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FOOD TECHNOLOGY

Acid resistance of *Salmonella Typhimurium* ATCC 14028 after desiccation stress during peanut storage

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ABSTRACT: *Salmonella* is a relevant pathogen, which causes foodborne outbreaks associated with both high and low moisture foods (LMF). This study evaluated the effect of previous desiccation stress on the acid resistance of *S.* Typhimurium ATCC 14028 using blanched peanut kernels as an LMF model. *Salmonella* was recovered from the peanut samples throughout 180 days of blanched peanut kernels storage at 28 ºC. During this period two death rates were verified, 0.04 log cfu/g/day in the first 30 days and 0.007 log cfu/g/day between 30 and180 days. Regarding acid resistance, there was no difference (P > 0.05) in the *Salmonella* growth/death kinetics between the undesiccated sample (TSB)and the cells recovered from peanut samples over 180 days of storage after 4 h at pHs 3.0, 3.5, 4.5 and 7.2. The average growth rate observed for pH 7.2 was 0.44 log cfu/ml/h. At pH 4.5, the *Salmonella* counts did not change significantly over 4 h. In contrast, *Salmonella* populations declined by 0.14 to 0.29 log cfu/ml/h at pH 3.5. At pH 3.0 declines were estimated to be 0.65 log cfu/ml/h for the undesiccated sample and 2.07 log cfu/ml/h for *Salmonella* recovered from peanuts stored for 120 days. Therefore, our data indicated that desiccation stress caused during the peanut storagedid not influence the *Salmonella* acid resistance.
Key words: cross-protection, low moisture food, acid resistance, peanuts, desiccation stress.

Avaliação da resistência ácida de *Salmonella Typhimurium* ATCC 14028 após o estresse dessecativo durante o armazenamento de amendoim

RESUMO: Em alimentos de baixa umidade (LMF), o primeiro desafio encontrado por patógenos como a *Salmonella* é o estresse de dessecação. Neste estudo, o efeito prévio do estresse de dessecação sobre a resistência ácida de *S.* Typhimurium ATCC 14028 foi avaliado utilizando amendoim blancheado como um modelo de LMF. *Salmonella* foi recuperada das amostras de amendoim após 180 dias de estocagem a 28 ºC. Durante este período foram verificadas duas taxas de mortalidade, 0,04 log ufc/g/dia nos primeiros 30 dias e 0,007 log ufc/g/dia entre 30 e 180 dias. Com relação à resistência ácida, não houve diferença (P > 0,05) na cinética de crescimento/morte de *Salmonella* entre a amostra sem estresse dessecativo (TSB) e as amostras de amendoim após 4 h em pHs 3,0, 3,5, 4,5 e 7,2. A taxa média de crescimento observada para o pH 7,2 foi de 0,44 log cfu/ml/h. No pH 4,5, a contagem de *Salmonella* não mudou significativamente durante 4 h. Em contraste, a população de *Salmonella* diminuiu de 0,14 a 0,29 log cfu/ml/h no pH 3,5. No pH 3,0, a queda foi estimada em 0,65 log cfu/ml/h para a amostra sem estresse dessecativo e 2,07 log cfu/ml/h para a *Salmonella* recuperada das amostras de amendoim estocadas por 120 dias. Portanto, nossos dados indicaram que o estresse dessecativo causado durante a estocagem do amendoim não influenciou a resistência ácida de *Salmonella*.
Palavras-chave: proteção cruzada, alimentos com baixa umidade, resistência ácida, amendoim, estresse dessecativo.

INTRODUCTION

Peanuts and peanut butter have been linked tosalmonellosis outbreaks around the world (CDC, 2009, 2016, 2022; KIRK et al., 2004). Some studies have demonstrated the ability of *Salmonella* to survive in low moisture foods (LMF), such as peanut products (BEUCHAT et al., 2013). *Codex Alimentarius* defines LMF as foods with water activity (a_w) ≤ 0.85 (CAC, 2015). In a previous study, this pathogen was recovered from raw in-shell peanuts (a_w 0.29) and unblanched peanut kernels (a_w 0.54) after 240 and 330 days of storage at 28 °C, respectively (NASCIMENTO et al., 2018). In peanut hull compost mixture, *Salmonella* survived for 6 weeks (ERICKSON et al., 2015), and in peanut butter and peanut butter spreads (a_w 0.20 and 0.30) for at least 24 weeks (BURNETT et al., 2000). However, it is important to emphasize that the survival time of the pathogen depends on multiple factors, such as the initial contamination load.

