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Heat resistance of *Staphylococcus aureus*, *Salmonella* sp., and *Escherichia coli* isolated from frequently consumed foods in the Lebanese market

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ABSTRACT

The purpose of this study was to determine the heat resistance of isolated *Staphylococcus aureus*, *Salmonella* sp., and *Escherichia coli* bacterial strains obtained from frequently consumed foods (chicken sandwiches, cheese, raw chicken, tahini, and raw meat) in Lebanon. The isolated bacterial strains were heat-treated at 55, 60, 65, 70, and 75°C. The colony-forming unit per gram (CFU.g⁻¹) was recorded for each temperature every minute for 10 min. Survival curves were plotted, and D- and z-values were calculated and compared using a two-sample T-test. All bacterial strains exhibited total resistance to heat at 55°C. Their D₆₀ ranged from 3.00 to 28.80 min, D₆₅ from 0.47 to 1.78 min, D₇₀ from 0.19 to 1.91 min, and D₇₅ from 0.11 to 0.46 min. Their z-values ranged from 4.74 to 19.65°C. When comparing these results to previous years, the need for combined interventions, such as active packaging solution, seems necessary as *S. aureus*, *Salmonella* sp., and *E. coli* are continually developing resistance to thermal processing. This study highlights emerging concerns regarding the safety of certain food products in the market. These results suggest further revising of the associated processing conditions to ensure compliance with food safety standards and avoid foodborne disease outbreaks.

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Introduction

According to the Centers for Disease Control and Prevention (CDC), “a foodborne disease outbreak is defined as an incident in which two or more persons experience a similar illness resulting from the ingestion of a common food”.^[1] Those diseases involve a large and diverse range of agents, the number of which continues to increase yearly as new agents are identified. Agents like, but not limited to, *Staphylococcus aureus*, *Salmonella* sp., *Clostridium perfringens*, *Campylobacter*, *Listeria monocytogenes*, *Vibrio parahaemolyticus*, *Bacillus cereus*, and *Escherichia coli* are considered to be involved in diseases transmitted by foods.^[2] C-1: References should be number format? It is estimated that in the United States, around 5 million people get sick each year due to foodborne illnesses, of which 31 known species of pathogens cause around 10 million cases, more than 100,000 are hospitalized, and 3,000 result in fatalities.^[3] In Lebanon, the number of food poisoning marked an increase going from 57 cases in 2005, to 510 cases in 2010 and 1,204 cases in 2021. These numbers still hide scores of unreported cases.^[4–7]

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Public health researchers are of specific interest as they are still strongly associated with foodborne illnesses triggered by consuming contaminated raw food and cross-contamination.^[8] When investigating the source of those foodborne diseases, studies indicated the presence of *S. aureus*, *Salmonella*, and *E. coli* in food products from developing countries,^[8] including Lebanon.^[9–14] *Staphylococcus aureus* is the cause of 2.6% of foodborne illnesses each year in the U.S. In Europe, the number of staphylococcal food poisoning incidents is also rising. In 2018, there were 114 reported outbreaks caused by staphylococcal toxins, with 1,124 human cases, where only 167 required hospitalizations.^[15] *Salmonella* sp. and *E. coli* were the cause of 13.3% and 3.6% of the infections in the U.S. in 2020, respectively.^[3]

Pasteurization is a relatively mild heat treatment in which liquid foods are mostly heated to below 100°C. It is used to minimize public health hazards from pathogenic microorganisms and to extend the shelf-life of foods for several days or weeks. In general, it has shown to be effective in destroying mesophilic microorganisms such as *S. aureus*, *Salmonella*, and *E. coli*.^[16] However, studies showed that bacteria develop resistance to heat when exposed to sub-lethal levels of stress.^[17–19] For example, in 2010, a partial increase in resistance of *S. aureus* was observed in the presence of many inhibitors (pH, desiccation hydrogen peroxide, etc.) during heat treatment.^[17] In fact, during thermal treatment, those three bacterial strains have a heat resistance response to protect themselves from cell destruction. It consists of the production of Heat Shock Proteins that help cells avoid protein denaturation and stabilization of membrane fluidity.^[20–22]

