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Original Article

Assessing the growth of *Staphylococcus aureus* and *Escherichia coli* on fruits and vegetables

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Abstract

Introduction: The number of registered foodborne diseases involving fresh produce is a preoccupation in many countries. For this reason, the aim of this study was to better understand the growth of *Staphylococcus aureus* and *Escherichia coli*, two indicators of hygienic and sanitary conditions, on fruits and vegetables that were exposed at different temperatures.

Methodology: The main salads served at the buffets of commercial restaurants were artificially contaminated with separate pools of both pathogens and subsequently exposed at 10, 20 and 30 °C and at different time intervals. Then, the growth potential of *S. aureus* and *E. coli* on each fruit and vegetable was determined.

Results: There was no significant *S. aureus* and *E. coli* growth on all evaluated foods exposed at 10 °C until 6 hours. When comparing both microorganisms, *E. coli* demonstrated higher growth potential than *S. aureus* on all analysed salads. Peculiarly, *E. coli* had the highest growth rate for the tomato ($\alpha = 6.43$ at 30 °C), a fruit with low pH.

Conclusion: We suggest that fruits and vegetables should be distributed at temperatures equal to or lower than 10 °C and should not be kept for more than 2 hours at room temperature.

Key words: indicator microorganisms; growth; time; temperature; salads.

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Introduction

The consumption of fresh fruits and vegetables is expected to increase worldwide between 8-9 % over the next 5 years [1]. As consumption increases, the number of registered foodborne diseases involving fresh produce is also becoming more common in many countries, including Brazil [2,3].

According to official records on foodborne diseases kept between 2000 and 2018, food services (restaurants and bakeries) in Brazil were the second most frequent establishments involved with foodborne outbreaks, only behind private residences, where the Sanitary Surveillance Services has no access. Among the main etiological agents involved with these outbreaks have been *Escherichia coli* and *Staphylococcus aureus* [2], both frequently used as indicators of hygienic and sanitary conditions [4,5]. Even though the majority of *E. coli* is not pathogenic, some strains can cause foodborne diseases. For example, pathotype enterotoxigenic *E. coli* (ETEC) is responsible for infantile diarrhea in developing countries, while the pathotype enterohaemorrhagic *E. coli* (EHEC) is regularly associated with food poisoning outbreaks in the developed world [6]. Fresh fruits and vegetables that are exposed to these strains could represent a risk for public health. *S. aureus* and both *E. coli* (ETEC and EHEC) have been isolated on fruits and salads, including strawberries, fruit salads, radish, cabbage, lettuce, cucumber, carrot, spinach and sprouts [7-13]. These foods can be contaminated and microbial multiplication can occur at various stages throughout the processing chain, including during harvest, storage, transport and distribution [14]. Inadequate temperatures during these stages can allow pathogenic bacteria growth, which is one of the main reasons for the occurrence of foodborne outbreaks involving fruits and vegetables [15,16].

According to Brazilian food-service legislation [17], cold food should be maintained at < 5 °C, while hot food should be maintained at > 60 °C for less than 6 hours. This legislation does not mention the cold chain distribution time and is an important topic to be explored. Therefore, the aim of this study was to assess the *S. aureus* and *E. coli* growth on fruits and vegetables in order to achieve a better understanding of what may happen during their distribution in food services.

Methodology

Commercial restaurants data

An informal interview approach was used to identify the fruits and vegetables most frequently served in 50 commercial restaurants in Porto Alegre, Southern Brazil. We elaborated questions regarding the number of daily meals, the fruits and vegetables most frequently served at buffets, and the served or distributed regularity of these foods per week. The data were grouped and ordered, and a descriptive statistical analysis was carried out to identify the frequency, means and standard deviations of each fruit and vegetable served/distributed at buffets.

Bacterial cultures

Five strains of *S. aureus* and five strains of *E. coli* were selected to compose two bacterial pools, which were artificially and separately inoculated on fruits and vegetables. The strains used were *S. aureus* 4668/03 (isolated from a foodborne outbreak that occurred in Southern Brazil), *S. aureus* S6 (isolated from a stainless-steel surface in a poultry slaughterhouse), *S. aureus* S8 (isolated from a cutting board of a poultry slaughterhouse), *S. aureus* ATCC 2998 and *S. aureus* ATCC 25923. The strains of *E. coli* were *E. coli* CQ (isolated from a hot dog in Southern Brazil), *E. coli* ECHC (isolated from human cystitis), *E. coli* DH5α (laboratory strain), *E. coli* ATCC 8739 and *E. coli* ATCC 25922.

Inoculum preparation

Each strain of *S. aureus* and *E. coli* was grown in Brain Heart Infusion broth (BHI, HiMedia, Mumbai, India) at 37 °C for 18-24 hours. After incubation, 2 mL of each strain of *S. aureus* and *E. coli* were mixed separately in a sterile tube. Next, each bacterial pool was centrifuged for 5 min at 14000 rpm; the supernatants were then discharged, and the pellets were washed with 0.1% peptone water. This procedure was repeated twice. In the second repetition, cells were re-suspended in 0.1% peptone water and the final cell concentration was adjusted through optical density 0.5 (OD_{630nm}) and plate count at 10^8 CFU/mL. Decimal serial dilutions using 0.1% peptone water were prepared and the pools were separately inoculated, reaching final concentrations on fruits and vegetables of approximately 1000 CFU/g (3 log CFU/g).

