BRAZILIAN JOURNAL OF FOOD TECHNOLOGY

ISSN 1981-6723
Online version

ORIGINAL ARTICLE

Determination of D and z values for *Salmonella* Typhimurium inoculated in an egg-based pastry

Determinação do valor D e z para *Salmonella* Typhimurium inoculada em produto de pastelaria à base de ovo

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Cite as: Alves, M. M., Machado, C., Saraiva, C., & Silva, J. A. (2020). Determination of D and z values for *Salmonella* Typhimurium inoculated in an egg-based pastry. *Brazilian Journal of Food Technology*, *23*, e2019122. https://doi.org/10.1590/1981-6723.12219

Abstract

Eggs, often used in pastry products, are one of the major transmission vehicles of *Salmonella*. The main purpose of this study was to evaluate *Salmonella* Typhimurium thermal resistance, by D and z values determination, in a traditional Portuguese egg-based pastry and its microbial and physical-chemical characterization. *Salmonella* Typhimurium ATCC 14028 (OXOID C6000L) (Ca. 1.33×10^8 cfu g^{-1}) was inoculated in a liquid batter consisting of 8 whole eggs, 7 egg yolks, 280 g of wheat flour and 250 g of sugar. D and z values determinations were performed after applying a water bath to vacuum-packed samples of liquid batter (5 g), following thermal treatments: 52 °C (45, 90, 135, 180 and 225 min); 55 °C (15, 30, 45, 60, 70 and 80 min); 58 °C (5, 10, 15, 20, 25 and 30 min) and 61 °C (1, 2, 3, 4 and 5 min). Physico-chemical (a_w, pH, moisture, sugar, ashes, protein and free fat) and microbiological determinations (mesophilic microorganisms, psychrotrophic, molds and yeasts, Enterobacteriacea, *E. coli*; *Salmonella* spp., *Bacillus cereus*, *Listeria monocytogenes* and *Staphylococcus aureus*) were also conducted in raw liquid batter. It was possible to investigate different combinations of time and temperature for *Salmonella* Typhimurium inactivation, compared to other studies on egg-based batter, often used to make pastry products. Liquid batter a_w before inoculation was 0.94 and pH 7.26. The D values were obtained 53.19 min, 20.45 min, 6.95 min and 1.60 min, at 52 °C, 55 °C, 58 °C e 61 °C, respectively. From the calculated D values, the corresponding z value was 5.96 °C.

Keywords: Thermal resistance; Thermal inactivation; Raw batter; Contamination; Microbial characterization; Physical-chemical characterization.

Resumo

Utilizados com frequência em produtos de pastelaria, os ovos são um dos principais veículos de transmissão de *Salmonella*. O presente estudo teve como objetivo a avaliação da resistência térmica de *Salmonella* Typhimurium, através da determinação dos valores D e z na massa crua de produto de pastelaria de tradição portuguesa à base de ovo, bem como a sua caracterização microbiológica e físico-química. *Salmonella* Typhimurium ATCC 14028 (OXOID C6000L) (Ca. 1,33×108 ufc/g) foi inoculada na massa crua constituída por 8 ovos inteiros, 7 gemas, 280 gramas de farinha e 250 gramas de açúcar. A determinação dos valores D e z foi realizada após submeter as amostras embaladas a vácuo (5 g) em banho-maria aos seguintes tratamentos térmicos: 52 °C (45, 90, 135, 180 e 225 min.); 55 °C (15, 30, 45, 60, 70 e 80 min.); 58 °C (5, 10, 15, 20, 25 e 30 min.) e 61 °C (1, 2, 3, 4 e 5 min.). Também foram realizadas determinações físico-químicas (a_w, pH, humidade, açúcar, cinzas, proteína e gordura livre) e microbiológicas (microbiota deteriorativa: microrganismos mesófilos, psicrotróficos, bolores e leveduras, Enterobacteriacea, *E. coli*; microbiota patogénica: *Salmonella* spp., *Bacillus cereus*, *Listeria monocytogenes* e *Staphylococcus aureus*) na massa líquida crua. Foi possível investigar diferentes combinações de tempo e temperatura para *Salmonella* Typhimurium, comparativamente a outros estudos realizados em massa à base de ovo, frequentemente utilizada para a produção de produtos de pastelaria. Antes da inoculação, a a_w foi de 0,94 e pH de 7,26. Os valores D obtidos foram 53,19 min, 20,45 min, 6,95 min e 1,60 min, para 52 °C, 55 °C, 58 °C e 61 °C, respetivamente. A partir dos valores D calculados, o valor z correspondente foi de 5,96 °C.