Treating foods with higher levels of heat may not constitute an optimal solution, as it tends to negatively alter the sensory and nutritional quality of the food product and therefore hinder consumer acceptance.^[16] Therefore, this study aims at determining the current heat resistance attributes of pure *S. aureus*, *Salmonella* sp., and *E. coli* cultures isolated from frequently consumed foods (chicken sandwiches, cheese, chicken, tahini, and raw meat) in Lebanon by exposing them to five temperature levels ranging from 55°C to 75°C, over 10 min. This intends to identify recent changes in the heat level and exposure time needed to eliminate those foodborne pathogens. If such levels were found unreasonable, exploring alternative or synergistic methods to end this vicious thermal resistance cycle would prove necessary.

Materials and methods

Materials

Staphylococcus aureus, *Salmonella* sp., and *E. coli* were isolated from highly consumed food products in Lebanon. *Staphylococcus aureus* and *E. coli* were isolated using Rapid'Staph Agar (BIO-RAD, France) and RAPID *E. coli* 2 agar (BIO-RAD, France), respectively. For *Salmonella* sp., Rappaport Vassiliadis Soya broth (RVSB) (BIO-RAD, France) for enrichment, RAPID'Salmonella agar plates (BIORAD – France) for isolation, Triple Sugar Iron Agar (TSI) (Oxoid Ltd., U.K.) and Lysine Iron Agar (LI) (Oxoid Ltd., U.K.) slants, and API 20E (BioMerieux, France) for confirmation. Latex agglutination test (Pastorex Staph Plus) was used as a confirmation test for *S. aureus* isolates.

The medium used to grow the conserved bacterial strains was the Brain Heart Infusion (BHI) broth (BIO-RAD, France) and Plate Count Agar (PCA) (BIO-RAD, France) for colony counting. Tryptose Soya Broth (TSB) (OXOID Ltd., U.K.) was the medium employed during the thermal resistance experiment, and peptone water (BIO-RAD, France) was utilized for dilution purposes.

Methods

Food samples collection

A random sample collection of highly consumed food products was accumulated from several sources in the Beirut area. A total of 20 samples were collected from retail and food services, including raw chicken, minced meat, ready-to-eat chicken and meat sandwiches, and tahini in addition to cheese.

Bacterial isolation from food samples. Samples were tested according to ISO 16140. Samples were analyzed for the presence of *Staphylococcus aureus*, *Salmonella* sp., and *E. coli*.

For the enumeration of *Salmonella* sp., selective enrichment was performed in RVSB and incubated at 41.5°C for 8 h. After incubation, 10 µl of the enrichment broth was streaked on RAPID'*Salmonella* agar plates in duplicates and incubated at 37°C for 24 h. Presumptive colonies were further identified biochemically on TSI, and LI slants were incubated at 37°C for 24 h. Additional confirmation for positive *Salmonella* spp. colonies were performed by API 20E.

For the enumeration of *S. aureus* and *E. coli*, 10 g of the samples were weighed in stomacher bags and homogenized with 90 ml buffered peptone water for 2 min. Serial dilutions were prepared, 0.1 ml was spread in duplicates on Rapid'*Staph* Agar, and presumptive colonies were further confirmed on the latex agglutination test. Additionally, for the isolation of *E. coli*, the pour plate technique was adapted; an aliquot of 1 ml was added into Petri dishes using RAPID *E. coli* 2 agar. All plates were incubated at 37°C for 48 h. Confirmed *S. aureus*, *Salmonella* sp., and *E. coli* isolates were stored in 30% glycerol solution at -20°C to be used in the following tests.

Culture growth

A loop of the bacterial isolate was taken from each frozen isolate, streaked onto PCA, and incubated at 37°C overnight. From the growing bacterial colonies, 5 ml were incubated in a BHI broth test tube at 37°C for 24 h.

Microbial thermal tolerance

A preliminary study was conducted to determine the thermal tolerance of the bacterial isolates at 73°C for 15 s. Among the isolates, the ones that survive this process are considered tolerant and will be tested for thermal resistance. A 10⁻² dilution from the BHI tube was done in a TSB that served as a control. A 10⁻⁶ dilution in peptone water was prepared to count the colony-forming unit per ml (CFU/ml) in the initial medium; 0.1 ml of the solution was spread on a PCA plate and incubated at 37°C for 24 to 48 h. On the other hand, to test the isolates' heat tolerance, another 10⁻² dilution was done in a TSB medium heated at 73°C for 15 s, and the count of the CFU/ml was done following a serial dilution in peptone water. Viable colonies (25–250) were enumerated, and the most tolerant ones were isolated. Data were obtained in duplicates, and the experiment was done in triplicates.

