The Microbial Safety of Dried Fruits

By

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THESIS

Submitted in partial satisfaction of the requirements for the degree of

MASTER OF SCIENCE

in

Food Science and Technology

in the

OFFICE OF GRADUATE STUDIES

of the

UNIVERSITY OF CALIFORNIA

DAVIS

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2021

Acknowledgements

When I decided to attend graduate school, I had no idea what I was getting myself into. I knew nothing about food science nor did I really know what getting a master's degree would entail. While I did end up learning the ins and outs of food science these past 2 years, I ended up learning so much more about myself and life in general. With all its twists and turns, graduate school has been an unreplaceable experience, and there are so many people I have to thank for making it all possible.

I would first like to thank my family. They have always believed that I can reach excellence in whatever I set my mind to; and it is because of their support I have achieved the things I have. Thank you to my parents, Sri and Anila, for their constant phone calls which make being away from home bearable. They are always able to provide the comfort and advice I need, even from hundreds of miles away. Thank you to my brother, Tadvana, for the sage advice he manages to sneak in between messages of dumb memes and jokes.

Next, I would like to thank my coworkers and cohort. Thank you to my lab mates Chao Liao and Lina Sheng who always manage to find answers to my incessant questions and have taught me so much about the day-to-day functions of working in a laboratory. What was equally important to learning these technical skills, was learning how to build a community, which is what I have to thank my cohort for. They were not only there to commiserate with me about our struggles, but were there to problem solve and piece together our food science knowledge into use for the real world.

I would also like to thank the California Department of Food and Agriculture for funding this project. Without them, none of the work I have done would have been possible.

Finally, I would like to thank the amazing women that make up my thesis committee. I could not think of a more brilliant group of people to work with. They have not only been able to impart their wisdom to me, but have created a fostering environment that makes one crave to learn more. Thank you to Dr. Linda J. Harris for all the insights she has provided to my research. Her understanding of science communication has helped give my work direction and has motivated me to think about the way I communicate science with others. Thank you to Dr. Erin DiCaprio for helping me develop the survey that shaped my thesis. Not only did she spend months helping me design and disperse a survey for dried fruit processors, but she took every moment to talk me up, instilling me with confidence in my abilities as a scientist. Last, and most certainly not least, I have to thank Dr. Luxin Wang for everything she has done for me. It is rare that you can find a mentor that strikes the perfect balance between drive and compassion, but Dr. Wang has accomplished this. She has always managed to keep us working passionately towards our goals, while constantly learning to adapt to the curveballs that have been thrown at us these past years. I have loved working in her lab and the community that we have built there. I could not envision a better mentor and feel so fortunate that she got to be mine.

Abstract

Dried fruits are one of the most economically valuable specialty crops in California. Dried fruits contain essential nutrients and health-promoting bioactive compounds such as antioxidative phenolic compounds and phytoestrogens. Unfortunately, there have been outbreaks associated with dried fruits that have sickened people worldwide. The limited literature about the behavior of common foodborne pathogens on various dried fruits and the intrinsic and extrinsic factors impacting their behavior has hindered the development of microbial food safety risk assessments of dried fruits.

To better address the knowledge gaps associated with dried fruit safety, a survey was first designed and conducted to identify current common practices that are being used by different sizes of processors. Results showed that the majority of processors use dehydrators to dry their fruits while the rest of the processors use oven or sun-drying. Pre-drying treatments, including dipping or soaking fresh fruits in sulfur, lemon juice, or citric acid solutions, are being used by some processors. Unfortunately, most processors do not have a validated method for determining if their products are adequately dried or not, but rather go by what processors before them have said.

To investigate the behavior of common foodborne pathogens on dried fruits, a challenge study was conducted, in which 5-strain cocktails of *Salmonella* spp., *Escherichia coli* O157:H7, and *Listeria monocytogenes* were artificially inoculated onto dried fruits and their survival was monitored for 6 months. Dried peaches, dried peaches processed with sulfur, dried pluots processed with sulfur, sundried tomatoes, high-moisture Medjool dates, and low-moisture Medjool dates were obtained from local farmers markets. Two inoculation carriers

(sand and phosphate buffered saline) were first tested for their potential to be used for the inoculation of dried fruits. Based on the measurement of the chemical and physical properties of inoculated dried fruits, sand as a dry carrier was determined to be appropriate to use with dried peaches and Medjool dates and phosphate buffered saline (PBS) as a wet carrier was determined to be appropriated to use with dried peaches, dried pluots, and sundried tomatoes. The sand inoculation led to initial *Salmonella* levels of 6.43 ± 0.07 log CFU/g to 7.26 ± 0.14 log CFU/g while PBS inoculation lead to initial *Salmonella* levels of 9.39 ± 0.32 log CFU/g to 9.73 ± 0.14 CFU/g. Since the drying of the liquid inoculum happened on the dried fruits, the properties of the dried fruits impacted the initial inoculation level after drying. For example, in dried pluots the initial inoculation of *Salmonella* of 9.39 ± 0.31 log CFU/g dropped to 8.09 ± 0.07 log CFU/g after 48 h of drying. Sand inoculation led to lower initial inoculation level, as up to 3.38 log CFU/g of reduction was observed during the preparation of the *Salmonella* sand inoculum.

Inoculated dried fruits were stored at refrigerated and ambient temperatures. Pathogens populations were determined 0, 5, and 15 days after inoculation, and every 30 days for 6 months. The limit of detection (LOD) by direct plating was 1.9 Log CFU/g; samples that fell under the limit of detection were enriched following FDA protocols. *Salmonella* survived longer than the other two pathogens. From high-moisture dates, *Salmonella* was recovered at 5.31 ± 0.06 log CFU/g after 180 days of storage at 5 °C. *E. coli* O157:H7 was recovered at 4.14 ± 0.31 log CFU/g after 150 days of storage and dropped below the LOD by 180 days. *L. monocytogenes* was recovered at 5.90 ± 0.07 log CFU/g after 120 days of storage and dropped below the LOD by 150 days. The three pathogens survived better in storage at refrigerated temperature than at ambient temperature. When stored at 5 °C*, Salmonella* on low-moisture dates was recovered

at 5.30 ± 0.16 log CFU/g after 180 days. When stored at 20 °C, the recovery was 4.43 ± 0.09 log CFU/g after 60 days and dropped below the LOD by 90 days. Intrinsic factors influenced pathogen survival as well, with pathogens surviving longer in dried fruits with lower pH and higher water activities. Sulfur treatment also had an impact on pathogen survival. *L. monocytogenes* wet-inoculated onto dried unsulfured peaches was recovered at 4.26 ± 0.18 log CFU/g up to 120 days. In contrast, on dried peaches processed with sulfur, recovery of *L. monocytogenes* was 7.21 ± 0.46 log CFU/g up to 5 days and dropped below the LOD by 15 days.

Primary linear models were built to describe the behavior of pathogens during storage. Among the three pathogens, *Salmonella* had the largest difference in rate of decline between the two storage temperatures. It declined at a rate of 242 days/log reduction when dry inoculated onto low-moisture dates in refrigerated storage and at a rate of 15 days/log reduction at ambient temperature. *E. coli* O157:H7 declined with a rate of 54 days/log reduction at refrigerated storage temperature and 22 days/log reduction at ambient temperature. *L. monocytogenes* declined at a rate of 35 days/log reduction at refrigerated temperature and 18 days/log reduction at ambient temperature. Pathogens declined more quickly on the sulfured dried fruits, peaches and pluots, followed by sundried tomatoes, non-sulfured peaches, and dates. Taking *Salmonella* as an example, the sulfured dried fruits had too rapid of die-off to calculate a D-value at ambient storage. The rates of decline in the sundried tomatoes, dried peaches, low-moisture dates, and high-moisture dates were 7.86, 10.89, 14.50, and 14.02 days/log reduction respectively.

In summary, common foodborne pathogens can survive on a range of dried fruits. The behavior of pathogens is impacted by intrinsic factors associated with dried fruits (e.g. pH,

water activity, sulfur, and available nutrients) and extrinsic factors (e.g. storage temperatures). In general, pathogens declined faster at ambient temperature than refrigerated temperature. *Salmonella*, a pathogen with well-known history of association with low moisture foods, survived the best amongst the three tested pathogens. Pre-drying treatments (e.g. sulfur treatment) can have long-lasting antimicrobial effects during storage. Additional research that can systematically illustrate the antimicrobial effects of various pre-drying treatments as well as post-drying strategies is still needed to better control the potential food safety risks associated with dried fruits.

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Introduction

Low-moisture foods and dried fruits. Water activity is a ratio between the vapor pressure of the food itself, and the vapor pressure of distilled water under identical conditions. It is a representation of how much "free" water (water that is not bound to solutes in the food matrix) is available in a food. A low-moisture food is a food that has a water activity (a_w) of 0.85 or lower (US Food and Drug Administration, 2014). When there is limited free water, microbes are unable to access water for use in biological processes, such as cellular growth (Podolak and Black, 2017). While the food industry often uses the terms moisture content and a_w interchangeably, moisture content alone does not dictate a microbe's ability to grow. Moisture content is the total amount of water in a food regardless of whether the water is free or bound. Thus, two foods of the same moisture content could have dissimilar water activities, making one more susceptible to bacterial growth than the other.

Fresh fruits are good sources of nutrients and their consumption can contribute to a decreased risk of obesity, diabetes, heart disease, and cancer (CDC, 2011). However, common fruits are mostly produced on a seasonal basis. Fresh fruits contain more than 70% of water (Gebhardt & Thomas, 2002) and they are considered perishable food with a limited shelf life (Mercier et al., 2019). The estimated total loss of fresh fruits at retail and consumer levels were 13.9 billion pounds in 2010, contributing to 37% of the total food supply of fresh fruits (Buzby, Farah-Wells, & Hyman, 2014). Therefore, fruits are dried with various techniques to extend shelf-life, retrain nutritional values, decrease packaging and shipping cost, and ultimately reduce food waste. The global production of dried fruits increased from 2,246,739 metric tons in 2009/2010 to 3,22,767 metric tons in 2019/2020 (International Nut & Dried Fruit Council,

2020). In particular, table dates, which accounted for 35% of world dried fruits production, showed the most significant increase over the last decade (International Nut & Dried Fruit Council, 2020). In California, dried fruits are economically valuable specialty crops and 1,174,000 tons of raisins, 325,500 tons of dried prunes, 11,000 tons of dried apricots, and 6,900 tons of dried freestone peaches were produced in 2017 (California Department of Food and Agriculture, 2020).