Palavras-chave: Resistência térmica; Inativação térmica; Massa crua; Contaminação; Caracterização microbiológica; Caracterização físico-química.

1 Introduction

Salmonella is known as a versatile microorganism, that adapts to pH, a_w and temperature conditions beyond its growth range. Optimal temperature growth for *Salmonella* is about 35 to 37 °C, however, it can develop between 5 to 47 °C. Furthermore, this microorganism can grow at pH ranges of 3.8 to 9.5 (6.5-7.5 optimal) and a_w of 0.94 to 0.99, however, below 0.94 it can remain at a latency state (D' Aoust & Maurer, 2007; International Commission on Microbiological Specifications for Foods, 1996; Jay et al., 2005).

The main causes of *Salmonella* contamination in foods with low a_w are inadequate hygiene practices, poor quality of facilities, and insufficient maintenance of equipment and its design (Bell & Kyriakides, 2002; Carrasco et al., 2012). The presence of *Salmonella* in bakery products has been a concern in the last years. Outbreaks associated with this microorganism have been reported in custards, pies, bread and cakes, among others (Bonner & Schweiger, 1994; Evans et al., 1996; Lathrop et al., 2014; Zhang et al., 2007). Most reported outbreaks involving bakery products are associated to eggs that are insufficiently cooked (Lathrop et al., 2014). Contamination by *Salmonella* through raw ingredients is a reality, as they are usually used in bakery receipts, such as eggs, chocolate, flour, peanut butter and dairy products (Arvanitoyannis & Bosinas, 2006; Saranraj & Geetha, 2012; Smith et al., 2004).

Salmonella resistance to stress factors as low a_w, lethal temperatures and acidic conditions has been approached in several studies (Bell & Kyriakides, 2002). Thermal resistance tends to increase with the addition of humectants such as sucrose and salt with the consequent decrease of a_w. In addition, other factors influence the increase of thermal resistance, such as lack of oxygen, use of sub lethal incubation temperatures before heat treatment and food composition, where the presence of lipids has been mentioned as having a protective effect on microbial cells (Bell & Kyriakides, 2002; Doyle & Mazzotta, 2000; Peña-Meléndez et al., 2014).

The majority of reported foodborne outbreaks are associated with low a_w products (Finn et al., 2013). According to Majowicz et al. (2010), 98.3 million cases of *Salmonella* gastroenteritis are estimated to occur worldwide yearly, with 80.3 million associated with foodborne illness. The possibility of *Salmonella* disease

with a reduced number of cells (<10) has been reported (Finn et al., 2013; Smith et al., 2004). Most of the outbreaks associated with *Salmonella* due to consumption of bakery products were caused by *S*. Enteritidis PT4, *S*. Enteritidis PT7, and *S*. Typhimurium. Studies confirmed that eggs are the main transmission vehicle (Smith et al., 2004).

The present study aimed to evaluate thermal resistance of *S.* Typhimurium ATCC 14028 (Oxoid C6000L), a serotype often associated with foodborne outbreaks, by inoculation in a Traditional Portuguese egg-based pastry "Cavacas de Resende".

2 Materials and methods

2.1 Sampling of "Cavacas de Resende" batter

Raw liquid batter of "Cavacas de Resende" was obtained from a local confectioner in Resende, Portugal. Its preparation was carried out with 8 eggs, 7 egg yolks, 280 g of flour and 250 g of sugar.

2.2 Physico-chemical determinations

All physico-chemical determinations were performed at the Laboratory of Technology, quality and food safety (Lab. TeQSA) from the University of Trás-os-Montes e Alto Douro (UTAD), Vila Real, Portugal, except for sugar evaluation that was analysed in an external laboratory.

2.2.1 a_w determination

Batter a_w determination was performed with 8 g of sample (in duplicate) using a Rotronic Hygroskop-DT a_w meter with a probe at 25 °C (temperature controlled by water circulator).

2.2.2 pH determination

pH determination was performed in duplicate, directly into samples, with a WTW 330i pH meter.

