Survival of Listeria monocytogenes during storage on dried apples, strawberries, and raisins at 4°C and 23°C

Ryan Cuzzi, Vivian Ly, Valeria R. Parreira, Alma Fernanda Sanchez-Maldonado, Jeffrey M. Farber

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Research Article

Title: Survival of *Listeria monocytogenes* during storage on dried apples, strawberries, and raisins at 4°C and 23°C

Authors: Ryan Cuzzi, Vivian Ly, Valeria R. Parreira*, Alma Fernanda Sanchez-Maldonado, Jeffrey M. Farber

Canadian Research Institute for Food Safety, Department of Food Science, University of Guelph, Guelph, Ontario, Canada

Abstract

The survival of *Listeria monocytogenes* was assessed during long-term storage on three dried fruits: dried apples, raisins and dried strawberries. Using sand as a carrier, the dried fruits were dry-inoculated with a four-strain cocktail of *L. monocytogenes* to achieve numbers of 4.0 to 4.6 log CFU/g. The inoculated foods were stored at 4°C, 25–81% relative humidity (RH) and 23°C, 30–35% RH for 336 days. Colonies of *L. monocytogenes* could not be recovered from the dried apples after inoculation, i.e., day 0. Concentrations of *L. monocytogenes* decreased rapidly on the raisins and dried strawberries during storage at 23°C, with enhanced survival observed at 4°C. Linear rates of decline for populations of *L. monocytogenes* during storage at 4°C on the raisins and dried strawberries were 0.1 and 0.2 log CFU/g/month, respectively. The relative distribution of the four *L. monocytogenes* strains making up the cocktail was determined by multiplex PCR at the beginning of storage and after 336 days on the dried fruits. At day 0, *L. monocytogenes* populations were predominantly composed of the serotype 1/2a and 3a strains on both the raisins and dried strawberries. After long-term storage at 4°C, a relative decrease in serotype 1/2a was observed on both fruits, coupled with relative increases in the serotype 3a strain during storage on both fruits, in addition to the serotype 1/2b strain on the raisins. These results demonstrate

that *L. monocytogenes* is rapidly inactivated during storage on raisins and dried strawberries at 23°C, but capable of long-term survival at 4°C. Improved knowledge on the survival of *L. monocytogenes* on these commodities is important for predictive modeling and can be used to better inform microbial health risk assessments.

Highlights

- *L. monocytogenes* survives for at least 1 year on dried strawberries and raisins stored at 4°C
- *L. monocytogenes* could not be recovered from artificially contaminated dried apples
- Rates of decline of *L. monocytogenes* at 4°C on dried apples > dried strawberries > raisins
- Capacity for survival on fruits varied between different *L. monocytogenes* strains

Keywords: inactivation, dry inoculation, low-moisture, dried fruit, long-term storage

1. Introduction

Over the past decade, low-moisture foods (LMFs) have been identified as having the capacity to pose a human health risk (FAO/WHO, 2015; Jordan et al., 2015). Although *Listeria monocytogenes* has been more commonly associated with sliced deli meats and dairy products, recent listeriosis outbreaks have also involved foods such as whole cantaloupe and pre-packaged salads (Buchanan et al., 2017; Jordan et al., 2015; Laksanalamai et al., 2012). In 2014, caramel-coated apples were determined to be the causative agent in a multi-state outbreak of listeriosis in the U.S.A. involving 35 cases and 3 deaths (Glass et al. 2015).

Information on the *L. monocytogenes* dose-response is lacking because of factors such as its long incubation period, poor animal models and the rarity of listeriosis outbreaks (D'Orazio,

2014; Farber and Peterkin, 1991). Importantly, recent outbreaks involving ice cream and stone fruits may have been associated with a low dose-response, particularly for high-risk population groups, as low numbers of *L. monocytogenes* were recovered from the implicated foods (Chen et al., 2016; Pouillot et al., 2016).

LMFs have been described as those foods with a water activity (a_w) < 0.85 (FAO/WHO, 2015). The growth of bacterial pathogens is inhibited in these products, but their capacity to survive for long periods on these foods has been demonstrated. While the majority of these studies focus on *Salmonella,* other pathogens such as *L. monocytogenes* and *Escherichia coli* O157:H7 have demonstrated long-term survival in LMFs such as nuts, nut products and powders (e.g., protein powder, powdered infant formula) (Bowman et al., 2015; Brar et al., 2015; Fong and Wang, 2016; Kenney and Beuchat, 2004; Moussavi et al., 2019; Santillana Farakos et al., 2014). Dehydrated fruits are typically considered to be low risk for foodborne illness due to their a_w (< 0.46) (Beuchat and Mann, 2014). However, the contamination of these products with *L. monocytogenes* could be hazardous for vulnerable people, as these foods are normally consumed in their raw state and often used as ingredients in ready-to-eat products (FAO/WHO, 2015).

The objectives of this study were to determine whether *L. monocytogenes* is capable of long-term survival on dried fruits and whether its capacity for survival varies among different strains.

2. Materials and methods

2.1. Preparation of dried fruits

Jumbo Thompson raisins (South Africa), dried strawberries (Dallas, OR) and natural, dehydrated diced apples (Argentina) were sourced from Johnvince Foods (North York, Canada). All the dried fruits used in this study were stored in the dark at room temperature (RT) prior to

use.

