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Desiccation resistance of a large set of *Salmonella enterica* strains and survival on dry- and wet-inoculated soybean meal through storage

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ABSTRACT

This study determined the desiccation resistance of 37 *Salmonella* strains belonging to 16 serotypes isolated from the soybean meal production chain. Besides, the survival of strains from three *Salmonella enterica* serovars (*S.* Typhimurium, *S.* Schwarzengrund, and *S.* Havana) on dry- and wet-inoculated soybean meal through storage at 25 °C and 37 °C was evaluated. Desiccation resistance varied within strains of the same serotype and amongst strains of different serotypes. On the other hand, the isolation source did not affect desiccation resistance. The inoculation method did not influence the survival of the three *Salmonella enterica* strains in soybean meal, but the effects of serovars and temperature were statistically significant ($p < 0.05$). The Weibull model was fitted to *Salmonella* survival in this matrix data, with the time for the first decimal reduction (δ) ranging from 21.1 to 50.8 days at 25 °C and from 2.7 to 7.9 days at 37 °C, respectively. The increase in storage temperature led to a decrease in survival regardless of the variability among the three isolates. The ability of *Salmonella enterica* to resist desiccation and to survive long-term on soybean meal reinforces the need for strategies to control this pathogen in the soybean production chain.

1. Introduction

Salmonella is one of the leading causes of foodborne diseases around the world. More than 80 million cases of gastroenteritis and 155,000 deaths occur globally each year due to the consumption of foods contaminated with this pathogen (CDC, 2021; Majowicz et al., 2010). According to the Centers for Disease Control and Prevention (CDC), 1.35 million cases of salmonellosis, 26,500 hospitalizations, and 420 deaths are reported annually in the USA (CDC, 2021). In 2019, Salmonella was the second most common cause of foodborne diseases in humans in the European Union, with 87,923 confirmed cases (EFSA, 2021). In Brazil, more than 30% of the cases/outbreaks of foodborne diseases reported between 2000 and 2017 were attributed to *Salmonella* (SVS, 2018).

Traditionally foodborne salmonellosis is frequently linked to food of animal origin such as eggs, meat, and poultry meat (Antunes et al., 2016; Ferrari et al., 2019; Moffatt et al., 2016). Even though *Salmonella* can contaminate foods of animal origin during processing (Møller et al., 2016) and preparation by consumers (Kusumaningrum et al., 2004; Møretrø et al., 2021), the introduction of this bacterium into livestock via feeds is critical and still challenging to manage (Habimana et al., 2014; Parker et al., 2022; Wierup, 2017). When livestock harbor *Salmonella*, the probability for the spread and contamination of foods of animal origin in different steps of the food production chain is expected to increase. One of the primary potential sources of *Salmonella* introduction into feeds comprise the use of contaminated ingredients (Sargeant et al., 2021). Amongst feed ingredients, soybean meal stands as an essential source of protein in animal feed, from which *Salmonella* has been frequently isolated (Österberg et al., 2006; Wierup & Häggblom, 2010; Wierup & Kristoffersen, 2014).

While soybean meal presents a low moisture content (Ibáñez, de Blas, Cámara, & Mateos, 2020), *Salmonella* is among the most highly adaptable pathogens to stressful conditions. As such, *Salmonella* can survive in low moisture substrates for extended periods (Beuchat et al., 2013; Podolak et al., 2010; Spector & Kenyon, 2012; Crucello et al., 2019). Factors such as food composition, water activity (a_w), storage temperature, among others, influence the survival of *Salmonella* in low

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moisture substrates, including foods (Farakos et al., 2014). In addition, physiological and genetic expression changes of the bacterial cells due to desiccation and low a_w may lead to increased resistance to heat treatments and other intervention strategies (Fong & Wang, 2016; Gruzdev et al., 2011) or may enter a viable non-cultivable (VBNC) state, challenging analytical methods (Gruzdev et al., 2012; Santillana Farakos et al., 2013). Because of these characteristics, *Salmonella* has also been responsible for numerous foodborne outbreaks linked to low moisture foods or ingredients (Beuchat et al., 2013; Finn et al., 2013; Russo et al., 2013; Van Doren et al., 2013).