Survey for dried fruit processors. To gain insight into current practices for dried fruit production, a survey was developed for dried fruit processors (Appendix 1). The survey questionnaire and protocol was submitted to the UC Davis Institutional Review Board (IRB) for approval of human subject research. A contact list was developed from a public records request to the California Department of Public Health Food and Drug Branch. The list included all contacts holding a Processed Food Registration in California and listed the types of products made, including dried fruits and vegetables. Additionally, a list of registered Farmers' Market managers was collated with the aim of these managers distributing the survey to vendors selling dried fruits. Upon approval (deemed Exempt) the survey was built in Qualtrics (Qualtrics, Provo, UT) and distributed via newsletters, social media channels, and email. The survey's target demographic was dried fruit processors in California, however, the survey was publicly available for anyone to take. The following is a summary of insights gathered from the 47 responses to the survey:

There are a wide variety of fruits being dried in California. While some of the major commodities are peaches and apricots, others include plums, pluots, various berries, tomatoes, various citrus fruits, apples, dates, nectarines, bananas, pineapples, and watermelons. In most

cases, these crops are grown and harvested by the same people who dry them, rather than sourcing from retailers or other distributors. However, some will purchase fruit to fill orders if they cannot meet the demand. The harvesting seasons, dependent on type of fruit, occur most often in early and late summer. After harvest, most processors wash their fruit in potable water and store them at refrigerated temperatures. Typically, pre-dried storage does not last longer than 1 to 3 days, but in some cases can last as long as 28 days. Over 50% of the processors that were surveyed add some sort of preservative to their fruits before drying. Preservatives include citric acid, lemon juice, Fruit Fresh®, and sulfur.

The most commonly-reported drying method was using a dryer or dehydrator. While not everyone knew exactly what kind of dryer they were using, tunnel dryers, cabinet dryers, and ovens were most commonly reported. A few processors also use sun drying, while one processor reported the use of freeze drying. About 60% of the processors measure temperature during drying either using the built-in sensor of the dryer or a standard thermometer. For sun drying, the ambient temperature is important, as that can change the drying time by a matter of days. Along with (or instead of) temperature, a small number of processors check for the a_w of their drying fruits for a target value dependent on the type of fruit (for instance, one date processor reported checking for a_w values between 0.7 and 0.79). Other processors mentioned validating the drying process by checking moisture content, brix, crispness, a pinch test, and wind feel. By far the most common way to check that fruits are done drying, was to check the visual appearance. Most processors did not apply any post-drying treatments to their fruit, but one did use ozone fumigation.

Once the fruits are dried, the most common storage method is bulk refrigeration (or in a freezer for long-term storage). Those not stored in bulk are either packaged by hand directly into their retail bags or stored in airtight containers or jars until selling. Many of these processors have use historical shelf lives for their products. Few mentioned using microbial tests to determine shelf life, though dried fruits should not spoil from microbial growth if properly dried. Andress and Harrison (2014) suggest that dried fruits can be introduced to moisture once its packaging is opened, which could lead to spoilage. None of the processors stated what kind of microbial tests they used, but an increase in moisture after opening packaging may be a reason why they performed those tests. Most processors relied on appearance/taste of the fruits, as well as what they called "experience with the product" to determine shelf life. The shelf life for many of these products in cold storage is years, but many claimed that their products are sold well before that. Using the survey as a guide, the following reviews the literature of common practices in dried fruit processing.

Drying. Dryers, or dehydrators, are common devices used for fruit drying. A dehydrator can be as simple as using a home oven, or a more dedicated device can be used such as a cabinet or tunnel dryer. Both cabinet and tunnel dryers use convective air drying, where hot air is circulated to pull moisture out of the fruit and create water vapor (Bourdoux et al., 2016). In cabinet dryers the fruits are placed on shelves and remain stationary through the drying process; in tunnel drying the fruit is continually moved through the tunnel on some sort of conveyor. A constant temperature can be selected on these devices (often upwards of 37 °C, which allows for a predictable time of the drying process. Sun drying is another way to dry fruits that can be as simple as exposing the produce to direct or indirect sunlight (Bourdoux et al.,

2016). The heat allows for loss of water to occur from the fruits (Bourdoux et al, 2016). Sun drying can be much more variable than using a convective dryer as it is dependent on ambient conditions (Bourdoux et al., 2016). A change in temperature, UV radiation or humidity can change drying times by a magnitude of days (Bourdoux et al., 2016). Other weather qualities, including precipitation and windspeed, can affect drying as well. However, one processor who participated in the survey suggested that sun drying has advantages compared to convective drying as no special equipment or monitoring is required.

Different drying methods have different impacts on the microbial populations present on dried fruits. Compared to the use of convective drying instruments, sun drying does not show reduction in native microbial populations (Karabulut et al., 2007; Eze and Agbo, 2011). During sun drying of apricots at 38 °C for 182 h, the total aerobic mesophilic bacteria counts increased from 2.75 log CFU/g to 5.16 log CFU/g. When the apricots were dried using convective air drying at 70 °C for 20 h, the mesophilic bacteria counts decreased to less than 2 log CFU/g (Karabulut, et al 2007). Sun drying of unpeeled ginger at 30.8 °C for 11 d showed no reduction from the initial 3 log CFU/g in total aerobic counts. When using a convective dryer, the unpeeled ginger dried at 43.2°C for 11 d decreased the total aerobic count by 1 log CFU/g (Eze and Agbo, 2011).

Several studies evaluate the impact of drying on pathogenic bacteria. DiPersio et al. (2006) observed that a 1.6-1.7 log CFU/g reduction (from an initial level of 7.8 log CFU/g) of *Salmonella* Typhimurium in carrots was achieved when the inoculated carrots were dried using convective air drying at 60 °C for 6 h. Phungamngoen et al. (2011) showed that *Salmonella* Anatum declined from approximately 6 log CFU/g to 3 log CFU/g during convective drying of

cabbage at 70 °C for 6 h. Yoon et al. (2004) observed that a 3.2-4.5 log CFU/g reduction of *Salmonella* Typhimurium in Roma tomatoes was achieved when the inoculated tomatoes (at 7.1-7.4 log CFU/g) were dried using convective air drying at 60 °C for 14 h. Wachuku et al. (2003) showed that *Salmonella* Typhimurium declined, from an approximate initial level of 4 log CFU/g, by 2.5 log CFU/g during sun drying of cowpeas at 33 °C for 3 d.

Pre-drying and post-drying treatments. Dried fruits can be treated in different ways before or after drying. The purposes of applying such treatments include preventing discoloration, texture retention, and reduction of the microbial populations in the final product (Table 1) (Kendall and Sofos, 2012).

Pre-Drying treatments. One common pre-drying treatment is to expose fresh fruits to sulfur dioxide gas. In dried fruit processing, sulfur dioxide is not usually used as an antimicrobial but rather is used as an antioxidant to prevent browning. However, the molecular form of SO_2 can penetrate the cell membranes of microbes and disrupt enzymatic activities (Agricultural Marketing Service, 2011). Sulfur dioxide's efficacy as an antimicrobial is dependent on pH, as there is more molecular SO_2 present at lower pH (Wedzicha, 1984). Before drying, SO_2 can be added through a process called sulfuring, where sublime sulfur is burnt and the sulfur dioxide fumes are able to enter the fruit (Schmutz and Hoyle, 1999; Sen et al., 2015). During this process, fruits to be dried are placed on trays inside a large box or container. The trays are placed at least 5 cm (2 inches) away from each other and at least 15 cm (6 inches) away from the sublimed sulfur, which is placed underneath the trays. The sulfur can be wrapped in paper so that it is easily lit. Once all the sulfur is done burning, the container is closed, giving the sulfur gas a chance to penetrate the fruit. The time for sulfuring depends on the type of fruit. For

example, 0.5 kg (1 pound) of apples should be sulfured for 30 min, while 0.5 kg (1 pound) of peaches should be sulfured for 1 h (Sivanandan, 2017). On average, 5 mL (1 teaspoon) of sublimed sulfur is used per 0.5 kg (1 pound) of fruit (Sivanandan, 2017).

While specifically used to preserve the appearance of dried fruits for commercial appeal, SO_2 treatment also can act as an antimicrobial similar to its use in winemaking (Agricultural Marketing Service, 2011; Nicholas and Cruess, 1932). Sulfur dioxide treatments have been shown to inactivate pathogenic bacteria on table grapes (Carter et al., 2015). Table grapes inoculated with low (4 Log CFU/grape) or high (6 log CFU/grape) of either *Escherichia coli* O157:H7, *Salmonella* Thompson, or *Listeria monocytogenes* were fumigated with different concentrations of sulfur dioxide (100, 200 or 300 ppm-h). All three treatment levels successfully inactivated all *L. monocytogenes* regardless of the inoculation levels, while 300 ppm was needed for inactivation of all *Salmonella* Thompson cells. When *E. coli* O157:H7 was inoculated at the high (6 Log CFU/grape) level, 300 ppm-h treatment did not inactivate all *E. coli* O157:H7. Results from Carter et al. (2015) showed that the sulfur treatment efficacy was determined by pathogen types. One significant drawback associated with gastric sulfur treatment is that this treatment is not environmentally friendly. Using sulfur dioxide treatments can release SO_2 emissions into the atmosphere, which can easily partition due to it being a highly water-soluble gas (Craig, 2018). Craig (2018) states that this production of atmospheric SO_2, such as from fumigation of produce, can cause many types of environmental damages, including acidification of water, injury to plants, and harmful sulfate particles accumulating in animals. Fumigation is the largest use of SO_2 in the state of California and is showing a trend of increase for the future.

Another method for applying sulfur is to soak fruits in solutions of sodium metabisulfite ($Na_2S_2O_5$). To do so, sodium metabisulfite is first dissolved in cold water (one recommendation is 21 g of sulfite per 1000 mL of water) and then fruits (peeled and cut as desired) are submerged in the solution and soaked for approximately 10 min (Kendall and Sofos, 2012). After that, fruits are drained and are ready to be dried (Kendall and Sofos, 2012). (DiPersio et al., 2004) evaluated the antimicrobial effects of such treatments by using peaches inoculated with 7.81 log CFU/g of *L. monocytogenes*. Results showed that the reduction of *L. monocytogenes* was 1.13 log CFU/g greater when the peaches were exposed to 4.18% sodium metabisulfite prior to drying, compared to untreated peaches (DiPersio, 2004) (Table 1).

The use of acids, in the form of ascorbic acid, citric acid, or lemon juice, is also a common pre-drying treatment for dried fruits (Kendall and Sofos, 2012). Application of these acids is very similar to application of sodium metabisulfite, in which fresh fruits are soaked for approximately 10 min in a dilution of the acid in cold water. For every 1000 mL of cold water, recommended amounts of citric acid and ascorbic acid are 5 g and 34 g, respectively (Kendall and Sofos, 2012). Lemon juice can be mixed with equal parts of water; 1,000 mL of solution is enough for approximately 10 L (10 quarts) of fruit (Kendall and Sofos, 2012). Derrickson-Tharrington et al. (2005) evaluated the antimicrobial effect of these different acid pre-treatments by using apple slices inoculated with 8 log CFU/g of *E. coli* O157:H7. Results showed that the reduction of *E. coli,* when compared to untreated apples slices was, 3.60, 3.60, or 4.00 log CFU/g greater when the apple slices were exposed (prior to drying) to 1.7% citric acid, 2.8% ascorbic acid, or 50% lemon juice respectively (Derrickson-Tharrington et al., 2005).