2.2. Bacterial strains

Four strains of *L. monocytogenes* were used: ILSI 4 (serotype 4b), ILSI 18 (serotype 3a), ILSI 34 (serotype 1/2a) and ILSI 39 (serotype 1/2b). All strains were obtained from Dr. Martin Wiedmann (Ithaca, NY) from the International Life Sciences Institute (ILSI) North America *Listeria* strain collection (Fugett et al., 2006). Stock cultures were stored at -80°C in 12% (w/v) skim milk (Nestle Carnation Inc., Carnation, WA), 1% (v/v) glycerol (Fisher Scientific, Fair Lawn, NJ) and 1% (v/v) dimethyl sulfoxide (DMSO; Corning, Tewksbury, MA).

2.3. Culture-dependent bacterial profiling

The absence of *Listeria* spp. from all the LMFs used in this study was confirmed using the Health Canada method MFHPB-30 (Pagotto et al., 2011). The overall bacterial diversity was analyzed as described by Gelda et al. (2020). Briefly, all the dried fruits were washed at a 1:1 ratio of food to 0.1% (w/v) peptone water (PW; Fisher Scientific). The food particulate was collected by centrifugation at 1000 x *g* for 2 min and discarded. The bacterial pellet was collected from the supernatant by centrifugation at 9000 x *g* for 15 min, re-suspended in 1 mL of PW and plated onto Columbia agar with 5% (v/v) sheep blood (blood agar; Oxoid). Plates were incubated at RT or 37°C for 24 h. Single colonies representing distinct phenotypes on blood agar were identified to the genus or species level by 16S-rRNA PCR identification as described by Gelda et al. (2020) to obtain a bacterial profile for each of the fruits in this study.

2.4. Preliminary study of dry carriers

To determine the most efficient carrier for the 1-year study, chalk, talc and sand were selected based on previous studies using these materials to dry-inoculate foods (Beuchat and Mann, 2014; Blessington et al., 2013; Hoffmans and Fung, 1993). All carriers were first

inoculated with only PW and dried as described in section 2.5. Secondly, talc and sand were inoculated with *L. monocytogenes* and dried as described in section 2.5. Recovery and quantification of *L. monocytogenes* from the carriers was conducted as described in section 2.8 to determine which carrier supported the greatest survival of *L. monocytogenes*.

2.5. Dry inoculum preparation

L. monocytogenes inocula were prepared as described by Blessington et al. (2013), with some modifications. All incubations were performed at 37°C. Briefly, each *L. monocytogenes* strain was grown in tryptic soy broth, then spread-plated on TSA-YE to obtain bacterial lawns. The lawns were re-suspended in 9 mL of PW and combined to obtain approximately equal numbers of each strain using OD_{600} to estimate *L. monocytogenes* concentration.

The 4-strain cocktail was mixed with sand (SiO_2, pure, 40-100 mesh; Acros Organics, Morris Plains, NJ) at 20% (v/w) in sterile Stomacher bags (Labplas Inc., Sainte-Julie, Canada). Each bag was shaken vigorously for 2 min to evenly distribute the cocktail. The inoculated sand was then dried at 37°C for 24 h until the a_w reached a value equal to or lower than the dried fruits. The inoculated dried sand was transferred into a sterile Whirl-Pak bag (15" x 20"; Nasco, Modesto, CA) and placed in an environmental chamber at 23°C; 30–35% RH for 72 h to allow for a_w equilibration.

2.6. Inoculation of dried fruits and storage

Dried inoculated sand was combined with the dried apples, strawberries and raisins at a ratio of 168 g sand to 1.34 kg of fruit (12.5% w/w) inside sterile Stomacher bags and shaken for 2 min to mix. Excess sand was removed from the fruits by sieving with a U.S. standard No. 12 sieve (Verder Scientific GmbH, Germany). Inoculated fruits were transferred into Stomacher bags and sealed closed. Samples were stored in an environmental growth chamber at 23°C, 30–

35% RH and an incubator at 4°C, 25–81% RH for 336 days.

2.7. Measurement of a_w and pH

A_w was determined for the dried fruits using an AquaLab 4TE Benchtop Water Activity Meter (METER Group Inc., Pullman, WA). Dried strawberries were manually sliced prior to measuring. For each experimental replicate, three sub-samples were obtained at each sampling time to determine the a_w. The measurements of pH for the LMFs were obtained using an Accumet AB150 Benchtop pH Meter (Fisher Scientific, Hampton, NH). Measurements were performed on 10 g samples of LMF in distilled water according to recommendations in Health Canada MFHPB-03 (2014).

2.8. Quantification of L. monocytogenes

Samples of inoculated sand were obtained for *L. monocytogenes* quantification immediately after wet inoculation, after drying and after equilibration. Inoculated sand (1 g) was combined with 1 mL of PW in a 1.5 mL microcentrifuge tube and mixed by vortexing for 30 sec. The sand was pelleted by centrifugation at 1000 x *g* for 1 min. The supernatant was transferred into a fresh 1.5 mL tube and the bacterial pellet was collected by centrifugation at 13 000 x *g* for 5 min. The supernatant was discarded and the pellet was re-suspended in 250 µL of PW. Ten-fold serial dilutions were conducted in PW, plated onto TSA-YE and incubated at 37°C for 24 h.