2.2.3 Moisture determination

Moisture content was determined by dehydrating 5 ± 0.001 g of samples in duplicate, at 105 °C for 24 hours (Tan & Mittal, 2006). The results were expressed as percentage of initial sample batter.

2.2.4 Ashes determination

According to the experimental procedure AACC, 08-01.01 (American Association of Cereal Chemists, 1999b), ashes were determined with 2 ± 0.001 g of sample in duplicate, incinerated in muffle Nabertherm Model 180 until a light grey or constant weight was obtained. Results are expressed as percentage of initial sample.

2.2.5 Crude protein determination

Total nitrogen determination was performed with 1 ± 0.001 g, duplicate samples, by Kjeldhal method (Association of Official Analytical Chemists, 1990). The sample was initially digested in a Bejtest Inkjel apparatus, by the addition of 1 catalyst tablet-(Merck 1.15348), and 12 mL of concentrated sulphuric acid. Total digestion time was 1 h and 30 min (20 min at 38% of apparatus heating capacity, 20 min at 75%, and 60 min at 100%). Subsequently, 70 mL of 35% NaOH, 50 mL of distilled H_2O and 30 mL of 4% H_3BO_3 with indicators (bromocresol green and methyl red) were used for distillation. Titration was carried out with HCl 0.1 N solution. Distillation and titration were conducted with a VELP UDK 159 automatic apparatus.

For the conversion from nitrogen content to protein content, Jones factors were used, 6.25 for eggs and 5.70 for wheat flour (Food and Agriculture Organization of the United Nations, 2003), taking into account the proportion of protein content of raw materials used in the batter. Results are expressed as percentage of initial sample batter.

2.2.6 Free fat determination

According to AACC Method 30-25.01 (American Association of Cereal Chemists, 1999a), the dehydrated sample resulting from moisture content procedure was used for free fat content determination, by extraction with *n*-hexane for 6 hours in a Soxhlet apparatus. After solvent recovery by distillation, the Soxhlet flask was dried in an oven at 100 °C for intervals of 30 min, with intercalate cooling and weighing operations, until the weighing differences did not differ more than 0.1% of the sample batter. Results are expressed as percentage of initial sample batter.

2.3 Microbiological determinations

All microbiological determinations were performed at the University of Trás-os-Montes e Alto Douro (UTAD), Vila Real, Portugal. The detection and enumeration of deteriorative and pathogenic microorganisms was performed before heat treatment. for serial (10-fold) dilutions we used0.9% isotonic saline, except when it required a selective medium. Results are expressed in log cfu g^{-1}.

2.3.1 *Salmonella* spp. detection

According to the Australian Standards (2009a), *Salmonella* spp. detection was performed by diluting the sample in Buffered Peptone Water medium (OXOID CM0509), subsequently incubated at 37 °C for 18 hours. After that, 0.1 mL was withdrawn into a 10 mL Rappaport Vassiliadis Broth with soya (Liofilchem 610175) and 1 mL into a tube with 10 mL Muller Kauffmann Tetrathionate-novobiocin broth medium (Biokar Diagnostics BK169HA), incubated at 41.5 °C for 24 hours and 37 °C for 24 hours, respectively. From the solutions obtained, aliquots of 0.1 mL were spread on Xylose Lysine Deoxycholate agar (VWR CHEMICALS) and Hektoen Enteric Agar (VWR CHEMICALS) with incubation at 37 °C for 24 hours.

Five colonies of the two selective media were streaked to Nutrient Agar (Biokar Diagnostics BK185HA) and incubated at 37 °C for 24 hours. The colonies obtained were spiked on Triple Sugar Iron (Liofilchem 610055) and Urea agar. Tubes were incubated at 37 °C.

2.3.2 *Bacillus cereus* enumeration

Based on ISO Standard 7932 (British Standards Institution, 2004), serial (10-fold) dilutions were performed and spread on Mannitol-Egg-yolk-Polymyxine agar medium (Biokar Diagnostics BK116) supplemented with egg yolk emulsion (Biokar Diagnostics BS066) and polymyxin (Biokar Diagnostics BS007). Plates were incubated in duplicate at 30 °C for 24 hours.

Five typical as well as presumptive colonies of *Bacillus cereus* were streaked on Nutrient Agar (Biokar Diagnostics BK185HA). Plates were incubated at 30 °C for 72 hours. The confirmation of the grown colonies was performed by streaking onto Columbia agar (OXOID CM0331) supplemented with 5% sheep blood. Plates were incubated at 30 °C for 24 hours. After this period, a hemolytic reaction development around the colonies was indicative of the presence of *B. cereus*.