Given the above, several studies have investigated the resistance and adaptive responses of *Salmonella* to desiccation, thermal inactivation, and persistence in low moisture matrixes such as peanut, almonds, walnuts, whey protein powder, milk chocolate, powdered milk, powder infant formula, wheat flour, black pepper, and dried pet food (Abdelhamid & Yousef, 2020; Blessington et al., 2013; Crucello et al., 2019; Fong & Wang, 2016; Lambertini et al., 2016; Mahmoud et al., 2018; Pereira et al., 2020; Prestes et al., 2019; Santillana Farakos et al., 2013). However, to the best of the author's knowledge, there are no studies about the survival of *Salmonella* in soybean meal. Furthermore, the studies that evaluate the desiccation resistance of *Salmonella* tested a limited number of strains or serotypes, while a considerable diversity of serotypes can be present in low moisture substrates (Abdelhamid & Yousef, 2020; Gruzdev et al., 2012; Habimana et al., 2014). Therefore, the objectives of this study were to determine the desiccation resistance and evaluate the influence of the inoculation method and storage temperature on the survival of different *Salmonella enterica* serotypes in soybean meal.

2. Material and methods

2.1. Salmonella enterica strains and preparation of a suspension of cells

The desiccation resistance assays were conducted with thirty-seven *Salmonella enterica* strains previously isolated from soybean meal and soybean meal processing premises (Chaves, 2017, p. 92). These strains were selected based on their ability to survive osmotic stress from a set of 190 *Salmonella* strains previously isolated from the soybean production chain (Furtado, 2017, p. 95).

Salmonella enterica strains were grown in Tryptic Soy broth (TSB) at 37 °C for 24 h and, afterward, centrifuged (Sorvall Legend XTR, Thermo Scientific, Waltham, USA) at 5000×*g* for 5 min, following washing twice in sterile 0.1% peptone water (Kasvi, Italy). Cells obtained from each strain were resuspended in sterile 0.1% peptone water (Kasvi, Italy), followed by adjustment of the concentration of final inoculum to approximately 7 log CFU/ml as previously described (Sant'Ana et al., 2013).

2.2. Desiccation resistance assays

Soybean meal samples (5 g) previously decontaminated in an autoclave at 121 °C/15min were individually inoculated with 5 ml of each *Salmonella enterica* strains bacterial suspension. According to the preliminary experiments, this proportion 1:1 allowed adequate homogenization of the soybean meal samples (data not shown). The samples were allowed to dry in a laminar flow hood at 25 °C. *Salmonella enterica* counts were determined immediately after inoculation and after 18 h of drying, when soybean meal reached an a_w ~0.60. This temperature was chosen to assess the strains' desiccation resistance, calculated as the difference between counts of *Salmonella enterica* strains before and after the drying period. The results were expressed as log CFU/g.

2.3. Salmonella survival on wet- and dry-inoculated soybean meal

This experiment was done using the Salmonella enterica serovar Typhimurium (ATCC 14028) and two *Salmonella* strains that were highly resistant to desiccation as determined in 2.2.

2.3.1. Inoculum preparation

Salmonella enterica strains selected for this experiment were individually cultured in TSB at 37 °C for 24 h three times in a row, increasing culture medium volumes (100, 250, and 700 mL). After the last incubation, cells were centrifuged at 5000×*g* for 5 min (Sorvall Legend XTR, Thermo Scientific, Germany) and washed three times in sterile 0.1% peptone water (Kasvi, Italy). The pellets were resuspended to obtain a final concentration of 7–9 log CFU/ml, measured as absorbance at 600 nm (Spectrophotometer Model, City, Brazil) (Sant'Ana et al., 2013) and confirmed by *Salmonella enterica* plate count as described in 2.1.

2.3.2. Wet- and dry-inoculation methods

For wet inoculation, *Salmonella enterica* suspensions (7 log CFU/ml) were individually added to soybean meal samples in a proportion of 1:1 (ml: ml) (Gabriel, Tongco, and Barnes, 2017). The inoculated samples were allowed to dry in a laminar flow chamber for up to 20h until a_w 0.65, which is the regular a_w of the soybean meal.

In the dry inoculation, sand (0.2–0.6 mm; Dinâmica, São Paulo, Brazil) was autoclaved at 121 °C/15 min for decontamination. Then, the sand was inoculated with *Salmonella enterica* as a vehicle for soybean meal inoculation (Blessington et al., 2013; Furtado et al., 2020). Briefly, *Salmonella enterica* suspensions (10^9 CFU/ml) were individually added to sand in a proportion of 1:2 (ml:g). After complete homogenization, sand samples were put on aluminum trays, covered with paper, and allowed to dry in an incubator at 35 °C for approximately 24 h. When needed, the drying of the samples was completed in a laminar flow chamber until a_w ~0.65. Afterward, the soybean meal was inoculated with sand artificially contaminated with *Salmonella enterica* in a proportion of 1:2 (g:g).