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In addition, the uses of acids as additives in food formulations (pH ~ 4-6) or the exposure of the bacteria to the gastric juice (pH ~ 2-3) during the digestion (HORN & BHUNIA, 2018) represent forms of acid stress that *Salmonella* needs to face. According to CAPOZZI et al. (2009) cross-protection is a phenomenon in which a pre-existing or acquired resistance to particular stress affords protection against other stress. In the context of LMF, cross-protection can be demonstrated primarily due to the desiccation stress. After a drastic reduction of the water activity (a_w) in an inert matrix, an increase in *S. Typhimurium* resistance to multiple stresses (high temperature, acidity, UV radiation, and oxidation) was observed (GRUZDEV et al., 2011). Nevertheless, this phenomenon can also be observed from other primary stresses, such as the pre-conditioning of *S. Typhymurium* at acid pH (6.5) followed by heat stress (50 ºC), which results in a death rate 10-fold lower (LEYER & JOHNSON, 1993).

In this way, evaluating the response to the different stresses to which *Salmonella* can be exposed is necessary to suit the food process to ensure consumer safety, especially in LMF industry. Therefore, this study evaluated the behavior of *S. Typhimurium* ATCC 14028 challenged under different pHs after exposure to desiccation stress using blanched peanut kernels as LMF matrix.

MATERIALS AND METHODS

Inoculum preparation

S. Typhimurium ATCC 14028 was stored at -80 ºC in Tryptic Soy Broth (TSB) (Difco, Becton Dickinson, MD, USA) plus 15% glycerol (v/v) (Synth, Brazil). It was reactivated in TSB and subcultured twice at 37 ºC for 18 h. Then, the culture was streaked onto Tryptic Soy Agar (TSA, Difco) and incubated at 37 ºC for 18 h.

Peanut inoculation

For inoculum preparation, a colony isolated from the TSA plate was transferred to a TSB tube and incubated at 37 ºC for 18 h. This procedure was repeated, and the second tube was used to prepare the inoculum. The bacterial suspension was centrifuged at 7740 *g* for 7 min at 4 ºC. The supernatant was discarded, and the pellet was resuspended in 0.85 % saline solution. The final bacterial population was around 8.0 log cfu.ml^{-1}. The count was determined by spread plating on Xylose Lysine Deoxycholate agar (XLD, Acumedia, MI, USA), incubating at 37 ºC for 18 h.

A sample of 1200 g of blanched peanut kernels (*Arachis hypogaea*) of Runner cultivar (a_w 0.43) was transferred to a sterile plastic bag and inoculated with 4.5 ml (0.38 % v/w) of the *S. Typhimurium* suspension supplemented with 0.6 % of Tween 80 (Merck, Germany). Then, the sample was mixed by hand for 1 min, transferred to sterile trays (35 x 25 x 5 cm),and held in a laminar flow cabinet (Vecco, Brazil) at room temperature up to bring the a_w back to 0.43 (PEREIRA et al., 2020). The a_w of the samples was measured every 3 min, in duplicate, at 25 °C in a water activity meter (Aqualab CX2, Decagon Device, Pullman, WA) (data not shown). The blanched peanut kernel samples were previously evaluated for the absence of *Salmonella* in 25 g, according to the International Organization for Standardization [ISO] 6579, 2017.

Peanut storage

After the inoculation step, the sample was fractionated into three portions of 400 g, transferred to sterile plastic bags (Twirl'em, Labplas, Canada), and were stored in desiccators containing potassium carbonate solution (a_w 0.43) (NASCIMENTO et al., 2018). The desiccators were kept in a bacteriological incubator (FANEM, mod 347 CD, Brazil) at 28 ºC for up to 180 days. The storage temperature chosen for the study was based on INMET meteorological data, which indicates that this is the average temperature of the Brazilian peanut production regions (Instituto Nacional de Meteorologia [INMET], 2016). The inoculated samples and the potassium carbonate solution had the a_w measured every 15 days, in duplicate, at 25 °C in a water activity meter. Three independent trials for each storage time were carried out (Figure 1).