Another pre-treatment for dried produce is blanching. While vegetables are more commonly blanched, fruits can also be blanched. This pre-treatment is often used to prevent discoloration and oxidation (Andress and Harrison, 2014). Blanching can be performed with steam or direct submersion in water. In both cases, water should be placed in a container and brought to a boil. For steam blanching, the water should be no more than 5 cm (2 inches) high, and the prepared fruit should be placed above the water in some sort of permeable container (i.e. a colander or wire basket). For water blanching, there should be enough liquid to cover the fruit, which can be added directly. For both types of blanching, the container of water should be covered once the fruit is added and left to blanch for the time recommended for each specific fruit. Blanching often only lasts several min. Andress and Harrison (2014) propose blanching fruits such as apples for 3-5 min, while peaches should be blanched for 8 min. Once done, the fruit should be dropped in cold water to cool down, which stops the blanching process (Andress and Harrison, 2014).

Post-Drying treatments: A post-drying treatment that can be used on dried fruits includes ozone fumigation. A single processor from the survey used ozone as a post-drying treatment for dried fruits. Though commonly used in fresh produce postharvest, ozone fumigation can also be used to treat dried fruits. An ozone generator and a chamber to hold the dried fruits while applying the ozone are needed (Oztekin et al., 2006; Chen et al., 2020). Oztekin et al. (2006) looked at the effect of ozone fumigation on the microflora of dried figs. Aerobic mesophilic counts dropped from 2.57 to 1.59 log CFU/g on dried figs fumigated with 10 ppm ozone for 5 h (Oztekin et al., 2006). Coliform counts dropped from 1.46 to 0.00 log CFU/g (Oztekin et al., 2006). Najafi and Khodaparast (2009) looked at the effect of ozone fumigation

on the microbial populations on dates. *Staphylococcus aureus* counts dropped from 3.52 to 0.41 log CFU/g on dates fumigated with 5 ppm ozone for 45 min (Najafi and Khodaparast, 2009). Coliform counts dropped from 3.54 to 0.44 log CFU/g (Najafi and Khodaparast, 2009).

Storage. Based on the survey results, most dried fruits are stored in bulk either under refrigerated (short term) or frozen (long-term) storage. A wide variety of containers can be used for storage but all are usually airtight. One packaging technology used for dried fruits is modified atmosphere packaging (MAP; Miranda et al., 2019). MAP works by replacing the air inside of the packaging of a final product with a mixture of gases that help preserve the quality and shelf life of the food (Brown et al., 2018). Passive MAP can be achieved by using a permeable packaging material that allows a certain percentage of different gasses to enter and leave the packaging (Oliveira et al. 2010). Randelovic et al. (2014) exposed packaged dried apricots to a modified atmosphere of 30% carbon dioxide, 60% nitrogen, and 10% oxygen, and monitored the quality of the peaches over 12 months. Across all types of packaging materials used (combinations of polyester, polyethylene, paper, and aluminum), the dried apricots had less change from their original a_w, moisture, and polyphenol content when packaged with MAP compared to normal atmospheric conditions (Randelovic et al., 2014). While there are limited studies on the impact of MAP on pathogens in dried fruits, MAP is often used to control foodborne pathogens (Brown et al., 2018). Oliveira et al. (2010) used passive MAP packaging on *E. coli* O157:H7 inoculated shredded lettuce. After 10 d of storage at 5 °C, the initial *E. coli* level of 4.48 log CFU/g dropped by 1 log CFU/g (Oliveira et al. 2010).

Dried fruits can last up to a year in refrigeration depending on the fruit and processing (Andress and Harrison, 2014). One response in the survey stated that dried fruits can last

indefinitely under frozen conditions. However, limited studies have evaluated impact of storage conditions (e.g. storage temperatures) on the behavior of pathogens and bacterial populations of dried fruits. Beuchat and Mann (2014) looked at the effect of storage temperature on the survival of *Salmonella* in dried cranberries and raisins. When stored at 4 °C, *Salmonella* inoculated on dried cranberries dropped from 6.87 to 1.8 log CFU/g and *Salmonella* inoculated on raisins dropped from 7.01 to 4.76 log CFU/g after 42 d (Beuchat and Mann, 2014). When stored at 25 °C, no *Salmonella* was detected on either of the dried fruits by the end of 42 d (Beuchat and Mann, 2014). Hyun et al. (2019) looked at the effect of storage temperature on the microbial populations of dried persimmons. With an initial mesophilic bacteria count of 4.60 ± 0.26 log CFU/g, dried persimmons stored for 70 d at 5 °C had an average of 3.18 ± 0.75 log CFU/g of total mesophilic bacteria, while dried persimmons stored at 25°C had an average count of 1.64 ± 1.50 log CFU/g (Hyun et al., 2019). The initial coliform count was 1.92 ± 0.47 log CFU/g, and the average coliform count over the 70 d was 0.87 ± 0.48 log CFU/g at 5 °C and was 0.77 ± 0.58 log CFU/g at 25 °C (Hyun et al., 2019). These studies suggest that the survival of bacteria is better at refrigerated temperatures compared to ambient temperatures.

In summary, there are many drying or pre- and post-drying treatments can be applied during the preparation and storage of dried fruits. However, the efficacy of many of these methods have not been validated (at least based on the literature search). For many processors, effectiveness of their techniques does not rely on agreed-upon values, but rather the know-how of the processors before them. Knowing when dried fruit is ready is often based on visual cues. Additional research is critically needed in order to bridge the knowledge gaps

associated with the preparation and storage of dried fruits and address the concerns associated with the microbial safety of dried fruits.

Foodborne pathogens of concern. According to the Centers for Disease Control and prevention (CDC), approximately 48 million people in the United States get sick from foodborne pathogens every year (Scallan et al., 2011). Produce is the largest cause of foodborne illness in the US, and accounts for approximately 39 billion dollars in economic loss every year (McDaniel and Jadeja, 2019). Some of the top foodborne pathogens associate with fruits include *Salmonella*, Shiga toxin producing *E. coli,* and *L. monocytogenes. Salmonella* is estimated to lead to approximately 19,000 hospitalizations per year and *E. coli* O157:H7 is estimated to lead 2,100 hospitalizations per year; both pathogens are amongst the top five pathogens contributing to foodborne illness resulting in hospitalization (CDC, 2018). *L. monocytogenes* is estimated to lead to approximately 1,500 hospitalizations per year, and has a high mortality rate, causing an estimate of 255 deaths per year (CDC, 2018; US Food and Drug Administration, 2020).

Salmonella. *Salmonella*, a member of the family *Enterobacteriaceae,* is a gram-negative, non-spore-forming bacteria that can cause salmonellosis in humans and is one of the most common foodborne pathogens (CDC, 2021a). While *Salmonella* can grow at temperatures ranging from 5.2 to 46.2 °C, the bacteria's optimal growth temperature ranges from 34 to 40 °C (National Advisory Committee on Microbiological Criteria for Foods, 2010; Bailey et al., 2010). The optimal pH for growth of *Salmonella* is 6.5-7.5, while the overall pH range for growth is 3.7-9.5 (National Advisory Committee on Microbiological Criteria for Foods, 2010; Bailey et al., 2010). *Salmonella* can grow in foods with a water activity of 0.94 or higher (National Advisory Committee on Microbiological Criteria for Foods, 2010). The reservoir of this bacteria is usually

the gastrointestinal tract of animals, such as livestock (Bailey et al., 2010). Contamination with fecal matter is what causes *Salmonella* to be found in so many other foods (CDC, 2021a). While a few serotypes of the bacteria can cause typhoid fever, most disease-causing serotypes of *Salmonella* cause an infection in humans that is referred to as salmonellosis. *Salmonella* infects by attaching to and entering intestinal epithelial cells with the use of fimbriae and the injection of proteins via a type-three secretion system. Symptoms include diarrhea, fever, and abdominal cramps. Most people who get infected recover within a week with no need for hospitalization. However, the elderly, infants, and people with compromised immune systems have a greater chance for severe illness that may lead to rare chronic conditions or death (Bailey et al., 2010).

Salmonella is one of the leading causes of foodborne illness around the world. According to the CDC, *Salmonella* bacteria causes an estimated 1.35 million infections every year in the United States. Outbreaks in the past several years have come from a range of foods, including poultry, ground meats, fresh produce, nut butters, mushrooms, and grain-based snacks (CDC, 2021b). Though the variety of foods linked to *Salmonella* outbreaks is wide, *Salmonella* is often associated with contaminated eggs and poultry. The association with eggs is due to an increase in illness from *Salmonella* Enteritidis in the USA during the 1980s to 1990s and the majority of outbreaks from this Enteritidis serotype were from undercooked eggs (Patrick et al., 2004). The association of *Salmonella* with poultry, particularly chicken, is due to the high rate at which poultry tests positive for *Salmonella*. However, the serotypes of *Salmonella* most frequently isolated from poultry are not the same as the most frequently isolated from humans with salmonellosis (Bailey et al., 2010).

Salmonella has been associated with many low-moisture food outbreaks. As shown in Table 2, in 2001 a large outbreak of 168 cases of salmonellosis was linked to consumption of raw almonds (Isaacs et al., 2005). This multistate outbreak led to the realization that there is a need for reassessment of the safety associated with consumption of raw low-moisture foods (Isaacs et al. 2005). *Salmonella* is a particular concern in low-moisture foods because of its increased thermal resistance in dry conditions (Podolak and Black, 2017; Bailey et al., 2010; Mutz et al 2020; Farakos et al., 2013). The mechanisms by which *Salmonella* survives under dry conditions is not completely understood, but multiple mechanisms are believed to have an impact on its survival. One such mechanism is filamentation. It has been shown that in culture, *Salmonella* can filament if exposed to less than optimal growth conditions (Podolak and Black, 2017). However, filamentation has not been shown to occur in solid food matrices, so this mechanism does not explain increased survival of *Salmonella* in low-moisture foods (Podolak and Black, 2017; Mattick et al, 2003). Osmoprotectants may help protect *Salmonella* from desiccation. These are compatible solutes that help maintain osmotic balance in the cell and prevent denaturation of proteins and lipids (Podolak and Black, 2017). *Salmonella* uses the transport systems *Pro*P, *Pro*P, and *Osm*U to bring osmoprotectants that it cannot make itself, such as glycine betaine, into the cell (Podolak and Black, 2017; Mutz et al., 2020). Other mechanisms that may help to combat osmotic shock include outer membrane proteins (*Omp*), biofilm formation, fimbriae, and sigma factors (Podolak and Black, 2017; Bailey et al, 2010; Mutz et al., 2020).

