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The Survival of *Salmonella enterica* Strains in Ready-to-Eat Fruit Purees under Different Storage Temperatures

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Abstract: *Salmonella enterica*, known for its resilience to acidic environments, has been linked to foodborne outbreaks of illness from fruit derivatives. This study aimed to assess the survival of five serovars of *Salmonella enterica* subsp. *enterica* in various fruit purees subjected to different storage temperatures. Among the studied serovars, *S. enteritidis* exhibited the most significant population decrease in all fruit purees. In contrast, *S.* Agona, *S.* Gaminara, *S.* Michigan, and *S.* Montevideo survived in peach puree at 4 °C for at least 3 days, and *S.* Agona, *S.* Gaminara, and *S.* Montevideo maintained their initial levels in pear puree under the same time/temperature conditions. However, none of the strains were detectable in plum and black currant purees after 2 days at 4, 15, or 25 °C. These findings highlight variations in the behaviour of *S. enterica* serovars within different fruit purees. Likewise, low-temperature conditions prolonged the survival of the tested strains in all fruit purees analysed.

Keywords: foodborne pathogen; storage temperature; *S. enterica*; pH; fruit puree; behaviour

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1. Introduction

Fruit puree is a semi-finished product obtained via a number of suitable procedures, such as sieving, crushing, or shredding the edible part of the whole or peeled fruit without removing the juice. This product can be consumed as a ready-to-eat product or used for further processing to obtain other foods.

Although fruit purees are acidic products, some acid-tolerant strains of foodborne pathogens can survive in acidic food matrices during cold storage [1]. Furthermore, the presence of pathogenic species in fruit and fruit juices is well documented. Krug et al. [2] highlighted the foodborne outbreaks associated with juices from 1922 to 2019. In these years, unpasteurized apple juice consumption was related to 18 outbreaks. Of them, 15 were caused by *Escherichia coli* O157:H7 and 3 by *Salmonella* Typhimurium. Regarding unpasteurized or reconstituted orange juice, eight outbreaks were reported and different serovars of *Salmonella* or *Shigella* spp. caused six of them. The presence of *Salmonella* spp. in fruit and fruit juices has been thoroughly substantiated. For example, Castillo et al. [3] detected *Salmonella* spp. in 14% of the orange juice samples sold in public markets and street stalls in Mexico. A lower incidence was noticed by Sospedra et al. [4] in orange juice samples from Spain, with 0.5% being positive samples.

In 2022, salmonellosis was the first identified cause of foodborne outbreaks in the European Union [5]. Most of the reported foodborne outbreaks of *Salmonella* were caused by *Salmonella enterica* subsp. *enterica*, with the *S.* Enteritidis serovar being the most frequently reported. This pathogen is of great concern in minimally processed and ready-to-eat fruits because of its ability to multiply on different fruit surfaces that are stored for long periods and to survive at low temperatures and acidic pHs [6,7]. While the general effect of fruit derivatives on the growth and survival of *S. enterica* is recognized, there exists a deficiency

in specific knowledge concerning how *S. enterica* strains, with different levels of virulence and prevalence, survive in fruit purees, and their response to different temperatures of storage. Although low temperature is essential to ensure food stability, temperature abuse can occur at different food processing, storage, and distribution stages, allowing microbial growth [8]. For example, under conditions of chronic temperature abuse (8 and 12 °C), *S. enterica* grew significantly in cut melons and watermelons and survived on radish and sliced pineapple stored at 4 °C [9].

Therefore, the present study aimed to evaluate the survival capacity of five *S. enterica* strains belonging to five different serovars in six fruit purees (plum, black currant, blueberry, peach, apple, and pear puree) under three different storage temperatures (4, 15, and 25 °C). Our work is the first that provides information about the impact on different serovars of *S. enterica* in ready-to-eat fruit purees.

2. Materials and Methods

2.1. Puree Samples and Their Characterization

Plum, black currant, blueberry, peach, apple, and pear purees were provided by fruit processing industries. pH was measured using a pH meter, Model GLP22 (XS Instruments, Carpi, Italy), with a pH electrode (2 PORE F TEMP BNC, XS Instruments, Carpi, Italy). Total soluble solids (TSSs) were measured using a digital refractometer (PAL-1 (Atago Co., Ltd., Tokio, Japan) and the results were expressed as °Brix. Titratable acidity (TTA) was determined by titrating 10 mL of fruit puree with 0.1 N NaOH until pH 8.1, and the results were expressed as g of the majority organic acid L^{-1}. Anthocyanin content (AC), total phenolic content (TPC), and antioxidant activity quantification were carried out according to Nicolau-Lapeña et al. [10]. AC was determined only in blackcurrant and blueberry purees due to their red/purple colour [11,12] and it was expressed as mg of cianidine-3-glucosyde L^{-1}. TPC results were expressed as mg of gallic acid equivalents (GAE) mL^{-1}, and antioxidant activity results were expressed as mg of ascorbic acid L^{-1}. Microbiological characterization was performed following ISO standards and included the enumeration of total mesophilic aerobic microorganisms (MAM), mould and yeasts, coliform bacteria, and *E. coli* following ISO 4833-1:2013 [13], ISO 21527-1:2008 [14], ISO 4832:2006 [15], and ISO 16649-1:2001 [16], respectively. The detection of *L. monocytogenes* and *Salmonella* spp. was performed in compliance with ISO 11290-1:2017 [17] and ISO 6579-1:2017 [18], respectively. There were three measurements of each parameter per fruit puree.

2.2. Salmonella Strains and Inoculum Preparation

Five serovars of *Salmonella enterica* subsp. *enterica* were used in this study: Agona (ATCC BAA 707), Michigan (ATCC BAA 709), Montevideo (ATCC BAA 710), Gaminara (ATCC BAA 711), and Enteritidis (CECT 4300). *Salmonella* strains were grown individually in 10 mL of tryptone soy broth (TSB, Biokar, Beauvais, France) for 22–24 h at 37 °C. Then, the culture media was centrifuged at 8900× *g* for 10 min at 20 °C and the pellet was re-suspended in 5 mL of sterile saline solution (SS; 8.5 g L^{-1} NaCl). For each strain, a cell suspension at a concentration of 10^7 colony-forming units (CFU) of *S. enterica* per millilitre was prepared by dilution in saline peptone (PS; 8.5 g L^{-1} NaCl and 1 g L^{-1} peptone). The concentration of *S. enterica* in the cell suspension was checked via a surface counting method using selective xylose lysine deoxycholate agar (XLD, Biokar, Beauvais, France) and non-selective tryptone soy agar (TSA, Biokar, Beauvais, France). The plates were incubated for 24 h at 37 °C and the results were expressed as CFU mL^{-1}.