Heat resistance test

Six serial dilutions from the BHI tube were done in cold peptone water sterile tube. 0.1 ml from the 6th dilution was then spread on a PCA plate and incubated at 37°C for 24 to 48 h for counting. In a preheated water bath, another 10⁻² dilution was prepared from the BHI tube in a TSB jar held at 55, 60, 65, 70, and 75°C. Every minute over 10 min, 1 ml was removed from the heated medium and transferred into 9 ml cold peptone water sterile tubes on ice. The bacterial count was done after dilution, plating, and incubation for each temperature and at every minute of sampling. Data were obtained in duplicates, and the experiment was repeated twice.

Calculation of D- and z-values and statistical analysis

The CFU/ml of all bacteria were calculated, and their log₁₀ was plotted against time to obtain their decimal reduction time (D-values) for each temperature. The D-values are the time required to kill 90% of the bacterial population at a specific temperature. In addition, the log D-values were plotted against temperature to obtain the thermal death time (z-values), which is the increase in temperature required to reduce to 10⁻¹ of its previous value, meaning to kill 90% of the bacterial population. Subsequently, the D- and z values were calculated using the following equation: -1/slope (Kennedy

et al., 2005). The thermal inactivation reactions of the studied bacteria at different temperatures followed different kinetic models. Statistical analysis using two sample T-test was computed using Excel (Microsoft, USA), considering significance when $p < .05$, to evaluate differences in bacterial resistance to heat treatments.

Results

Microbial thermal tolerance

Twenty-seven bacteria are isolated from the 20 food samples: 16 *Salmonella* sp., six *E. coli*, and five *S. aureus*. Under pasteurization conditions (73°C for 15 s), only seven out of twenty-seven isolates are considered heat tolerant, and the thermal tolerance data of the seven selected isolates are presented in Table 1. In addition, the limits of *S. aureus*, *Salmonella* sp., and *E. coli* in food are 3 to 4, 0, and 2 Log CFU. g⁻¹, respectively. Accordingly, a treatment at 73°C for 15 s is insufficient to reduce the incidence of those three contaminants from the media since the Log CFU. g⁻¹ of all the isolates is still higher than the accepted limits. Therefore, studying their behavior at different temperatures and times is of interest.

Microbial heat resistance

Staphylococcus aureus: Figure 1a illustrates a higher resilience exhibited by the strain St1 at all temperatures tested compared to St3 (Figure 1b). Heating *S. aureus* at 55 and 60°C did not affect

Table 1. Log CFU g⁻¹ of *S. aureus* (St), *Salmonella* sp. (S), and *E. coli* (F.C.) isolates before and after heat treatment at 73°C for 15s in TSB medium.

Bacterium	Food origin	Isolates	Log CFU.g ⁻¹	
			Initial*	At 73°C after 15s*
<i>Staphylococcus aureus</i>	Chicken	St1	10.36 ± 0.354	6.73 ± 0.852
	Cheese	St3	10.02 ± 0.688	5.83 ± 0.182
<i>Salmonella</i>	Tahini (sesame paste)	S1	9.09 ± 0.032	4.35 ± 0.526
	Chicken	S4	9.06 ± 0.122	4.66 ± 0.592
	Chicken	S7	8.90 ± 0.037	4.50 ± 1.498
<i>Escherichia coli</i>	Raw meat	FC2	8.83 ± 0.362	4.49 ± 1.749
	Raw meat	FC4	9.13 ± 0.032	5.66 ± 0.542