There are multiple detection methods for *Salmonella*. Traditional cultural methods for isolation include plating on selective agars (such as xylose lysine deoxycholate, bismuth sulfite,

or Hektoen agars) and incubating for 24 h at 35 °C (Bailey et al., 2010; FDA, 2021). Before plating, naturally-contaminated samples are often enriched with nonselective and/or selective broths such as lactose broth since a low concentration of *Salmonella* is expected. Standard methods and media used to isolate *Salmonella* can be found in the FDA Bacteriological Analysis Manual (BAM) (US Food and Drug Administration, 2021). In conjunction with traditional cultural methods, rapid biochemical or antigen-antibody-based methods can be used for quicker isolation and identification of *Salmonella* (Bailey et al., 2010). *Salmonell*a can also be identified through testing a combination of biochemical and serological reactions. Most *Salmonella* will provide a positive result for glucose (TSI), lysine decarboxylase (LIA), H_2S (TSI and LIA), lysine carboxylase broth, phenol red dulcitol broth, polyvalent flagellar test, polyvalent somatic test, and methyl red test; and provide a negative result for urease, potassium cyanide (KCN) broth, malonate broth, indole test, phenol red lactose broth, phenol red sucrose broth, and Voges-Proskauer test (US Food and Drug Administration, 2021) *Salmonella* can be further identified through phenotyping methods such as serotyping, phage typing, biotyping, and R typing (Bailey et al., 2010). Finally, *Salmonella* can be identified through genotyping by PCR or pulse-field gel electrophoresis (PFGE). PFGE was a highly used method to trace outbreaks, but whole genome sequencing (WGS) is now the current method used by PulseNet (Bailey et al., 2010; CDC, 2016). Whole genome sequencing is a laboratory procedure that determines the order of bases in the genome of an organism in one process (CDC, 2016). Because millions of bases make up the WGS for every organism, it is much more detailed method than Pulse Field Gel Electrophoresis (PFGE) which was the former gold standard method for differentiating among pathogen isolates (CDC, 2016). The CDC started implementing the use of WGS as its main way tracking foodborne

outbreaks in 2013 (CDC, 2016). They are able to compare genomes from outbreak strains to reference genomes from public data bases such as EnteroBase (Brown et al., 2019).

Shiga toxin producing *Escherichia coli*. Shiga toxin producing *E. coli* (STEC) is a gram-negative, non-spore-forming bacteria that can cause infection in humans. Like *Salmonella*, it belongs to the family *Enterobacteriaceae.* STEC can grow in temperatures ranging from 7 °C to 45 °C but has optimal growth from 35 °C to 42 °C (Sofos and Juneja, 2010). It can grow in a pH range of 4-10, and requires a water activity of 0.95 or higher (Sofos and Juneja, 2010).

STEC can be carried by many types of animals and is commonly associated with ruminants such as cattle (Persad et al., 2014). STEC will be passive in many of these hosts, but can cause disease in humans. Symptoms of infection by STEC include bloody diarrhea, vomiting, and in certain cases (often in children under 5 years old) hemolytic uremic syndrome (HUS) (Sofos and Juneja, 2010). STEC infects humans by using attachment and effacement lesions encoded for on their LEE pathogenicity island (Sofos and Juneja, 2010). As the name suggests, the main toxins used by STEC are Shiga toxins, which is what leads to cell death in the host.

Apart from being the most known disease-causing STEC serotype, *E. coli* O157:H7 informs most of what is known about STEC (CDC, 2014). The serotype *E. coli* O157:H7 was first identified in 1982 and was well studied during that decade (Rangel et al., 2005). The pathogen rose to infamy in 1993 when a large outbreak occurred across multiple locations of the fast food chain Jack in the Box (Food Safety News, 2017). The consumption of the chain's undercooked hamburgers led to illness in more than 600 people (Food Safety News, 2017). Because of this incident, the way food safety processes are handled, especially the inspection of meat and poultry, have drastically changed (Murano et al., 2018). This incident is also the

reason why O157:H7 has been so well-studied compared to other STEC serotypes. Among other STEC serotypes *E. coli* O26 is less likely to cause HUS compared to O157, even though its toxins are similar (CDC, 2014). Hemolytic uremic syndrome, or HUS, is a severe condition that damages the blood vessels of the kidneys and leads to renal failure. Once in the body, Shiga toxin can bind to globotriaosylceramide (Gb3) in vascular endothelial cells, and damages those cells by inhibiting protein synthesis. If those cells are part of the kidney, it can lead to HUS (Ko et al., 2016). In general, *E. coli* O157 is more likely to cause severe symptoms than other types of STEC (CDC, 2014).

STEC and may also be of concern in low-moisture foods. While the main reservoir for *E. coli* O157:H7 is cattle, the pathogen can easily spread through fecal contamination of water and other foods (WHO, 2018). According to the World Health Organization (WHO), this contamination can occur at many stages of growing and processing produce, which has led to increases in outbreaks of the pathogen in fruits and vegetables (WHO, 2018). Because of the recent outbreaks associated with STEC in low-moisture foods, and the various stages at which contamination can occur, it is important to explore its ability to survive in dried fruit, which can have many processing steps (Podolak and Black, 2017).

The detection of STEC can also be culturable or molecular based. Selective media often used for STEC plating include MacConkey agar, violet red bile agar, and Levine's Eosine methylene blue agar (Sofos and Juneja, 2010; FDA, 2020). To differentiate *E. coli* O157 from other *E. coli*, sorbitol can be added to the agar since O157 will not usually ferment sorbitol (Sofos and Juneja, 2010). Because the number of *E. coli* cells present in food is low, enrichment is very important to make sure that any cells present are detected. Common enrichments for

STEC include brain heart infusion broth, tryptic soy broth, and modified buffered peptone water with pyruvate (Sofos and Juneja, 2010; FDA, 2020). For identification in pure cultures, agglutination assays are useful for serotyping (Sofos and Juneja, 2010). The enzyme-linked immunosorbent assay (ELISA) is becoming more common for identifying STEC. Use of this assay has led to a better understanding of the most common serotypes of STEC. While O157:H7 is the most common STEC serotype associated with disease, there is a decrease in proportion of that serotype when using ELISA compared to culture-based methods (Sofos and Juneja, 2010). When screening with biochemical tests, most pathogenic *E. coli* will have negative test results for H_2S, urease, arabinose non-fermenting, and indole (FDA, 2020). To further determine if pathogenic *E. coli* is STEC specifically, real-time PCR can be used. The genes that should be targeted during PCR are *stx*1, *stx*2, and *uid*A, with the latter being highly conserved in O157:H7 strains (US Food and Drug Administration, 2020a). As mentioned with *Salmonella*, the main outbreak identification tool used by PulseNet is WGS. It has more differentiation capability than past methods used like PFGE. Abdelhamid et al. (2019) looked at a recent outbreak of *E. coli* O157:H7 from cattle to human and found that WGS was able to distinguish which isolates from the cattle matched (and did not match) the isolates in the infected patients, while the use of PFGE was unable to differentiate between all the isolates tested.

Listeria monocytogenes. *L. monocytogenes* is a gram-positive, non-spore forming bacteria belonging to the family *Listeriaceae.* It can grow in a temperature range of from -0.4 to 45 °C with optimal growth from 30 to 37 °C and can grow within a pH range of 4.4-9.6, but has optimal growth at 6-8 (Porto-Fett et al., 2009). *L. monocytogenes* can grow in foods with a water activity of 0.9 or higher (Podolak and Black, 2017).

L. monocytogenes is found in a variety of places, including plants, animals, soil, water, and humans (Porto-Fett et al., 2009). It can cause listeriosis, which can be a very serious infection in high-risk groups (pregnant women and people with weakened immune systems) but is unlikely to manifest severely in other groups of people (CDC, 2016). Foodborne *Listeria* needs only a few cells to infect and once in the digestive tract *Listeria* can invade cells and use cell-to-cell transmission to spread to the rest of the body. Symptoms of listeriosis in high-risk individuals can include miscarriage, sepsis, and meningitis, while in the rest of the population people may experience only mild gastroenteritis.

There is some debate of whether *L. monocytogenes* poses a significant risk in low-moisture foods. There have been no documented outbreaks of *L. monocytogenes* associated with low-moisture foods and the current prevalence of the pathogen in low-moisture foods is likely low (Ly, et al., 2019). However, *L. monocytogenes* can survive for long periods of time in low-moisture foods and there have been recalls associated with *L. monocytogenes* in these foods, including in dried fruits, nuts, biscuits, and oats (Ly et al., 2019). *L. monocytogenes* is notorious for its ability to grow in cold environments. This is why outbreaks of this pathogen are often found in refrigerated, ready-to-eat foods (RTEs), as they do not require heating before consumption. Dried fruits are an RTE and are often stored at refrigerated temperatures by processors, but due to the inability of pathogens to grow at low water activities, *L. monocytogenes* growth should not be a concern in dried fruits. Pathogen survival is still a concern though, as *L. monocytogenes* has been shown to have a desiccation tolerance of up to 1 year in certain low moisture foods. For instance, Kimber et al. (2012) found that 6 log CFU/g

of *L. monocytogenes* inoculated onto raw almonds did not decline significantly when the almonds were stored at 4 °C for 12 months.

Agar used for selective plating of *Listeria* include Oxford, Modified Oxford, PALCAM, Chromogenic Listeria agar, and lithium chloride-phenylethanol-moxalactam (US Food and Drug Administration, 2017). Selective enrichment can be done with buffered *Listeria* enrichment broth (US Food and Drug Administration, 2017). Proper subtyping is particularly important in identifying *Listeria,* as many different strains can have similar phenotypic qualities (Porto-Fett et al., 2009). The most common serotypes of *L. monocytogenes* isolated from patients are type 1 and type 4 (FDA, 2017). There can also be strains that have qualities that are unusual to *Listeria* that make identification more difficult. The FDA BAM mentions as examples isolates of *Listeria innocua* that are hemolytic and *L. monocytogenes* and *Listeria welshimeri* isolates that are rhamnose negative (FDA, 2017). When trying to differentiate *L. monocytogenes* specifically, the species should usually test negative for mannitol and xylose, and should test positive for rhamnose, virulence, and beta hemolysis (FDA, 2017). Again, sequencing plays an important role in identification of many pathogens such as *Listeria.* In fact, *Listeria* was the first bacteria the CDC began using WGS with and has since then spread its use to other organisms including *Salmonella* and *E. coli* (CDC, 2016).