Inoculation and preparation of vegetables and fruits

All fruits and vegetables were acquired from a supermarket in Porto Alegre, Brazil. The water activity (a_w) was performed using an AquaLab water activity meter (Model 3TE, Decagon Devices, USA). For the pH, the foods were homogenized at the ratio of 1:2 (food:water) and were then measured in a pHmeter (Model Q400A, Quimis, Diadema, Brazil).

Each food was submitted to different treatments and processing (Table 1). Then, 10 g of each sample was transferred to a sterile plastic bag. Each fruit and vegetable surface-piece was separately contaminated with 1 mL of each bacterial pool. Subsequently, samples were incubated at different temperatures, simulating the following scenarios: (1) refrigeration using inadequate temperature (10 °C), (2) distribution at room temperature (20 °C) and (3) distribution at room temperature during a Brazilian summer day (30 °C). Samples were taken at 0, 2, 4, 6 hours (simulating the time exposure for these foods at a buffet) and 24 hours (considering inadequate foods reuse). In order to evaluate the microbial contamination of fruits and vegetables, 10 g of each food was homogenized in 90 mL of 0.1% peptone water in a stomacher plastic bag, which was followed by decimal dilution. *S. aureus* counts were carried out using Baird-Parker Agar (BP, Oxoid, Hampshire, United Kingdom). *E. coli* counts were done using Violet Red Bile Agar (VRB, Merck, Darmstadt, Germany) and mesophilic microorganisms were also quantified using Brain Heart Infusion Agar (BHI, HiMedia, Mumbai, India). The microorganisms were quantified by drop method [18] with a detection limit of 1.69 log CFU/g (50 CFU/g). Not-inoculated controls were included to verify the initial microbial quality of fruits and vegetables that were used. Experiments were performed at least three times with three repetitions and the results were expressed as log CFU/g.

Table 1. Treatments, processing applied, pH and water activity (a_w) of foods analyzed.

Vegetable/ Fruit	Treatments	Processing	pH	a_w
Carrot	Wash in running water for removal of dirt, sanitized with chlorine 200 ppm for 15 minutes and rinse under running water to remove the chlorine residue	Grated (0.2 cm)	6.54 ± 0.15	0.975 ± 0.006
Tomato		Slices (0.5 cm)	4.21 ± 0.04	0.991 ± 0.006
Cucumber		Slices (0.5 cm)	5.59 ± 0.17	0.983 ± 0.005
Cabbage		Slices (0.2 cm)	6.24 ± 0.42	0.959 ± 0.004
Broccoli	Cooked for 3 minutes in boiling water	Floret (4.0 cm)	6.71 ± 0.29	0.991 ± 0.003
Watermelon	The fruits peel was removed	Pieces (4.0 cm^2)	5.28 ± 0.03	0.986 ± 0.006
Papaya			5.43 ± 0.29	0.989 ± 0.003

Table 2. *S. aureus* and mesophilic microorganism populations (log CFU/g) on fruits and vegetables stored at 10, 20 and 30 °C.