*Results are means ± S.D. of triplicate measurements

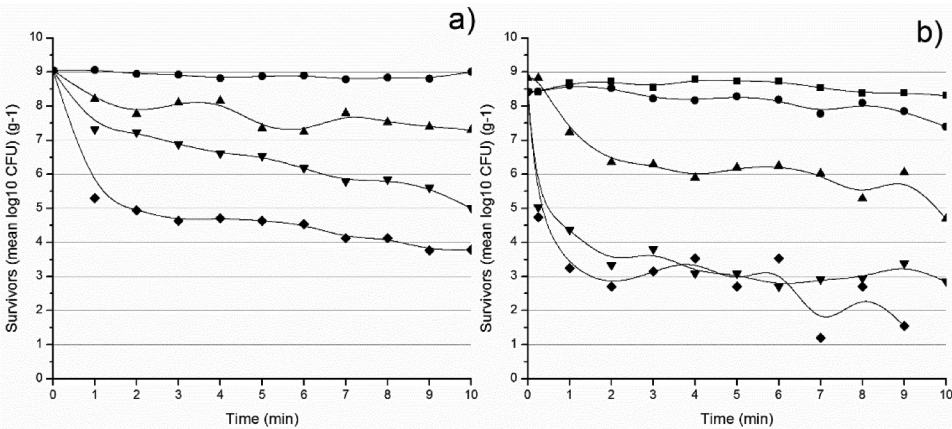


Figure 1. Survivor curves of *Staphylococcus aureus* isolates a) St1 and b) St3 at 55 (—■—), 60°C (—●—), 65°C (—▲—), 70°C (—▼—) and 75°C (—◆—).

the death of the two tested isolates ($p > .05$), after 2 min, a decrease of 1 log cycle was observed at 65 and 70°C with their D-values recordings at 1.91 and 1.78 min, respectively ($p < .05$). Moreover, St1 did not die off even after 10 min of heating up at 75°C; its D_{75} was equal to 0.44 min. It was also required to increase the temperature by 12.36°C to inactivate 90% of viable cells of this strain (Table 2). The thermal inactivation of ST1 at 60 and 65°C followed a respective 4th and 3rd order polynomial trajectory with an R^2 of 0.8, while at 70 and 75°C, it manifested a respective 2nd and 3rd order kinetics with an R^2 of 0.9.

The isolate St3 was considered resilient; however, it did not completely die off at any combination of time and temperature (Figure 1b) as well as it was until the temperature reached 65°C that a significant drop in Log CFU.g^{-1} took place ($p < .05$). Thermal inactivation of ST2 at 60°C followed a linear trend with a D-value of 11.38 min and continued with 2nd order for 65°C with a D-value of 1.47 min and 3rd order for 70 and 75°C with D_{70} 0.45 min and D_{75} 0.19 min. R^2 was equal to 0.8 for all temperature trajectories. As for the z-value, a rise in temperature of 8.57°C was needed to induce a drop of 1 log cycle for this strain (Table 2).

Salmonella sp.: Regarding the *Salmonella* sp. isolates, Figure 2a shows only a slight decrease in the survival rate at 60°C of the S1 isolate, but it becomes more pronounced with the increase in temperature ($p < .05$). It eventually dies off after 2 min at 70°C. The thermal inactivation of S1 at 60°C followed first-order kinetics with $R^2 = 0.9$ and a D-value of 9.45 min. It continues with a 3rd-order polynomial at 65, 70, and 75°C with R^2 of 0.8, 0.9, and 0.9 and D-values of 0.49, 0.19, and 0.19, respectively. The z-value obtained from those D-values was 9.14°C (Table 2).

While 55°C still did not affect the death rate of S4 ($p > .05$), the decreasing trend at 60°C was shown to be a bit steeper; however, it persisted till 6 min at 75°C to completely die off (Figure 2b). The 65°C trajectory represented a 3rd-degree polynomial giving an R^2 of 0.9 and a D-value of 0.47 min. The thermal inactivation at 60°C followed a 2nd-order kinetics with R^2 equals 0.9 and D-value recording 3.64 min, while 70 and 75°C followed a 5th degree polynomial with their R^2 0.8 and 0.9 and their D-values 0.19 and 0.13 min respectively; their calculated z-value was equal to 10.73°C (Table 2).