2.3. Salmonella Survival in Fruit Purees

The fruit purees were inoculated individually with 0.2 mL of the corresponding *S. enterica* strain cell suspension, to achieve a final concentration of 10^5 CFU mL^{-1}, and stored at 4, 15, and 25 °C. The plate count method on XLD was used to determine bacterial counts at each sampling point (0, 1, 2, 3, 7, and 13 days). Plates were incubated at 37 °C for 24 h. When the bacterial count approached the limit of detection (LOD), 1 mL of sample

was transferred to 9 mL BPW (Buffered peptone water, Biokar, Beauvais, France), followed by incubation for 24 h at 37 °C. Then, it was streaked onto XLD agar and incubated at 37 °C for 24 h.

2.4. Data Processing and Statistical Analysis

Microbial data were log-transformed ($\log_{10}$ CFU mL^{-1}). The term "survival" was used when the population of the *S. enterica* strains maintained its level in the samples. Results were expressed as below the LOD when viable cells could not be enumerated but were detected. An Analysis of variance test (ANOVA) was carried out, and the criteria for statistical significance were $p < 0.05$. Tukey Honest Significant Difference test (HSD) of the means was applied to observe significant differences between a single strain over time in a distinct type of puree. Pearson correlation coefficients between a *S. enterica* strain population ($\log_{10}$ CFU mL^{-1}) and the physicochemical characteristics of fruit purees were calculated. All data were analysed using JMP Pro 16 software (SAS Institute Inc., Cary, NC, USA)

3. Results

3.1. Physicochemical and Microbiological Characterization

The physicochemical properties of fruit purees are shown in Table 1. The pH values ranged from 3.23 ± 0.09 (black currant puree) to 4.42 ± 0.07 (peach puree). The TSSs varied from 9.5 ± 0.1 °Brix in blueberry puree to 15.1 ± 0.0 °Brix in black currant puree. The TTA values ranged from 2.55 ± 0.12 g malic acid L^{-1} in pear puree to 21.18 ± 0.19 g tartaric acid L^{-1} in black currant puree. The AC in blueberry puree (110.88 ± 6.03 mg cianidine-3-glucosyde L^{-1}) was almost five times higher than in black currant puree (25.14 ± 9.51 mg cianidine-3-glucosyde L^{-1}). The lowest TPC was observed in peach puree (42.01 ± 0.12 mg GAE mL^{-1}), while the black currant and blueberry purees exhibited the highest TPC values (486.79 ± 0.23 and 446.99 ± 0.47 mg GAE mL^{-1}, respectively). Black currant puree presented the highest values for DPPH elimination activity (7347.7 ± 31.7 mg ascorbic acid L^{-1}) and the FRAP assay (12,665.3 ± 7.8 mg ascorbic acid L^{-1}), while pear puree exhibited the lowest values (1081.4 ± 12.3 and 1023.5 ± 3.4 mg ascorbic acid L^{-1} in the DPPH and FRAP assays, respectively). Counts of MAM, moulds and yeasts, coliform bacteria, and *E. coli* were below the LOD in all fruit puree samples (LOD = 5 CFU mL^{-1} for the moulds and yeasts count and 1 CFU mL^{-1} for the others). *L. monocytogenes* and *Salmonella* spp. were not detected in the analysed samples.

Table 1. Physicochemical characteristics of fruit purees. Values are means of triplicates ($n = 3$) ± standard deviations. Different lowercase letters in the same column indicate significant differences ($p < 0.05$) according to the HSD test.

	pH	TSS	TTA	AC	TPC	Antioxidant Capacity	
						DPPH	FRAP
Plum	3.40 ± 0.06 ^{cd}	12.5 ± 0.0 ^{b}	8.80 ± 0.49 ^{b}	nd	103.21 ± 0.20 ^{d}	2909.7 ± 17.3 ^{c}	3110.6 ± 6.8 ^{c}
Black currant	3.23 ± 0.09 ^{d}	15.1 ± 0.0 ^{a}	21.18 ± 0.19 ^{a}	25.14 ± 9.51 ^{b}	486.79 ± 0.23 ^{a}	7347.7 ± 31.7 ^{a}	12,665.3 ± 7.8 ^{a}
Blueberry	3.57± 0.16 ^{c}	9.5 ± 0.1 ^{e}	3.93 ± 0.11 ^{c}	110.88 ± 6.03 ^{a}	446.99 ± 0.47 ^{b}	4964.2 ± 165.2 ^{b}	7490.1 ± 7.8 ^{b}
Peach	4.42 ± 0.07 ^{a}	12.5 ± 0.0 ^{b}	3.11 ± 0.18 ^{d}	nd	42.01 ± 0.12 ^{e}	1746.1 ± 23.4 ^{d}	1656.4 ± 3.9 ^{d}
Apple	4.11 ± 0.03 ^{b}	11.8 ± 0.1 ^{d}	2.57 ± 0.10 ^{d}	nd	137.64 ± 7.59 ^{c}	1283.5 ± 97.2 ^{e}	1229.7 ± 3.9 ^{e}
Pear	4.06 ± 0.01 ^{b}	12.1 ± 0.1 ^{c}	2.55 ± 0.12 ^{d}	nd	98.42 ± 4.25 ^{d}	1081.4 ± 12.3 ^{e}	1023.5 ± 3.4 ^{f}

TSS = total soluble solids, °Brix. TTA = titratable acidity, g organic acid L^{-1}, (tartaric (black currant and blueberries) or malic acid (plum, peach, apple and pear)). AC = anthocyanin content, mg cianidine-3-glucoside L^{-1}. TPC = total phenolic content, mg gallic acid mL^{-1}. Antioxidant capacity, mg ascorbic acid L^{-1}, DPPH = 2,2-diphenyl-1-picrylhydrazyl. FRAP = Ferric Reducing Antioxidant Power. nd: not determined.

3.2. Survival of S. enterica Strains in Fruit Purees

Figures 1 and 2 show the survival of *S. enterica* strains in plum and black currant puree, respectively. The initial population of the CECT 4300 strain was the lowest in both purees (2.3 ± 0.6 and 2.9 ± 0.9 $\log_{10}$ CFU mL^{-1}, respectively), while the populations of the other

strains ranged from 3.7 ± 0.6 to 4.3 ± 0.1 $\log_{10}$ CFU mL^{-1}. The populations of all strains in both purees decreased dramatically, and, after 2 days, no strain of *S. enterica* was detected under the three storage temperatures.