Fruit/ Vegetables	Temperature (°C)	Storage (h)									
		0		2		4		6		24	
		S. aureus	**Mesophilic**	***S. aureus***	**Mesophilic**	***S. aureus***	**Mesophilic**	***S. aureus***	**Mesophilic**	***S. aureus***	**Mesophilic**
Tomato	10	3.83 ± 0.26 ^{a}	3.80 ± 0.30 ^{a}	3.54 ± 0.55 ^{a}	3.67 ± 0.41 ^{a}	3.48 ± 0.37 ^{a}	3.73 ± 0.42 ^{a}	3.50 ± 0.44 ^{a}	3.81 ± 0.24 ^{a}	3.67 ± 0.38 ^{a}	4.10 ± 0.27 ^{a}
	20	3.83 ± 0.16 ^{a}	3.41 ± 0.46 ^{a}	3.73 ± 0.24 ^{a}	3.49 ± 0.41 ^{a}	3.48 ± 0.37 ^{a}	3.77 ± 0.23 ^{a}	3.59 ± 0.26 ^{a}	3.70 ± 0.20 ^{a}	3.57 ± 0.49 ^{a}	7.75 ± 0.82 ^{b}
	30	3.69 ± 0.43 ^{a}	3.67 ± 0.46 ^{a}	3.48 ± 0.35 ^{a}	3.88 ± 0.46 ^{a}	3.48 ± 0.37 ^{a}	4.29 ± 0.22 ^{b}	3.54 ± 0.47 ^{a}	4.29 ± 0.24 ^{b}	3.92 ± 0.16 ^{a}	9.00 ± 0.22 ^{c}
Grated carrot	10	3.51 ± 0.12 ^{a}	3.71 ± 0.15 ^{a}	3.26 ± 0.28 ^{a}	3.84 ± 0.40 ^{a}	3.24 ± 0.46 ^{a}	3.75 ± 0.44 ^{a}	3.00 ± 0.26 ^{b}	3.89 ± 0.46 ^{a}	2.73 ± 0.10 ^{b}	3.84 ± 0.49 ^{a}
	20	3.54 ± 0.22 ^{a}	3.72 ± 0.24 ^{a}	3.08 ± 0.31 ^{a}	3.93 ± 0.56 ^{a}	3.13 ± 0.28 ^{a}	3.75 ± 0.24 ^{a}	3.13 ± 0.20 ^{a}	4.20 ± 0.71 ^{a}	2.85 ± 0.21 ^{b}	6.36 ± 1.09 ^{c}
	30	3.42 ± 0.19 ^{a}	3.74 ± 0.25 ^{a}	3.33 ± 0.13 ^{a}	4.20 ± 0.49 ^{a}	3.41 ± 0.15 ^{a}	4.38 ± 0.64 ^{a}	3.75 ± 0.48 ^{a}	6.83 ± 0.66 ^{b}	6.72 ± 0.75 ^{b}	9.33 ± 0.58 ^{c}
Green cabbage	10	3.62 ± 0.13 ^{a}	3.66 ± 0.07 ^{a}	3.60 ± 0.12 ^{a}	3.68 ± 0.22 ^{a}	3.44 ± 0.19 ^{a}	3.66 ± 0.18 ^{a}	3.13 ± 0.39 ^{a}	3.61 ± 0.10 ^{a}	3.54 ± 0.12 ^{a}	4.94 ± 0.70 ^{b}
	20	3.60 ± 0.15 ^{a}	3.69 ± 0.08 ^{a}	3.45 ± 0.19 ^{a}	3.66 ± 0.09 ^{a}	3.43 ± 0.18 ^{a}	3.60 ± 0.30 ^{a}	3.57 ± 0.21 ^{a}	3.89 ± 0.31 ^{a}	3.93 ± 0.33 ^{a}	7.30 ± 0.62 ^{b}
	30	3.52 ± 0.08 ^{a}	3.71 ± 0.11 ^{a}	3.57 ± 0.20 ^{a}	3.59 ± 0.13 ^{a}	3.78 ± 0.24 ^{a}	4.15 ± 0.19 ^{b}	4.44 ± 0.15 ^{b}	4.98 ± 0.11 ^{c}	6.35 ± 0.57 ^{d}	8.11 ± 0.20 ^{e}
Papaya	10	3.71 ± 0.16 ^{a}	3.80 ± 0.13 ^{a}	3.74 ± 0.17 ^{a}	3.84 ± 0.09 ^{a}	3.66 ± 0.23 ^{a}	3.88 ± 0.16 ^{a}	3.79 ± 0.19 ^{a}	3.89 ± 0.25 ^{a}	3.84 ± 0.15 ^{a}	3.87 ± 0.20 ^{a}