2.3.3 *Escherichia coli* enumeration

According to ISO 16649-2 (International Organization for Standardization, 2001), serial (10-fold) dilutions were performed and incorporated into Trypone Bile X-Glucuronide agar (VWR CHEMICALS) medium. Plates were incubated in duplicate at 44 °C for 18 to 24 hours. Typical blue coloration colonies were counted.

2.3.4 *Listeria monocytogenes* detection

According to Australian Standards (2009b), the first step for *L. monocytogenes* detection was performed using primary enrichment of half Fraser broth (VWR CHEMICALS) and incubation at 30 °C for 24 hours. In the second step, aliquots of 0.1 mL were transferred to Fraser broth (VWR CHEMICALS) with incubation at 37 °C for 48 hours. From the cultures obtained in the first and second step, aliquots of 0.1 mL were spread on supplemented Oxford Agar Base (VWR CHEMICALS) and supplemented PALCAM Agar (VWR CHEMICALS) with incubation at 37 °C for 24 to 48 hours.

Five typical as well as presumptive colonies were isolated and cultured on tryptone soya yeast extract agar (Biokar Diagnostics) and incubated at 37 °C for 18 to 24 hours. Catalase reaction, Gram staining, haemolysis test and carbohydrate utilization were performed for confirmation.

2.3.5 *Staphylococcus aureus* isolation

For the procedure, ISO 6888-2 (International Organization for Standardization, 1999), were followed. Serial (10-fold) dilutions were performed and spread into Baird Parker agar (Biokar Diagnostics BK055) supplemented with egg yolk tellurite (Biokar Diagnostics BS060) and sulfamethazine (Biokar Diagnostics BS02808). Plates were incubated in duplicate at 37 °C for 48 hours. Five typical as well as presumptive colonies were isolated and cultured in Brain Heart Infusion broth (Biokar Diagnostics BK015HA) for 24 hours at 37 °C. Further isolates were tested for coagulase production. Clotting of rabbit plasma (Biokar Diagnostics BR00208) was verified after 4 to 6 hours and when the test was negative, after 24 hours of incubation at 37 °C.

2.3.6 Enterobacteriaceae enumeration

According to ISO 21528-2 (British Standards Institution, 2004), serial (10-fold) dilutions were performed and incorporated into VRBG (Violet Red Bile Glucose Agar) (VWR CHEMICALS). Plates were incubated in duplicate at 37 °C for 24 hours. Typical colonies of purple staining were counted and 5 of them were spiked to Nutrient Agar (Biokar Diagnostics BK185HA) and incubated at 30 °C for 24 hours. Then, they were subjected to biochemical confirmation by oxidase (negative reaction) and glucose fermentation in Glucose agar medium (HIMEDIA M1589) (positive reaction).

2.3.7 Molds and yeasts enumeration

Based on Portuguese Standard 3277-1 (Instituto Português da Qualidade, 1987b), serial (10-fold) dilutions were performed and spread on Chloramphenicol Glucose Agar culture medium (VWR CHEMICALS). Plates were incubated in duplicate at 25 °C for 5 days.

2.3.8 Mesophilic enumeration

According to ISO 4833 (International Organization for Standardization, 2003), serial (10-fold) dilutions were performed and incorporated into Plate Count Agar culture medium (VWR CHEMICALS). Plates were incubated in duplicate at 30 °C for 72 hours.

2.3.9 Psychrotrophic enumeration

According to Portuguese Standard 2307 (Instituto Português da Qualidade, 1987a), serial (10-fold) dilutions were performed and incorporated into Plate Count Agar culture medium (VWR CHEMICALS). Plates were incubated in duplicate at 7 °C for 10 days.