Acid stress

After each storage period (0, 14, 30, 60, 120, and 180 days), portions of 20 g of blanched peanut kernels from each of the three stored bags were composited into a 60 g sample and mixed by hand. Then, 10 g of this composed sample were transferred to a bottle containing 90 ml of 0.1 % peptonated water (Acumedia, MI, USA), and homogenized at 400 rpm for 1 min in a shaker (Lab-Line Orbit Environ - 3527 Shaker, Lab Line Instruments, IL, USA). Four milliliters (4 ml) of the cell suspension were transferred to tubes containing 4 mlof TSB double concentration with different pH values (3.0, 3.5, 4.5, and 7.2). The pH was adjusted using a 10% HCl solution. After that, the tubes were incubated for 1, 2, 3, and 4 h at 37 ºC. The *Salmonella* population was determined after each incubation time. Three independent trials for each pH treatment were carried out (Figure 1).

For comparative purposes, the inoculum without desiccation stress (in TSB) was subjected to the same pH treatments.

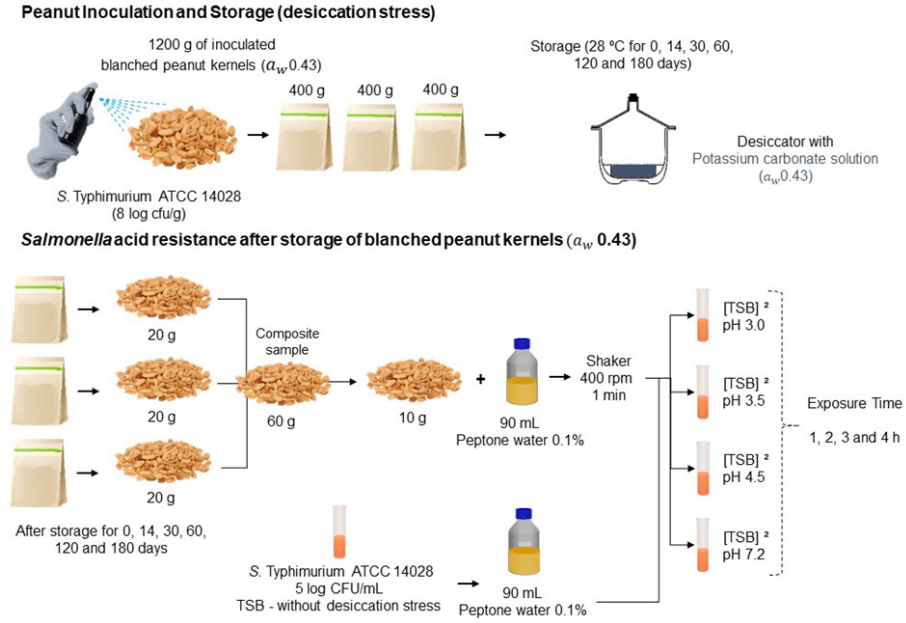

Figure 1 - Experimental procedures.

Determination of Salmonella

The *Salmonella* enumeration was determined after each storage time and acid treatment. Serial dilutions were made in 0.1 % peptone water followed by spread plating on XLD agar with incubation at 37 ºC for 24 h. The colonies were confirmed by biochemical and serological tests (ISO 6579, 2017). Results were expressed as log of colony forming unit per gram or ml, and the limit of detection was 10 cfu/g for the peanut samples and 1 cfu/ml for TSB.

S*tatistical analysis*

The fate of *S. Typhimurium* inoculated on blanched peanut kernels and its acid resistance was modeled using two primary predictive models. The former was built to estimate the effect of the peanut storage time on the *Salmonella* population, and the latter was made to estimate the impact of the pH exposure time on the *Salmonella* population previously subjected to desiccation stress during the peanut storage. Three different distributions were tested for each model: normal, Weibull and Gamma. To evaluate the goodness-of-fit of the models, the residual analyses were performed using a quantil-quantil plot and the Shapiro-Wilk normality test (SHAPIRO & WILK, 1965). The Gamma distribution showed the best goodness-of-fit (data not shown).