	20	3.72 ± 0.19 ^{a}	3.77 ± 0.14 ^{a}	3.81 ± 0.16 ^{a}	3.84 ± 0.18 ^{a}	3.82 ± 0.27 ^{a}	3.65 ± 0.45 ^{a}	3.79 ± 0.18 ^{a}	3.89 ± 0.25 ^{a}	4.39 ± 0.23 ^{b}	4.90 ± 0.85 ^{b}
	30	3.67 ± 0.16 ^{a}	3.67 ± 0.24 ^{a}	3.68 ± 0.30 ^{a}	3.81 ± 0.17 ^{a}	3.89 ± 0.25 ^{a}	3.93 ± 0.24 ^{a}	4.05 ± 0.22 ^{b}	4.49 ± 0.14 ^{b}	7.23 ± 0.07 ^{c}	8.85 ± 0.25 ^{d}
Cucumber	10	3.72 ± 0.18 ^{a}	3.77 ± 0.19 ^{a}	3.67 ± 0.35 ^{a}	3.87 ± 0.15 ^{a}	3.71 ± 0.08 ^{a}	3.78 ± 0.12 ^{a}	3.64 ± 0.16 ^{a}	3.82 ± 0.18 ^{a}	3.80 ± 0.09 ^{a}	3.93 ± 0.09 ^{a}
	20	3.65 ± 0.09 ^{a}	3.70 ± 0.16 ^{a}	3.60 ± 0.22 ^{a}	3.84 ± 0.15 ^{a}	3.81 ± 0.14 ^{a}	3.87 ± 0.11 ^{a}	3.83 ± 0.24 ^{a}	4.09 ± 0.14 ^{b}	5.43 ± 0.22 ^{c}	7.26 ± 0.32 ^{d}
	30	3.79 ± 0.20 ^{a}	3.74 ± 0.19 ^{a}	4.01 ± 0.09 ^{a}	4.04 ± 0.06 ^{ab}	4.81 ± 0.15 ^{b}	4.80 ± 0.15 ^{b}	5.79 ± 0.16 ^{c}	6.00 ± 0.07 ^{c}	8.34 ± 0.30 ^{d}	9.17 ± 0.11 ^{e}
Watermelon	10	3.71 ± 0.16 ^{a}	3.82 ± 0.22 ^{a}	3.77 ± 0.13 ^{a}	3.84 ± 0.11 ^{a}	3.79 ± 0.19 ^{a}	3.85 ± 0.24 ^{a}	3.75 ± 0.07 ^{a}	3.91 ± 0.12 ^{a}	3.89 ± 0.05 ^{a}	4.02 ± 0.05 ^{a}
	20	3.65 ± 0.18 ^{a}	3.60 ± 0.10 ^{a}	3.95 ± 0.10 ^{a}	3.88 ± 0.15 ^{a}	3.85 ± 0.12 ^{a}	4.00 ± 0.14 ^{a}	3.97 ± 0.20 ^{a}	4.05 ± 0.18 ^{a}	6.43 ± 0.70 ^{b}	8.63 ± 0.06 ^{c}
	30	3.71 ± 0.17 ^{a}	3.82 ± 0.22 ^{a}	3.97 ± 0.27 ^{a}	4.07 ± 0.10 ^{ab}	4.36 ± 0.43 ^{b}	4.25 ± 0.61 ^{b}	4.41 ± 0.85 ^{b}	4.80 ± 0.58 ^{b}	8.49 ± 0.24 ^{c}	9.44 ± 0.39 ^{d}
Broccoli	10	3.75 ± 0.13 ^{a}	3.78 ± 0.18 ^{a}	3.89 ± 0.16 ^{a}	3.93 ± 0.12 ^{a}	3.83 ± 0.16 ^{a}	3.91 ± 0.10 ^{a}	3.94 ± 0.18 ^{a}	3.97 ± 0.11 ^{a}	3.98 ± 0.06 ^{a}	4.03 ± 0.07 ^{a}
	20	3.76 ± 0.13 ^{a}	3.81 ± 0.17 ^{a}	4.00 ± 0.13 ^{b}	4.03 ± 0.10 ^{b}	4.29 ± 0.08 ^{c}	4.23 ± 0.08 ^{c}	4.50 ± 0.23 ^{c}	4.62 ± 0.12 ^{c}	8.14 ± 0.09 ^{d}	8.12 ± 0.04 ^{d}
	30	3.76 ± 0.23 ^{a}	3.87 ± 0.08 ^{a}	4.25 ± 0.11 ^{b}	4.29 ± 0.09 ^{b}	5.24 ± 0.09 ^{c}	5.22 ± 0.07 ^{c}	6.24 ± 0.27 ^{d}	6.36 ± 0.18 ^{d}	9.44 ± 0.05 ^{e}	9.29 ± 0.09 ^{e}