2.4 Thermal inactivation of *S.* Typhimurium

2.4.1 Inoculum preparation

S. Typhimurium ATCC 14028 (OXOID C6000L) culture was preserved at -20 °C in BHI (Brain Heart Infusion) (Biokar Diagnostics BK015HA) supplemented with 25% (v/v) of glycerol (Panreac). Bacterial strain was subcultured in BHI (Brain Heart Infusion) (VWR CHEMICALS) and incubated at 37 °C for 24 hours, followed by subculture to XLD (Xylose Lysine Deoxycholate agar) (VWR CHEMICALS) and HKT (Hektoen Enteric agar) (VWR CHEMICALS) followed by incubation at 37 °C for 24 hours. Isolated colonies were subcultured in BHI (Brain Heart Infusion) (VWR CHEMICALS) and incubated at 37 °C for 24 hours. Then, cultures were transferred to sterile centrifugal tubes and centrifuged at 10.000x g for 10 min at 4 °C in a Sigma 3k18 centrifuge. The supernatant was decanted and the pellet suspended in sterile 0.9% isotonic saline. The washing procedure was repeated 3 times. Standardized concentration (6.64×10^9 cfu mL^{-1}) was determined by optical density (O.D. 1.2) at 600 nm,followed by successive decimal dilutions (1:10) in 0.9% isotonic saline and confirmation of viable cells of the microorganism in XLD (Xylose Lysine Deoxycholate agar) (VWR CHEMICALS). The plates were incubated at 37 °C for 24 hours.

2.4.2 Inoculation and heat treatment

The samples were prepared in triplicate with 5.0 ± 0.1 g and placed in COMBITHERM bags (93 mm width x 200 mm length x 0.08 mm thickness). Inoculation was performed with 0.1 mL from *S.* Typhimurium suspension (6.64×10^9 cfu mL^{-1}), resulting in 1.33×10^8 cfu g^{-1}, homogenizing the sample with the inoculated culture. Then, the bags were sealed under vacuum (99%) with a SAMMIC V-420 SGA vacuum sealer machine.

Subsequently, samples were submitted to a water bath at 52 °C during 45, 90, 135, 180, 225 min, at 55 °C during 15, 30, 45, 60, 70 and 80 min, at 58 °C during 5, 10, 15, 20, 25 and 30 min and at 61 °C during 1, 2, 3, 4 and 5 min. Sample core temperature was monitored continuously using thermocouples inserted into a non-inoculated control sample.

2.4.3 *S.* Typhimurium enumeration

After samples cooling in an ice bath (4 °C) for 10 min, enumeration of *S.* Typhimurium heat treatment survivors was carried out by dilution of each sample in 22.5 mL of sterile 0.9% isotonic saline, followed by maceration in a stomacher for 90 seconds at room temperature. Sample dilutions (1:10) were spread plated on XLD (Xylose Lysine Deoxycholate agar) (VWR CHEMICALS). The plates were incubated at 37 °C, for 24 hours. Results of the typical colonies enumeration are expressed as log cfu g^{-1}.

2.4.4 D and z values determination

D values were determined by plotting the $\log_{10}$ of the survived microorganisms against the correspondent heating times, for each temperature, to obtain a regression line ($y = mx + b$). The slope was used to determine the D value by the expression $D = -1/m$. A minimum set of heating times was needed in order to obtain a linear relationship with more than 5 points, with correlation coefficients (r) > 0.90 (International Organization

for Standardization, 2006). The expression z = -1/m was used to calculate z values and it was obtained from the negative slope of the regression line over the $\log_{10}$ of D values at the corresponding temperatures.

3 Results and discussion

3.1 Microbiological determinations before heat treatment

For non-inoculated samples without heat treatment, pathogenic microorganisms such *as L. monocytogenes*, *B. cereus*, *Salmonella* spp. and *S. aureus* were not detected. No counts were obtained for *E. coli*, which demonstrates a good microbiological quality of the batter before heat treatment application. These parameters are good indicators of the raw materials quality, mainly eggs, that presents greater susceptibility of pathogenic microorganisms' development. Similarly, Pajohi-Alamoti et al. (2016) reported non-contamination of *L. monocytogenes*, *E. coli* and *Salmonella* in 80 pastry cream samples.

On the other hand, the counts obtained for mesophilic microorganisms were 4.02 ± 0.10 log cfu g^{-1}; 2.57 ± 0.04 log cfu g^{-1}, for Enterobacteriaceae; for psychrotrophic microorganisms, 3.51 ± 0.06; and for molds and yeasts, 2.31 ± 0.06 log cfu g^{-1} (mostly molds). Comparatively, similar counts were reported in cream pastry samples by Pajohi-Alamoti et al. (2016), 3.90 ± 0.29 log cfu g^{-1} for mesophilic microorganisms, 2.70 ± 1.43 log cfu g^{-1} for coliforms and 2.68 ± 1.20 cfu g^{-1} for molds. However, in that study the products were heat-treated.