Both wet- and dry-inoculated soybean meal samples were transferred to metallic moisture barrier bags and stored at 25 °C and 37 °C. At least ten samples from each experimental condition were taken at regular intervals to determine *Salmonella enterica* counts. Up to ten colonies per experimental conditions were further submitted to serology using agglutination test with anti-*Salmonella* polyvalent serum (Probac, São Paulo, Brasil).

2.3.3. Water activity determination

The a_w of the soybean meal samples was determined immediately after inoculation and drying and during storage using an a_w meter (Aqualab CX2 instrument (Decagon Devices, Washington, USA). This last measurement was carried out to ensure that a_w was not a factor of variation in the experiment during storage.

2.3.4. Salmonella enterica enumeration

The drop plate method (Herigstad et al., 2001) was used to determine *Samonella enterica* counts in the samples throughout the storage period of soybean meal. Samples were serially diluted (10-fold), following inoculation onto Xylose Lysine Deoxycholate Agar (XLD, Acumedia, Michigan, USA), following incubation at 37 °C for 24 h. *Salmonella enterica* counts were expressed in log CFU/g for each experimental condition.

2.3.5. Salmonella enterica survival curves

Salmonella enterica survival curves were plotted as a function of time, considering the different storage temperatures tested, strain, and inoculation methods. The survival curves were then fitted to the Weibull predictive model (Mafart et al., 2002) using GInaFiT (Geeraerd et al., 2005). The following equation describes this model:

$$\mathrm{Log}S_{(t)} = -(t/\delta)^{\rho}$$

Where $S_{(t)}$ is the surviving fraction, t is the time; ρ is the shape parameter representing the curvature of the survival curve, and δ is the time for the

first decimal reduction.

2.4. Statistical analysis

A one-way analysis of variance (ANOVA) was performed using the R software (version 3.3.1, The R Foundation for Statistical Computing Vienna, Austria) to analyze the experimental data at a 5% significance level ($p < 0.05$).

Significant differences among desiccation resistances of the *Salmonella enterica* strains were evaluated using Scott-Knott's test ($p < 0.05$). The student's t-test (unpaired, two-tailed) was used to determine the significant differences between the resistance of *Salmonella enterica* strains isolated from soybean meal and processing environment groups ($p < 0.05$). The difference in the δ of *Salmonella enterica* strains was analyzed by ANOVA ($p < 0.05$) followed by multiple comparisons using *t*-Test (Fisher's LSD, $p < 0.05$).

Network graphs were used to show the relationship between the desiccation resistance and *Salmonella enterica* serotypes, isolation sources, or industries. These graphs were created in Gephi v0.9.2 software using the Fruchterman Reingold force-based algorithm (Fruchterman & Reingold, 1991). The nodes correspond to *Salmonella enterica* strains linked by edges relating to each parameter.

3. Results

3.1. Desiccation resistance

A significant difference in desiccation resistance was observed among some of the thirty-seven *Salmonella enterica* strains belonging to 16 serotypes studied (Table 1). The reductions of *Salmonella enterica* counts in the artificially contaminated soybean meal ranged from 0.6 to 2.3 log CFU/g. The a_w of the samples reduced from 0.98 to 0.60 after 18 h of drying in the laminar flow chamber. No significant differences were found regarding the number of decimal reductions observed for different strains belonging to eight serotypes: *S.* Mbandaka, *S.* Tennessee, *S.* Agona, *S.* Akuafo, *S.* Anatum, *S.* Brooklyn, *S.* Derby and *S. enterica* (O:16) ($p > 0.05$) (Table 1). Nonetheless, some of the strains belonging to these serotypes presented a higher number of decimal reductions when compared to some strains of serotypes *S.* Havana, *S.* Infantis, *S.* Montevideo, *S.* Morehead, *S.* Ohio, *S.* Rugosa, *S.* Schwarzengrund and *S.* Senftenberg ($p < 0.05$). Overall, the highest number of decimal reductions observed were ~2 log CFU/g, while the lowest γ was observed for specific strains belonging to serotypes *S.* Havana, *S.* Montevideo, *S.* Ohio, *S.* Rugosa, and *S.* Schwarzengrund were around 0.6–0.9 log CFU/g (Table 1).