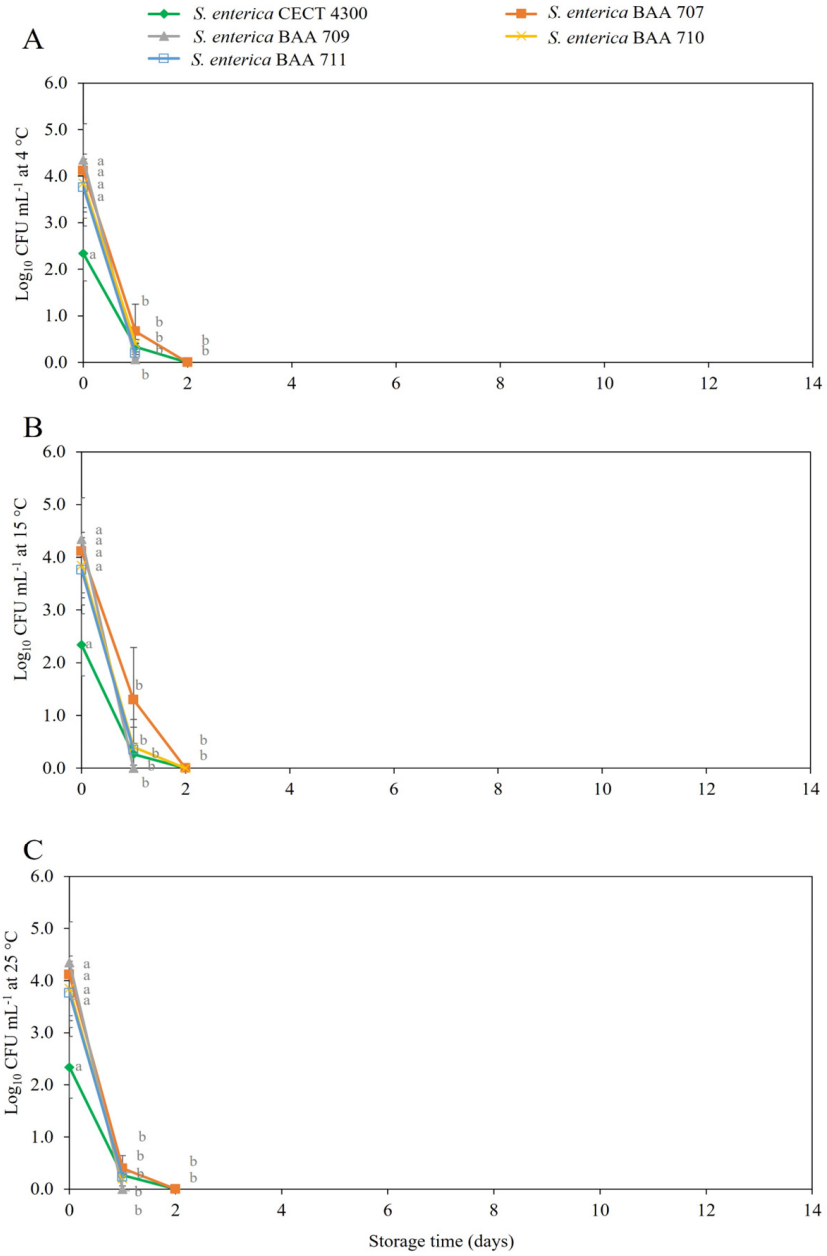

Figure 1. Population of *Salmonella enterica* subsp. *enterica* strains (CECT 4300 (◆), BAA 707 (■), BAA 709 (▲), BAA 710 (×), and BAA 711 (□); $\log_{10}$ CFU mL^{-1}) in plum puree throughout storage at 4 °C (**A**), 15 °C (**B**), and 25 °C (**C**). Symbols represent means and error bars represent standard deviation of the mean (n = 6). Different letters represent significant differences over storage time, according to analysis of variance (ANOVA) and Tukey's test ($p < 0.05$).

In the blueberry puree, the initial populations of *S. enterica* were above 4.4 ± 0.2 $\log_{10}$ CFU mL^{-1} (Figure 3). After 3 days of storage, *S. enterica* BAA 707 maintained the highest population, while the strain CECT 4300 was below the LOD (0.4 $\log_{10}$ CFU mL^{-1}) at 4 °C and 15 °C, and not detected at 25 °C. After 7 days at 4 °C, the strains BAA 707 and 711 were reduced by almost 2.5 log cycles, the strain BAA 710 was reduced by around 4 log cycles, the strain BAA 709 was below the LOD, and the strain CECT 4300 was not detected. At the same time, at 15 °C, the strain BAA 707 was reduced by 1.2 ± 0.2 log cycles, the

strains BAA 710 and 711 were reduced by almost 3.5 log cycles, and the strains CECT 4300 and BAA 709 were below the LOD. After 7 days at 25 °C, the strain BAA 707 was reduced by almost 4 log cycles, the BAA 710 and 711 strains were below the LOD, and the strains BAA 709 and CECT 4300 were not detected.