Data represent mean ± standard deviation of minimum 3 experimental repetitions; Means with the same letter within a row are not significantly different ($p > 0.05$); Means with different lowercase letters in the same row (same temperature condition) are significantly different ($p < 0.05$).

Table 3. *E. coli* and mesophilic microorganism populations (log CFU/g) on fruits and vegetables stored at 10, 20 and 30 °C.

Fruit/ Vegetables	Temperature (°C)	Storage (h)									
		0		2		4		6		24	
		E. coli	Mesophilic	*E. coli*	Mesophilic	*E. coli*	Mesophilic	*E. coli*	Mesophilic	*E. coli*	Mesophilic
Tomato	10	3.24 ± 0.21 [a]	3.33 ± 0.17 [a]	3.28 ± 0.17 [a]	3.29 ± 0.19 [a]	3.32 ± 0.27 [a]	3.41 ± 0.10 [a]	3.06 ± 0.25 [a]	3.28 ± 0.13 [a]	3.27 ± 0.11 [a]	3.59 ± 0.05 [b]
	20	3.29 ± 0.19 [a]	3.24 ± 0.18 [a]	3.17 ± 0.22 [a]	3.32 ± 0.16 [a]	3.39 ± 0.06 [a]	3.48 ± 0.15 [a]	3.55 ± 0.16 [b]	3.76 ± 0.21 [b]	8.09 ± 0.12 [c]	8.14 ± 0.11 [c]
	30	3.27 ± 0.19 [a]	3.36 ± 0.11 [a]	3.50 ± 0.13 [a]	3.42 ± 0.32 [a]	4.94 ± 0.11 [b]	5.09 ± 0.17 [b]	6.49 ± 0.25 [c]	6.38 ± 0.22 [c]	9.70 ± 0.15 [d]	9.76 ± 0.07 [d]
Grated carrot	10	3.26 ± 0.17 [a]	3.27 ± 0.26 [a]	3.12 ± 0.29 [a]	3.25 ± 0.16 [a]	3.23 ± 0.33 [a]	3.17 ± 0.23 [a]	3.27 ± 0.17 [a]	3.33 ± 0.26 [a]	3.50 ± 0.20 [a]	3.53 ± 0.26 [a]
	20	3.26 ± 0.16 [a]	3.38 ± 0.27 [a]	3.30 ± 0.17 [a]	3.22 ± 0.38 [a]	3.46 ± 0.37 [ab]	3.50 ± 0.20 [ab]	3.78 ± 0.18 [b]	3.84 ± 0.24 [b]	8.16 ± 0.16 [c]	8.40 ± 0.19 [c]
	30	3.17 ± 0.26 [a]	3.32 ± 0.29 [a]	3.40 ± 0.22 [a]	3.80 ± 0.22 [a]	4.19 ± 0.20 [b]	4.60 ± 8.65 [b]	5.56 ± 0.65 [c]	6.37 ± 0.33 [d]	9.61 ± 0.15 [e]	9.42 ± 0.46 [e]
Green cabbage	10	2.97 ± 0.31 [a]	3.09 ± 0.32 [a]	2.91 ± 0.30 [a]	3.20 ± 0.25 [a]	3.15 ± 0.24 [a]	3.10 ± 0.20 [a]	3.09 ± 0.10 [a]	3.21 ± 0.27 [a]	2.93 ± 0.19 [a]	3.00 ± 0.25 [a]
	20	3.06 ± 0.32 [a]	3.13 ± 0.30 [a]	3.27 ± 0.18 [a]	3.16 ± 0.20 [a]	3.27 ± 0.12 [a]	3.39 ± 0.12 [ab]	3.45 ± 0.26 [b]	3.55 ± 0.21 [b]	6.24 ± 0.35 [c]	6.32 ± 0.31 [c]
	30	2.91 ± 0.30 [a]	3.18 ± 0.27 [a]	3.13 ± 0.30 [a]	3.36 ± 0.29 [a]	3.80 ± 0.30 [b]	4.14 ± 0.24 [b]	4.83 ± 0.79 [c]	5.40 ± 0.51 [d]	8.10 ± 0.10 [e]	8.17 ± 0.09 [e]
Papaya	10	3.05 ± 0.23 [a]	3.48 ± 0.15 [a]	3.04 ± 0.33 [a]	3.37 ± 0.23 [a]	2.89 ± 0.21 [a]	3.44 ± 0.22 [a]	2.91 ± 0.18 [a]	3.47 ± 0.17 [a]	3.82 ± 0.19 [b]	4.00 ± 0.15 [b]
	20	3.34 ± 0.25 [a]	3.53 ± 0.15 [a]	3.50 ± 0.09 [a]	3.45 ± 0.19 [a]	4.00 ± 0.13 [b]	3.99 ± 0.15 [b]	4.44 ± 0.25 [c]	4.45 ± 0.33 [c]	8.12 ± 0.17 [d]	8.16 ± 0.15 [d]
	30	3.00 ± 0.25 [a]	3.50 ± 0.14 [a]	4.05 ± 0.08 [b]	4.01 ± 0.10 [b]	5.42 ± 0.11 [c]	5.44 ± 0.17 [c]	6.89 ± 0.10 [d]	6.82 ± 0.22 [d]	9.06 ± 0.20 [e]	9.11 ± 0.29 [e]
Cucumber	10	3.21 ± 0.18 [a]	3.35 ± 0.18 [a]	3.04 ± 0.26 [a]	3.37 ± 0.16 [a]	3.24 ± 0.10 [a]	3.36 ± 0.17 [a]	3.09 ± 0.22 [a]	3.48 ± 0.23 [a]	3.60 ± 0.11 [b]	3.76 ± 0.10 [b]
	20	3.20 ± 0.17 [a]	3.32 ± 0.15 [a]	3.38 ± 0.19 [a]	3.51 ± 0.30 [a]	3.84 ± 0.13 [b]	4.11 ± 0.13 [b]	4.04 ± 0.14 [b]	4.05 ± 0.10 [b]	8.30 ± 0.14 [c]	8.28 ± 0.06 [c]
	30	3.25 ± 0.20 [a]	3.24 ± 0.20 [a]	3.80 ± 0.21 [b]	3.87 ± 0.14 [b]	5.51 ± 0.20 [c]	5.65 ± 0.27 [c]	7.00 ± 0.14 [d]	7.07 ± 0.18 [d]	9.21 ± 0.14 [e]	9.34 ± 0.26 [e]
Watermelon	10	3.45 ± 0.21 [a]	3.54 ± 0.16 [a]	3.08 ± 0.18 [a]	3.45 ± 0.14 [a]	3.04 ± 0.09 [a]	3.44 ± 0.13 [a]	3.43 ± 0.21 [a]	3.57 ± 0.12 [a]	4.05 ± 0.09 [b]	4.04 ± 0.13 [b]
	20	3.35 ± 0.19 [a]	3.44 ± 0.22 [a]	3.32 ± 0.16 [a]	3.49 ± 0.19 [a]	3.90 ± 0.10 [b]	4.01 ± 0.12 [b]	4.39 ± 0.15 [c]	4.45 ± 0.15 [c]	8.71 ± 0.22 [d]	8.77 ± 0.11 [d]
	30	3.38 ± 0.18 [a]	3.45 ± 0.21 [a]	3.92 ± 0.09 [b]	4.04 ± 0.06 [b]	5.36 ± 0.03 [c]	5.36 ± 0.04 [c]	7.12 ± 0.21 [d]	7.10 ± 0.13 [d]	9.49 ± 0.19 [e]	9.40 ± 0.20 [e]
Broccoli	10	3.33 ± 0.26 [a]	3.40 ± 0.23 [a]	3.35 ± 0.17 [a]	3.60 ± 0.19 [a]	3.30 ± 0.27 [a]	3.55 ± 0.27 [a]	3.57 ± 0.22 [a]	3.52 ± 0.36 [a]	3.80 ± 0.14 [b]	3.81 ± 0.46 [b]
	20	3.36 ± 0.25 [a]	3.44 ± 0.22 [a]	3.56 ± 0.14 [a]	3.69 ± 0.29 [a]	3.83 ± 0.14 [b]	4.03 ± 0.30 [b]	4.22 ± 0.24 [c]	4.82 ± 0.55 [c]	7.69 ± 0.21 [d]	9.14 ± 0.19 [e]
	30	3.28 ± 0.31 [a]	3.42 ± 0.13 [a]	4.00 ± 0.20 [b]	4.13 ± 0.17 [b]	5.61 ± 0.29 [c]	5.62 ± 0.32 [c]	6.92 ± 0.27 [d]	7.06 ± 0.13 [d]	9.55 ± 0.27 [e]	9.89 ± 0.12 [e]

Data represent mean ± standard deviation of minimum 3 measurements; Means with the same lowercase letter within a row are not significantly different ($p > 0.05$); Means with different lowercase letters in the same row (same temperature condition) are significantly different ($p < 0.05$).

Determination of growth potential (α)

The growth potential (α) of *S. aureus* and *E. coli* on each fruit and vegetable was estimated by calculating the log difference between the initial counts and the counts at 24 hours. *S. aureus* and *E. coli* were considered unable to grow on the foods when α value was negative or lower than 0.5 log [19].