Samples of inoculated fruit were obtained for *L. monocytogenes* quantification immediately after inoculation and throughout the storage period. Concentrations of *L. monocytogenes* were determined by mixing 10 g of dried strawberries and raisins with 15 mL of PW and 10 g of dried apples with 30 mL of PW in filtered Stomacher bags (Labplas Inc.). The bags were manually massaged for 2 min and the liquid food wash was transferred into 15 mL

centrifuge tubes. The tubes were vortexed for 30 s and food particulate was allowed to settle for 2 min. The supernatant was transferred to fresh 15 mL tubes and centrifuged at 10 000 x *g* for 10 min. The bacterial pellet was re-suspended in 6 mL of PW. Ten-fold serial dilutions were made in PW which were then spread-plated onto *Listeria* selective Oxford agar (Oxoid, Nepean, Canada). Plates were incubated at 35°C for 48 h.

2.9. L. monocytogenes serotyping by multiplex PCR

A total of 390 presumptive *L. monocytogenes* colonies were randomly picked from the agar used for *L. monocytogenes* quantification and re-suspended in brain heart infusion (BHI; BD). Cultures were incubated overnight (~18 h) at 37°C. DNA extraction was performed on the bacterial pellet from 100 µL of overnight culture using InstaGene matrix (Bio-Rad, Hercules, CA) and single colonies were serotyped by multiplex PCR as described in Ly et al. (2020), according to distinct banding profiles differentiating each serotype.

2.10. Experimental design, data and statistical analysis

Inoculation of the dried fruits with *L. monocytogenes* was repeated twice with independently-raised cocktails. At each sampling time, three sub-samples of contaminated fruits were collected for *L. monocytogenes* quantification from each storage condition. The a_w measurements were also determined from triplicate sub-samples of fruit. Measurements of pH were determined from duplicate sub-samples. All statistical analyses were conducted using IBM SPSS Statistics Version 26, 64-bit edition (SPSS Inc., Chicago, IL). Using the General Linear Model Univariate procedure, an analysis of variance (ANOVA) followed by a Games-Howell post-hoc test was used to determine significant changes in *L. monocytogenes* concentration during storage on the dried fruits. Significant pairwise differences between the proportions of *L. monocytogenes* strains recovered from the raisins and dried strawberries at the beginning and end

of storage were determined using a chi-square test for independence followed by post-hoc tests with the Bonferroni adjustment.

3. Results and discussion

3.1. Bacterial profiling of dried fruits

Listeria monocytogenes was not detected on any of the dried fruits used in this study. Additionally, indigenous bacteria (microbiota) were not recovered from the dried strawberries by the culture-dependent methods used in this study. Aerobic plate counts (APCs) were highest on the raisins, i.e., approximately 5.0 log CFU/g, which also demonstrated a greater species diversity as compared to the dried apples. The most abundant bacteria recovered from the raisins were *Bacillus* spp., but determination of the exact species could not be obtained with confidence by 16S rRNA gene comparison, because of the high degree of similarity among species of this genus (Mclauchlin and Rees, 2009). Examples of raisin isolates identified to the species level included *B. cereus/toyonensis/thuringiensis, B. flexus/megaterium, B. haynesii/licheniformis/paralicheniformis,* and *B. zhangzhouensis/pumilus/safensis.* The genera *Paenibacillus, Solibacillus* and *Terribacillus* were also represented among these isolates, although at a comparatively smaller proportion.

APCs from the dried apples were approximately 3.6 log CFU/g. Bacterial isolates recovered from the dried apples belonged to the genera *Aneurinibacillus, Bacillus, Lysinibacillus, Paenibacillus* and *Psychrobacillus*. Apple isolates that were identified to the species level included *A. migulanus/aneurinilyticus, B. humi/timonensis/onubensis, B. niacini, Lysinibacillus macrolides/pakistanensis, Paenibacillus terreus/puldeungensis/massiliensis* and *Psychrobacillus psychrodurans/lasiicapitis.*

These species have all been isolated from soil and thus may have originated from the

farm environment (Jung et al., 2003; Markande et al., 2018; Reva et al., 2002). However, some of these species could have also been introduced at the processing plant given the ubiquitous and hardy nature of *Bacillus* spores (Ryu and Beuchat, 2005; Soni et al., 2016). Interestingly, some of these isolates have demonstrated anti-listerial activity, i.e., *L. macrolides/pakistanensis* from the dried apples, and *Paenibacillus illinoisensis, B. flexus/megaterium* and *B. pumilus/safensis* from the raisins (Gelda et al., 2020). However, whether the presence of these species affect the survival of pathogens during storage on foods that do not support bacterial growth, such as LMFs, is unknown. In this study, *L. monocytogenes* demonstrated the greatest survival during storage at 4°C on the raisins, from which more anti-listerial bacteria were isolated, as compared to the apples and strawberries.

3.2. Preliminary comparison of three carriers for dry-inoculation

Chalk, talc and sand were assessed to determine the most effective carrier for transferring *L. monocytogenes* to the LMFs. Chalk was eliminated as a viable option due to its inability to dry within a reasonable time period as compared to the sand and talc, which both required approximately 24 h. Both the inoculated sand and talc were dried to a_w levels approximating that of the dried fruits (i.e., 0.4 to 0.6) within 24 h, while chalk remained at approximately 0.9. The chalk volume was nearly double that of the talc and sand of equivalent weight, which is most likely a factor in the drying capabilities of chalk (Blessington et al. 2013).