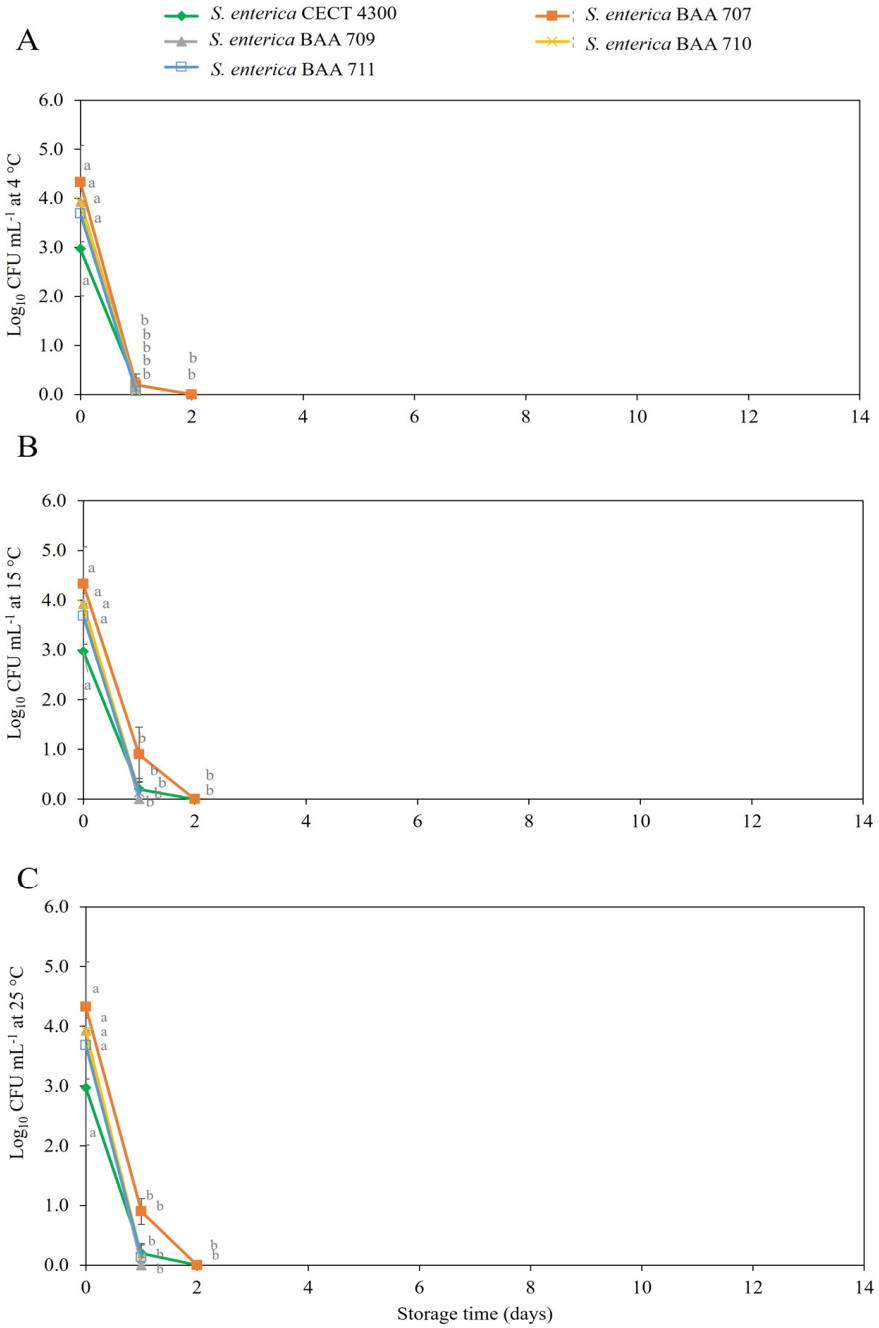

Figure 2. Population of *Salmonella enterica* subsp. *enterica* strains (CECT 4300 (◆), BAA 707 (■), BAA 709 (▲), BAA 710 (×), and BAA 711 (□); Log_{10} CFU mL^{-1}) in black currant puree throughout storage at 4 °C (**A**), 15 °C (**B**), and 25 °C (**C**). Symbols represent means and error bars represent standard deviation of the mean (n = 6). Different letters represent significant differences over storage time, according to analysis of variance (ANOVA) and Tukey's test ($p < 0.05$).

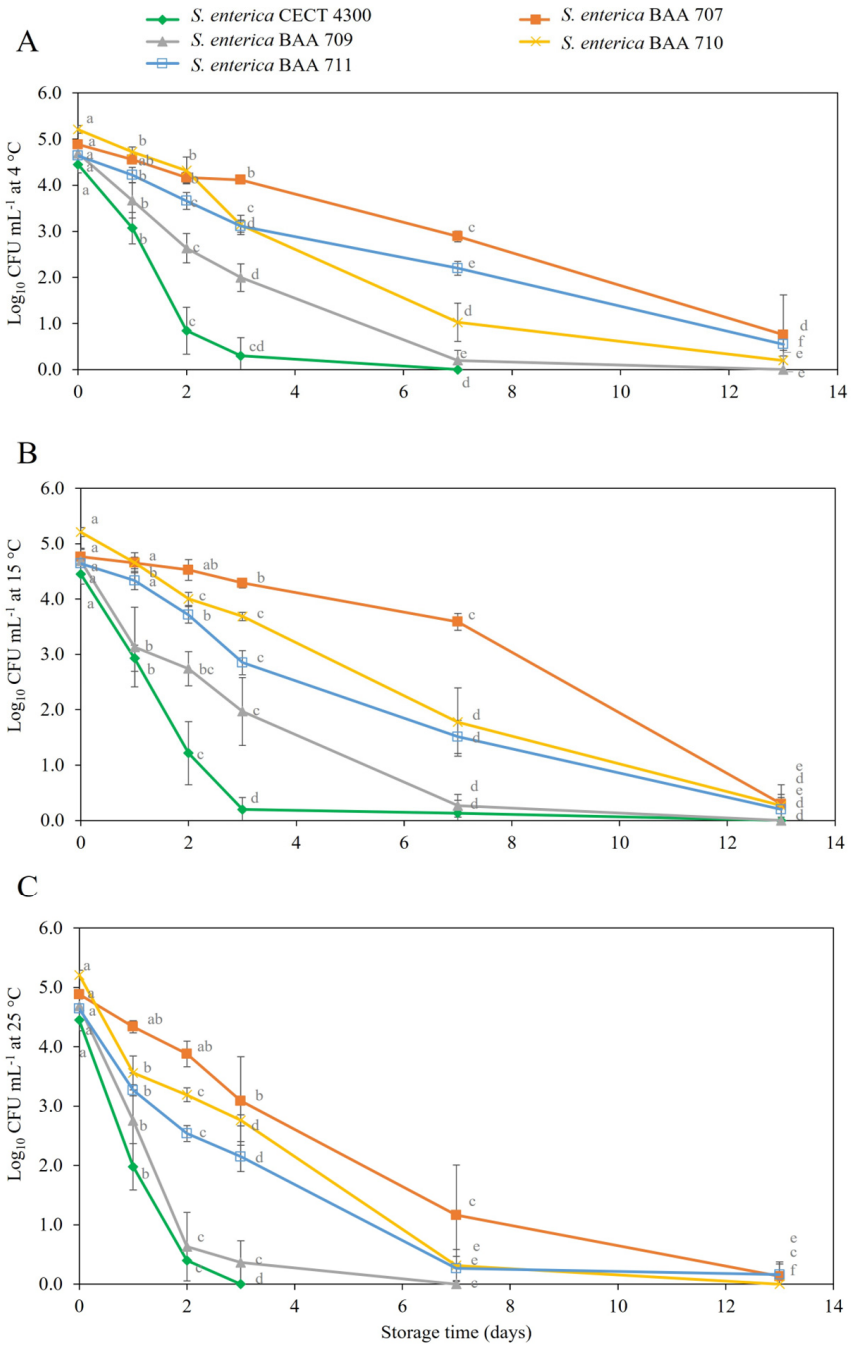

Figure 3. Population of *Salmonella enterica* subsp. enterica strains (CECT 4300 (◆), BAA 707 (■), BAA 709 (▲), BAA 710 (×), and BAA 711 (□); Log_{10} CFU mL^{-1}) in blueberry puree throughout storage at 4 °C (**A**), 15 °C (**B**), and 25 °C (**C**). Symbols represent means and error bars represent standard deviation of the mean (n = 6). Different letters represent significant differences over storage time, according to analysis of variance (ANOVA) and Tukey's test ($p < 0.05$).