Intrinsic factors influencing pathogen survival. Pathogen survival can be influenced by many factors, including a_w and pH. In general, the ability of microorganisms to survive common food processes increase when a_w is lowered. However, while higher a_w promotes growth, high a_w also enhances lethality of thermal treatments (Chitrakar et al., 2019; Villa-Rojas et al., 2017). The mechanisms for thermal resistance are not completely agreed upon but are shown to be

strongly influenced by a_w (Chitrakar et al., 2019). The lower the a_w, the more difficult it is for the number of cells present to decline (Mugnier et al., 1985; Keller et al., 2018). For example, Keller et al. (2018) found that *Salmonella* inoculated onto pumpkin seeds became increasingly resistant to thermal inactivation when the a_w decreased from its original value of 0.97 to below 0.20. The pumpkin seeds began with a *Salmonella* population of 7.48 ± 0.57 log CFU/g and dropped to 0.68 ± 0.81 log CFU/g after 6 h or drying at 60 °C (Keller et al., 2018). After 6 h, the a_w dropped to below 0.20 and no more significant decrease in the *Salmonella* population was seen during 12 more h of drying at 60 °C (Keller et al., 2018). Just knowing the a_w alone is not enough information to understand pathogen survival, as water activity is often working in conjunction with other factors such as temperature (Mugnier et al., 1985; Keller et al., 2018).

pH is also known to have some effect on bacterial survival. While bacteria have a specific (and often narrow) pH range in which they can grow, they can survive outside that pH range. Thermal resistance is decreased at lower pH, so pathogens are generally easier to inactivate in more acidic food matrices (Podolak and Black, 2017). Deng et al. (1998) inoculated dry infant cereals of pH 4.0 and 6.8 with 6 log CFU/g of *E. coli* O157:H7. After 24 weeks of storage at 5 °C the cereal with a pH of 4.0 had 3.19 log CFU/g of *E. coli*, while no *E. coli* was detected in the cereal at pH 6.8 (Deng et al., 1998).

Another intrinsic factor of dried fruits that may impact pathogen survival are antimicrobial properties. The phytochemicals found in dried fruits, including alkaloids, flavonoids, and phenolic compounds, can exhibit antibacterial activity (Jagathambal et al., 2011). Jagathambal et al. (2011) screened various phytochemicals from dried figs to see if they had any inhibitory effects on various bacteria. The phytochemicals extracted from dried figs

were able to inhibit *Salmonella* spp., *Klebsiella* spp., *Haemophilus* spp., and *Serratia* spp. with a minimum inhibitory concentration of 1.0 mg/mL (Jagathambal et al., 2011). Mainasara et al. (2019) screened phytochemical from dates to see how inhibitory they could be against pathogens. The extracted phytochemicals created inhibition zones against *Bacillus subtilis*, *Staphylococcus aureus, E. coli*, and *Salmonella* spp. of 13, 15, 9, and 11 mm respectively (Mainasara et al., 2019). These results suggest that the phytochemicals found in dried fruits could play a role in the survival of pathogens.

Dry fruit related outbreaks. While not many, there have been several foodborne outbreaks associated with low moisture foods (Table 2). In 2020, an outbreak of the hepatitis A virus associated with dates occurred in the United Kingdom (Food Safety New, 2021). Twenty eight people were infected and the dates, which were imported from Jordan, were subsequently voluntarily recalled. Another outbreak of hepatitis A occurred in England from semi-dried tomatoes, which infected two people (Carvalho et al., 2012). An outbreak of salmonellosis in Norway was associated with consumption of a *Salmonella* Agbeni-contaminated dried fruit and nut mix (Food Safety New, 2019). In this outbreak, 39 people were infected. Another outbreak was linked to *Salmonella* Phage type 13a in a dried vegetable spice mix, in which 108 people were infected in Sweden (Jernberg et al., 2015).

Objectives. As discussed above, dried fruits are of great economic importance to California and different processors follow distinct protocols to prepare their dried fruits. In addition to the various drying methods used, pre- and post-drying treatments can also be applied. Unfortunately, there has not been a systematic evaluation of the antimicrobial efficacy of different drying methods or pre- and post-drying treatments or a combination of them.

Pathogen contamination of the final products can happen at any processing point. Once happened, it is critical to better understand the behavior of these pathogens in dried fruits. The goal of this study is to fill in the current knowledge gaps associated with microbial safety risks of dried fruit by conducting a challenge study. The three pathogens selected include *Salmonella, E. coli* O157:H7, and *L. monocytogenes*. Dried fruits, including peaches, pluots, tomatoes, and dates were purchased from local farmers markets for this study.

Table 1. Log reduction of pathogens achieved by pre-drying treatments.

Treatment	Fruit	Treatment Concentration	Bacteria	Reduction after drying (log CFU/g)		Reference
				WITH TREATMENT	WITH NO TREATMENT	
Blanching	Carrot slices	Steam for 3 min	*Salmonella* Typhimurium	4.01	1.33	DiPersio et al. (2006)
	Carrot slices	Hot water for 3 min	*Salmonella* Typhimurium	3.46	1.33	DiPersio et al. (2006)
Sodium Metabisulfite	Peach slices	4.18%	*L. monocytogenes*	4.28	3.15	DiPersio et al. (2004)
	Gala apple slices	1.7%	*E. coli* O157:H7	6.70	3.10	Derrickson-Tharrington et al. (2005)
	Roma tomato halves	0.21%	*Salmonella* spp.	5.10	3.20	Yoon et al. (2004)
Lemon Juice	Gala apple slices	50%	*E. coli* O157:H7	7.10	3.10	Derrickson-Tharrington et al. (2005)
Ascorbic Acid	Peach slices	3.4%	*L. monocytogenes*	5.01	3.15	DiPersio et al. (2004)
	Gala apple slices	2.8%	*E. coli* O157:H7	6.70	3.10	Derrickson-Tharrington et al. (2005)
	Roma tomato halves	3.4%	*Salmonella* spp.	≥ 6	3.20	Yoon et al. (2004)
	Gala apple slices	3.4%	*E. coli* O157:H7	8.00-8.30	2.90-3.50	Burnham et al. (2001)

Table 2. Select outbreaks from 2001-2021 associated with low-moisture foods.

Category	Food	Pathogen	Cases	Year	Country	Reference
Dairy	Powdered milk	*Staphylococcus aureus*	36	2006	USA	CDC (2018)
	Whey powder	*Salmonella* Typhimurium	2	2018	USA	CDC (2018)
Dried Fruits	Dates	Hepatitis A	28	2020	USA	Food Safety New (2021)
	Dried coconut	*Salmonella* Typhimurium	14	2017	USA	CDC (2018)
	Semi-dried tomatoes	Hepatitis A	2	2011	England	Carvalho et al. (2012)
	Puffed rice and wheat cereal	*Salmonella* Agona	36	2008	USA	CDC (2018)
Grains	Flour	*Escherichia coli* O121; O26:NM	56	2015	USA	CDC (2018)
	Flour	*Escherichia coli* O121	7	2017	USA	CDC (2018)
	Flour	*Escherichia coli* O26:H11	21	2018	USA	CDC (2018)
	Flour	*E. coli* O26	29	2019	USA	CDC (2018
	Puffed wheat cereal	*Salmonella* Mbandaka	136	2018	USA	CDC (2018)
	Vegetable flavored rice and corn snack	*Salmonella* Wandsworth	65	2007	USA	CDC (2018
Herbs/Spices	Moringa leaf powder	*Salmonella* Virchow	35	2015	USA	CDC (2018)
	Pepper	*Salmonella* Seftenberg	11	2009	USA	CDC (2018)
Mixed fruit and nut	Dried fruit and nut mix	*Salmonella* Agbeni	39	2019	Norway	Food Safety News (2019)
	Dried vegetable spice mix	*Salmonella* Phage type 13a	108	2015	Sweden	Jernberg et al. (2015)
Nuts/Seeds	Almonds	*Salmonella* Enteritidis	3	2001	USA	CDC (2018)
	Almonds	*Salmonella* Enteritidis	168	2001	USA/Canada	Isaacs et al. (2005)
	Almonds	*Salmonella* Enteritidis	42	2003	USA	CDC (2018)
	Almond and peanut butter	*Salmonella* Braenderup	6	2014	USA	CDC (2018)
	Chia seed powder	*Salmonella* Newport; Hartford; Oranienburg; Gaminara	45	2014	USA	CDC (2018)
	Hazelnuts	*Escherichia coli* O157:H7	8	2010	USA	CDC (2018)
	Peanuts	*Salmonella* Thompson	100	2006	USA	CDC (2018)
	Peanut butter	*Salmonella* Tennessee	425	2007	USA	CDC (2018)
	Pine nuts	*Salmonella* Enteritidis	53	2010	USA	CDC (2018)
	Pistachio	*Salmonella* Montevideo; Senftenberg	11	2016	USA	CDC (2018)
	Soy nut butter	*Escherichia coli* O157:H7	32	2021	USA	CDC (2018)

Chapter 1: Effects of various carriers on intrinsic factors of dried fruits

When conducting challenge studies and preparing artificially contaminated food items, there are different carriers that can be used. The currently available inoculation methods can be grouped into three major categories: a liquid-carrier method, a dry-carrier method, and a no-carrier method. Examples of liquid carriers include peptone water, saline buffers, and ultrapure water. Cocoa butter oil has also been used to carry out inoculation (Komitopoulou and Peñaloza, 2009). Dry carriers that have been tested include sand, chalk, and talc (Blessington et al., 2013; Liu et al., 2021; Beuchat and Mann, 2014). The no-carrier method utilizes freeze- or vacuum-dried cultures or cell pellets and directly applies them to products (Santillana-Farakos et al., 2014a; Santillana-Fatillana-Farakos et al., 2014b). Choosing the correct inoculation carrier is critical. Although liquid carriers, such as buffered peptone water or ultrapure water, have been more widely used for delivering pathogens onto product surfaces, the addition of liquid or the introduction of additional moisture into the products (especially low-moisture foods) changes the moisture content and a_w of a dry substrate and may requires additional or extended drying steps (Blessington et al., 2013). As indicated by Palipane and Driscoll (1993), moisture adsorption/desorption isotherms are inherently non-equivalent, the a_w of the product after an additional drying step may not be the same as the original food. Beuchat and Mann (2014) used two different methods for inoculating dried cranberries, raisins, and strawberries and date paste. No difference in *Salmonella* behavior was observed between two inoculation methods (moist vs. sand). Similar observation was made by Blessington et al. (2013), in which no difference in *Salmonella* decline was observed between dry-inoculated and wet-inoculated nut kernels. Both studies indicated that when choosing the proper carriers for

dried products, the key features or changes that need to be monitored are the physical or chemical properties. In addition, inoculation methods should try to mimic real life contamination scenarios to give the most accurate representation of survival after a contamination event.