In addition, the interaction between the peanut storage time (desiccation stress) and the pH exposure time (acidic stress) was evaluated by a pairwise comparison via 95% confidence intervals with Bonferroni correction (WRIGHT, 1992). All the analysis was performed using the software R version 4.0.3 (R CORE TEAM, 2022).

RESULTS AND DISCUSSION

In our study, blanched peanut kernels were used as an LMF matrix model to evaluate the acid resistance of *S.* Typhimurium ATCC 14028 after exposure to desiccation stress. First, the effect of the peanut storage time on the inoculum population was determined and subsequently the acid resistance of the *Salmonella* cells recovered from the peanut samples was evaluated.

Storage time

The a_w of the blanched peanut kernels remained stable throughout the storage at 28 °C (a_w 0.43). To study the effect of the peanut storage time on the *Salmonella Typhimurium* ATCC 14028 death kinetics, i.e., the effect of the desiccation stress, the following model was proposed (1):

$$log(\mu_j) = \beta_0 + \beta_1 \times I + \beta_2 \times j + \beta_3 \times I \times j \quad (1)$$

Where, μ_j is the average of *Salmonella* population at storage time *j* (without desiccation stress [TSB], after 0, 14, 30, 60, 120, and 180 days), *I* is an indicator variable such that $I = 0$ if $j \leq 30$ days

and $I = 1$ if $j > 30$ days, β_2 represents the death rate in log cfu/g per storage time in the interval from 0 to 30 days, and $\beta_2 + \beta_3$ represents the death rate in log cfu/g per storage time in the interval from 30 to 180 days. The model assumes a linear relationship between the log cfu/g and the storage time, with different intercepts and slopes for the periods from 0 to 30 days and from 30 to 180 days. Table 1 presents the estimates of the parameters for the model in equation (1).

The initial *Salmonella* population in the samples was around 5 log cfu/g. It decreased as the peanut storage time increased (Figure 2), most markedly in the first 30 days, followed by a slowing down phase. In the 0-30-day interval, the estimated death rate of *Salmonella* was approximately 0.04 log cfu/g/day, whereas in the 30-180-day interval, the value was 0.007 log cfu/g/day. BRAR et al. (2015) evaluated the fate of *Salmonella* in raw peanut kernels stored for 365 days at 22 ºC. The authors also calculated two death rates, one considering the entire storage period (0-365 days) and another for the first 30 days. The rate of decline obtained for 0-365 days was the same verified in our study (0.007 log cfu/g/day), whereas the death rate for 0-30 days was 0.22 log cfu/g/day. In the current study, at the end of the storage period (180 days), reductions of 2.60 log cfu/g in the *Salmonella* population inoculated on the peanut samples were observed. NASCIMENTO et al. (2018) observed reported similar reduction rates for unblanched peanut kernels ($a_w = 0.54$) and for roasted peanuts ($a_w = 0.39$) inoculated with *Salmonella Typhimurium* and stored for 180 days at 28 ºC, 2.51 log cfu/g and 2.22 log cfu/g, respectively. Reductions of 3.13 log cfu/g after 7 days and 1.24 log cfu/g after 168 days were obtained in peanut butter (a_w 0.29) inoculated with a pool of five *Salmonella* serotypes (Agona, Enteritidis, Michigan, Montevideo, and *Typhimurium*) and stored at 21 ºC (BURNETT et al., 2000). In contrast, peanut paste (47 %fat, $a_w = 0.3$) inoculated with *S.* Tennessee showed reductions of 0.50 and 1.30 log cfu/g after 30 and 365 days of storage at 20 ºC, respectively (KATAOKA et al., 2014). Although the long-term storage can reduce the *Salmonella* population, our study showed that a small number of cells might persist in the end product. It represents a risk to the consumers since low infectious doses around 1-3 cfu/g of *Salmonella* have been reported in some LMF outbreaks (WERBER et al., 2005).

Table 1 - Estimates of the parameters for the model adjusted to study the effect of the peanut storage time on the *Salmonella* survival.