3.2 Physic-chemical determinations before heat treatment

Table 1 shows physic-chemical results of raw liquid batter of "Cavacas de Resende".

According to the range of a_w, 0.94-0.99 (International Commission on Microbiological Specifications for Foods, 1996), for *Salmonella* growth, 0.944 obtained for "Cavacas de Resende" is at the minimum growth limit. Furthermore, the ability of some *Salmonella* serotypes to survive in environments with $a_w \leq 0.85$ and dry environments has already been demonstrated (Kapperud et al., 1990; Peña-Meléndez et al., 2014). The pH value obtained, 7.26, is in the range of optimum pH for *Salmonella* grow (6.5-7.5) (D' Aoust & Maurer, 2007; International Commission on Microbiological Specifications for Foods, 1996; Jay et al., 2005).

Channaiah et al. (2017), reported a_w value of 0.924 ± 0.001, and pH of 6.61 ± 0.12 before cooking in Muffins batter. The higher a_w values obtained in raw liquid batter of "Cavacas de Resende" can be explained by the reduced content of dry ingredients comparatively to those used in Muffins, like flour, granulated sugar, baking powder, salt, non-fat dry milk, whole egg solids and all-purpose shortening.

Table 1. Physico-chemical parameters of "Cavacas de Resende" raw liquid batter.

pH	a_w	Moisture (%)	Sugar (%)	Free Fat (%)	Crude Protein (%)	Ash (%)
7.26	0.944	39.92	26.45	12.13	8.51	1.04

a_w – Water activity.

3.3 D and z values

Thermal inactivation for *S.* Typhimurium ATCC 14028 in the batter of "Cavacas de Resende at 52 °C, 55 °C, 58 °C e 61 °C is shown in Figure 1. D values obtained from the negative inverse of the regression line slope were: D_{52} = 53.19 ± 0.16 min, D_{55} = 20.45 ± 0.96 min, D_{58} = 6.95 ± 0.67 min, and D_{61} = 1.60 ± 0.04 min.