Sand and talc were inoculated with *L. monocytogenes* at a target concentration of 10 log CFU/g. Bacterial enumerations were conducted on both TSA-YE and Oxford agar. Right after inoculation, the decrease in numbers of *L. monocytogenes* was 0.6–0.7 and 2.8–3.3 log CFU/g for sand and talc, respectively (**Fig. 1**; time 0). After 24 h of drying, populations of *L. monocytogenes* decreased by an additional 3.7–4.5 and 2.7–4.0 log CFU/g, for sand and talc,

respectively. After the 3-day equilibrium period at 23°C which followed the drying period, *L. monocytogenes* populations increased by 0.9–1.6 and 1.4–2.3 log CFU/g for sand and talc, respectively. Higher concentrations of *L. monocytogenes* (by approximately 1 log) were consistently recovered from the sand as compared to talc, regardless of the growth media used for quantification (**Fig. 1**).

Previous studies have compared the efficiency of chalk, talc and sand to transport *Salmonella enteritidis*, *Listeria monocytogenes* and *Klebsiella aerogenes* for bacterial inoculation of pecans, walnuts and various dried fruits (Beuchat and Mann, 2014; Blessington et al., 2013; Hoffmans and Fung, 1993). Consistent with Blessington et al. (2013) findings, we also found the talc powder to clump more than the sand. As such, sand was chosen for its ease of use and high *L. monocytogenes* recovery. Compared to wet-inoculation methods, the use of a dry carrier for LMF inoculation is advantageous for a number of reasons. Primarily, it does not affect the a_w of the product or the properties of the food surface (Blessington et al., 2013). By its very nature and design, dry-inoculated foods do not require the pre-storage drying and stabilization/equilibrium period necessary when using a wet-inoculum. If prepared in bulk, the same dry-inoculum can also be used across multiple experiments and easily transported to facilitate interlaboratory collaboration (Enache et al., 2015). On the other hand, comparatively higher bacterial numbers have been achieved on nut kernels using a wet inoculation technique, as demonstrated with *Salmonella* (Blessington et al., 2012, 2013). However, Beuchat and Mann (2014) observed no difference in rates of inactivation for *Salmonella* on dried cranberries, strawberries, raisins and date paste contaminated by misting (wet-inoculation) and via a sand carrier. Future studies investigating the effect of wet vs. dry inoculation on pathogen survival on LMFs would provide additional valuable information on the effect, if any, of the inoculation method on *L.*

monocytogenes survival.

3.3. Effect of storage on the a_w of dried fruits

There was no significant change in a_w for the fruits after long-term storage at 4°C ($P > 0.05$), i.e., the a_w values remained generally stable throughout the 336 days of storage (**Fig. 2**). The a_w of the raisins and dried strawberries remained within the range of 0.4 to 0.5 over the 336 days, which is consistent with a_w dynamics in previous studies examining the survival of pathogens on raisins and dried strawberries during long-term storage (Beuchat and Mann, 2014). Therefore, changes in the a_w of the foods during storage was likely not a factor affecting the inactivation of *L. monocytogenes*.

3.4. Survival of L. monocytogenes during storage on dried fruits

Due to the potential presence of an interfering natural microbiota, Oxford agar was used to inhibit the growth of the background microbiota, while allowing for *L. monocytogenes* to be recovered and quantified. LMFs were inoculated with *L. monocytogenes* with a target concentration of approximately 6.8 log CFU/g. The loss associated with inoculating the raisins and strawberries using the inoculated sand was determined to be 1.5 to 2.8 log CFU/g (**Fig. 2**). At the beginning of storage, initial concentrations of *L. monocytogenes* on the raisins and strawberries were approximately 4.6 and 4.0 log CFU/g, respectively. *L. monocytogenes* could not be recovered from the inoculated dried apples at day 0 (inoculation day).

During storage at 23°C, *L. monocytogenes* decreased rapidly in concentration on both fruits by > 4 and > 3.6 log CFU/g, falling below the limit of detection (i.e., 0.4 log CFU/g) after 14 and 7 days of storage on the raisins and dried strawberries, respectively (**Fig. 2**). However, during storage at 4°C, *L. monocytogenes* populations only decreased by approximately 0.1 and 0.2 log CFU/g/month on the raisins and dried strawberries, respectively (**Table 1**). After 336

days of storage on the raisins and strawberries, concentrations of *L. monocytogenes* had decreased by 1.4 and 3.1 logs, respectively (**Fig. 2**). However, population declines on the raisins at 4°C were not statistically significant ($P > 0.05$). Populations of *L. monocytogenes* recovered from the raisins were generally higher than that recovered from the strawberries at equivalent sampling times. For LMF stored at 4°C, the levels of *L. monocytogenes* recovered were in the following order: raisins > strawberries > apples, throughout the entire storage period.

The underlying mechanism(s) for the observed difference in *L. monocytogenes* survival when stored at the two storage temperatures is/are unknown, but it is well documented that bacteria in general, survive much better on foods at 4°C than at 23°C. The U.S. National Center for Home Food Preservation Storage recommends storing dried fruits for one year at 60ºF (15ºC) or 6 months at 80ºF (27ºC) (NCHFP, 2014). The U.S. Department of Agriculture Food Safety and Inspection Service has also provided a recommendation to store dried fruits in the refrigerator for up to 6 additional months or to freeze them for one month after opening (USDA-FSIS, 2019). As such, if contaminated, these LMFs could remain in consumers' homes for a long time. In addition to temperature and a_w, water mobility, which is a measure of the translocation of water molecules in a food, could possibly be a factor affecting *L. monocytogenes* survival on LMFs. However, Santillana Farakos et al. (2013) found that water mobility did not significantly influence the survival of *Salmonella* spp. in LMF independent of a_w. Currently, little is known about the role of water mobility in influencing the survival of *L. monocytogenes* in LMF.

