella* serotypes grew in carrot juice at 12 ± 1, 20 ± 1, 30 ± 1 and 37 ± 1°C, reaching counts of approximately 4.3, 6, 6.8 and 7.5 log CFU/mL, respectively after 24 h. At 3 ± 1°C, the *Salmonella* growth was inhibited at least for 7 days.

Key words: *Salmonella* Typhimurium, *Salmonella* Typhi, *Salmonella* Agona, *Salmonella* Montevideo, carrot juice.

INTRODUCTION

Salmonella is one of the most common causes of foodborne disease worldwide (Gómez-Aldapa et al., 2012). A range of fresh fruit and vegetable products have been implicated in *Salmonella* infection, most frequently lettuce, sprouted seeds, melons and tomatoes (CDC, 2009). *Salmonella* spp. are often isolated during routine surveys from raw vegetable products and unpasteurized juices (Thunberg et al., 2002; CDC, 2008, 2009), In Mexico, *Salmonella* has been isolated from different raw vegetables (Quiroz-Santiago et al., 2009; Castro-Rosas et al., 2011). *Salmonella* spp. and *Salmonella* Typhi infections are endemic in México; indeed, in the 2009 and 2011 period, 381,320 salmonellosis cases and 139,000 typhoid fever cases were reported in Mexico (Secretaria de Salud, 2012). Both *Salmonella* Gaminara and *Salmonella* Montevideo have also been associated with several cases of human illness in Mexico (Gutiérrez-Cogco et al., 2000).

Unpasteurized juice has been associated with outbreaks for many years (Vojdani et al., 2008). Although non- sporulating foodborne bacteria can be destroyed by pasteurization, consumption of unpasteurized juice

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occurs frequently due to consumer preferences. Fruits juice producers have traditionally relied on the acidity of their products to assure microbiological safety. However, the ability of pathogens to survive in acid juices has been documented (Ryu and Beuchat, 1998; Uljas and Ingham, 1998; Linton et al., 1999). Different pathogenic bacteria have been isolated from unpasteurized juice (Harris et al., 2003). Recently, we isolated *Salmonella* strains from fresh carrot juice samples purchased in restaurants in Mexico (Torres-Vitela et al., 2013). Determining the behavior of pathogenic bacteria as *Salmonella* in carrot juice is important because frequently carrot juice is consumed unpasteurized in México. Frequently prepared at home, fresh unpasteurized carrot juice can also be purchased in restaurants, public markets and from street vendors. Independent of place of preparation, carrot is usually only washed with water before juices are obtained, and rarely is carrot disinfected. Carrot grows in soil, meaning washing with water is insufficient to eliminate any pathogenic microorganisms that might be present on the external surface. As a result, contamination by any microorganisms on the peel can occur during peel removal, cutting of the pulp or juice preparation. In addition, carrot juice is often left at room temperature for 2 to 4 h, providing ideal conditions for microbial growth. No reported data exist on the behavior of *Salmonella* serotypes on unpasteurized carrot juice at different temperatures. The objective of this study was to determine the behavior of *Salmonella* Typhimurium, *S.* Typhi, *Salmonella* Agona and *S.* Montevideo in unpasteurized carrot juice at 3 ±1, 12 ±1, 20 ±1, 30 ±1 and 37 ±1°C.

MATERIALS AND METHODS

Bacterial strains

Bacteria which are six strains of *Salmonella enterica* were included in this study: three *S.* Typhimurium [ATCC 14028, one isolated from tomato (J1) and the other one from carrot juice, (RS20) (Torres-Vitela et al., 2013)], one *S.* Typhi (RB15), one *S.* Gaminara (RF1-30) and one *S.* Montevideo (RF3-10) isolated to from carrot juice (Torres-Vitela et al., 2013). Rifampicin (Rif; Sigma-Aldrich St. Louis, MO, USA) resistance was induced in all six strains (Castro-Rosas et al., 2010). Rifampicin resistant strains were streaked on Tryptic Soy Agar (TSA, Bioxon, México) slants and maintained at 3 to 5°C, with weekly transfers onto TSA.