The analysis of the network graphics showed differences in desiccation resistance between some *Salmonella enterica* strains of the same serotype (Fig. 1A). A relation between desiccation resistance and the isolation sources or industries was not observed (Fig. 1B and C). The source of the serotypes (environment or soybean meal) did not impact their counts ($p = 0.2615$) after desiccation (Fig. 2).

3.2. Salmonella enterica survival on wet- and dry-inoculated soybean meal

The counts of *S.* Typhimurium ATCC 14028, *S.* Schwarzengrund IOC 5691, and *S.* Havana IOC 2307 in the soybean meal decreased during storage. However, these strains survived at least 30 and 130 days in the samples stored at 37 °C and 25 °C, respectively. The inoculation method did not influence *S.* Typhimurium ATCC 14028, *S.* Schwarzengrund IOC 5691, and *S.* Havana IOC 2307 survival in soybean meal samples ($p > 0.05$). However, the effects of strains and temperature and the interaction between these two factors were statistically significant ($p < 0.001$).

The survival of *S.* Typhimurium ATCC 14028, *S.* Schwarzengrund IOC 5691, and *S.* Havana IOC 2307 in soybean meal did not follow the first-order kinetics. Therefore, the Weibull model was used to fit the data (Fig. 3). The increase in storage temperature from 25 °C to 37 °C led to a decrease in *S.* Typhimurium ATCC 14028, *S.* Schwarzengrund IOC 5691, and *S.* Havana IOC 2307 survival in soybean meal, which was observed through the first decimal reduction (δ). Considering the wet-inoculation method, the δ values of *S.* Schwarzengrund IOC 5691, *S.* Typhimurium ATCC 14028, and *S.* Havana IOC 2307 were 40, 22, and 46 days at 25 °C, and 2.7, 3.5 and 4.0 days at 37 °C, respectively. Similar behavior was observed for dry inoculation when *S.* Schwarzengrund IOC 5691, *S.* Typhimurium ATCC 14028, and *S.* Havana IOC 2307 showed δ values of 50.8, 21.1, and 36.4 days at 25 °C and 7.9, 3.3 and 4.3 days at 37 °C, respectively (Fig. 4).

Table 1
Desiccation resistance of *Salmonella enterica* strains of different serotypes in the soybean meal after drying process at 25 °C for 18 h (a_w reduction from 0.98 to 0.60).

Serotype	Strain[a]	Isolation Source	Reduction (log CFU/g)[c]
S. Agona	5676	Environment	1.9 ± 0.26[a]
	5641	Environment	1.9 ± 0.02[a]
	2305	Environment	1.8 ± 0.18[a]
	2325	Soybean meal	1.7 ± 0.05[a]
	5650	Environment	1.6 ± 0.19[a]
S. Akuafo	5604	Environment	1.9 ± 0.22[a]
	4268	Environment	1.7 ± 0.16[a]
	5638	Environment	1.4 ± 0.34[a]
S. Anatum	2322	Soybean meal	1.4 ± 0.10[a]
S. Brooklyn	2280	Environment	1.6 ± 0.04[a]
S. Derby	5593	Environment	2.3 ± 0.22[a]
	5601	Environment	2.0 ± 0.24[a]
	5592	Environment	1.9 ± 0.05[a]
S. enterica (O:16)	2224	Environment	1.6 ± 0.20[a]
S. Havana	2310	Soybean meal	1.9 ± 0.07[a]
	2307[b]	Soybean meal	0.6 ± 0.03[c]
S. Infantis	2309	Soybean meal	2.0 ± 0.20[a]
	4263	Environment	1.1 ± 0.80[b]
S. Mbandaka	5666	Environment	1.7 ± 0.08[a]
	4272	Environment	1.4 ± 0.37[a]
	2317	Soybean meal	1.4 ± 0.30[a]
S. Montevideo	5677	Environment	1.9 ± 0.15[a]
	5720	Soybean meal	1.7 ± 0.56[a]
	5690[b]	Environment	0.8 ± 0.22[c]
S. Morehead	5728	Soybean meal	1.2 ± 0.28[b]
S. Ohio	5653	Environment	1.8 ± 0.11[a]
	5651	Environment	1.8 ± 0.05[a]
	5688	Environment	1.5 ± 0.28[a]
	5659	Environment	1.3 ± 0.02[a]
	5694[b]	Environment	0.9 ± 0.01[b]
S. Rugosa	2294	Environment	1.1 ± 0.02[b]
	2320[b]	Soybean meal	0.6 ± 0.14[c]
S. Schwarzengrund	**5691**[b]	Environment	0.6 ± 0.11[c]
S. Senftenberg	2319	Soybean meal	1.7 ± 0.11[a]
S. Tennessee	4269	Environment	1.8 ± 0.05[a]
	5680	Environment	1.5 ± 0.12[a]
	4274	Environment	1.4 ± 0.24[a]