The observed differences in *L. monocytogenes* survival between the fruits during refrigerated storage could be due to a number of factors. Compared to the dried diced apples, the outer layer of the raisins and strawberries was not compromised, which may have presented a more hospitable environment for the presumed surface-associated *L. monocytogenes* cells.

During storage on the dried apples, exposure to the inner fruit tissues may have exposed *L. monocytogenes* to a lower pH as compared to that of the other intact dried fruits. Strawberries, apples and raisins typically have a pH in the range of 3 to 4 (Beuchat and Mann, 2014; Bower et al., 2003; Cordenunsi et al. 2003; Wu et al., 2007). The dried fruits were measured for pH at both the beginning (day 0) and end (day 336) of the experiment and can be ranked by their pH values as follows: raisins (4.2–4.3) > strawberries (3.8–3.9) > apples (3.7), which appears to be negatively correlated with rates of *L. monocytogenes* inactivation during storage at 4°C.

The dried apples used in this study were more acidic than both the strawberries and raisins which could partially explain the rapid inactivation of *L. monocytogenes* observed on the apples. Conway et al. (2000) demonstrated *L. monocytogenes* inactivation during storage at 26°C in apple juice at a similar pH to the dried apples used in the present study (pH = 3.4), but growth of *L. monocytogenes* during storage on apple slices (pH = 4.7) at 10°C and 20°C. This is consistent with our understanding of *L. monocytogenes* in high-a_w foods such as juice and fresh fruit, where listerial growth is not expected to occur at pH < 4.4 (ICMSF, 1996). Malic acid is the predominant organic acid in apples and has demonstrated enhanced dose-dependent inactivation of *L. monocytogenes* in combination with low pH (< 5.5) (Buchanan and Golden, 1998; Wu et al., 2007). The greatest relative effects observed against *L. monocytogenes* occurred between pH 3.0 and 4.0. This may be an additional factor that could explain the lack of *L. monocytogenes* recovery from the dried apples. However, this does not explain the successful recovery of *L. monocytogenes* from the dried stawberries, whose pH is comparable to that of the apples. Citric acid, followed by malic acid, are the most abundant organic acids in strawberries and have demonstrated similar efficacy against *L. monocytogenes* in BHI broth and on whole red apples (Buchanan and Golden, 1994; Kallio et al., 2000; Park et al., 2011).

Lastly, using a wet-inoculation method, Hasani et al. (2020) achieved numbers of 4.5 to 4.8 log CFU/g *L. monocytogenes* on dried apples, strawberries and raisins with a broth-cultivated inoculum. In the current study, the authors used an agar-grown inoculum that was pre-adapted on sand to low a_w. In this stressed state, it seems that the *L. monocytogenes* cells were not capable of tolerating additional acid stress upon introduction onto the apples. As such, a wet-inoculation method may be preferable in future work investigating the behaviour of *L. monocytogenes* on dried fruits.

Beuchat and Mann (2014) demonstrated that acid-adapted *Salmonella* are capable of long-term survival on both raisins and dried strawberries during storage at 4°C, with comparatively faster inactivation occurring on the strawberries. Consistent with this study for *L. monocytogenes*, inactivation of *Salmonella* occurred faster at 25°C as compared to 4°C. The enhanced survival of *Salmonella* demonstrated in foods with high levels of sucrose suggests that sugar may have protective effects against cell death (Beuchat and Mann, 2014). Interestingly, sugar was listed as an ingredient in the dried strawberries used in this study, which would be present on the surface of the whole berry. Flessa et al. (2005) found that *L. monocytogenes* exhibited a higher survival rate when 20% sucrose had been applied to sliced strawberries, potentially adding another factor as to why *L. monocytogenes* also survived on dried strawberries.

Although little difference was observed between the Oxford *Listeria* selective agar and the non-selective TSA-YE agar for *L. monocytogenes* quantification from the inoculated sand (**Fig. 1**), we cannot discount the potential role of selective media as an additional stress factor on cells, which could contribute to an underestimation of the true *L. monocytogenes* population during storage on the raisins and dried strawberries.

During the drying of the sand, *L. monocytogenes* levels decreased rapidly (**Fig. 1**). Sand is rough

and devoid of nutrients or compounds that could be used to protect against cell death. However, the use of a dry carrier for *L. monocytogenes* in this study was done to try and simulate real-life conditions in the food processing environment. The potential for sand or dust to serve as a vehicle for *L. monocytogenes* contamination has been demonstrated (De Roin et al., 2003). However, the limitation of doing survival studies using carriers such as sand for the dry-inoculation of foods, is that this approach may not accurately represent the survival of the bacterial pathogen on the food itself if residual carrier particles remain on the food during storage, which was the case for this study, even after thorough sieving. Bagi and Buchanan (1993) inoculated silica gel with *L. monocytogenes* at 6.5 log CFU/g and demonstrated its culturability after storage at 5°C and 22-24°C for 168 days. In the present study, rapid inactivation of *L. monocytogenes* was demonstrated on all the dried fruits investigated during storage at 22°C. This suggests that although there was residual silica sand, the observations in this study were not exclusively the result of *L. monocytogenes* survival on the carrier. However, the nature of the interaction between the carrier particulate and the bacteria is still unknown. The study done by Bagi and Buchanan also recovered their bacteria by incubating their gel in BHI broth while this study did not use broth to recover *L. monocytogenes* adding an additional stress factor to its recovery on Oxford agar.