Whole carrots preparation

Fresh carrots were purchased from a market in the city of Pachuca, Hidalgo State, Mexico. After purchase, carrots were placed in sterile bags, transported to the laboratory and processed within 1 h. In the laboratory, carrots were manually cleaned with a cloth to remove dust. Only the carrots free of visible defects (e.g., bruises, cuts, abrasions, etc.) were used. Before inoculation, the carrots were maintained at room temperature (approximately 25°C).

Carrot juice

Carrot juice was obtained with a juice extractor (Uso Rudo, Turmix, Mexico, Mex.), producing approximately 50% of the weight of the fresh carrots as juice. The juice was filtered through filter paper (No. 4, WhatmanTM International Ltd., U.K.) and then used as a sample. Carrot juice samples (100 mL) were collected in separate sterile stomacher bags.

Inocula preparation and inoculation

TSA broth (3 ml) was inoculated with individual rifampicin *S.* Typhimurium, *S.* Typhi, *S.* Agona and *S.* Montevideo strains and incubated at 35°C for 18 h. The cultures were washed twice in sterile isotonic saline solution (ISS) by centrifugation at 1507 x*g* for 20 min, and then the pellets were resuspended in sterile peptone water at about 10^9 CFU/mL. A cocktail of inocula of the same bacteria was prepared for each of the three *S*. Typhimurium strains (ATCC 14028, J1 and RS20) by mixing 1 ml of each washed suspension (1 x 10^9 CFU/ml) in a sterilized tube.

Whole carrots were inoculated with approximately 3 and 6 log CFU of individual or cocktail suspension of *Salmonella* serotypes by placing 10 µl inside a circle (approximately 0.5 cm diameter) marked on the external surface. Inoculated carrots were placed on a sterile stainless steel tray and stored at 3±1 or at 30 ± 1°C and 80% of relative humidity, this incubation was in a humidity chamber with constant temperature (Model LHT-0250E, Daihan Labtech Co., LTD, Korea). Carrot juice was inoculated with a final concentration of approximately 2 log CFU/mL of each *S.* Typhi, *S.* Agona and *S.* Montevideo individual strains or *S.* Typhimurium cocktail, and stored at 3 ± 1, 12 ± 1, 20 ± 1, 30 ± 1 and 37 ± 1°C. All trials were made three times (three replicates) in independents experiments.

Microbiological counts

In whole peppers, quantification of *Salmonella* serotypes survival was carried out by removing the inoculated area (marked circle) with a sterile knife to a depth of approximately 0.5 cm. Each extracted piece was placed in a sterile bag containing 10 ml of 0.1% sterile peptone water and manually rubbed for 2 min while inside the sealed bag. The stomacher bags containing the inoculated juice were pummeled in a stomacher for 1 min, and sample dilutions, for microorganism counts, were prepared using peptone as a diluent (0.1%). Counts were performed with two replicate by plate count using appropriate dilutions of the bacterial suspensions spread on TSA plates containing 100 mg of Rif/L. Plates were incubated at 35 ± 2°C for 24 to 48 h. In order to confirm the presence of rifampicin resistant, *Salmonella* serotypes strains, three to five colonies from selected Rif plates were transferred to xylose lysine desoxycholate (XLD, Bioxon, México) agar plates containing 100 mg of Rif/L and incubated at 35 ± 2°C for 24 h. The typical colonies that were isolated from XLD agar were confirmed using both the somatic polyvalent (O) test and *Salmonella* serological flagellar (H) test (Antiserum O and H were purchased from the Institute of Epidemiological Diagnosis and Reference of the Secretariat of Health, Mexico).

Finally, behavior of Rif-resistant and non-resistant strains of the six *Salmonella* strains was compared by culturing in TSB at 37°C in a Bioscreen C machine (Growth Curves, Ab Ltd).