Statistical analysis

The *S. aureus* and *E. coli* growth on all fruits and vegetables was statistically analysed via examination of variance (ANOVA) by applying the Tukey test ($p < 0.05$). The experimental data were analysed by using Statistic 13.0 software (STATSOFT Inc., Tulsa, OK, USA).

Results

Commercial restaurants evaluation

Among the restaurants visited, 34.0% served up to 150 daily meals; 38.3% served between 150 and 300 daily meals, and 27.7% served more than 300 daily meals. Observing that if the restaurants had maximum attendance five days a week, then fruits and vegetables would have high distribution an average of four days a week. The fruits and vegetables more often exposed at the buffets included cucumber (3.61 ± 1.45 days), green cabbage (3.96 ± 1.33 days), cooked broccoli (4.06 ± 1.20 days), watermelon (4.21 ± 1.32 days), grated carrot (4.31 ± 1.16 days), papaya (4.67 ± 0.88 days) and tomato (4.91 ± 0.41 days). Due to these high rates of exposure, these foods were chosen for this study.

S. aureus, E. coli and mesophilic microorganism growth on fruits and vegetables

Samples from each fruit and vegetable used in this study were analysed for the presence of *E. coli* and *S. aureus* before inoculation. These pathogens were not detected on any of the analysed samples. Results shown in Table 2 indicate that *S. aureus* did not grow on any evaluated fruit or vegetable stored at 10 °C until 24 hours. For tomatoes, *S. aureus* did not grow at any evaluated temperature, indicating that the pool used for this pathogen did not adapt to this food (Table 2). At 20 °C, there was not *S. aureus* growth on grated carrot and cabbage during 24 hours. At the same temperature, *S. aureus* growth was detected in cucumber and watermelon after 6 hours; however, at 30 °C, the growth of this pathogen was significant after only 2 hours in both foods. For broccoli, at 20 and 30 °C, *S. aureus* growth began in less than 2 hours ($p < 0.05$), with 24 hours demonstrating the highest final populations (8.14 log CFU/g at 20 °C and 9.44 log CFU/g at 30 °C). Growth for mesophilic bacteria was similar for all foods evaluated at 10 °C in relation to *S. aureus*, but at 20 and 30 °C the total count was higher.

There was not *E. coli* growth in all evaluated fruits and vegetables exposed at 10 °C until 6 h (Table 3). In contrast to *S. aureus* growth, *E. coli* was able to grow on tomatoes in only 4 hours at 30 °C. Like with the tomatoes, there was *E. coli* growth on grated carrot and green cabbage after 4 hours at 30 °C and after 6 hours at 20 °C. Papaya, cucumber, watermelon and broccoli all demonstrated faster growth of *E. coli*. At 20 °C, this microorganism was able to grow on these fruits and vegetables in 4 hours, while *E. coli* was able to grow at 30 °C in 2 hours ($p < 0.05$). Mesophilic bacteria growth was similar to *E. coli* growth for these foods (Table 3).

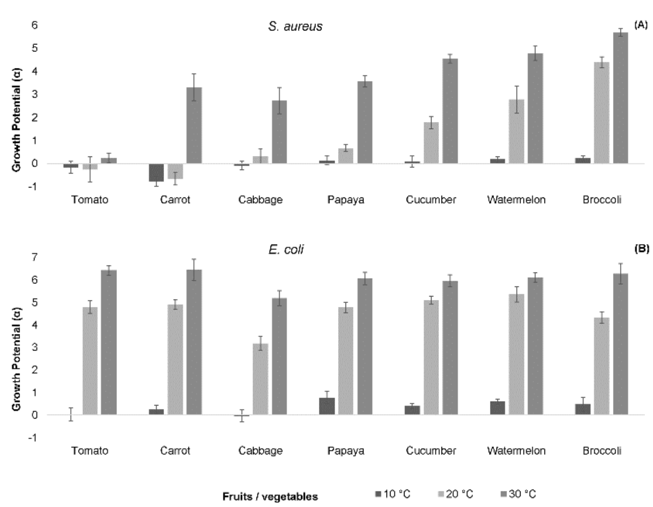
S. aureus and E. coli growth potential on fruits and vegetables

At 10 °C, no *S. aureus* growth potential was observed until 24 hours for all fruits and vegetables evaluated (Figure 1A). At the same temperature, the *E. coli* growth potential was insignificant for tomato, carrot and cabbage (Figure 1B). However, *E. coli* had a surprisingly growth rate for tomatoes at 20 and 30 °C, with $\alpha = 4.80$ and $\alpha = 6.43$, respectively. The carrot also drew attention because it had negative *S. aureus* growth potential at 10 and 20 °C.

Discussion

The interaction between intrinsic and extrinsic factors is fundamental to guarantee the quality and safety of food. In this work, foods with different pH and a_w were kept for 24 hours at different temperatures to determine safe parameters for their distribution in food

Figure 1. *S. aureus* (A) and *E. coli* (B) growth potential (α) on fruits and vegetables at 10, 20 and 30 °C.



services. We chose to start with the temperature 10 °C because it is more easily attained in food services. The results showed that both *S. aureus* (Table 2) and *E. coli* (Table 3) did not grow at this temperature for 24 hours. Similarly, Likotrafiti *et al*. [20] showed that *Listeria monocytogenes* and *Escherichia coli* O157:H7 also did not grow on cucumber stored at 10 °C for 24 hours, suggesting that this temperature can be used to maintain fruits and vegetables for a short period of time.