Furthermore, the existing experimental design may be representative of a particulate contamination situation, but the use of acid-adapted cells, as described by Beuchat and Mann (2014) for instance, would have provided further insight as a "worst-case" scenario condition. Cell death may not be the only reason that a decline in the numbers of culturable *L. monocytogenes* was observed on the raisins and dried strawberries used in this study. Ly et al. (2020) provided evidence for a viable but non-culturable (VBNC) sub-population of *L.*

monocytogenes after 12 plus months of storage on chocolate liquor, corn flakes and dry-roasted pistachios at 4°C. Similarly, entry into a VBNC state was demonstrated after a few days for *L. monocytogenes* stored on parsley leaves stored at 20°C at low RH conditions (Dreux et al., 2007). Thus, in addition to cell death, transition into a VBNC state could have contributed to the declines in culturable *L. monocytogenes* observed during long-term storage on the dried fruits in this study. However, the significance of the VBNC state for *L. monocytogenes* survival on food and for foodborne listeriosis is not well understood. In contrast, Nicolò et al. (2011) demonstrated complete inactivation, i.e., elimination of both culturability and viability, of *L. monocytogenes* after storage of the cells in grapefruit juice (pH 3) at 4°C for 24 h.

3.5. Distribution of 4 different L. monocytogenes strains during storage on dried fruits

The majority of methods used to evaluate differences in the survival of different *L. monocytogenes* strains make use of single-strain inocula, which are both tedious and inefficient. We have previously described a multiplex PCR method to distinguish individual strains within a multi-strain cocktail based on serotype identities (Ly et al., 2020). Strain distribution analysis for *L. monocytogenes* was only done for those samples stored at 4°C, as the populations of *L. monocytogenes* on fruits stored at 23°C declined too rapidly for information on strain distribution to be captured.

At day 0, proportions of serotypes 1/2a and 3a were the highest among populations of *L. monocytogenes* recovered from the raisins and dried strawberries (**Fig. 3**). The serotype 4b strain represented the smallest proportion of colonies, i.e., 0% and 1%, respectively, recovered from the raisins and dried strawberries at this sampling point. Using the same *L. monocytogenes* strains described in this study, Ly et al. (2020) demonstrated the serotype 4b strain to be the most sensitive to stresses experienced during the drying-equilibrium period on wet-inoculated

chocolate liquor, corn flakes and dry-roasted pistachios. The fruits in this study were dry-inoculated, thus eliminating the need for a drying-equilibrium period prior to storage. However, rapid declines of the serotype 4b strain during the drying period on the sand, likely explains the population distribution observed on the fruits at day 0.

Population declines after long-term storage on the fruits at 4°C were strain dependent. During storage at 4°C on both dried fruits, the proportion of serotype 1/2a declined ($P < 0.05$), while the proportions of serotype 1/2b and 3a on the raisins and serotype 3a on the strawberries increased ($P < 0.05$; **Fig. 3**). Changes in the *L. monocytogenes* population distribution observed after 336 days of storage on the raisins and dried strawberries at 4°C are consistent with that observed by Ly et al. (2020) during 4°C storage on various LMFs. Importantly, end-point populations of *L. monocytogenes* were predominantly composed of the serotype 3a strain, which was a clinical isolate from a sporadic listeriosis case. These results support the potential relevance of "low-risk" serotypes, particularly for research focused on potentially emerging vehicles for listeriosis.

4. Conclusion

In conclusion, *L. monocytogenes* was capable of survival on raisins and dried strawberries during storage for up to 336 days at 4°C, a common practice used to extend the shelf-life of dried fruits (Beuchat and Mann, 2014). However, rapid population declines were observed during storage at 23°C on both dried fruits. Regardless of the storage temperature, *L. monocytogenes* was not able to survive on diced dried apples. Strain-level analysis of *L. monocytogenes* survival demonstrated that serotype 3a may be better suited for survival on the dried fruits as compared to the serotype 4b strain, which showed the lowest survivability. Of the 3 materials tested, sand appeared to be the best carrier for the dry-inoculation method, not only because its a_w was the

most similar to the dried apples, strawberries, and raisins, but it also showed a higher transfer rate of *L. monocytogenes* to the fruits, as compared to the chalk and talc. Future studies should consider alternatives to carriers for the dry-inoculation of foods to avoid adding foreign material that may influence pathogen behaviour (e.g., freeze-dried bacteria, pre-inoculated food to be used as a seeding vector). It may also be useful for future studies to use of a number of different strains representing various serotypes, to understand whether certain serotypes may be better adapted to long-term survival on dried fruits.

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Table 1. Calculated linear rates of change for *L. monocytogenes* during long-term storage on raisins and dried strawberries stored at 4°C, 25–81% RH, using best-fit models.