Lastly and again, heating S7 at 55°C for 10 min did not decrease its bacterial log CFU.g^{-1} ($p > .05$). However, the 60°C trajectory, as shown in Figure 2c, marked a sharper decline until ultimate death was reached after 4 min at 70°C and after 3 min at 75°C ($p < .05$). Thermal inactivation at 55°C followed the 4th-order kinetics and continued with 2nd order for 60, 65, 70, and 75°C. All inactivation curves generated a common R^2 of 0.8 and D-values of 3.00, 0.69, 0.54, and 0.46 min, respectively, and a z-value of 19.65°C (Table 2).

Table 2. D-values calculated using the equation $D = -1/\text{slope}$ for the plots of surviving cell numbers (\log_{10} CFU) vs. treatment times and z values calculated using the equation $z = -1/\text{slope}$, for the plots of \log_{10} D vs. temperature of *S. aureus* (St), *Salmonella* sp. (S) and *E. coli* (F.C.) isolates at 55, 60, 65, and 75°C. The results of this study were compared to previous studies.

		D-values (min) at					z values (°C)	Date	Reference
Isolate		55°C	60°C	65°C	70°C	75°C			
<i>S. aureus</i>	St1	No effect	10.1	1.91	1.78	0.44	12.36	2022	This study
	St3	No effect	11.38	1.47	0.45	0.19	8.57	2022	This study
		6.59 to 14.3 13 to 21.7	4.8 to 6.5				3.37 to 6.06 7.7 to 8	2011 2005	23 24
<i>Salmonella</i>	S1	No effect	7.32	0.65	0.5	0.38	4.74	2022	This study
	S4	No effect	28.8	1.02	0.11	0.11	6.06	2022	This study
	S7	No effect	9.45	0.49	0.19	0.19	9.14	2022	This study
		7.08 to 7.5	0.32 to 0.52 0.45 to 0.86	0.03 to 0.06			3.78 to 4.58 4.3	2013 2011	25 26
<i>E. coli</i>	FC4	No effect	3.64	0.47	0.19	0.13	10.73	2022	This study
	FC2	No effect	3	0.69	0.54	0.46	19.65	2022	This study
		23.2	>70					2020	27
		4.17						2013	28
		7 to 9.4	4.74 to 0.93	0.055 to 0.07			4.3	2011	29 26