The absence of *S. aureus* growth at 20 °C on grated carrot and cabbage may be partially explained by these vegetables composition. Babic *et al.* [21] have showed that extracts of peeled and shredded carrots have an antimicrobial effect against foodborne microorganisms like *S. aureus* and *L. monocytogenes*. They have suggested that this antimicrobial activity is due to apolar compounds present in purified and active extracts of carrots. As for green cabbage, Rúa *et al.* [22] have demonstrated that various phenolic compounds naturally present in plants showed antimicrobial activity against several *S. aureus* strains. Although there was not *S. aureus* growth on the grated carrots and green cabbage, there was a significant mesophilic bacteria growth in 24 hours (Table 2). According to McLandsborough [23], *S. aureus* is a relatively poor competitor with food microbiota. Thus, besides the food matrix, the growth of other bacteria may have inhibited *S. aureus* growth. In the same way, *S. aureus* did not grow on papaya at 20 °C for 6 hours, which can be explained by this fruit composition. Studies have suggested that the high quantity of proteolytic enzymes present in papaya, including papain and chymopapain, can inhibit *S. aureus* growth [24,25]. However, *S. aureus* was able to grow on cucumber, watermelon and broccoli and this growth may be explained by the high presence of vitamins, high water activity and pH of these vegetables and the lack of inhibiting enzymes (Table 1). Broccoli demonstrated higher final populations and was the food that had pH closer to neutrality (6.71), thus probably favouring *S. aureus* growth. Another factor that may have contributed to this result was the fact that the broccoli was cooked, which may have resulted in competitive flora elimination.

For the tomatoes, *S. aureus* did not grow at any evaluated temperature. Although the tomato has high water activity, its low pH (Table 1) may constitute an unfavourable environment for microorganism growth. However, the *E. coli* was able to grow on this food, surprisingly reaching a final population of 9.7 log CFU/g at 30 °C in 24 hours (Table 3). While in our study, *E. coli* grew after 4 hours at 20 ° C, the Food and Drug Administration (FDA) [26] has reported greater lag times for *Salmonella* on cut Beefsteak tomatoes (pH = 4.54), 5.29 to 7.49 hours at 22.2 °C. Such findings demonstrate the importance of the time and temperature controls for fruits and vegetables in food services.

We calculated the *S. aureus* and *E. coli* growth potential on the evaluated fruits to better understand their microbial behaviour in 24 hours (Figure 1). According to Sant'Ana *et al.* [19], the pathogens growth is not considered if growth potential (α) is equal to or lower than 0.5 log CFU/g. When comparing both microorganisms, *E. coli* demonstrated higher growth potential than *S. aureus* among the fruits and vegetables analysed (Figure 1). The negative growth potential with carrots (Figure 1A) may have occurred because the *S. aureus* population was reduced in 24 hours, probably because of the competition with mesophilic bacteria, which presented higher values at 10 and 20 °C (Table 2). We suggest that the *E. coli* strains used in this work have more significant growth in fruits and vegetables than the *S. aureus* strains. Furthermore, *E. coli* showed to be more resistant against the competitive microbiota existing in the analysed foods.

To ensure food microbiological safety and quality, predictive microbiology can forecast the response of microorganism growth in relation to factor variations such as temperature, storage conditions, humidity and pH. Preliminary studies highlight the necessity for improving the use of predictive microbiology because this tool can assist food services and regulatory agencies, thus maintaining the quality and microbiological safety of fresh produce [27,28]. From this global study, we can choose the fruits and vegetables most susceptible to pathogen growth in order to build predictive models and to better control the time and temperature of distribution in food services.

Conclusion

Based on our results, fruits and vegetables kept at 10 °C did not demonstrate *S. aureus* and *E. coli* growth, suggesting that foods maintained at temperatures equal to or lower than 10 °C would be safely conserved, at least during the period of distribution (6 hours). However, our results demonstrated that *E. coli* was able to grow in less than 2 hours at 30 °C, indicating fruits and vegetables have to be kept at refrigeration temperatures, especially on warm days. This approach may be also a suggestion to food safety managers for better defining the control measures to be adopted in food services to prevent foodborne diseases transmitted by fruits and vegetables. However, it is important to note that other pathogens, especially psychrotolerantes like *Listeria monocytogenes*, should be explored.