LMF	Temperature (°C)	Model[a]	R^2	Linear rate of change (log CFU/g/mth)[b]	Reference
Raisins	4	Log-linear	0.8072	-0.1140	(Bigelow and Esty, 1920)
Dried strawberries	4	Weibull	0.3827	-0.2036	(Mafart et al., 2002)

[a] Best-fit models were chosen based on the R^2 values, shape and model complexity as determined using GInaFiT v.1.6 (Geeraerd et al., 2005).
[b] Rates of change were calculated based on the linear part(s) of the model. 'mth' indicates 'month'. For the Weibull model, the rate of change was approximated using the slope of the tangent line.

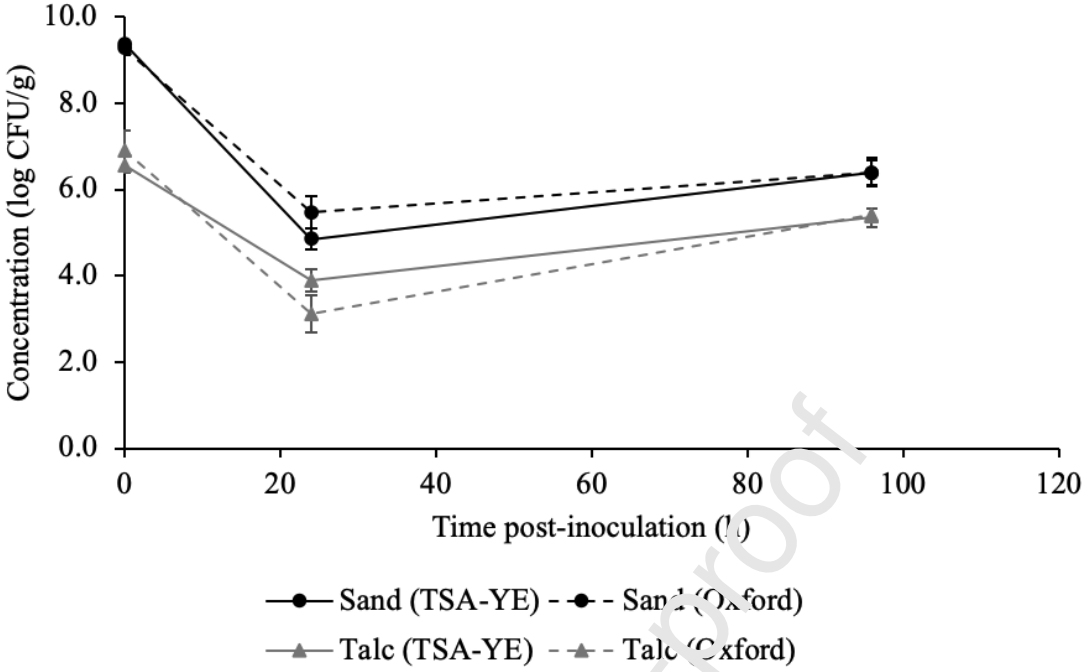

Fig. 1. Recovery of *L. monocytogenes* from inoculated carriers comparing selective (Oxford) and non-selective (TSA-YE) media (n = 3). Each dry carrier was inoculated and monitored over a 4-day period which included drying (37°C) as well as a 3-day equilibrium period (23°C; 30–35% RH).