Parameter	Estimates	SE	P-value
β_0	4.989	0.071	< 0.001
β_1	- 0.191	0.209	< 0.001
β_2	- 0.040	0.004	< 0.001
β_3	0.033	0.004	< 0.001

Acid resistance

To study the effect of the exposure time at different pH values on *S. Typhimurium* previously inoculated on blanched peanut kernels, we assumed that: $Y^k_{ij}(t)$ is the *Salmonella* count (log cfu/ml) for the replicate i (i = 1, 2, 3), at storage time j (j = without desiccation stress, and after 0, 14, 30, 60, 120, 180 days), pH = k and exposure time t (t = 0, 1, 2, 3, 4 h). $Y^k_{ij}(t)$ follows a Gamma distribution with a mean equal to μ^k_{jt}. Thereby the following model was proposed (2):

$$log\,(\mu^k_{jt}) = \alpha^k_j + \beta^k_j \times t \quad (2)$$

where $\alpha^k_j = log\,(\mu^k_{j0})$, i.e., *Salmonella* count (log cfu/ml) estimated at time zero for the storage time = j and pH = k; and β^k_j represents the rate of change in log cfu/ml per hour of exposure at each pH value for storage time = j and pH = k. Then a linear relation for the *Salmonella* count (log cfu/ml) and the exposure time for a given pH value in a specific storage time was assumed. Table 2 shows the parameters estimate with confidence intervals of 95% and Bonferroni correction. When the confidence interval (CI 95%) did not contain the value "0" the parameter estimated was statistically significant ($P < 0.05$) (corresponding to data from the same table row). Moreover, the CI 95% of the storage times for the same pH value were compared two-by-two. Intersections between the Cis mean no significant difference ($P > 0.05$).

In the optimum growth condition, i.e., pH 7.2 (Figure 3D), an increase in the *Salmonella* population was observed from 2 h, with an average growth rate estimated by the model at 0.44 log cfu/ml/h. There was no significant difference ($P > 0.05$) among the cells recovered from the peanut samples and from the undesiccated sample (in TSB). After 4 h at 37 °C at pH 7.2, the bacteria population increased between 1.52 and 1.93 log cfu/ml. At pH 4.5 (Figure 3C), the *Salmonella* counts did not change significantly over time ($P \geq 0.05$), ranging between 0.02 and 0.36 log cfu/ml over 4 h exposure for the peanut samples and up to 0.08 log cfu/ml for the undesiccated sample (Table 2). At pH 3.5, the *Salmonella* death rate over the exposure time ranged from 0.14 to 0.29 log cfu/ml/h for the undesiccated sample and the 120-day peanut sample, respectively. Figure 3B shows a drop in the *Salmonella* count between 0.38 and 1.02 log cfu/ml