Determination of pH

The pH value of carrot juice was determinate with a pH meter

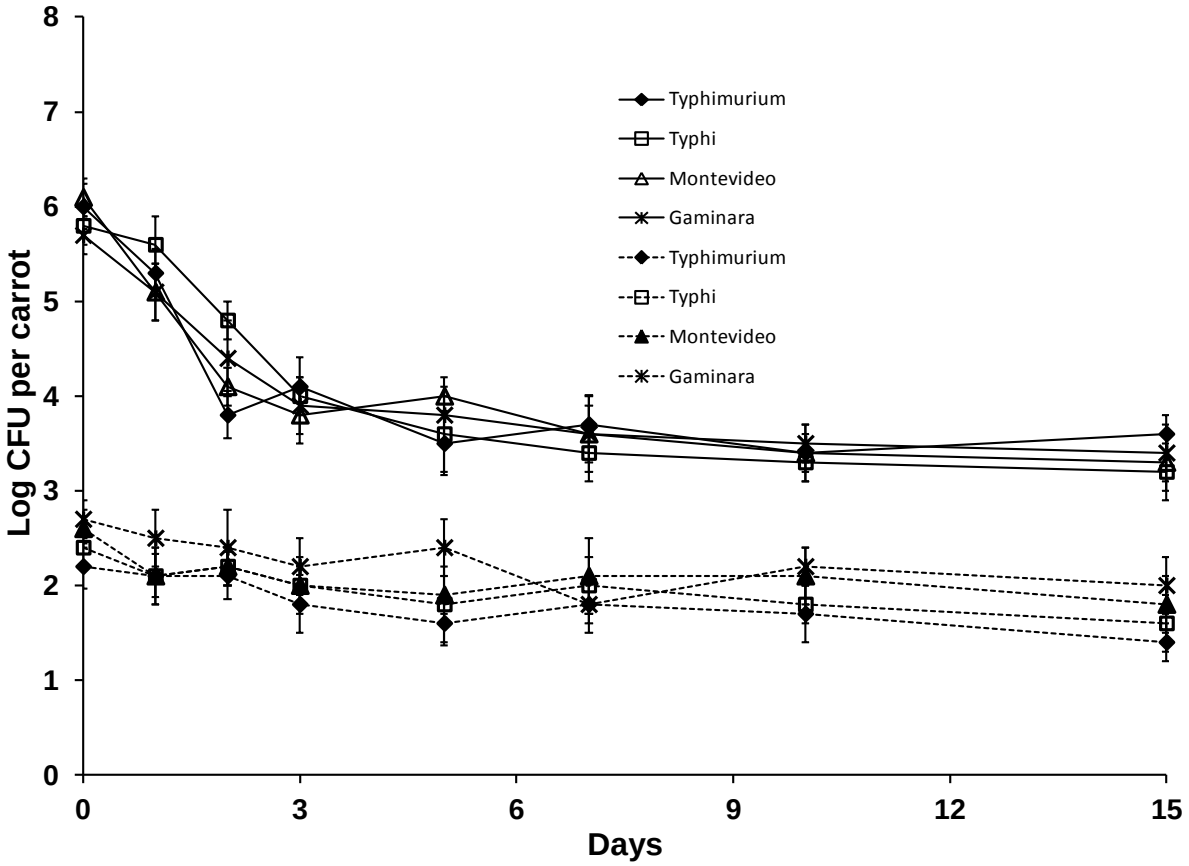

Figure 1. Behavior of *S.* Typhimurium, *S.* Typhi, *S.* Montevideo and *S.* Gaminara on whole carrots at 3 ± 1°C, 30 ± 1°C and 80% of relative humidity. The vertical bars are the standard deviations of the means (n=3).

(Orion 3 star, Thermo Electron Co. USA) following the ISO 1842:1991 test.