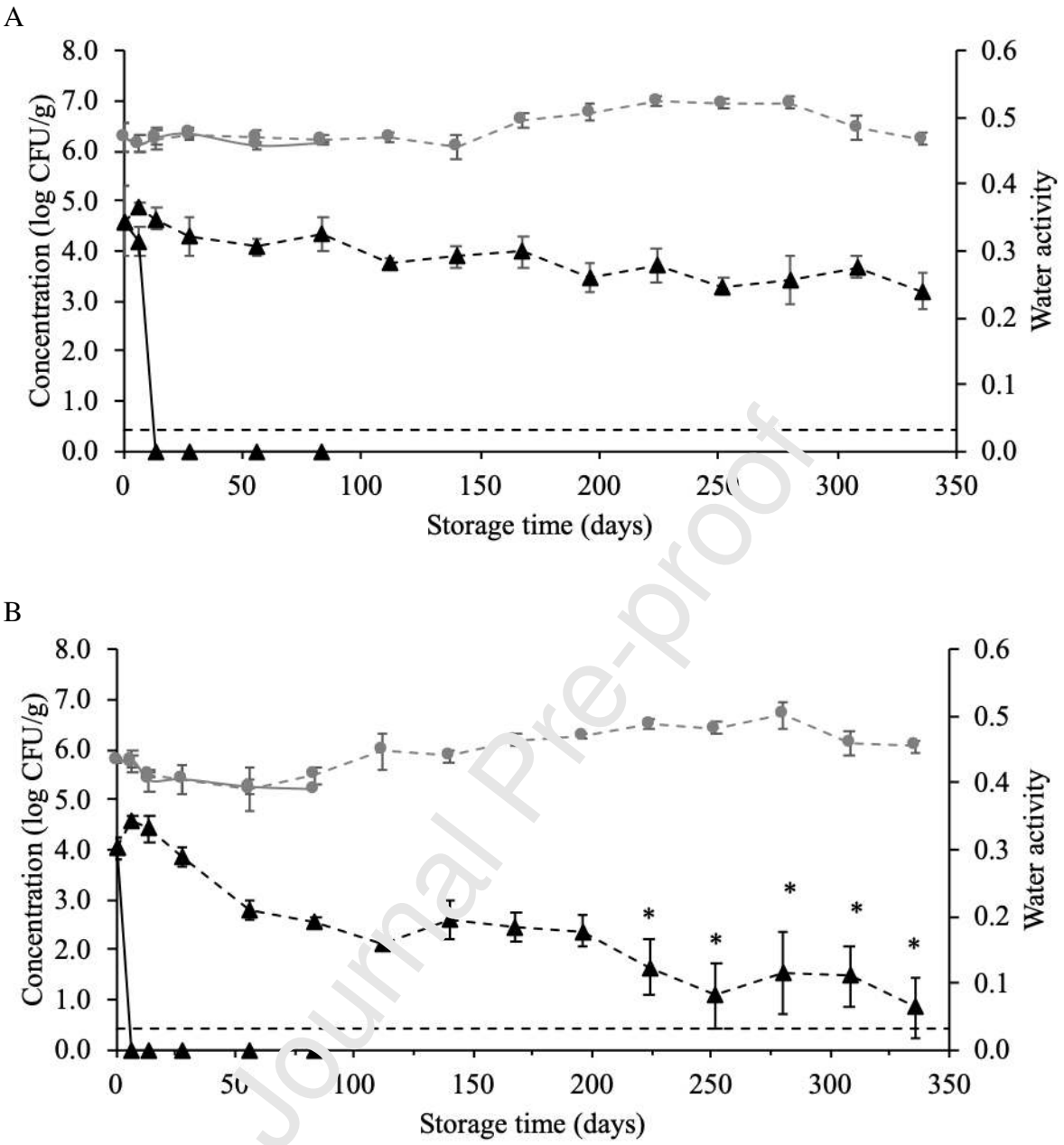

Fig. 2. Survival of *L. monocytogenes* during storage on inoculated (A) raisins and (B) dried strawberries stored at 23°C, 30–35% RH (solid) and 4°C, 25–81% RH (dashed), over 336 days. Black lines (triangle) represent *L. monocytogenes* concentration; gray lines (circle) represent the water activity of the LMFs. Experiments were conducted in biological duplicate. Error bars represent standard deviations from the mean (n = 6). The limit of detection was 0.4 log CFU/g (dotted line). Asterisks indicate population estimates (i.e., below the limit of quantification).

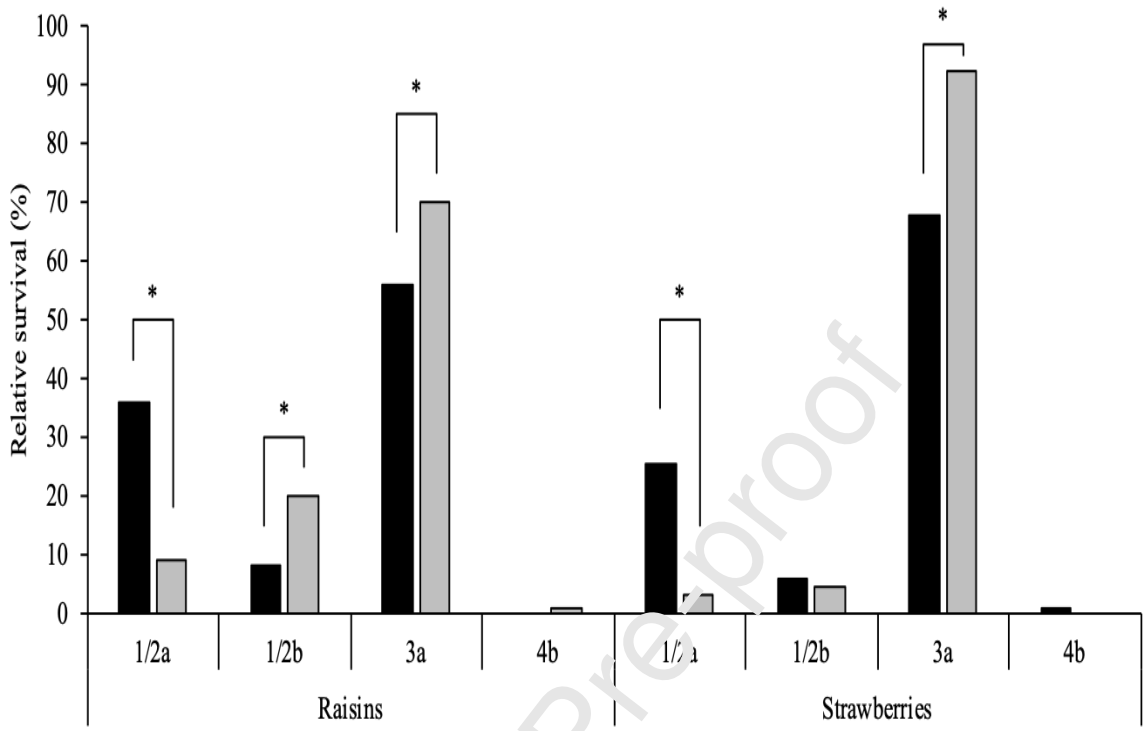

Fig. 3. Relative survival of four *L. monocytogenes* serotypes on inoculated raisins and dried strawberries at day 0 (black), and after storage at 4°C, 25–81% RH for 336 days (grey). Bars indicate the average of two experimental replicates. Asterisks indicate significant pairwise differences according to a chi-square test for independence followed by post-hoc tests with the Bonferroni adjustment ($P < 0.05$).