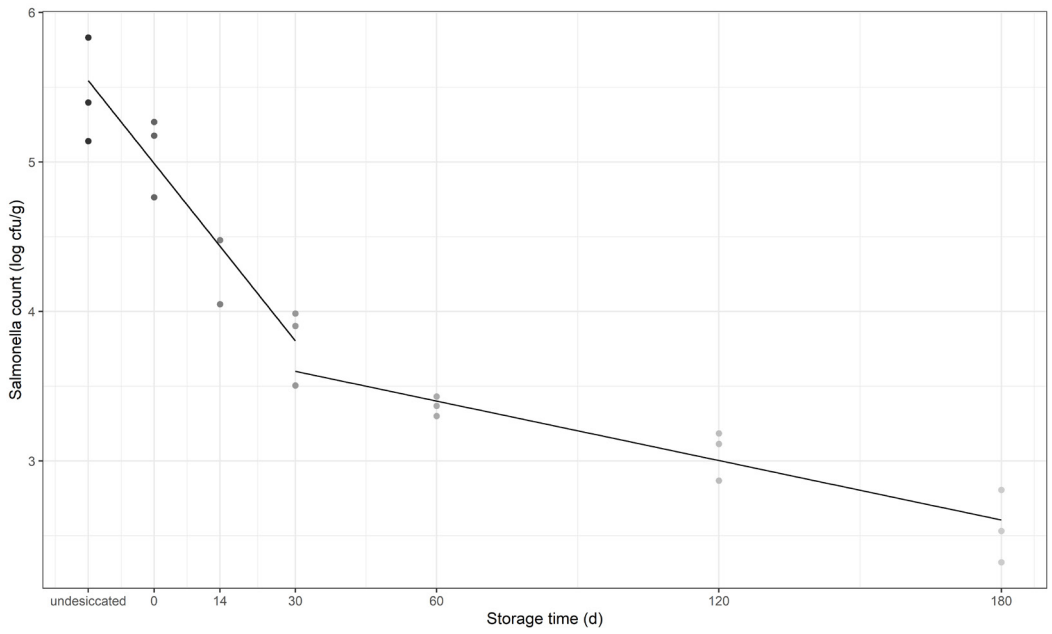

Figure 2 - Evolution of the *Salmonella Typhimurium* ATCC 14028 counts in blanched peanut kernel samples throughout the storage time at 28 ºC. Undesiccated (inoculum without desiccation stress). The gray scales on the graph represent the experiments performed on the specified storage days. Each point on the graph represents an independent experiment performed.

in the first hour of exposure to pH 3.5, followed by a phase of stability. A significant difference ($P < 0.05$) among the pH exposure times was noted for the cells recovered from the peanut samples stored for 120 and 180 days (Table 2). However, there was no significant difference ($P \geq 0.05$) between the *Salmonella* population recovered from the peanut samples and the sample without desiccative stress (Table 2). At pH 3.0 (Figure 3A), only the data referring to the first hour of exposure for the peanut samples stored at 120 and 180 days were considered for the statistical analysis, since from this time on the *Salmonella* counts reached the limit of detection (1 log cfu/ml). Figure 3A shows a decrease in the bacteria population after 1 h of exposure at pH 3.0 for all samples, with *Salmonella* reductions of around 1.50 log cfu/ml on the undesiccated and the 180-day sample and between 2.05 and 2.37 log cfu/ml on the other samples. In addition, only *Salmonella* population recovered from the peanut sample without storage (day 0) and the undesiccated sample had counts above the limit of detection after 4 h. Indeed, the pH 3.0 exposure time showed a significant effect ($P < 0.05$) on *Salmonella* count for all samples. The *Salmonella* death rate estimated by the model was 0.65 log cfu/ml/h for the undesiccated sample and 0.77, 0.79, 0.67, 0.55, 2.07 and 1.57 log cfu/ml/h for the peanut samples stored for 0, 14, 30, 60, 120 and 180 days at 28 ºC, respectively. There was no significant difference ($P > 0.05$) in the *Salmonella* death rates of all analyzed samples. However, comparing the confidence intervals (CI 95%, Table 2) for the cells recovered from the undesiccated sample [-0.91; -0.39] with the 120-day sample [-3.26; -0.85], it is possible to note that the intersection is tiny, i.e., it is on the threshold between statistically significant or not.

According to some studies, a primary, adaptative or acquired resistance to any injury process can trigger a secondary resistance mechanism or cross-protection to other subsequent stresses, such as heat, cold, salts, UV, and reactive oxygen species (YE et al., 2019; STACKHOUSE et al., 2012; GRUZDEV et al., 2011). During the stress condition, the microbial molecular machinery starts to operate to try to adapt the cell to the new environmental condition. *Salmonella* inoculated in peanut oil ($a_w = 0.30$) or granulated sugar ($a_w = 0.50$) showed increased expression of genes linked with regulatory function, DNA protection, and biosynthesis of unsaturated fatty acids in response to desiccation stress (CHEN et al., 2014). From a mechanistic perspective of cross-protection in *Salmonella*, YE et al. (2019) reported that an earlier adaptation of *S.* Enteritidis

Table 2 - Parameters of the model fitted for the fate of *Salmonella Typhimurium* ATCC 14028 during exposure to different pH values after been stored up to 180 days at 28 °C on blanched peanut kernels (confidence intervals of 95% with Bonferroni correction).