Statistical analysis

Statistically significant differences ($p<0.05$) were calculated with an analysis of variance (ANOVA) and a Duncan's test, using the Statistica 8 program (StatSoft, Inc., Tulsa, version 8).

RESULTS AND DISCUSSION

We used *S.* Typhimurium, *S.* Typhi, *S.* Agona and *S.* Montevideo strains resistant to rifampicin, a broad-spectrum antibiotic against bacteria, because the carrot juice is not sterile. We found that selective culture media for *Salmonella* (for example, XLD agar) counts produced abundant spurious bacteria colonies with a similar colony of *Salmonella* (for example, lactose negative). We therefore had to use rifampicin resistant *Salmonella* strains throughout the study. Rifampicin completely inhibited (<10 CFU) the native bacteria of carrot juice in TSA plates containing 100 mg/L of Rifampicin. No difference ($p>0.05$) was observed between the growth patterns of resistant and non-resistant pathogenic strains in Tryptic Soy Broth monitored by Bioscreen C® (data not shown).

All *Salmonella* strains exhibited a similar behavior on whole carrots and in carrot juice. On whole carrots stored at 20 ± 1 or 3 ± 1°C, no growth was observed for *Salmonella* strains (Figure 1). After 15 days at 30 ± 2°C, *Salmonella* serotypes had decreased from an initial inoculum level of approximately 6 log CFU to 3.4 log on whole carrots; and at 3 ± 1°C they had decreased from approximately 2.6 log CFU to 1.7 log (Figure 1). In general, survival among the five *Salmonella* serotypes on whole carrots did not significantly differ ($p>0.05$) at 30 ±1 or 3 ±1°C.

Salmonella serotypes grew after inoculation in carrot juice and incubation at 12 ± 1, 20 ± 1, 30 ± 1 and 37 ± 1°C (Figures 2 to 5), after a short lag period, *Salmonella* populations increased from approximately 2 log up to approximately 4.3, 6, 6.8 and 7.5 log CFU/mL, respectively (Figures 2 to 5). These results are in agree-

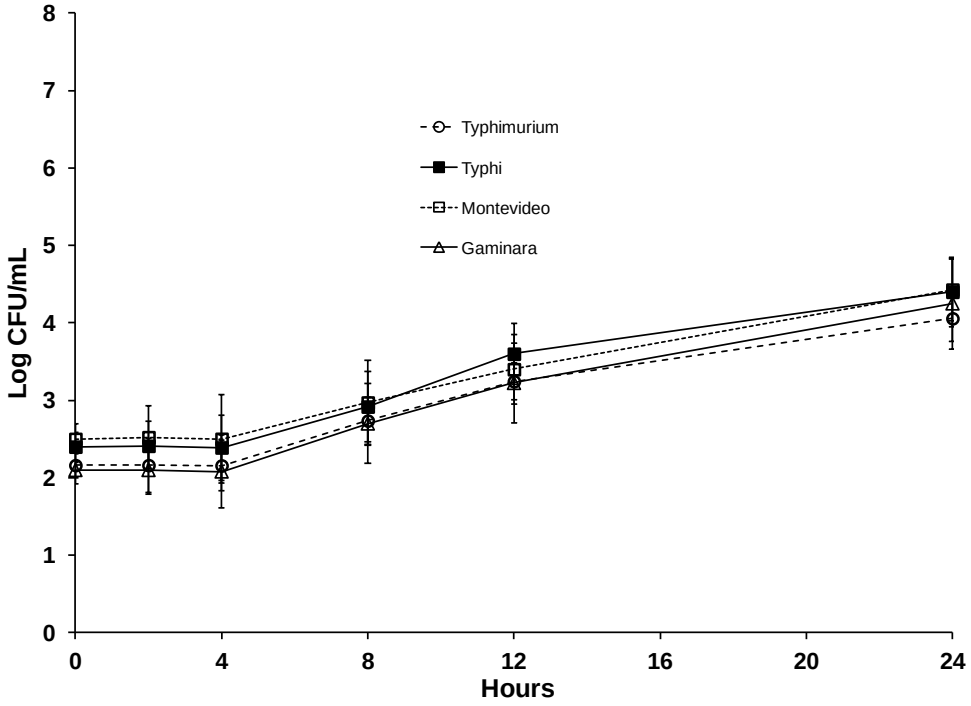

Figure 2. Behavior of *S.* Typhimurium, *S.* Typhi, *S.* Montevideo and *S.* Gaminara in unpasteurized carrot juice at 12 ± 1°C. The vertical bars are the standard deviations of the means (n=3).