References

1. Produce for Better Health Foundation (2015) Study on America's consumption. State of the plate. Available: http://www.pbhfoundation.org. Accessed: 21 April 2018.
2. Brazilian Department of Health Surveillance (2016) Outbreaks of foodborne diseases in Brazil. Available: http://portalarquivos.saude.gov.br/images/pdf/2016/junho/08/Apresenta----o-Surtos-DTA-2016.pdf. Acessed: 10 June 2019. [Available in Portuguese].
3. European Food Safety Authority (EFSA) (2013). Scientific opinion on the risk posed by pathogens in food of non-animal origin. Part 1 (outbreak data analysis and risk ranking of food/pathogen combinations) EFSA J 11: 3025-3163.
4. Ho J, Boost M, O'Donoghue M (2015). Sustainable reduction of nasal colonization and hand contamination with *Staphylococcus aureus* in food handlers, 2002-2011. Epimediol Infect 143: 1751-1760.
5. Lambrechts AA, Human IS, Doughari JH, Lues JFR (2014) Bacterial contamination of the hands of food handlers as indicator of hand washing efficacy in some convenient food industries in South Africa. Pak J Med Sci 30: 755–758.
6. Roussel C, Cordonier C, Livrelli V, Wiele TV, Blanquet-Diot S (2017) Enterotoxigenic and enterohemorrhagic *Escherichia coli*: Survival and modulation of virulence in the human gastrointestinal tract. In Samie A, editor. *Escherichia coli*: recent advances on physiology, pathogenesis and biotechnological applications. London: IntechOpen. 3-24.
7. Harris LJ, Farber JN, Beuchat LR, Parish ME, Suslow TV, Garrett EH, Busta FF (2003) Outbreaks associated with fresh produce: incidence, growth, and survival of pathogens in fresh and fresh cut produce. Compr Rev Food Sci F 2: 78 – 141.
8. Seo YH, Jang JH, Moon KD (2010) Occurrence and characterization of enterotoxigenic *Staphylococcus aureus* isolated from minimally processed vegetables and sprouts in Korea. Food Sci Biotechnol 19: 313-319.
9. Laidler MR, Tourdjman M, Buser GL, Hostetler T, Repp KK, Leman R, Samadpour M, Keene WE (2013) *Escherichia coli* O157:H7 infections associated with consumption of locally grown strawberries contaminated by deer. Clin Infect Dis 57: 1129-1134.
10. Estrada CS, Alcaráz LE, Satorres SE, Manfredi E, Velázquez LC (2014). Presence of enterotoxigenic *Staphylococcus aureus* in artisan fruit salads in the city of San Luis, Argentina. Braz J Microbiol 44: 1155-1161.
11. Shah MS, Eppinger M, Ahmed S, Shah AA, Hameed A, Hansan F (2015). Multidrug-resistant diarrheagenic *E. coli* pathotypes are associated with ready-to-eat salad and vegetables in Pakistan. J Appl Biol Chem 58: 267–273.
12. Hong J, Kim Y, Kim J, Heu S, Kim SR, Kim KP, Roh E (2015). Genetic diversity and antibiotic resistance patterns of *Staphylococcus aureus* isolated from leaf vegetables in Korea. J Food Sci 80: 1526-1531.
13. Al-Kharousi ZS, Guizani N, Al-Sadi AM, Al-Bulushi IM, Shaharoona B (2016). Hiding in fresh fruits and vegetables: opportunistic pathogens may cross geographical barriers. Int J Microbiol 2016: 14.
14. Tian J, Bae YM, Choi NY, Kang DH, Heu S, Lee SY (2012) Survival and growth of foodborne pathogens in minimally processed vegetables at 4 and 15 °C. J Food Sci 71: 48-50.
15. Cunha DT, Oliveira ABA, Saccol ALF, Tondo EC, Junior EAS, Ginani VC, Montesano FT, Castro AKF, Stedefeldt E (2014) Food safety of food services within the destinations of the 2014 FIFA World Cup in Brazil: Development and reliability assessment of the official evaluation instrument. Food Res Int 57: 95-103.
16. Oliveira ABA, Cunha DT, Stedefeldt E, Capalonga R, Tondo EC, Cardoso, MRI (2014) Hygiene and good practices in school meal services: Organic matter on surfaces, microorganisms and health risks. Food Control 40: 120-126.
17. National Health of Surveillance Agency (2004). Resolution nº 216, September 15th 2004. Technical Regulation of Good Practices for Food Services. Diário Oficial da União, Brasília. [Available in Portuguese].
18. Miles AAL, Misra SS (1938) The estimation of the bacterial power of the blood. J Hyg 38: 732–749.
19. Sant'Ana AS, Landgraf M, Destro MT, Franco BD (2013). Growth potential of *Salmonella* and *Listeria monocytogenes* in ready-to-eat lettuce and collard greens packaged under modified atmosphere and in perforated film. J Food Prot 76: 888-891.
20. Likotrafiti E, Smirniotis P, Nastou A, Rhoades J (2013) Effect of relative humidity and storage temperature on the behavior of *Listeria monocytogenes* on fresh vegetables. J Food Res 33: 545-551.
21. Babic I, Nguyen C, Amiot MJ, Aubert S (1994) Antimicrobial activity of shredded carrot extracts on food-borne bacteria and yeast. J Appl Bacteriol 76: 135-141.
22. Rúa J, Fernández-Alvarez L, Gutiérrez-Larraínzar M, Valle P, Arriaga D, García-Armesto MR (2010) Screening of phenolic antioxidants for their inhibitory activity against foodborne *Staphylococcus aureus* strains. Foodborne Pathog Dis 7: 695-705.
23. McLandsborough L (2004) Food Microbiology Laboratory, 1st edition. Florida: CDC Press 196 p.
24. Emeruwa AC (1982) Antibacterial substance from *Carica papaya* fruit extract. J Nat Prod 45: 123-127.
25. Vij T, Prashar Y (2015) A review on medicinal properties of *Carica papaya* Linn. Asian Pac J Trop Dis 5: 1-6.
26. Food and Drug Administration (2015) Program information manual retail food protection-storage and handling of tomatoes. Available: http://www.fda.gov/Food/GuidanceRegulation/RetailFoodProtection/IndustryandRegulatoryAssistanceandTrainingResources/ucm113843.htm. Accessed: 21 May 2018.
27. Caleb OJ, Mahajan PV, Al-Said FAJ, Opara UL (2013) Modified atmosphere packaging technology of fresh and fresh-cut produce and the microbial consequences - A review. Food Bioprocess Tech 6: 303-329.
28. Martins RC, Lopes VV, Vicente AA (2008) Computational shelf-life dating: complex systems approaches to food quality and safety. Food Bioprocess Tech 1: 207-222.

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