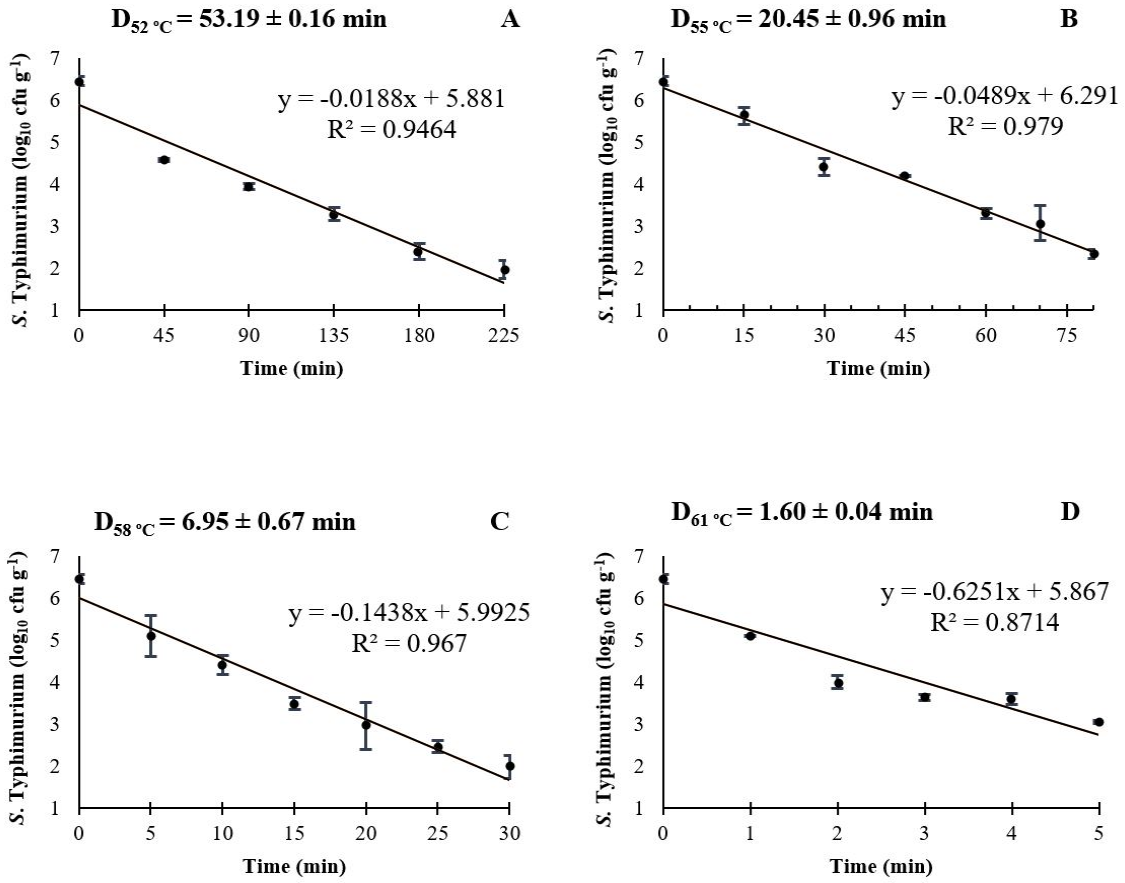

Figure 1. Thermal inactivation for S. Typhimurium ATCC 14028 in "Cavacas de Resende" batter (A, B, C, D). R^2: R Square value; Slope and y intercept equation (y= mx + b).

Figure 2 represents the linear regression constructed for z value determination of *S.* Typhimurium ATCC 14028 in "Cavacas de Resende" batter, obtained from the negative slope of the regression line over the $\log_{10}$ of D values at the corresponding temperatures (y = -0.1678x + 10.501). The calculated z value was 5.96 ± 0.06 °C.

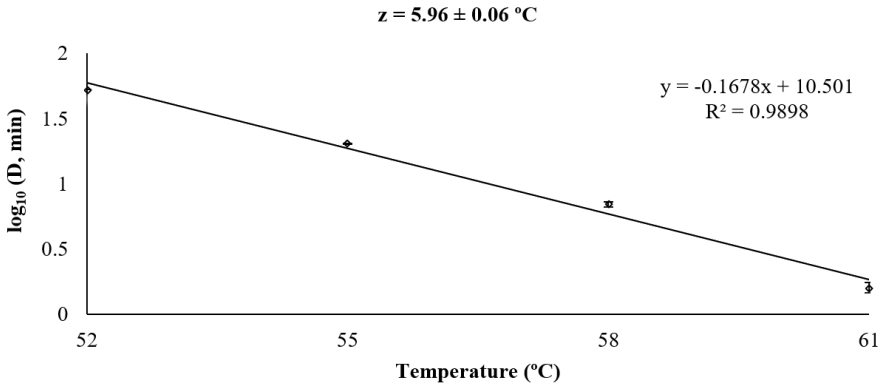

Figure 2. z value for *S.* Typhimurium ATCC 14028 in "Cavacas de Resende" batter.

Baking or cooking should destroy any *Salmonella* spp. present, but the degree of inactivation will depend on a number of inter-related factors. Several studies have demonstrated the influence of intrinsic and extrinsic factors in *Salmonella* thermal resistance. Decrease of a_w, exposure to sublethal temperatures before heat

treatment, low pH, absence of oxygen and food composition play an important role, increasing the microorganism's thermal resistance. An increase in *Salmonella* thermal resistance has been mentioned as related with the addition of humectants agents like sugar, as well as the presence of lipids in food composition that may cause a protective effect on the microorganism (Bell & Kyriakides, 2002; Doyle & Mazzotta, 2000; Peña-Meléndez et al., 2014).