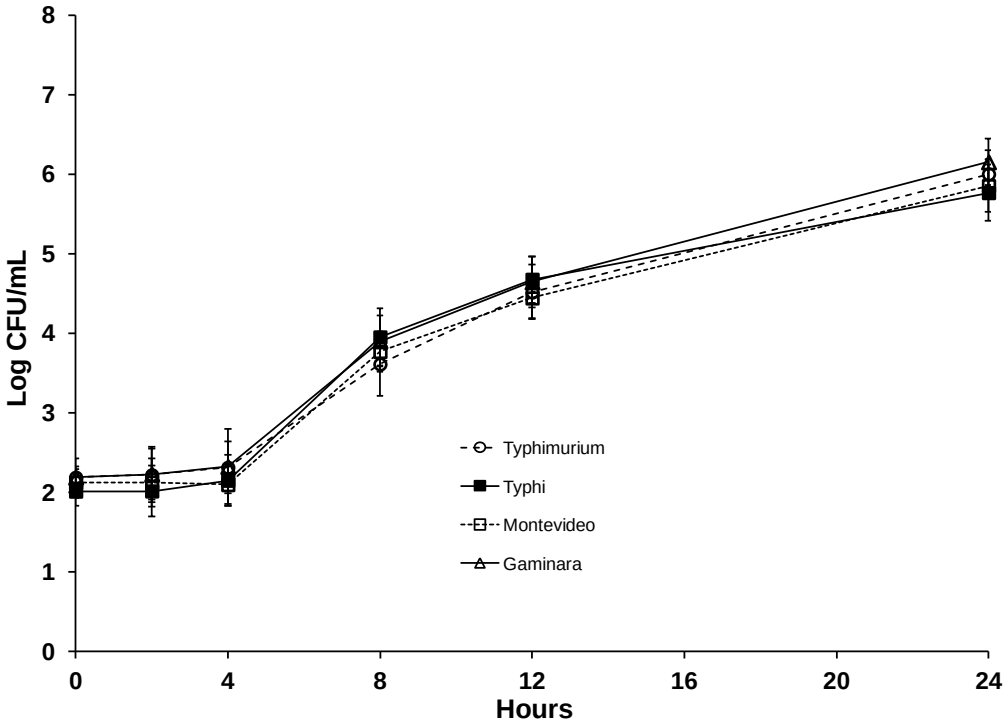

Figure 3. Behavior of *S.* Typhimurium, *S.* Typhi, *S.* Montevideo and *S.* Gaminara in unpasteurized carrot juice at 20 ± 1°C. The vertical bars are the standard deviations of the means (n=3).