Phosphate-buffered saline (PBS) is a buffer solution commonly used in biological research. It is a water-based salt solution containing sodium chloride, potassium chloride, disodium hydrogen phosphate, and potassium dihydrogen phosphate. It helps maintain the osmolarity balance of bacteria when being used as the carrier for inoculation. However, since another key function if this buffer is to help maintain a constant pH, there is a concern over the use of PBS for inoculation and sample homogenization when measuring the pH of inoculated dried fruits. In this case, before the long-term survival study, the impact of PBS on the pH measurement needs to be studied.

An efficient recovery method that can release and recover target bacteria from food surfaces, is the foundation for accurate detection and enumeration. Stomaching, shaking, rubbing, sonication, pummeling, pulsing, as well as blending have been tested and used for releasing and recovering bacteria from food or environmental samples (Beuchat et al., 2001; Kim et al., 2012). Based on the various physical and chemical properties of the samples, the efficacy of these cell recovery method change. As shown by Kim et al. (2012), bacterial populations recovered from pummeled and pulsed iceberg lettuce, perilla leaves, cucumber, and green pepper samples were higher than those recovered from sonicated and hand-shaken samples. However, this trend was not observed on cherry tomatoes. Thus, which bacteria recovery method is more appropriate for sand-inoculated dried fruit needs to be determined.

The selected method needs to have the best recovery efficacy while generating no damage to the bacteria cells.

1.1 Objective

The objective of this chapter is to select the proper carriers (wet or dry) for different dried fruits, select the proper liquid for carrying out the wet-inoculation and pH measurement, and determine an efficient cell recovery method for samples that need to be dry (sand) inoculated.

1.2 Materials and Methods

Dried fruits. Dried fruits used for this survival study were purchased from local farmers markets. Fruits used included sundried tomatoes, peaches, peaches processed with sulfur, pluots processed with sulfur, low-moisture Medjool dates, and high-moisture Medjool dates (Figure 1.1). High-moisture dates are harvested directly from the tree; low-moisture dates are dates allowed to continue drying in nets after falling off the tree and have a harder texture compared to the high-moisture dates. Tomatoes and the sulfured peaches and pluots were sun dried. The peaches that were not processed with sulfur were dried using a dehydrator. Once purchased, the dried fruits were stored at room temperature for up to 1 week prior to use in experiments.

Inoculation with wet and dry carriers. Dried fruits were combined with either water or sand by the following methods. Briefly, ultrapure water (15 mL; Milli-Q Advantage A10, MilliporeSigma, Burlington, MA) was added to every 100 g of each of dried fruits and massaged by hand for 1 min. The dried fruits were then transferred to plastic containers with drying racks lined with filter paper (Fisherbrand, filter paper P5, Fisher Scientific, Pittsburg, PA). The lids of

the containers were taped down slightly ajar with a piece of mesh to cover the opening (Figure 1.2). The containers of fruit were set out to dry at room temperature for 48 h. For sand inoculation, 20 g of sand (Pure white, Fisher Scientific, Hanover Park, IL) was added to 100 g of each dried fruit and massaged and shaken for 1 min. The dried fruits were then transferred to gallon storage zipper bags (Ziploc, Pleasanton, CA) and stored at room temperature. For the water-inoculated samples, the pH and a_w was measured before and right after the inoculation. After 48 h, the pH and a_w of both the wet and dry inoculated dried fruits were both measured. A pH meter (FiveEasy pH Meter F20, Mettler Toledo, Schwerzenbach, Switzerland) and water activity meter (Aqualab model 4TE, Decagon Devices, Pullman, WA) were used to take the measurements.

Effect of phosphate buffered saline on pH measurement of dried fruits. Phosphate buffered saline (PBS, pH 7.4) was chosen as the wet carrier with which to inoculate the dried fruits. Since PBS is a buffer solution, the impact of PBS on the pH measurement of dried fruits was tested. To do so, dried fruits (100 g) were combined with either ultrapure water or PBS (15 mL) and massaged and shaken by hand for 1 min. The pH of the sample was measured before the addition of the liquid, immediately after massaging, and after 48 h of drying. To measure the pH, each fruit sample was combined with water or PBS equal to 40% of the sample mass and then stomached for 1 min at the fast setting to homogenize. The pH meter was used to take the measurements.

Bacterial cultures and inoculum preparation. The strains of bacteria used for this study were provided courtesy of Dr. Linda J. Harris at the University of California, Davis. Five strains of rifampicin-resistant *Salmonella* were used. The strains are summarized in Table 1.1.

Individual frozen stock cultures were streaked onto tryptic soy agar (TSA, Becton, Dickinson and Company (BD), Sparks, MD), supplemented with 50 µg/mL of rifampicin (TSAR; Biosynth International, Itasca, IL), and incubated at 37 °C overnight. Each isolated colony was transferred into 10 mL of tryptic soy agar (TSB, BD) supplemented with rifampin at 50 µg/mL (TSBR), and then incubated at 37 °C overnight. One 10-µL loopful of the overnight culture was transferred to 10 ml of fresh TSBR and incubated at 37 °C for another 24 h. The newly inoculated broth was spread onto TSAR plates, 250 µL per plate, one plate per strain, and incubated for 24 h at 37 °C. To recover bacterial lawns from plates, 1 mL of phosphate-buffered saline (PBS, pH=7.4) was pipetted onto each plate, and an L-shaped plastic cell spreader (Cole-Parmer, Swedesboro, NJ) was used to loosen and scrape the lawn. The re-suspended cells were then pipetted into a 15-mL Falcon™ tube (Corning, Pittsburgh, PA). The addition of PBS and lawn scraping was repeated two more times for each plate, for a total of 3 mL of PBS used per plate. Approximately 2.5 mL of culture was recovered from each plate. Once all plates were scraped, 15 mL of the recovered culture from each strain were combined to make the 5-strain cocktail of *Salmonella*. The cocktail was diluted and plated onto TSAR for calculating the inoculum level.

Evaluation of homogenization methods for recovering pathogenic cells from inoculated sand. *Salmonella*-inoculated sand was used to test the recovery method used for dried fruit (stomaching vs. shaking). Twenty grams of sand was inoculated with 1 mL of the 5-strain *Salmonella* cocktail and they were mixed together by hand for 1 min. Samples of the inoculated sand were sampled immediately after mixing and after 48 h of drying. The drying process was done at 40 °C for 48 h in a gravity oven (Fisher Scientific Model 725 G, Hampton, NH). At each sampling point, three 10-g sub-samples were taken for the analysis of *Salmonella*

counts. Each 10-g inoculated sand sample was divided into two portions (5 g each). These two portions were added to two 24-oz filter bags (WHIRL-PAK, Nasco, Milton, WI) together with 95 mL of PBS in each bag. One bag was stomached for 1 min using a smasher (Smasher™, BioMérieux Industry, Hazelwood, MO), while the other bag was shaken by hand for 1 min. The contents were then serially diluted appropriately with PBS and two 100 µL suspensions from each dilution were spirally plated onto TSA with rifampicin and Xylose Lysine Tergitol 4 agar (XLT-4, BD) with 50 µg/mL rifampicin (XLT-4R). After 24 h of incubation at 37 °C, colonies were counted and the populations determined.

Statistical Analysis. One trial was conducted for every test performed in this section. At each sampling point, three samples were analyzed (n=3). Means comparison were performed using Excel (Excel 16.50, Microsoft, Redmond, WA) to determine whether carrier type had a significant impact on pH and water activity of dried fruits as well as if homogenization methods for inoculated sand had a significant impact on recovery of pathogenic cells. Differences between mean values were considered significant at $P < 0.05$.

1.3 Results

Inoculation with a wet or dry carrier. Table 1.2 shows the pH and water activity of dried fruits before and after the addition of either water as a wet-carrier or sand as a dry carrier. In low-moisture dates, the initial pH was 5.83 ± 0.06. With both wet and dry-inoculation, the change in pH was statically significant, dropping to 5.24 ± 0.05 and 5.59 ± 0.04 respectively. The initial a_w of the low-moisture dates was 0.62 ± 0.03 and showed no significant change after either wet or dry-inoculation, with their values being 0.64 ± 0.00 and 0.61 ± 0.00, respectively. In high-moisture dates the initial pH was 5.59 ± 0.04. The pH did not change significantly after

wet-inoculation. The pH dropped after the dry-inoculation to 5.39 ± 0.04. The initial a_w of the high-moisture dates was 0.55 ± 0.02. No significant change was observed after either wet or dry inoculation.

The initial pH of the dried peaches was 3.94 ± 0.07, and neither the wet nor the dry inoculation generated significant change on the pH. Additional loss of moisture might have occurred during the 48-h of drying after wet inoculation, which might be the reason why there was differences between the a_w before and after wet-inoculation. A similar observation was made in dried peaches made with sulfur treatment, as the a_w decreased after wet-inoculation. Both the wet- and dry-inoculation slightly reduced the pH value of the products, changing from 3.59 to 3.51 and 3.48 respectively.

In dried pluots, although neither wet- nor dry-inoculation generated any impact on a_w, dry-inoculation reduced the pH of the products (3.45 ± 0.02 before inoculation vs. 3.36 ± 0.02 after dry-inoculation). For sundried tomatoes, inoculation had no impact on pH but the dry inoculation significantly reduced the a_w of the products (0.78 ± 0.00 before inoculation vs. 0.62 ± 0.00 after inoculation).

Effect of phosphate buffered saline on pH measurement of dried fruits. Table 1.2 shows the pH values of dried fruits before and after using PBS or MilliQ water as the wet carrier and then homogenizing with PBS or MilliQ water. As shown in Table 1.3, no difference was observed between pH measurements taken from samples inoculated with PBS or MilliQ water and homogenizing with PBS or MilliQ water for most of the samples. The only significant difference in pH measurement was observed from dried peaches that were inoculated with PBS and dried for 48 h. When these samples were homogenized with PBS or MilliQ water for pH

measurement, the pH taken from samples homogenized with MilliQ water were higher than the pH taken from samples homogenized with PBS (4.04 ± 0.09 vs. 3.63 ± 0.04).