The initial populations of *S. enterica* in peach puree were above 4.6 ± 0.0 log_{10} CFU mL^{-1} (Figure 4). At 4 °C (Figure 4A), there were no significant reductions in the bacterial populations until 7 days of storage, except for the strain CECT 4300, which was reduced by 1.0 ± 0.3 log cycles after 3 days. The strains BAA 707 and 711 showed final populations of 1.4 ± 0.1 and 2.5 ± 0.5 log_{10} CFU mL^{-1}, respectively, while the populations of the other strains were below the LOD (1.4 log_{10} CFU mL^{-1}). At 15 °C (Figure 4B), *S. enterica* BAA 707 and 711 maintained stable populations for 3 days. In contrast, the strains BAA 709 and 710 decayed to around 0.5 log cycle, and CECT 4300 was reduced by 1.0 ± 0.4 log

cycle. After 13 days, the BAA 707 population was 1.2 ± 0.3 $\log_{10}$ CFU mL^{-1}, BAA 710 and 711 were below the LOD (0.4 $\log_{10}$ CFU mL^{-1}), and BAA 709 and CECT 4300 were not detected. After 3 days at 25 °C (Figure 4C), the populations of the BAA 707, 709, 710, and 711 strains decreased to below 1.5 log cycles, while the CECT 4300 population was reduced by more than 3 log cycles. After 7 days, all the strains decreased to values below the LOD (1.4 $\log_{10}$ CFU mL^{-1}), except BAA 711, which was not detected. No *S. enterica* serovars were detected at the end of storage.

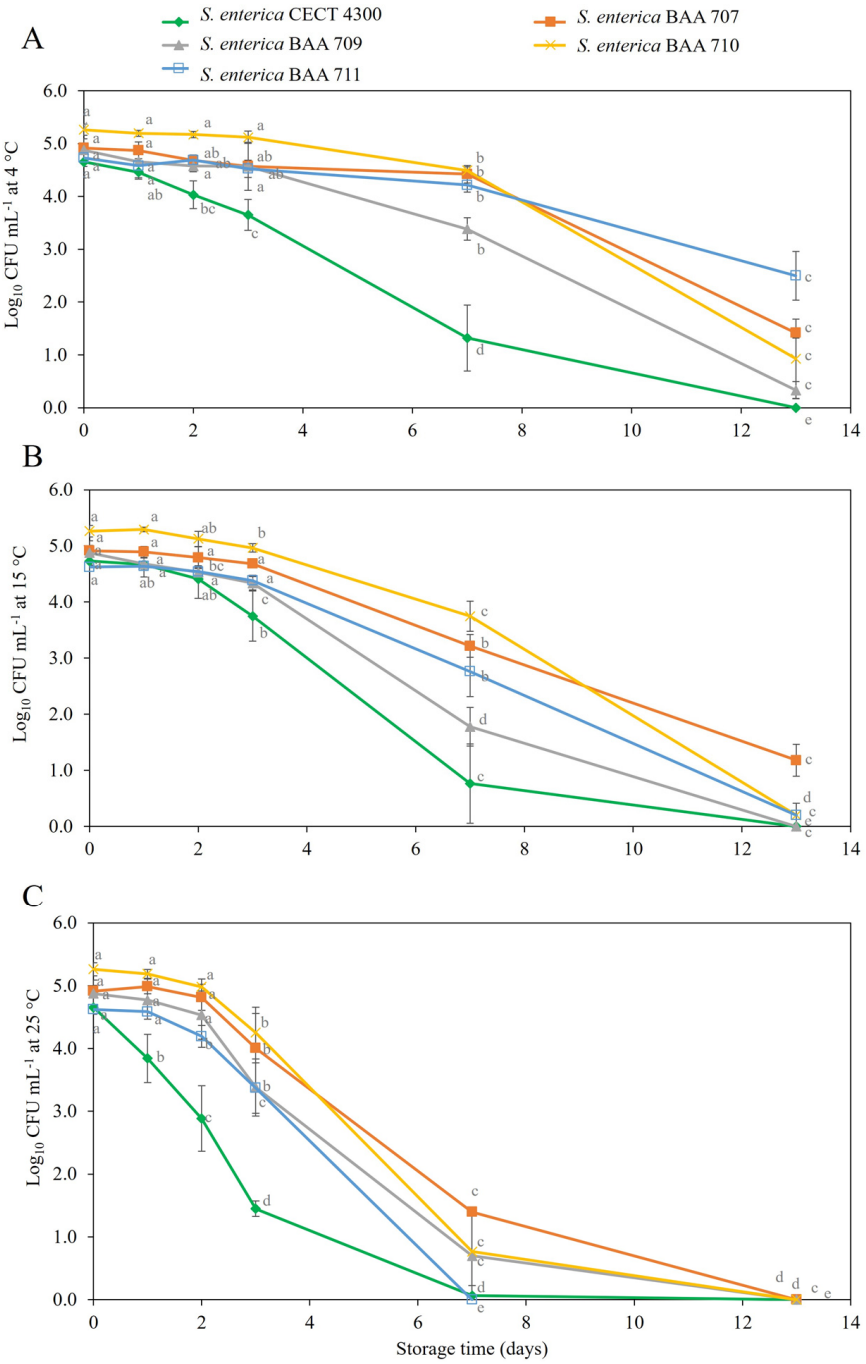

Figure 4. Population of *Salmonella enterica* subsp. *enterica* strains (CECT 4300 (◆), BAA 707 (■), BAA 709 (▲), BAA 710 (×), and BAA 711 (□); $\log_{10}$ CFU mL^{-1}) in peach puree throughout storage at 4 °C (**A**), 15 °C (**B**), and 25 °C (**C**). Symbols represent means and error bars represent standard deviation of the mean (n = 6). Different letters represent significant differences over storage time, according to analysis of variance (ANOVA) and Tukey's test ($p < 0.05$).