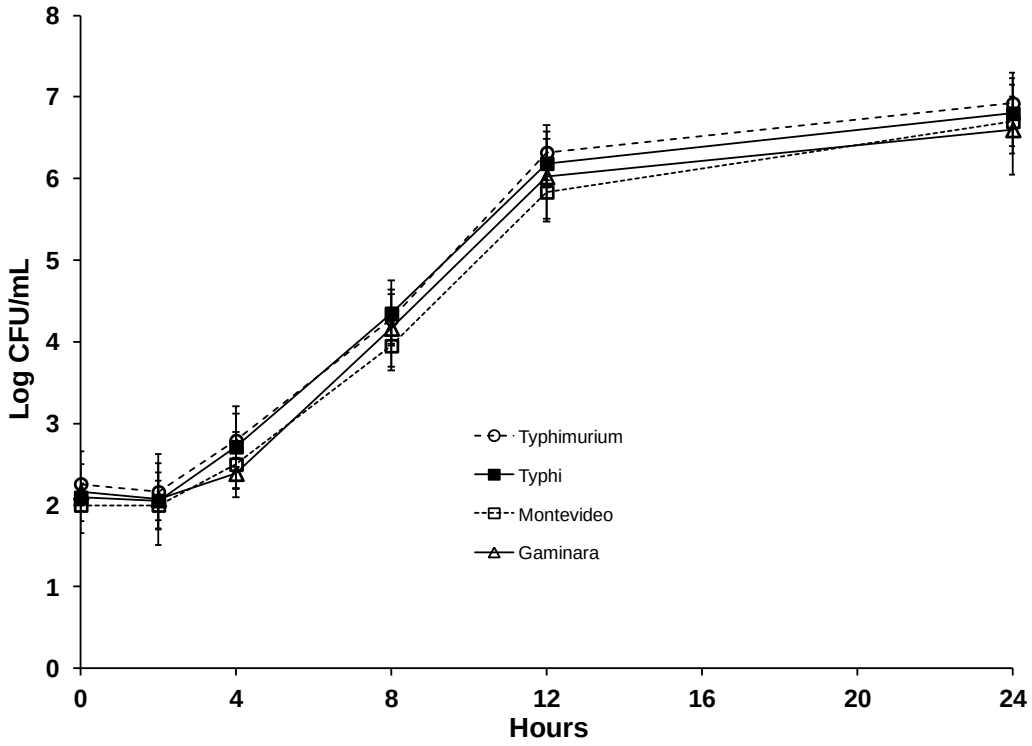

Figure 4. Behavior of *S.* Typhimurium, *S.* Typhi, *S.* Montevideo and *S.* Gaminara in unpasteurized carrot juice at 30 ± 1°C. The vertical bars are the standard deviations of the means (n=3).

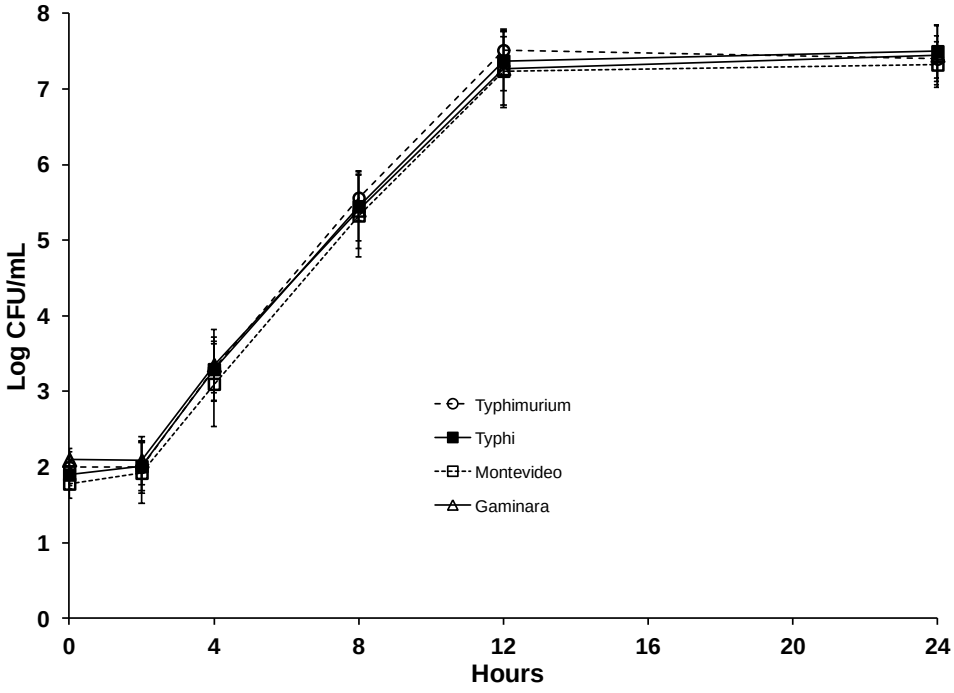

Figure 5. Behavior of *S.* Typhimurium, *S.* Typhi, *S.* Montevideo and *S.* Gaminara in unpasteurized carrot juice at 37 ± 1°C. The vertical bars are the standard deviations of the means (n=3).