[a] IOC – Reference code of *Salmonella* strain in the Culture Collection of the Oswaldo Cruz Foundation, Rio de Janeiro, Brazil.
[b] *Salmonella* strains highlighted in bold showed reductions in the count ≤1 log CFU/g.
[c] Values followed by the same letter are not significantly different ($P > 0.05$) using Scott-Knott's test.

4. Discussion

Soybean meal is an essential product from soybean processing that may harbor *S. enterica* (Österberg et al., 2006; Wierup & Häggblom, 2010; Wierup & Kristoffersen, 2014). Dust present in external and internal areas of soybean processing premises is a potential source of *S. enterica* (Chaves, 2017, p. 92). Even though contamination of soybean meal via dust may occur during soybean processing, understanding whether different *S. enterica* serotypes originated from different sources may survive desiccation and in soybean meal during storage is essential

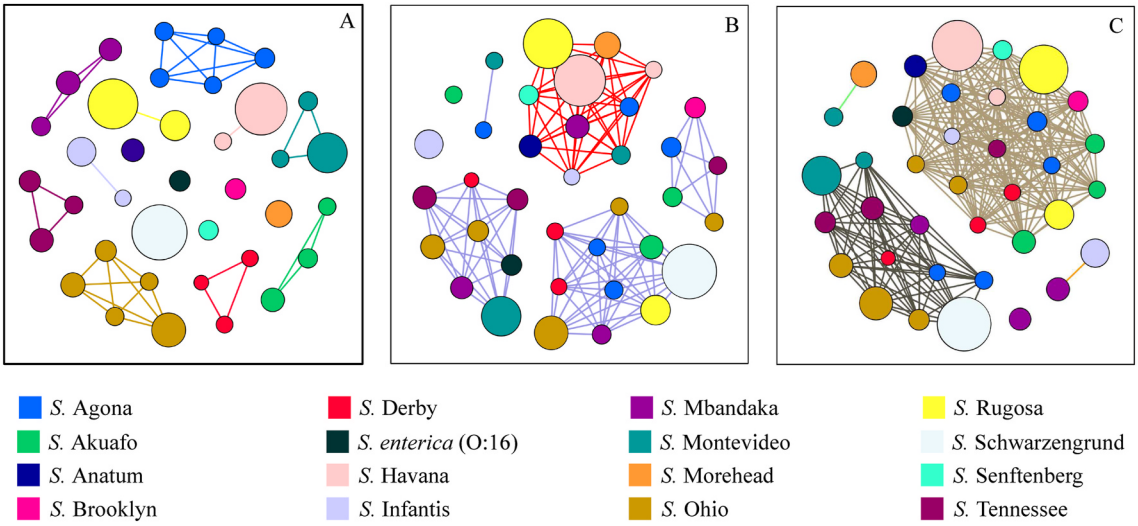

Fig. 1. Network analysis showing the relationship between the desiccation resistance of *S. enterica* strains isolated from soybean meal productive chain as a function of serovars (A) isolation sources (environment or soybean meal) (B), and industries from which the *S. enterica* strains were isolated (C). Network graphs were built in Gephi v0.9.2 software using the Fruchterman Reingold force-based algorithm. The nodes correspond to *S. enterica* strains linked by edges relating to each parameter. The size of the nodes is proportional to the relative resistance to desiccation of the isolates.