In apple puree (Figure 5), the initial *Salmonella* populations were above 4.5 ± 0.1 $\log_{10}$ CFU mL^{-1}. At 4 °C (Figure 5A), CECT 4300 was the most reduced (from 2.4 ± 0.5 log cycles after 3 days to not detected after 13 days). The other strains declined gradually. At the end of the experiment, the populations of BAA 707, 710, and 711 were 3.2 ± 0.2, 2.3 ± 0.3, and 1.7 ± 0.3 $\log_{10}$ CFU mL^{-1}, respectively. In contrast, BAA 709 was below the LOD (1.4 $\log_{10}$ CFU mL^{-1}). After 3 days at 15 °C (Figure 5B), the populations of BAA 707, 709, and 711 decayed the least, with reductions of 0.5 ± 0.1, 1.0 ± 0.2, and 1.2 ± 0.2 log cycles, respectively. The counts of BAA 710 and CECT 4300 decayed by 2.2 ± 1.8 and 3.4 ± 0.5 log cycles each. After 7 days, CECT 4300 was not detected and BAA 709, 710, and 711 were below the LOD (1.4 $\log_{10}$ CFU mL^{-1}), while BAA 707 was reduced by 2.6 ± 0.4 log cycles. After 3 days at 25 °C (Figure 5C), all the strains were reduced by approximately 3 log cycles except CECT 4300, which was not detected. No *S. enterica* serovars were detected after 7 days at 25 °C.

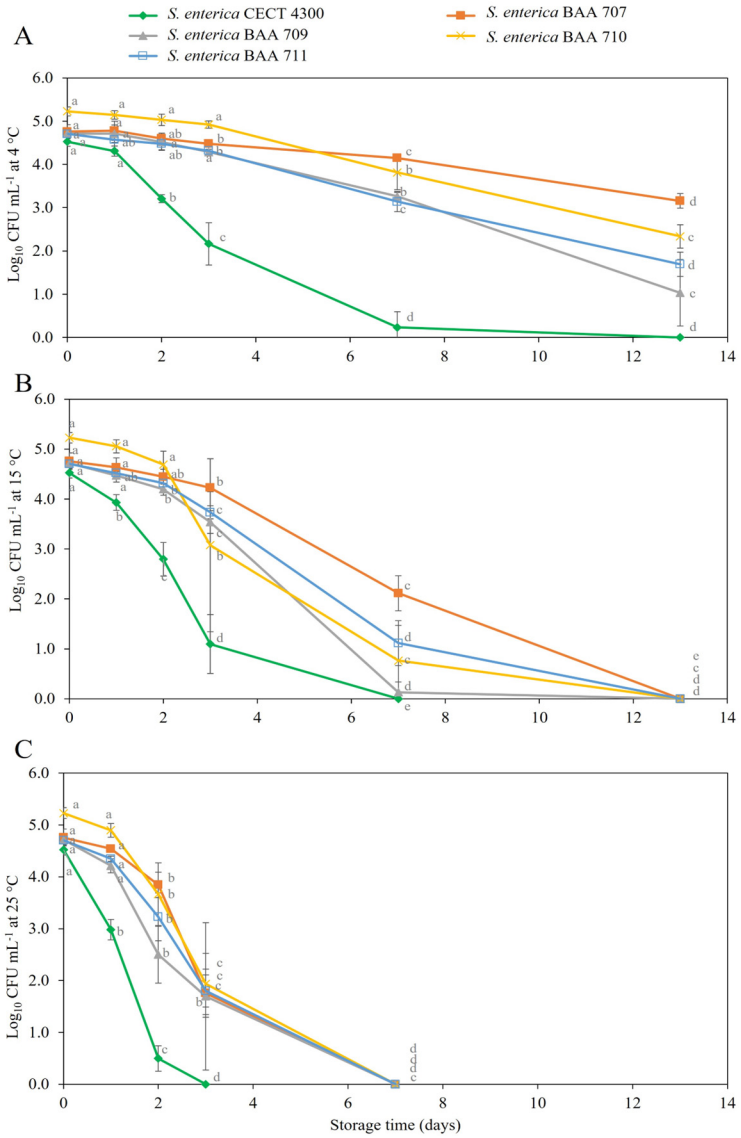

Figure 5. Population of *Salmonella enterica* subsp. enterica strains (CECT 4300 (◆), BAA 707 (■), BAA 709 (▲), BAA 710 (×), and BAA 711 (□); $\log_{10}$ CFU mL^{-1}) in apple puree throughout storage at 4 °C (**A**), 15 °C (**B**), and 25 °C (**C**). Symbols represent means and error bars represent standard deviations of the mean (n = 6). Different letters represent significant differences over storage time, according to analysis of variance (ANOVA) and Tukey's test ($p < 0.05$).

The initial populations of the *S. enterica* strains in pear puree ranged from 4.8 ± 0.2 to 5.2 ± 0.1 $\log_{10}$ CFU mL^{-1} (Figure 6). *S. enterica* BAA 707, 710, and 711 remained steady, without significant differences, for 3 days at 4 °C (Figure 6A), while the populations of BAA 709 and CECT 4300 decreased slightly. After 7 days, the populations of BAA 707, 709, 710, and 711 were maintained above 3.5 $\log_{10}$ CFU mL^{-1}, while that of CECT 4300 decreased by 2.9 ± 0.6 log cycles. After 13 days, the population of BAA 707 was 3.7 ± 0.2 $\log_{10}$ CFU mL^{-1}, the populations of BAA 709, 710, and 711 ranged between 2.1 ± 0.2 and 2.9 ± 0.2 $\log_{10}$ CFU mL^{-1}, and CECT 4300 was not detected. After 3 days at 15 °C (Figure 6B) the populations of BAA 707, 709, 710, and 711 were slightly reduced, by around 0.5 log cycles, while CECT 4300 was reduced by 1.0 ± 0.4 log cycles. After 7 days, the populations of BAA 707, 710, and 711 remained over 3 $\log_{10}$ CFU mL^{-1}, but the population of BAA 709 decreased by almost 3 log cycles, and the strain CECT 4300 was not detected. All the final populations were below the LOD (0.4 $\log_{10}$ CFU mL^{-1}). At 25 °C (Figure 6C), the populations declined significantly throughout the first 3 days, with CECT 4300 being the most reduced (by 3.3 ± 0.1 log cycles). After 7 days, BAA 707, 710, and 711 presented levels below the LOD (0.4 $\log_{10}$ CFU mL^{-1}) and BAA 709 and CECT 4300 were not detected. No serovars were detected at the end of the experiment.