Evaluation of homogenization methods for recovering pathogenic cells from inoculated sand. The efficacy of recovering *Salmonella* from inoculated sand by stomaching or manually shaking was evaluated. Inoculated sand samples were taken right after inoculation and after 48 h of drying in the oven. The concentration of the *Salmonella* cocktail was 11.07 ± 0.04 log CFU/mL. Since 20 g of sand was mixed with 1 mL of liquid culture, the theoretical inoculation level in sand is 9.77 log CFU/g. As shown in Table 1.4, 9.51 ± 0.04 log CFU/g of *Salmonella* was recovered from freshly inoculated sand by stomaching. After 48 hours of drying, 6.93 ± 0.09 Log CFU/g of *Salmonella* was recovered from the inoculated sand by stomaching. Drying at 40 °C for 48 h caused an approximately 2.5 log reduction of *Salmonella*. The differences between counts obtained from TSAR and XLT-4R indicated the formation of injured cells during drying. Comparing the cell numbers recovered by stomaching with the cell numbers recovered by shaking methods, no difference in TSAR counts was observed from inoculated sand that has been dried for 48 h (6.93 ± 0.09 log CFU/g vs. 6.88 ± 0.04 log CFU/g). When looking at the XLT-4R counts, stomaching method had higher counts on XLT-4R than shaking method (3.43 ± 0.00 vs. 2.82 ± 0.01 Log CFU/g). Similar trend was observed from freshly inoculated sand. Based on this result, stomaching was used in the following studies for recovering pathogens from inoculated sand or sand-inoculated dried fruits.

1.5 Discussion

The objective of this chapter was to determine the inoculation carriers for different dried fruits, the buffer system for pH measurement, as well as the pathogen recovery method

for sand-inoculated dried fruits. Based on the results, both wet and dry inoculation changed the pH of low-moisture dates, while generating no significant impact on the a_w of the samples (comparing wet or dry-inoculated samples with non-inoculated ones). A similar observation of a_w before and after inoculation was found in high-moisture dates. Dry inoculation reduced the pH of high-moisture dates more than the wet-inoculation. However, when looking at the physical properties of the inoculated dates, wet inoculation caused the skin to start to peel from the dates. Based on these observations, dry inoculation was chosen to inoculate dates. In addition, since dates are typically grown in sandy regions where sand storms are common and some dates are cleaned by air pressure without any contact with water before packaging (Glasner, Botes, Zaid, & Emmens, 2002).

For dried peaches, the wet inoculation reduced the a_w slightly compared to the non-inoculated ones (0.53 vs. 0.58 in dried peaches without sulfur treatment and 0.69 vs. 0.74 in dried peaches with sulfur treatment). This reduction in a_w might be caused due to the additional 48 h drying after inoculation. As indicated by Palipane and Driscoll (1993), moisture adsorption/desorption isotherms are inherently non-equivalent; the a_w of the product after an additional drying step may not be the same as in the original food. Dry inoculation did not impact the a_w of either dried peaches. Neither dry nor wet inoculation altered the pH of dried peaches without sulfur treatment significantly. They both reduced the pH of dried peaches made with sulfur treatment. Based on the measurement, there is no strong preference between two inoculation methods. In this case, it was decided to use both wet and dry carriers to inoculate dried peaches.

For dried pluots and sundried tomatoes, wet inoculation generated no impact on their pH or a_w. In addition, since pluots and tomatoes are also often processed in large quantities (based on the survey information gathered) and washed before drying like the peaches (Schmutz and Hoyle, 1999). Using a liquid carrier could help represent possible contamination from water during washing.

The impact of different inoculation methods on the behavior of inoculated pathogens can be determined by the types of products. As discussed earlier, both Blessington et al. (2013) and Beuchat and Mann (2014) did not find any significant differences between wet- and dry-inoculated products. Bowman et al. (2015) monitored the survival of *Salmonella* on black peppercorns and cumin seeds. The tested methods included wet-inoculation with *Salmonella* cells grown in TSB wet-inoculation with *Salmonella* grown on TSA biofilm inoculation (in which *Salmonella* and seeds were co-incubated in TSB for 24 h at 37 °C), and dry inoculation with TSA-grown-*Salmonella*-inoculated sand. Their results showed that the biofilm inoculation method had the least *Salmonella* reduction. When comparing the dry inoculation method vs. the wet inoculation methods (both using TSA-grown *Salmonella*), the dry inoculation method had no difference with wet inoculation method on black peppercorn but had less reduction of *Salmonella* on cumin seed. One potential explanation for these differences might be the content and release of different amounts of antimicrobials from the food during wet inoculation (Shelef, 1984; Waje et al., 2008).

Deng et al. (1998) investigated the impact of a_w, pH and temperature on the survival of *E. coli* O157:H7 in a commercial dry infant rice cereal. Results showed that much better survival was seen when the pH was at 6.8 than 4.0. In the current study, if PBS is chosen to carry out the

wet inoculation, how it impacts the pH and pH measurement needs to be understood before the experiment. PBS is more often used as a carrier than MilliQ water because of its ability to prevent cells bursting due to osmosis (Martin et al., 2006). Based on our results, wet inoculation with PBS did not have a significant impact on the pH of the dried fruit and using PBS for pH measurement also did not impact the result (Table 1.2). Thus, PBS was appropriate to use as the liquid carrier for the dried fruits as changes in pH can influence pathogen survival in dried foods.

In the Blessington et al. (2013) study, in which both sand and chalk were tested as dry carriers, bigger impact on the weight of the inoculated nuts was observed from the chalk inoculated nuts, especially almonds (0.18 g weight loss when inoculating with sand vs. 7.21 g weight gain when inoculating with chalk) (Blessington et al., 2013). Based on our own lab's data, chalk and talc are lighter and tend to fly around when conducting the inoculation. When dealing with pathogens, it is not safe. Thus, sand is a better option compared with other dry carriers. Unfortunately, sand is an abrasive substance and could potential damage cells during inoculation. Sahin (2016) used sand in their study to disrupt bacterial cell walls, indicating that sand can lead to cell abrasion if used in a particular way. When comparing stomaching and shaking by hand in this study for homogenization of samples, *Salmonella* was recovered at significantly higher levels from stomached samples than shaken samples (Table 1.4). When looking at the cell counts obtained from the selective agar, stomaching recovered a higher number of *Salmonella* from sand samples that had been dried for 48 h. The major difference observed in this study was the injured cells formed during drying as indicated by the differences

between counts on TSA and XLT-4 agars. Based on these results, stomaching was chosen for recovering pathogens from sand-inoculated samples.

Table 1.1. Pathogen strains used for the inoculation of sand. All isolates were resistant to 50 µg/L rifampicin.

Organism	Strain	Source	Date Received
Salmonella Enteritidis PT30	ATCCBAA-1045	Raw almonds associated with an outbreak	November 11th 2018
Salmonella Tennessee	K4643	Clinical isolate associated with peanut butter outbreak	November 11th 2018
Salmonella Montevideo	FDA-2010-149-pistachios2	Pistachios from FDA	November 11th 2018
Salmonella Saintpaul	LJH1375	DFA walnut Salmonella survey	November 11th 2018
Salmonella Gaminara	F2712	Orange juice outbreak 1995	November 11th 2018

Table 1.2. pH and water activity of dried fruits before and after inoculation with wet (water) or dry (sand) carriers. pH and a_w measurements were taken in triplicate. Different uppercase letters within a type of fruit represent significant difference between pH values ($P < 0.05$). Different lowercase letters within a type of fruit represent significant difference between a_w values ($P < 0.05$).

Dried Fruit	Carrier	average pH	average a_w
Date (low-moisture)	before	5.83 ± 0.06^{A}	0.62 ± 0.03^{ab}
	wet	5.24 ± 0.05^{B}	0.64 ± 0.00^{a}
	dry	5.59 ± 0.04^{C}	0.61 ± 0.00^{b}
Date (high-moisture)	before	5.59 ± 0.10^{A}	0.55 ± 0.02^{ab}
	wet	5.47 ± 0.05^{AB}	0.59 ± 0.00^{a}
	dry	5.39 ± 0.04^{B}	0.54 ± 0.00^{b}
Peach	before	3.94 ± 0.07^{AB}	0.58 ± 0.00^{a}
	wet	3.89 ± 0.04^{B}	0.53 ± 0.00^{b}
	dry	4.13 ± 0.01^{A}	0.57 ± 0.01^{a}
Peach (with sulfur)	before	3.59 ± 0.00^{A}	0.74 ± 0.00^{a}
	wet	3.51 ± 0.03^{B}	0.69 ± 0.00^{b}
	dry	3.48 ± 0.04^{B}	0.73 ± 0.00^{a}
Pluot (with sulfur)	before	3.45 ± 0.02^{A}	0.68 ± 0.00^{a}
	wet	3.46 ± 0.02^{A}	0.68 ± 0.00^{a}
	dry	3.36 ± 0.02^{B}	0.68 ± 0.00^{a}
Tomato	before	3.80 ± 0.12^{A}	0.78 ± 0.00^{a}
	wet	3.79 ± 0.08^{A}	0.76 ± 0.01^{a}
	dry	3.79 ± 0.17^{A}	0.62 ± 0.00^{b}

Table 1.3. pH measurement of dried fruits when inoculated and/or homogenized with PBS versus water. A: Dried peaches without sulfur; B: Dried peaches processed with sulfur; C: Sundried tomatoes; D: Dried pluots processed with sulfur; E: High-moisture dates; F: Low-moisture dates. *indicates a significant difference in pH between homogenization with PBS and homogenization with water.

A

		Dried Peach	
		Homogenized with PBS	**Homogenized with water**
Before inoculation		3.77 ± 0.04	3.89 ± 0.17
Immediately after inoculation	**Inoculated with PBS**	3.66 ± 0.06	3.78 ± 0.18
	Inoculated with water	3.79 ± 0.05	3.78 ± 0.21
48 h of drying after inoculation	**Inoculated with PBS**	3.63 ± 0.04*	4.04 ± 0.03*
	Inoculated with water	3.81 ± 0.14	4.00 ± 0.09

B

		Dried Peach (with sulfur)	
		Homogenized with PBS	**Homogenized with water**
Before inoculation		3.24 ± 0.03	3.31 ± 0.02
Immediately after inoculation	**Inoculated with PBS**	3.26 ± 0.06	3.33 ± 0.08
	Inoculated with water	3.36 ± 0.02	3.44 ± 0.06
48 h of drying after inoculation	**Inoculated with PBS**	3.47 ± 0.03	3.41 ± 0.03
	Inoculated with water	3.57 ± 0.06	3.38 ± 0.03

C

		Sundried Tomato	
		Homogenized with PBS	**Homogenized with water**
Before inoculation		3.69 ±0.12	3.76 ± 0.11
Immediately after inoculation	**Inoculated with PBS**	3.70 ± 0.13	3.86 ± 0.12
	Inoculated with water	3.70 ± 0.04	3.44 ± 0.13
48 h of drying after inoculation	**Inoculated with PBS**	3.51 ± 0.04	3.71 ± 0.03
	Inoculated with water	3.59 ± 0.07	3.95 ± 0.29