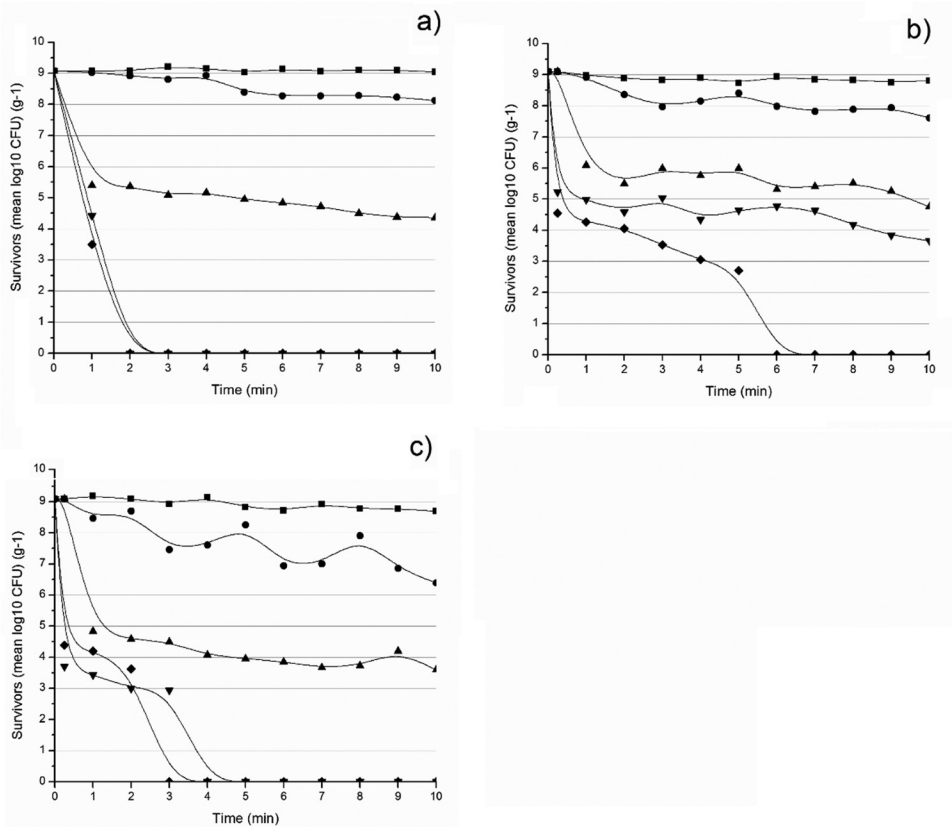


Figure 2. Survivor curves of *Salmonella* sp. isolates **a)** S1, **b)** S4, and **c)** S7 at 55°C (—■—), 60°C (—●—), 65°C (—▲—), 70°C (—▼—), and 75°C (—◆—).

Escherichia coli: Regarding the *E. coli* isolates, results of FC2 revealed no significant changes at 55 and 60°C ($p > .05$) in the bacterial behavior, unlike the effect of temperatures 65, 70, and 75°C ($p < .05$) presented in [Figure 3a](#). The thermal inactivation of FC2 at 60°C followed a linear trend with R^2 0.8 and a D-value of 28.8 min. At 65°C, it followed 2nd-order kinetics with an R^2 of 0.9 and D-value of 1.02 min, while at 70 and 75°C, it manifested a 6th-order polynomial trajectory with an R^2 of 0.8 and a calculated D-value of 0.11 min; their z-value recorded 6.06°C ([Table 2](#)).

Same as FC2, subjecting FC4 to 55 and 60°C for 10 min did not affect its death rate ($p > .05$); it was until the temperature increased to 65, 70, and 75°C that we noticed a clear drop in the log CFU.g⁻¹ across the 10 min period ($p < .05$), as shown in [Figure 3b](#). The thermal inactivation of FC4 at 60, 65, 70, and 75°C followed 3rd-order kinetics with R^2 of 0.8, 0.8, 0.8, and 0.9, respectively, and calculated D-values of 7.32, 0.65, 0.50 and 0.38 min respectively. The z-value was calculated from the obtained D-values of 4.74°C ([Table 2](#)).

Discussion

The studied bacterial isolates of *S. aureus*, *Salmonella* sp., and *E. coli* demonstrate increased heat resistance compared to results obtained in previous studies, which are in accordance with our results presented in [Table 2](#). In 2005, the thermal inactivation of *S. aureus* in TSB resulted in a D_{55} of 13 to 21.7 min, a D_{60} of 4.8 to 6.5 min, and a calculated z value ranging from 7.7 to 8.0°C.^[23] However, in 2011, the D_{54} and D_{55} of several *S. aureus* isolates obtained from ready-to-eat Indonesian traditional foods logged 13.42–23.8 min

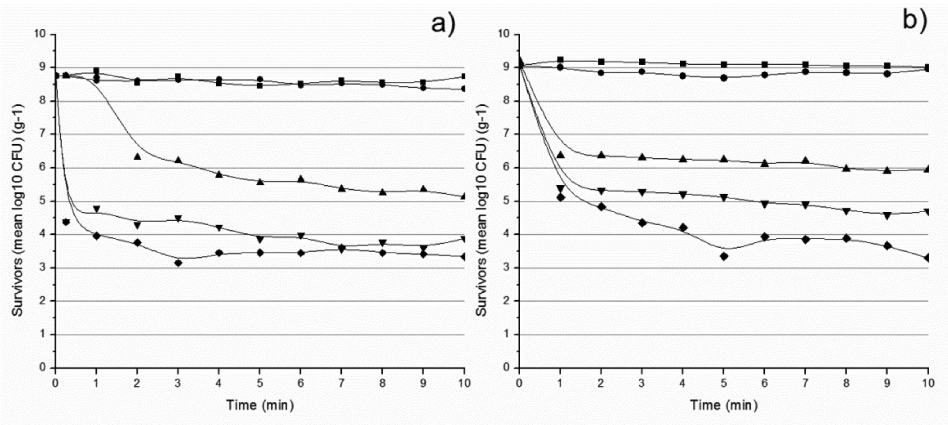


Figure 3. Survivor curves of *E. coli* isolates **a)** FC2 and **b)** FC4 at 55°C (—■—), 60°C (—●—), 65°C (—▲—), 70°C (—▼—) and 75°C (—◆—).

and 6.59–14.3 min, respectively, with their z -values ranging from 3.37 to 6.06°C.^[24] A recent study on the cell viability of *S. aureus* in orange juice showed that it needs 60 min to reduce by 5-log the microbial load when the product is treated at 55°C and 35 min to reduce the load by 10-log.^[25] In this study, the behavior of the *S. aureus* isolates at 55°C was similar to their behavior when no heating was involved, and their D_{60} values were recorded at 10.10 min for St1 and 11.38 min for St3.