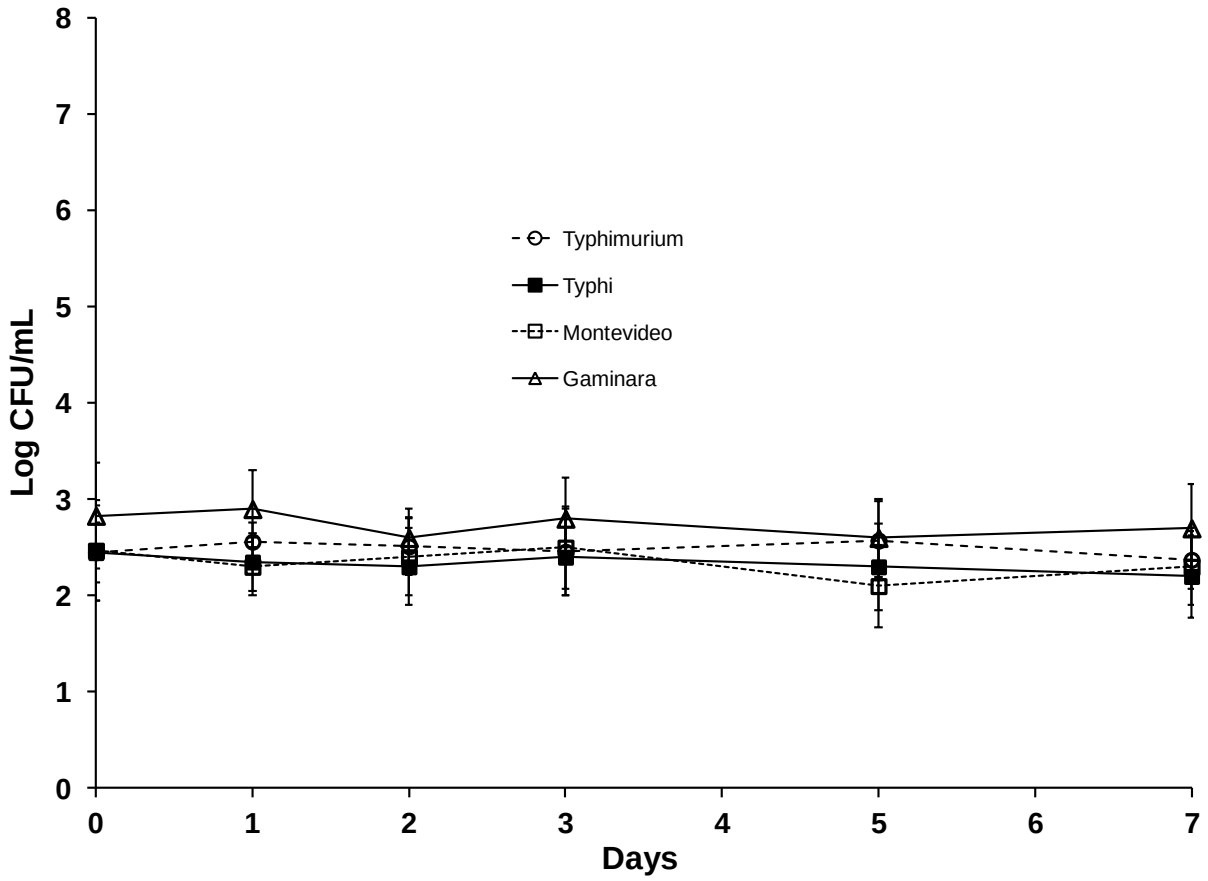

Figure 6. Behavior of *S.* Typhimurium, *S.* Typhi, *S.* Montevideo and *S.* Gaminara in unpasteurized carrot juice at 3 ± 1°C.