pH	Storage time	----------Intercept (α^k_j)----------		----------Growth rate (β^k_j)----------		
		estimates	SE	estimates	SE	CI 95%
3.0	undesiccated	5.196	0.262	-0.652	0.107	[-0.914; -0.390]
	0	4.555	0.262	-0.765	0.151	[-1.135; -0.395]
	14	3.819	0.262	-0.792	0.151	[-1.163; -0.422]
	30	3.257	0.262	-0.673	0.151	[-1.044; -0.303]
	60	2.870	0.262	-0.547	0.151	[-0.917; -0.176]
	120	3.065	0.338	-2.065	0.490	[-3.265; -0.865]
	180	2.573	0.338	-1.573	0.490	[-2.773; -0.373]
3.5	undesiccated	5.372	0.153	-0.136	0.062	[-0.289; 0.017]
	0	4.834	0.153	-0.157	0.088	[-0.373; 0.060]
	14	4.094	0.153	-0.195	0.088	[-0.411; 0.021]
	30	3.597	0.153	-0.185	0.088	[-0.401; 0.032]
	60	3.164	0.153	-0.199	0.088	[-0.416; 0.017]
	120	2.781	0.153	-0.288	0.088	[- 0.505; -0.072]
	180	2.340	0.153	-0.262	0.088	[-0.479; -0.046]
4.5	undesiccated	5.368	0.102	0.047	0.042	[-0.055; 0.149]
	0	4.873	0.102	0.068	0.059	[-0.076; 0.213]
	14	4.165	0.102	- 0.020	0.059	[-0.165; 0.124]
	30	3.753	0.102	-0.029	0.059	[-0.17′4; 0.116]
	60	3.323	0.102	-0.009	0.059	[-0.154; 0.135]
	120	2.973	0.102	-0.040	0.059	[-0.185; 0.104]
	180	2.449	0.102	0.043	0.059	[-0.102; 0.187]
7.2	undesiccated	5.190	0.150	0.476	0.061	[0.326; 0.625]
	0	4.812	0.150	0.506	0.086	[0.295; 0.718]
	14	4.113	0.150	0.376	0.086	[0.164; 0.587]
	30	3.624	0.150	0.421	0.086	[0.210; 0.633]
	60	3.185	0.150	0.399	0.086	[0.188; 0.611]
	120	2.790	0.150	0.465	0.086	[0.253; 0.676]
	180	2.334	0.150	0.438	0.086	[0.226; 0.649]

to acidic stress (pH 5.5 to 6.0) was a critical factor in promoting the expression of genes resistant to subsequent injuries, such as heating (e.g. htrA), cooling (e.g. cspA, cspC), and salinity (e.g. proP, proV). AVILES et al. (2013) reported that the high-fat levels and the low a_w of the peanut butter conferred resistance to *S.* Tennessee in an *in vitro* gastric simulation system (pH ~ 2-3). Another example of cross-protection was demonstrated when *Salmonella* was previously exposed to a 10% concentration of bile salts; subsequently the bacterium showed higher resistance at pH 2.0 (STACKHOUSE et al., 2012).

Although, our results could not show that previous desiccation stress caused by long-term storage on blanched peanut kernels can influence the acid resistance of *Salmonella Typhimurium*, they indicate a particular concern from a public health point of view since *Salmonella* counts above the limit of detection (1 log cfu/ml) were obtained in peanut samples stored for 60 days after exposure at pH 3.0 and in peanut samples stored for 180 days after exposure at pH 3.5 or 4.5 for 4 h. Epidemiological investigations of outbreaks linked to LMF have evidenced the presence of low infective doses (0.5 to 5 MPN/g) of this pathogen (WERBER et al., 2005). KIRK et al. (2004) obtained a *Salmonella* contamination level of 39% of peanut in-shell samples linked to an outbreak in Australia, Canada and UK, with counts ranged from <0.03 and 2 cfu/g. CALHOUN et al. (2013) verified a *Salmonella* positive rate of 1.63% of raw shelled peanuts, with counts ranged from <0.03 and 2.4 MPN/g. In Brazil a rate of 2.21% of *Salmonella* contamination in peanut samples throughout the production chain, with counts between 0.004 and 2.4 MNP/g was reported (NASCIMENTO et al., 2018). Nevertheless, it is important to emphasize that even though *S Typhimurium* ATCC14028 has been used in studies involving LMFs (PEREIRA et al.,