The *Salmonella* isolates S1 isolated from tahini exhibited the highest heat resistance, followed by S7 and S4 isolated from chicken. The former was not affected by thermal heating at 55°C for 10 min and behaved as if no heating intervened. It was until the 5th minute at 60°C that significance was detected, whereas S4 and S7 were significantly affected by heating at 60°C as of the 1st min (Table 2). Comparing D_{60} of S1 (9.45 min), S4 (3.64 min), and S7 (3.00 min) to those of *Salmonella* Typhimurium isolated from chicken in Jordan in 2013, we notice a remarkable increase in resistance with D_{58} ranging from 1.06 to 1.30 min, D_{60} ranging from 0.32 to 0.52 min and z -values ranging from 3.78 to 4.58°C.^[26] The z -values in our case were 9.14, 10.73, and 19.65°C for S1, S4, and S7, respectively, which means that it recently requires a minimum of a twofold increase in temperature degrees to reach a 90% decrease in bacterial count. In 2011, thermal inactivation of *Salmonella* seafood and meat isolates were tested at 55, 60, and 65°C, and their D -values ranged from 7.08 to 7.5 min, 0.45 to 0.86 min, and 0.03 to 0.06 min, respectively with a z value equal to 4.3°C.^[27] Moreover, in 2000, to induce a drop of 1 log of *Salmonella* survival, 1.2 s at 71°C was enough or an increase in temperature by only 5.3°C.^[28] In contrast, the heating time should reach 11.4 s for S1 and S4 and 32.4 s for S7 at 70°C to inactivate 1 log of *Salmonella* cells more than 10 folds.

In 2010, a study reported that at 65°C, *E. coli* O157:H7 isolated from a traditional South African sausage subsided by 7.5 log CFU in 1.3 min,^[29] while results of this study showed a decrease of the FC4 isolate by 3 log CFU in 10 min. Additionally, a temperature of 70°C was used to induce the same drop survival in 1 min, whereas, in this study, 4.5 log CFU decreased in 10 min at the same temperature. As for isolate FC2, the CFU/ml dropped 4 log cycles at 65°C and 5 log cycles at 70°C for up to 10 min. In another study describing the effects of culture conditions on the subsequent heat inactivation of *E. coli* O157:H7 in apple juice, the highest D_{55} value among the different treatments was recorded at 4.17 min,^[30] while heating at 55°C in this study did not affect the *E. coli* strains. They behaved as if no heating was involved. The same results were obtained by Gabriel (2012), where the D_{55} of *E. coli* from apple juice ranged from 0.9 to 4.43 min.^[31] Similarly, in 2011, another strain of *E. coli* displayed in 10 min a decrease in CFU of around 1 log at 54°C and of about 5 logs at 58°C^[32]; whereas FC4 and FC2 strains in this study were affected by thermal treatment neither at 55 nor at 60°C. Their log CFU.g⁻¹ remained constant throughout the 10 min. At 65°C, a clear heat development in heat-resistance of *E. coli* can be observed, which is explained by a decrease from a 5 log CFU.g⁻¹ drop in 2011^[33] to a 3–3.5 log CFU.g⁻¹ drop in the isolates in this study. More evidence of resistance is expressed by D -values