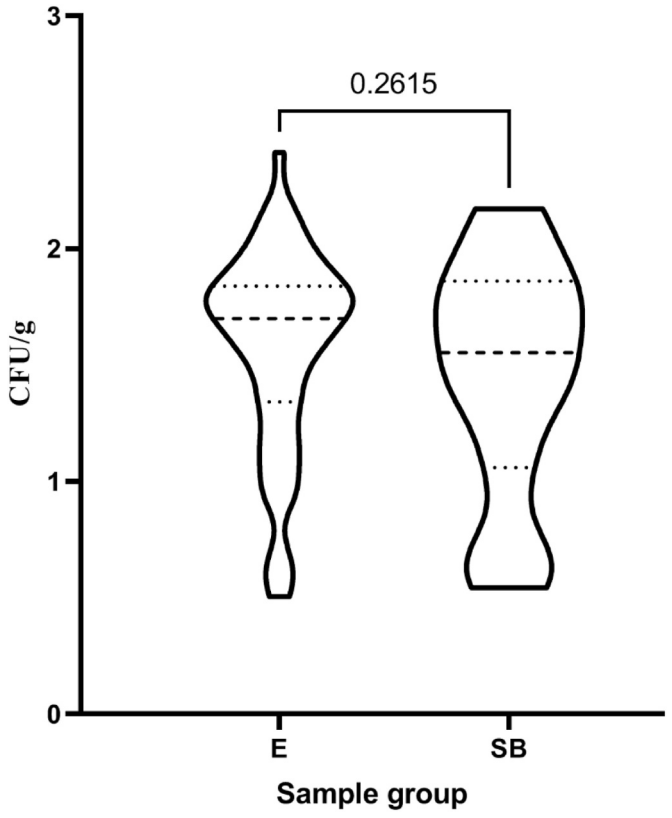

Fig. 2. Differences of desiccation resistance of *S. enterica* strains isolated from the environment and the soybean meal. Violin plots show the data distribution of desiccation resistance between two *S. enterica* groups. The dashed line (——) indicate median and dotted line (····) correspond to the Q1 and Q3 interquartile range; E – environment; SB - soybean meal. Group comparisons were performed by unpaired *t*-test (p = 0.2615).

for designing effective control measures.

The assessment of desiccation resistance of 37 strains of *S. enterica* strains belonging to 16 serotypes indicated no link with the source of isolation. Therefore, the source of *S. enterica* is not critical for resistance to desiccation, highlighting the difficulties in preventing the contamination of soybean meal by this bacterium through processing. *Salmonella* can survive various stress conditions, including starvation, acid, oxidative, osmotic, desiccation, and thermal stresses (Spector & Kenyon, 2012). The stress response involves structural and physiological changes in the bacterial cell controlled by various gene expression regulators (Hengge, 2014; Shen & Fang, 2012). *Salmonella* possesses several extracellular and intracellular defenses against desiccation stress. The O-antigen polysaccharide chain of LPS, extracellular cellulose, and curli appear to play an essential role in the extracellular defenses against desiccation (Garmiri et al., 2008; White et al., 2006). Trehalose, a disaccharide acting intracellularly as a compatible solute, can prevent denaturation of proteins and stabilize memmeale phospholipids (Howells et al., 2002). Gene expression patterns inherent to each *Salmonella* strain may explain the differences in the stress resistance profiles inter-serotypes and intra-serotypes (Barnhill et al., 2019; Crucello et al., 2019; Guillén et al., 2020).

All the five strains with the highest resistance to desiccation belonged to serotypes commonly isolated in Brazil from feed ingredients or poultry feed: *S.* Ohio, *S.* Havana, *S.* Schwarzengrund, *S.* Montevideo, and *S.* Rugosa (Hofer et al., 1998). This finding may indicate that the occurrence of some strains of these serotypes with higher resistance to desiccation may play a role in their frequent association with feed ingredients and feed. Besides, it should be emphasized that some of these serotypes are frequently identified in clinical and non-clinical non-human sources such as poultry and bovine (CDC, 2013).

The findings are shown in Table 1 add information to the literature because most of the studies focus on assessing a low number of *Salmonella* strains or serotypes (Issenhuth-Jeanjean et al., 2014). Understanding whether several *S. enterica* serotypes and strains respond differently to stress conditions such as desiccation is crucial because it may lead to selecting specific strains to be used as indicators of processing hygiene or lethality.

In order to assess the influence of inoculation method and storage temperature on the survival of *S. enterica* in soybean meal, two highly resistant strains to desiccation (*S.* Schwarzengrund IOC 5691 and *S.* Havana IOC 2307) (Table 1) and one reference strain (*S.* Typhimurium ATCC 14028) were selected. Literature has demonstrated a prolonged survival of *Salmonella* in low moisture substrates, estimated as months for cookies (Beuchat & Mann, 2015), peanut butter (Burnett et al., 2000), sesame seeds and tahini (Torlak et al., 2013), and over one year