In similar studies, Angelotti et al. (1961) reported a D value of 11.32 min at 60 °C for *S.* Senftenberg 775W inoculated in custard. Besides, Channaiah et al. (2017), inoculated a cocktail of *S.* Newport, *S.* Typhimurium and *S.* Senftenberg 775W, in flour that was mixed with the remaining ingredients to cook Muffins, obtained D values of 62.16 ± 2.99 min, 40.09 ± 0.88 min and 16.46 ± 1.71 min, at 55, 58 e 61 °C respectively, with a z value of 10.40 ± 0.63 °C. It can be verified that the values reported by these authors are higher than the values showed in Figures 1 and 2. However, considering the differences in thermal resistances of between *Salmonella* serotypes, mainly *S.* Senftenberg 775W, which is considered one of *Salmonella* serotypes most resistant to high temperatures (Ng et al., 1969), these differences are predictable. Food composition may also explain the differences in *Salmonella* thermal resistance reported in different studies. D'Aoust (1989) highlighted the influence of sugar concentration in *S.* Typhimurium and *S.* Senftenberg 775W thermal resistance between 55 °C and 75 °C. Thermal resistance increased in sucrose solutions as a_w decreased from 0.995 to 0.706. Studies using eggs have been also carried out on thermal resistance of *Salmonella*. Garibaldi et al. (1969) obtained a D_{60} values of 0.60 min and a z value of 4.6 °C for *S.* Typhimurium in eggs containing 10% sucrose. The same authors reported a D_{60} value of 1.0 min and a z value of 5.3 °C for *S.* Typhimurium in eggs containing egg yolk and corn syrup enhancing thermal resistance, increase with the addition of other ingredients. Garibaldi et al. (1969), also reported $D_{58.8}$ and D_{60} values of 0.7 and 0.4 min respectively, with a z value of 4.4 °C. Palumbo et al. (1995), also reported D_{60} values of 4.0 and 5.1 min with a z value of 4.8 °C for *S.* Typhimurium in egg yolk with the addition of 10% sucrose and in egg yolk with the addition of 10% NaCl, respectively. Comparing these studies, D and z values obtained for "Cavacas de Resende" batter are higher. This finding suggests that the addition of flour and sugar may have contributed to the increase in *S.* Typhimurium thermal resistance.

Likewise, wheat flour has been demonstrated to increase *Salmonella* thermal resistance compared to other foods. The destruction curves of *S.* Weltevreden at different a_w, showed a rapid death in the first 5 – 10 min, followed by a slower and more linear destruction (Archer et al., 1998). Even though flour is not the main ingredient in "Cavacas de Resende", a higher rate of destruction of the microorganism in the first minute, followed by a slower rate of destruction also occurs. This may be related to the changes of the physical state of the batter promoted by heat. So, at the beginning, the liquid batter allows a greater destruction of the microorganisms. Due to baking, the batter becomes more solid, providing protection to the microorganisms, since the increase of solid particles is a factor for increasing the resistance of microorganisms (Blackburn et al., 1997).

Some protective mechanisms in microorganisms that present grant superior resistance to stress factors have been studied. Finn et al. (2013) reported that one of the microorganisms resistance mechanisms is the accumulation of osmoprotective molecules (betaine (N,N,N-trimethyl glycine), proline for example), cell filamentation, the presence of regulatory genes, and biofilm formation. Under stress conditions, microorganisms can express specific genes to respond quickly to adverse conditions (Mattick et al., 2000). Tapia et al. (2007) suggested that the increase in thermal resistance occurs due to the reduced presence of water that diminishes molecular vibrations as the cells are heated. When the water content is high, the molecular vibrations increase, causing protein denaturation, ribosomal damage and enzymes inactivation, and consequently, the inactivation of microorganisms (Mackey et al., 1991; Nguyen et al., 2006).

4 Conclusions

Based on the results, reduction of *Salmonella* is expected to occur in bakery products for combinations of time and temperature that exceed the resistance of the microorganism. In this particular study, 1.60 min at 61 °C is sufficient to inactivate *Salmonella* Typhimurium. However, different food compositions may hinder *Salmonella* inactivation. Not reaching the appropriate temperatures and times, mainly in the thermal centre of the product, enhances the need to implement safety measures, especially in products that can support the development of *Salmonella*. Continuous improvement of manufacturing processes and use of good manufacturing practices should be encouraged in order to avoid post-processing contamination, since the resistance of some pathogenic microorganisms such as *Salmonella* increases the risk of causing consumer illness.

Acknowledgements

The authors would like to thank "Município de Resende" by their contributions, Mrs. Ana Leite and Mrs. Helena Santos for their contributions to this research and to CECAV-UTAD. Special thanks also to the sample supplier, Mrs. Manuela Machado. This paper was supported by the Portuguese Science and Technology Foundation (FCT) under the projects, UID/CVT/00772/2016 and UID/CVT/00772/2019.

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Funding: Ministério da Educação e Ciência > Fundação para a Ciência e a Tecnologia UID/CVT/00772/2016 and UID/CVT/00772/2019.

Received: May 10, 2019; Accepted: Sept. 23, 2019