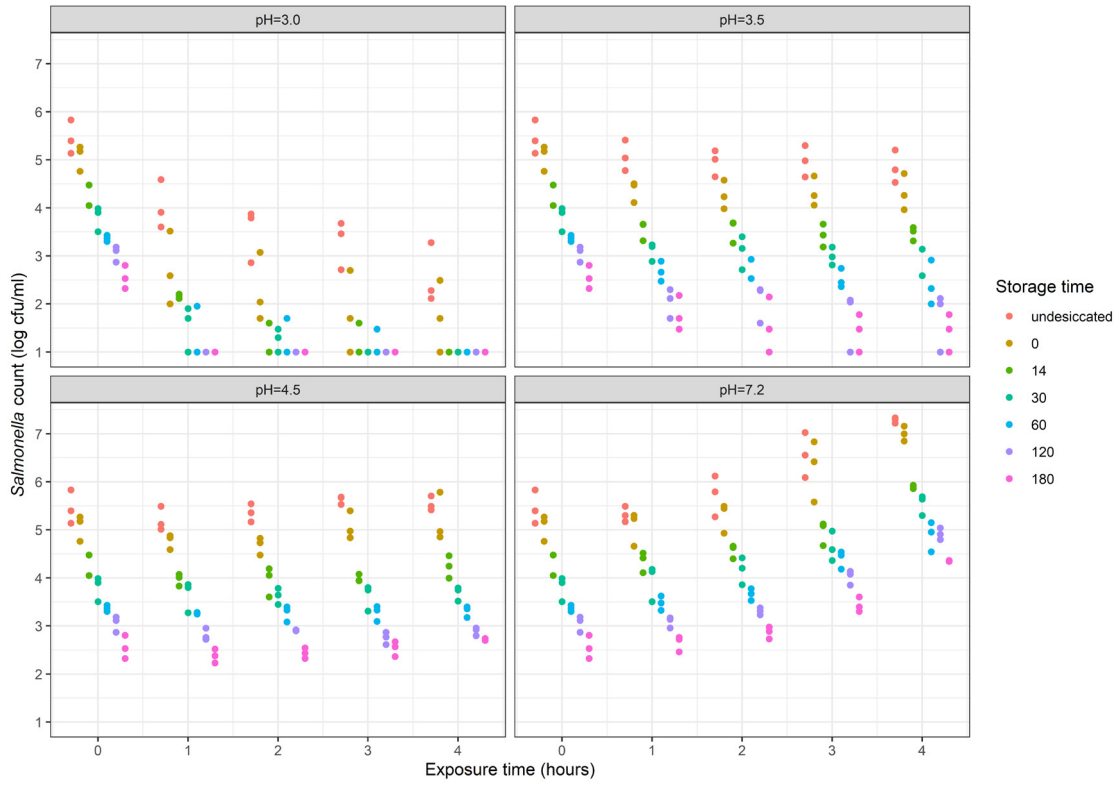

Figure 3 - *Salmonella Typhimurium* ATCC 14028 counts after exposure to different pH for up to 4 h at 37 °C after inoculation into culture medium or blanched peanut kernel stored up to 180 days at 28 °C. Ref (inoculum without desiccation stress). The different colors on the graph represent the days of storage, legend at the side of the graph. (a) pH = 3.0, (b) pH = 3.5, (c) pH = 4.5 e (d) pH = 7.2. Each point on the graph represents an independent experiment performed.

2020; NASCIMENTO et al., 2018; ROSSBACH et al., 2017; MATAK et al., 2010) other serotypes and strains need to be screened for their ability to develop cross-protection in this type of matrix.

CONCLUSION

The peanut storage time affected the *Salmonella* survival. However, it did not significantly influence the acid resistance of the pathogen. Our data can contribute to comprehend the *S. Typhimurium* ability to survive on LMF and also provide parameters to risk assessment studies. Nevertheless, further studies testing different strains are required to better understand the resistance mechanisms especially after subsequent exposures to different stress.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

AUTHORS' CONTRIBUTION

All authors contributed equally to the design and writing of the manuscript. All authors critically reviewed the manuscript and approved the final version.

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