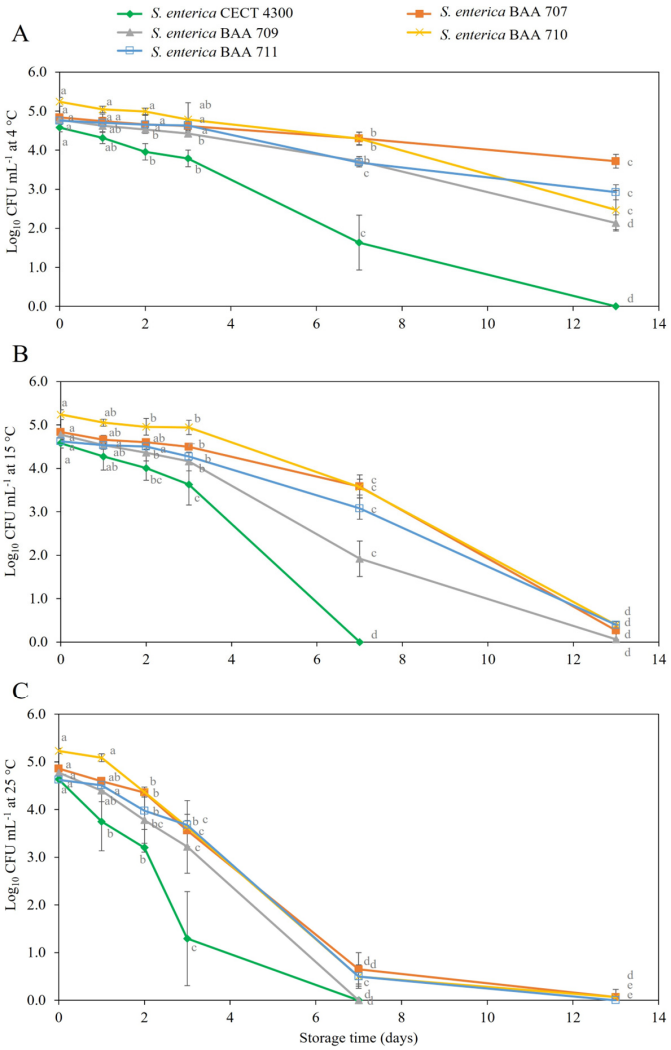

Figure 6. Population of *Salmonella enterica* subsp. *enterica* strains (CECT 4300 (◆), BAA 707 (■), BAA 709 (▲), BAA 710 (×), and BAA 711 (□); $\log_{10}$ CFU mL^{-1}) in pear puree throughout storage at 4 °C (**A**), 15 °C (**B**), and 25 °C (**C**). Symbols represent means and error bars represent standard deviations of the mean (n = 6). Different letters represent significant differences over storage time, according to analysis of variance (ANOVA) and Tukey's test ($p < 0.05$).

A correlation analysis of the results was conducted to ascertain any relationships between the population of the *S. enterica* strains (Log_{10} CFU mL^{-1}) and the physicochemical characteristics of the fruit purees (Figure 7). All evaluated *Salmonella* strains exhibited a significant correlation with the physicochemical characteristics of the fruit purees. The five strains exhibited positive correlations with puree pH and negatively correlated with the rest of the physicochemical evaluated characteristics. These correlations showed reduced significance with the increase in storage temperature. The most robust correlation was identified between pH and the populations of *S.* Gaminara (BAA 711) and the *S.* Agona (BAA 707) at 4 °C (correlation coefficients of 0. 5086 and 0.5145, respectively), and between TTA and the population of *S.* Agona at 4 °C (correlation coefficients of −0.5223). The strains *S.* Michigan (BAA 709) and *S.* Gaminara (BAA 711) exhibited a significant ($p < 0.005$) negative correlation with DPPH and FRAP, regardless of the evaluated temperature. The correlation analysis results align with the previously described pathogenic behaviour of these strains in ready-to-eat fruit purees.

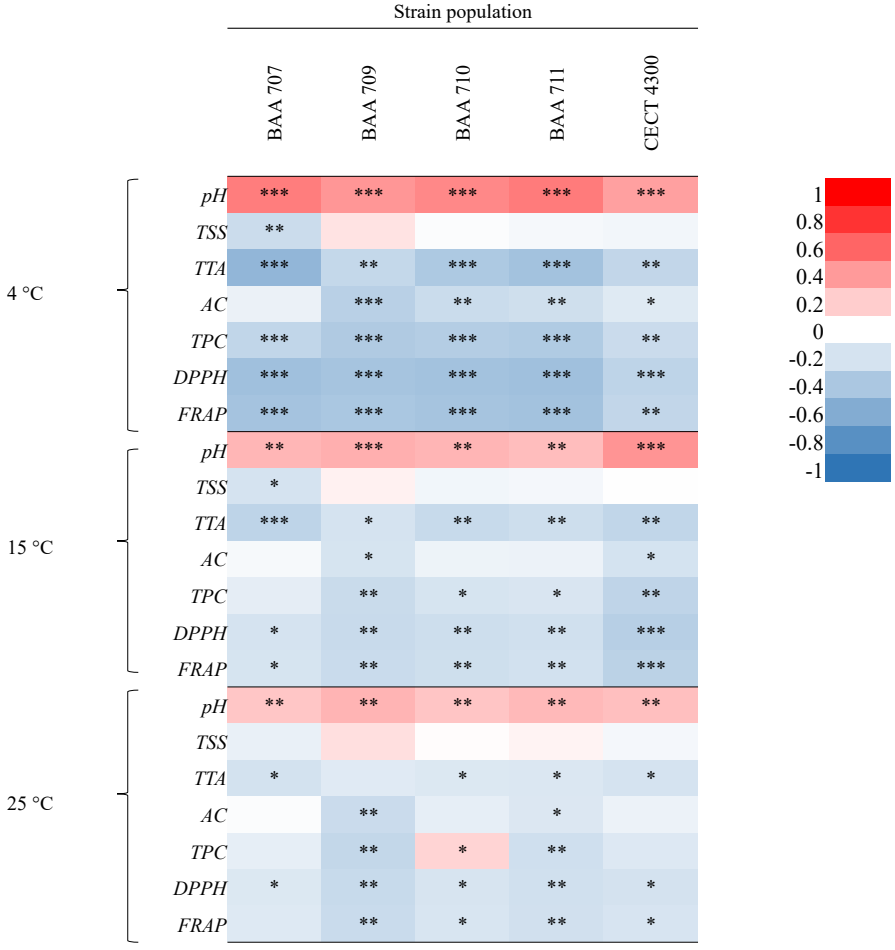

Figure 7. Correlation heat map analysis between *S. enterica* strains' populations (Log_{10} CFU mL^{-1}) and physicochemical characteristics of fruit purees. TSS = total soluble solids. TTA = titratable acidity. AC = anthocyanin content. TPC = total phenolic content. DPPH = 2,2-diphenyl-1-picrylhydrazyl. FRAP = Ferric Reducing Antioxidant Power. (*** = $p < 0.0001$; ** = $p < 0.005$; * = $p < 0.05$.)