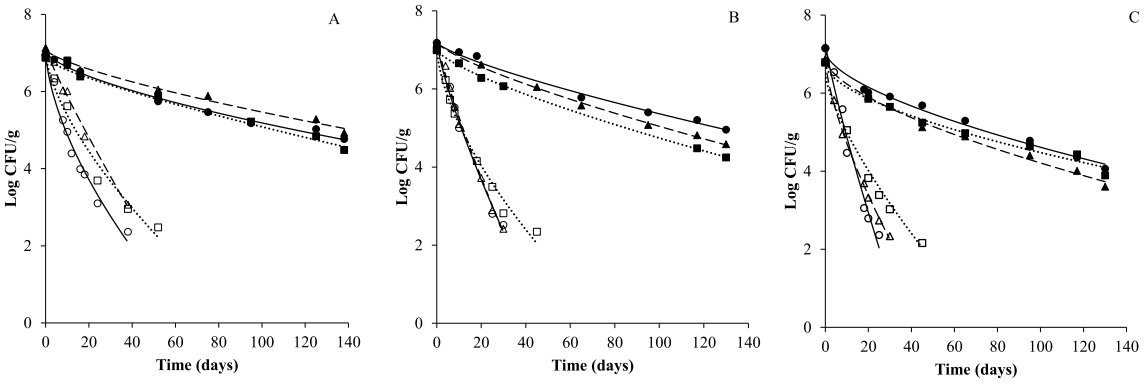

Fig. 3. Survival of *S. enterica* on soybean meal contaminated via wet-inoculation (circle) or dry-inoculation (triangle) and stored at 25 °C (closed symbols) and 37 °C (open symbols). Sand used as a carrier also was evaluated (square). *S.* Schwarzengrund IOC 5691 (A), *S.* Havana IOC 2307 (B), and *S.* Typhimurium ATCC 14028 (C). Lines represent the Weibull models fitted for each experimental condition: solid line (wet-inoculation), dashed line (dry-inoculation), and dotted line (sand). Results are mean ± standard deviation (S.D.).

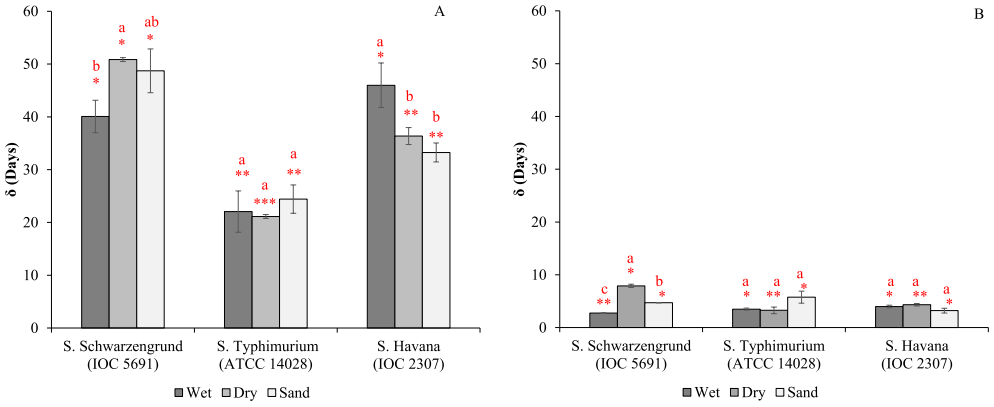

Fig. 4. Time for the first decimal reduction (δ) values of *S. enterica* strains in soybean meal as affected by the inoculation method and storage temperature [(A) 25 °C and (B) 37 °C]. Asterisk represent comparisons among the serotypes for the same inoculation method (wet or dry or sand). Small letters represent comparisons for the same serotype inoculated in wet, dry and sand. Values followed by the same letter or symbol are not significantly different ($P > 0.05$) using the *t*-test (Fisher's LSD, $p < 0.05$).

for nuts (Blessington et al., 2012), and powdered infant formula (Barron & Forsythe, 2007). Several factors such as temperature, a_w, substrate, culture media, inoculation methods, serotypes, and strain have been described to influence *Salmonella*'s survival in low moisture substrates (Farakos et al., 2014; Wiertzema et al., 2019, pp. 1082–1088). For studies to assess the behavior of microorganisms in low moisture foods, one of the remaining challenges comprises the inoculation procedure. The inoculation procedures employed in low moisture foods must be as realistic as possible, should not interfere with the intrinsic characteristics of the food, and should also preferably simulate the likely contamination routes. While the wet-inoculation method involves the addition of liquid carriers that can change the a_w of food, the dry inoculation method involves the preparation of a liquid suspension of cells of the target microorganisms, further inoculation, and homogenization in the carrier, drying to a_w of interest and inoculation of food.