comparison over the years. In 2012, D_{55} ranged from 7 to 9.4 min, D_{60} from 4.74 to 0.93 min, and D_{65} from 0.055 to 0.07 min, with a z-value of 4.3°C in a study measuring the inactivation of *E. coli* O157:H7 from catfish and tilapia^[27]; while this present study computed a D_{60} of 7.32 and 28.82 min and a D_{65} of 0.65 and 1.02 min, with a z-value of 4.74°C and 6.06°C for FC4 and FC2 respectively. The trend toward higher resistance is obvious as the thermal inactivation of *E. coli* O157:H7 in coconut liquid endosperm in 2013 was assessed, giving a D_{55} of 23.20 min.^[34] Ma et al. (2020) isolated three *E. coli* from human cases of acute gastroenteritis that resisted heat inactivation at 60°C; their D_{60} -values exceeded 70 min.^[35] When those isolates were tested in grilled ground beef patties, the cell reductions of heat-resistant isolates to 60°C and 71°C remained above 2.8 and 4.9 log CFU/mL, respectively, compared to reductions of 6.1 log CFU/mL and greater in heat-sensitive *E. coli*.^[35]

This study describes the behavior of *S. aureus*, *Salmonella* sp., and *E. coli* isolates in their pure and concentrated state in a liquid-heated medium. This makes them more sensitive and vulnerable to high temperatures as food layers do not protect them. A study on the effect of colony formation on the heat inactivation dynamics of *E. coli* and *Salmonella* proved that bacterial cells surrounded by a jellified environment are protected from heat stress and survive longer compared to their presence in a liquid medium.^[32] In general, the thermal resistance of bacteria varies due to the complexity of the food matrix. A study showed that the food composition, the water activity, and the drying conditions play a major role in the thermal resistance of *Salmonella* in food.^[36] In another study on an inoculated walnut shell, *S. aureus* showed higher resistance when the product's water activity was low. Its D_{64} value increased from 0.56 to 10.21 min when the product's water activity decreased from 0.931 to 0.586.^[37]

Critical limits are established for each bacterial species to determine the safety level of food. For *S. aureus* to induce poisoning and produce symptoms, a minimal amount of less than 1.0 µg of its toxin is enough to be present in the food. This level is reached when *S. aureus* concentration exceeds 10^5 CFU.g⁻¹.^[38] Provided the food was initially contaminated with 10^9 CFU.g⁻¹ before toxin release, as in this study, St3 needs to be heated at 70 or 75°C for more than 15 s and St1 at 75°C for more than 2 min to reach a bacterial population below 10^5 CFU.g⁻¹. However, *S. aureus* levels in food should not exceed 10^3 CFU.g⁻¹ to remain "acceptable" for consumption and should neither exceed 10^2 CFU.g⁻¹ to be considered "good" for consumption according to the FDA and NSW Food Authority guidelines.^[39,40] Therefore, a heat-treatment for ST1 at 75°C for more than 10 min is not enough to reach 10^3 CFU.g⁻¹. For ST7, a treatment at 70°C for 5 min or 75°C for 2 min is enough to reach the 10^3 CFU.g⁻¹. To reach 10^2 CFU.g⁻¹, the medium should be heated at 75°C for 7 min.

According to the Canadian Food Inspection Agency, *Salmonella* in poultry requires a minimum thermal process that induces a 7D decrease to eliminate this pathogen, as stated in the FDA guidelines.^[41] Accordingly, S1 should be treated at 70°C for 2 min, S4 at 75°C for 6 min, and S7 at 70°C for 4 min or 75°C for 2 min to be eliminated from the medium. As for *E. coli*, its levels should be less than 10^2 and less than 10^3 CFU.g⁻¹ respectively, to consider the food satisfactory or acceptable for consumption.^[39,40] Unfortunately, FC2 and FC4 could not reach 10^3 CFU.g⁻¹ even after 10 min heating at 75°C. Thus, the need for exploring and engaging alternative or synergistic methods which are advised to be more effective will ultimately end this vicious thermal resistance cycle and prevent post-cooking recontamination.

Conclusion

The resistance of pathogenic bacterial strains to heat treatments has been widely evaluated over the last few years. Although mesophilic bacteria are considered to be sensitive to pasteurization, they are becoming more resistant due to the heat shock response they are developing and their ability to adapt to environmental stresses such as desiccation, acid, salt, etc. When comparing these results to previous years, the need for combined interventions, such as active packaging solution, seems necessary as pathogens are continually acquiring resistance to heat treatment. This study shows emerging concerns regarding the safety of frequently consumed foods in the Lebanese market. These results highlight further revising of the associated processing conditions to ensure compliance with food safety standards and avoid foodborne disease outbreaks.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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