4. Discussion

Our results revealed a distinct pattern of pathogen survival depending on the storage temperature, fruit puree type, and strain. *S. enterica* strains survived in fruit purees stored at 4 °C for at least 3 days. The obtained results agreed with the previously reported ability of *S. enterica* to protect itself from cold stress/shock for long periods [19]. Sharma et al. [20] observed that some serovars of *S. enterica* maintained their levels (6.6–7.0 log_{10} CFU mL^{-1})

in orange juice stored at 4 °C for up to 32 days. The storage time analysed by the authors was longer than that evaluated in our study and indicated that *S. enterica* survives in fruit derivatives for long periods. Nogueira et al. [21] also reported the ability of *S. enterica* to survive for extended periods in frozen products (- 23 °C) such as fruit concentrates (apple, orange, pineapple, and white grape) and banana puree, with reductions of around 2 log cycles after 12 weeks of storage.

In our study, we discovered high levels of *S. enterica* populations in several fruit purees after 13 days of storage under refrigeration. The evaluated *S. enterica* strains were detected under conditions of chronic temperature abuse (15 °C) in blueberry, peach, and pear puree at the end of storage (13 days), and at room temperature (25 °C) in blueberry, peach, and pear puree for up to 7 days. However, *S. enterica* exhibited better survival at low temperatures than at room temperatures. Similar findings were reported by Savran et al. [22], stating that *S.* Enteritidis survived longer in yoghurt (pH = 4.5) when the temperature was lower (e.g., up to 14 days at 4 °C and approximately 3 days at 25 °C). Additionally, Álvarez-Ordóñez et al. [23] observed that the time required for a 5-log-cycle reduction in *S.* Typhymurium in orange juice was 10.2 days at 4 °C, 6.3 days at 10 °C, 0.6 days at 25 °C, and 0.10 days at 37 °C.

Microorganisms exposed to adverse conditions induce adaptive responses within their cell. When *S. enterica* is exposed to cold temperature, the production of saturated fatty acids in the membrane increases, making the membrane rigid and preventing the passage of molecules into the cell [24–26]. Consequently, the entry of protons and organic acids into the cell is inhibited, rendering the cells more tolerant to acidic conditions. This could explain the higher survival of *S. enterica* populations at the low temperatures observed in our study.

The behaviour of *Salmonella* spp. in the food matrix also depends on its intrinsic characteristics. The fruit purees used in this study had an acidic pH ranging from 3.23 $\pm$ 0.09 to 4.42 $\pm$ 0.07. The populations of all *Salmonella* strains decreased sharply in the black currant and plum purees, which had lower pHs and higher organic acid contents [27]. Several researchers have suggested that the high acidity and the low pH in fruit matrices can markedly inhibit foodborne pathogens [28–30]. The predominant acid in fruit puree may also affect the pathogen's survival. In our study, four out of six analysed purees presented malic acid as their main organic acid. Raybaudi-Massilia et al. [31] observed that adding 0.2 % v/v malic acid (corresponding to 3.22 g malic acid L^{-1} of juice) to apple and pear juices inhibited *S.* Enteritidis's growth at 20 and 35 °C. We observed a significant decline in the populations of *S. enterica* strains in plum puree after 24 h of storage at the three evaluated temperatures, possibly due to its malic acid content being almost three times higher than the minimum inhibitory concentration (MIC) observed by Raybaudi-Massilia et al. [31]. Black currant and blueberry purees, despite having the highest TPCs and antioxidant capacities, exhibited different *Salmonella* survival rates, possibly influenced by the varying phenolic compounds present in each puree [32]. The antimicrobial effect of other intrinsic factors, including phenolic compounds, have been studied in fruit and vegetable juices [32–34]. Nevertheless, despite these findings, they are insufficient to elucidate the conditions governing pathogen inhibition in fruit purees.

In the present study, we also observed differences in the survival of the five included *S. enterica* strains: two strains associated with foodborne outbreaks (*S.* Montevideo BAA 710 and *S.* Enteritidis CECT 4300) and three food isolates (*S.* Agona BAA 707 isolated from alfalfa sprouts, *S.* Michigan BAA 709 isolated from cantaloupe melon, and *S.* Gaminara BAA 711 isolated from orange juice). Although the populations of all five strains declined over time regardless of the puree and temperature evaluated, not all strains behaved equally. At 4 °C, BAA 710 (*S.* Agona) demonstrated the highest survival in peach, apple, and pear purees for 3 days. Consequently, if one of these fruit purees is contaminated with this strain, the product might pose a risk for up to 3 days if maintained at its recommended storage temperature. In contrast, *S.* Enteritidis (CECT 4300) was the most sensitive and decreased drastically in all the evaluated fruit purees, although it is the serovar which

causes most foodborne outbreaks in the European Union [5]. Therefore, the higher incidence of this serovar in the EU could be related to its high virulence [35]. The differences in the behaviour of *S. enterica* strains against different environmental stresses (acid, NaCl, low water activity or heat) are currently being studied [36,37]. However, the serovars *S. enteritidis* and *S. typhimurium* are the most studied worldwide. The lack of available information about the prevalence, survival, and stress behaviour of different *Salmonella* serovars is alarming, because such studies are not only necessary to understand their physiology, but also to help design inactivation processes and/or action plans that are more efficient throughout the food chain to prevent the health risk they represent.

5. Conclusions

The results obtained in this study provide insights into the implications of the presence of different strains of *Salmonella enterica* in fruit purees. According to our findings, if a fruit puree (such as peach, apple, and pear puree) is maintained at the correct storage temperature (4 °C), this pathogen could remain stable for up to 3 days. Additionally, not all fruit purees contaminated with the same concentration of *S. enterica* will pose a problem, except the serovar Agona, which may represent a greater risk compared to the other evaluated strains. Based on this, further studies will be necessary to confirm the virulence, infectivity, and survival of these strains in fruit purees.

6. Patents

There are no patents resulting from the work reported in this manuscript.

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