ment, in part, with the studies reported by Ibrahim et al. (2008), who observed growth of *Salmonella* in pasteurized carrot juice at 37°C. Ibrahim et al. (2008) reported increments of 5 log units in the population of *Salmonella* in pasteurized carrot juice after 12 h; we observed increments of approximately 5.5 log units in the population of all four *Salmonella* serotypes after 12 h at 37 ± 1°C (Figure 5).

Growth of the *Salmonella* strains in carrot juice was inhibited at 3 ± 1°C (Figure 6); *Salmonella* strains populations decreased slowly over time at 3 ± 1°C. *Salmonella* strains had decreased by 0.5 to 1 log CFU/mL of juice after seven days (Figure 6). Nonetheless, survival of even a small concentration of *Salmonella* under refrigeration poses a serious health hazard to consumers since salmonellosis outbreaks have been reported as originating in different foods at low pathogen concentrations (Greenwood and Hopper, 1983).

Finally, the outcome among the four *Salmonella* serotypes in carrot juice did not significantly differ ($p>0.05$) at 3 ± 1, 12 ± 1, 20 ± 1, 30 ± 1 or 37 ± 1°C (Figures 2 to 6).

In this study, all *Salmonella* serotypes strains were able to survive and grow in carrot juice. Microbial growth was clearly affected by temperature. In food, this behavior is also affected by each microorganism's intrinsic characteristics (ICMSF, 1998). Although temperature influences bacteria behavior in carrot juice during store, other factors may have a greater influence. For example, the native bacteria as acid lactic bacteria, from carrot can generate compounds with antimicrobial activity such as organic acids and bacteriocins (Wood and Holzapfel, 1995).

The pH value of carrot juice used in this study was five, and *Salmonella* serotypes were able to grow in these conditions. Most of *Salmonella* strains used in this study were isolated from fresh unpasteurized carrot juice samples purchased in restaurants. There are reports of *Salmonella* developing tolerance to low pH levels, suggesting the possibility that these pathogens could survive and grow in acidified foods or in foods with low

pH (Foster and Hall, 1990; Foster, 1991; Lin et al., 1995), as carrot juice. The behavior of *Salmonella* serotypes under these low pH conditions does not differ greatly from that of *Salmonella* strains in other fruit juices. For instance, *Salmonella* Hartford, *Salmonella* Gaminara, *Salmonella* Rubislaw and *S.* Typhmurium inoculated in orange juice at pH values of 3.5, 3.8, 4.1 and 4.4 survived for 27, 46, 60 and 73 days, respectively; survival times increased with increase of pH (Parish et al., 1997).

The results of this study demonstrate that *S.* Typhimurium, *S.* Typhi, *S.* Agona and *S.* Montevideo strains can survive and grow in carrot juice, making them effective transmission vehicles and posing a potential public health threat. The presence of bacterial growth in unpasteurized carrot juice shows that the minimally processed form of this product is an important and potential risk to consumers' health.

Conclusion

To the best of our knowledge, this is the first report on the behavior of *S.* Typhimurium, *S.* Typhi, *S.* Agona and *S.* Montevideo in fresh unpasteurized carrot juice. In the restaurants and at home, most of this risk can be mitigated through proper handling and correct food safety practices, such as thorough washing and disinfection, prevention of contamination by humans during preparation and storage, discarding of leftovers, storage of the unpasteurized juice under refrigeration (3 ± 1°C), or pasteurize the carrot juice before consumption. Avoiding temperature abuse may help to limit the growth of *S.* Typhimurium, *S.* Typhi, *S.* Agona and *S.* Montevideo in carrot juice.

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