Materials such as sand, talc, and chalk, among others, are employed as carriers of the target microorganisms for food inoculation (Blessington et al., 2013; Enache et al., 2015; Furtado et al., 2020; Shrestha & Nummer, 2016). The dry inoculum method precludes the post-inoculation drying required in the wet-inoculum method, leading to injuries in microorganisms (Blessington et al., 2013; Liu et al., 2019). While carriers such as sand, chalk, and talc inoculated with a target microorganism are used in the dry-inoculation method, it is known that depending on the material and conditions, the microbial viability and desiccation resistance can be impacted.

In this study, the Weibull model was fitted to the data, and the kinetic survival parameters of *S.* Schwarzengrund IOC 5691, *S.* Havana IOC 2307, and *S.* Typhimurium ATCC 14028 in soybean meal were determined. Previous studies with other low moisture substrates have also indicated that the survival pattern of *S. enterica* did not follow first-order kinetics (Farakos et al., 2014; Santillana Farakos et al., 2013). This survival pattern is likely the result of the *S. enterica* adaptation to an environment with low moisture content. Fig. 1 clearly shows that the higher the storage temperature (37 °C), the quicker *S. enterica* death. The impact of low temperature in enhancing the survival of pathogens under stressful conditions is known (Morey & Singh, 2012). The findings of this study indicated that an increase in storage temperature led to a decrease in *Salmonella enterica* survival, regardless of the strain or inoculation method. Other authors reported similar findings in dried milk, egg powder, alfalfa seeds, and nuts (Beuchat & Scouten, 2002; Beuchat & Mann, 2010; Jung & Beuchat, 1999; McDonough & Hargrove, 1968). Despite the resistance differences among serotypes, *S. enterica* strains assessed here could persist in soybean meal stored at 25 °C. This finding is of concern as this temperature is within the storage range that soybean meal is subjected to in most producing countries. Although rising the temperature to 37 °C considerably reduces *S. enterica* survival rate, it is not feasible in practical terms.

In this study, it has been found that the inoculation methods (wet or dry) did not influence the survival of *Salmonella enterica* in the soybean meal during storage. Other studies have demonstrated more remarkable bacterial survival with dry-inoculation (Podolak et al., 2010) or wet-inoculation (Farakos et al., 2014). Despite this, the dry-inoculation method using sand as a carrier was proved to be more pertinent for use in low moisture foods, maintaining the high viability of the cells during the drying step and mimicking dust as a source of *S. enterica* during soybean meal production. The dust has been reported as one of the most critical routes of contamination of *S. enterica* of soybean meal (Chaves, 2017, p. 92). For instance, it has been reported the presence of

Salmonella in the dust samples from all soybean shiploads from South America (Wierup & Kristoffersen, 2014) and internal and external areas of soybean processing premises (Chaves, 2017, p. 92). In addition, sand has been successfully used as a carrier for *S. enterica* inoculation low-a_w foods, with limited reductions in viable counts in the dry inoculum (ranging <1 to 2.5 log CFU/ml) (Blessington et al., 2013; Bowman et al., 2015; Furtado et al., 2020; Shrestha & Nummer, 2016).

5. Conclusion

The desiccation resistance of 37 *S. enterica* strains belonging to 16 serotypes isolated from soybean meal chain productive was assessed. These assays indicated that some strains were highly resistant to desiccation, which may favor their survival throughout soybean meal processing, storage, and transportation. Even though the inoculation methods have not influenced *S. enterica* survival during storage, dry inoculation was deemed more suitable for mimicking soybean meal's most likely contamination routes.

The differences regarding the survival to desiccation and soybean meal storage conditions suggest that studies on the resistance and survival of *S. enterica* in low moisture substrates should not be conducted with a single strain. Finally, the survival of strains of *S. enterica* for long periods reported in this study reinforces the potential role of soybean meal as a critical source of feed contamination by this pathogen.

CRediT authorship contribution statement

Ana Paula Norberto: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization. **Verônica O. Alvarenga:** Methodology, Validation, Formal analysis, Writing – original draft, Visualization. **Humberto M. Hungaro:** Visualization, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Anderson S. Sant'Ana:** Conceptualization, Methodology, Validation, Formal analysis, Writing – original draft, Writing – review & editing, Resources, Supervision, Project administration, Funding acquisition.

Declaration of competing 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.

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