D

		Dried Pluot (with sulfur)	
		Homogenized with PBS	**Homogenized with water**
Before inoculation		3.16 ± 0.02	3.16 ± 0.01
Immediately after inoculation	**Inoculated with PBS**	3.33 ± 0.01	3.35 ± 0.06
	Inoculated with water	3.07 ± 0.01	3.21 ± 0.03
48 h of drying after inoculation	**Inoculated with PBS**	3.08 ± 0.05	3.43 ± 0.03
	Inoculated with water	3.27 ± 0.01	3.19 ±0.10

E

		High-moisture Dates	
		Homogenized with PBS	**Homogenized with water**
Before inoculation		4.86 ± 0.06	4.83 ± 0.07
Immediately after inoculation	**Inoculated with PBS**	4.85 ± 0.06	4.96 ± 0.05
	Inoculated with water	4.88 ± 0.02	4.80 ± 0.14
48 h of drying after inoculation	**Inoculated with PBS**	4.81 ± 0.05	4.69 ± 0.11
	Inoculated with water	4.89 ± 0.07	4.71 ± 0.12

F

		Low-moisture Dates	
		Homogenized with PBS	**Homogenized with water**
Before inoculation		4.88 ± 0.04	4.80 ± 0.04
Immediately after inoculation	**Inoculated with PBS**	4.86 ± 0.06	4.83 ± 0.02
	Inoculated with water	4.75 ± 0.04	4.70 ± 0.03
48 h of drying after inoculation	**Inoculated with PBS**	4.97 ± 0.10	4.95 ± 0.15
	Inoculated with water	4.82 ± 0.08	4.76 ± 0.0

Table 1.4. *Salmonella* spp. recovered from inoculated sand by stomaching and shaking. The liquid inoculum level was 11.07 ± 0.04 log CFU/mL. Different uppercase letters within a row represent significant difference between *Salmonella* spp. counts ($P < 0.05$).

Time	Media	Average microbial count (Log CFU/g)	
		Stomached	Shaken
after Inoculation	TSAR	9.51 ± 0.04^{A}	9.42 ± 0.01^{B}
	XLT-4R	8.87 ± 0.01^{A}	8.61 ± 0.01^{B}
after 48 h of drying	TSAR	6.93 ± 0.09^{A}	6.88 ± 0.04^{A}
	XLT-4R	3.43 ± 0.00^{A}	2.82 ± 0.01^{B}

Figure 1.1. Dried fruits purchased from local farmers markets. A: Dried peaches; B: Dried peaches processed with sulfur; C: Sundried tomatoes; D: Dried pluots processed with sulfur; E: High-moisture Medjool dates; F: Low-moisture Medjool dates.

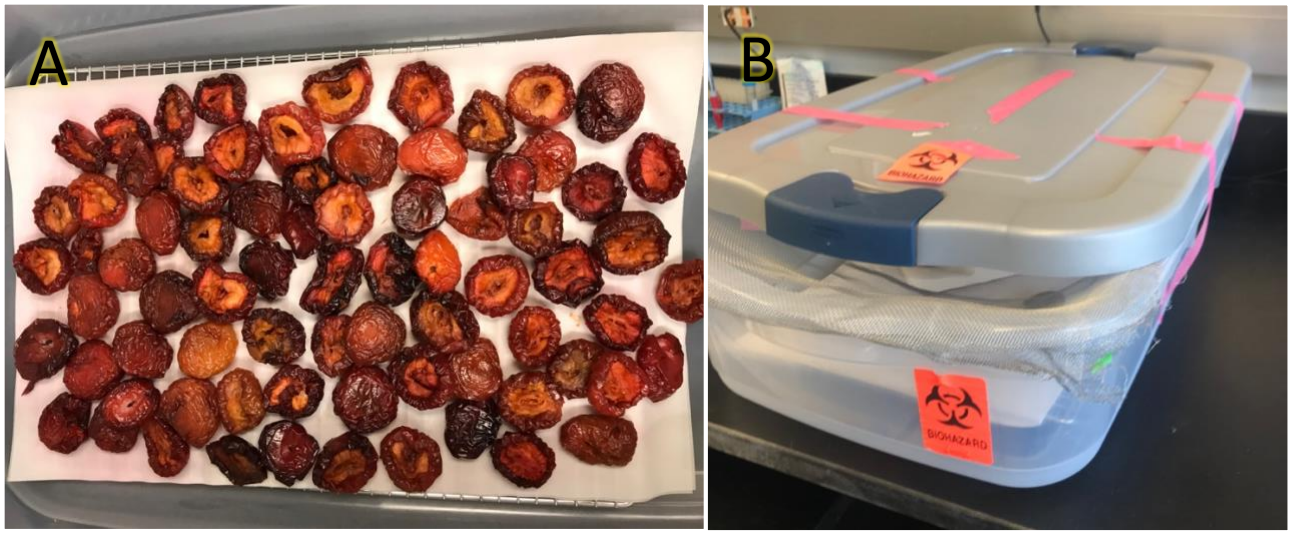

Figure 1.2. Drying container for wet inoculated dried fruit. A: Wet inoculated dried pluots are arranged in the box in a single layer on the filter paper. B: The container is set up with a metal rack placed at the bottom covered with a sheet of folded filter paper upon which the dried fruits are placed. Mesh is placed at the front of the container. The lid is taped to the container slightly ajar to allow air to pass through.

Chapter 2: Survival of pathogens on peaches made with or without sulfur treatment

Sulfur treatments are often used in dried fruits to preserve color (Schmutz and Hoyle, 1999). In other food applications, sulfur is often used as an antimicrobial but it has not been evaluated in this capacity for dried fruits. Based on a recent report (Report No. 527775R by ETS Laboratories), dried fruits produced in the U.S. contain free SO_2 ranging from 20 to 635 ppm. Among all the products tested, dried pineapple contains the least free SO_2 while dried apples contain the highest levels of SO_2. Dried peaches contain 355 ppm SO_2 when measured in slurry (pH 3.43-3.53). Witthuhn et al. (2004) evaluated the sulfur dioxide (SO_2) content in various commercial South African dried fruits and the microbial quality associated with these products. Results showed that raisins, Clingstone peaches, apricots, nectarines, and prunes contained approximately 1,302, 866, 1,318, and 806 mg/kg of SO_2 respectively. However, no correlation between the SO_2 content and the microbial counts (e.g. total plate counts and coliform counts) was identified. On the other hand, Karabulut et al. (2007) showed that the total number of microbes present on sulphurated dried apricots were 3 logs less compared to the non-sulfured dried apricots. Türkyılmaz et al. (2012) reported a significantly lower total microbial population on dried apricots made with the sulfur dioxide treatment compared to non-sulfured dried apricots. In the Liu et al. study (2021), the authors have shown that pathogens survived at higher levels for a longer period on dried apricots made without sulfur treatment.

Sulfur dioxide is considered by the FDA as generally recognized as safe (GRAS). The FDA does not set limits on the amount of sulfur dioxide (in ppm) permitted in foods. However, proper labeling is required for foods containing levels of sulfur dioxide that exceeds 10 ppm. In dried fruits, less than 100 ppm is typically found (USDA, 2011). However, when inhaled or

ingested by sensitive groups, it can induce asthma, even in low concentrations (Freedman, 1980). The California Office of Environmental Health Hazard Assessment (OEHHA) has developed a proposed MADL for SO_2 of 220 μg/day (OEHHA, 2012). Taking dried peaches (containing 355 ppm SO_2) as an example and assuming 26 grams of dried peaches are consumed each day, the estimated exposure to SO_2 is 191 μg/day (Interpretive guideline No. 2012-12).

2.1 Objective

The objective of this chapter is to determine the impact of sulfur treatment on the survival of common foodborne pathogens during storage of dried peaches.

2.2 Materials and Methods

Bacterial strains and preparation. The strains of bacteria used for this survival study were provided courtesy of Dr. Linda J. Harris at the University of California, Davis. Five strains each of rifampicin-resistant *Salmonella* spp., *Listeria*, and *E. coli* O157:H7 were used. The strains are summarized in Table 2.1.

Preparation of inocula. Individual frozen stock cultures were streaked onto TSAR and incubated at 37 °C overnight. Each isolated colony was transferred into 10 ml of TSBR, and then incubated at 37 °C overnight. One 10-μL loopful of the overnight culture was transferred to 10 mL of fresh TSBR and incubated at 37 °C for another 24 h. The newly inoculated broth was spread onto TSAR plates, 250 μL per plate, 6 plates per strain, and incubated for 24 h at 37 °C. To recover bacterial lawns from plates, 1 mL of phosphate-buffered saline (PBS, pH=7.4) was pipetted onto each plate, and an L-shaped plastic cell spreader was used to loosen and scrape the lawn. The re-suspended cells were then pipetted into a 15-ml Falcon™ tube. The addition of

PBS and lawn scraping was repeated 2 more times for each plate, for a total of 3 ml of PBS used per plate. Approximately 2.5 ml of culture was recovered from each plate and a total of 15 mL cell suspension was recovered for each strain. Once all plates were scraped, 15 mL of the recovered culture from each strain were combined to make the 5-strain cocktail. A 5-strain cocktail was made for each of the pathogens (*L. monocytogenes*, *E. coli* O157:H7, and *Salmonella*) and was then used to inoculate dried fruits. Each cocktail was diluted and plated onto TSAR for calculating the inoculum levels.

Inoculation of dried peaches with wet or dry carriers. Before inoculation, 3 samples of uninoculated dried peaches (25 g each) were homogenized with 100 mL of PBS. One hundred microliters of each of the homogenates were plated on to TSAR to check for the presence of rifampicin-resistant bacteria. This was done to ensure that any bacteria present on plates during the study were from the rifampicin-resistant inoculum used and not from background microbes.

Once the 5-strain cocktails were prepared, they were used to inoculate the dried peaches or sand. For sand inoculation, 1 mL of each 5-strain cocktail was added for every 20 g of fine white sand and mixed thoroughly. The inoculated sand was then spread as thinly as possible across a sheet of filter paper in a metal tray. The tray was placed in a gravity oven at 40 °C to dry for 48 h. Once dry, 160 g of the inoculated sand was used to inoculate 800 g of dried peaches (peaches or peaches processed with sulfur) and massaged into the fruit for 1 min by hand. The dry inoculated peaches were transferred into Ziploc bags and placed in a plastic container for storage at ambient or refrigerated temperature (5°C) for 6 months. For wet inoculation, the remaining 5-strain cocktail was added to enough PBS to make a 1:10 dilution.

One hundred and twenty milliliters of each diluted cocktail were added to 800 g of dried peach batch (peaches or peaches processed with sulfur) and massaged into the fruit for 1 min. The peaches were then transferred to the containers (see Figure 1.2) and dried at ambient temperature for 48 h. After drying, the wet-inoculated fruit was transferred into Ziploc bags and placed in a plastic container for storage at the designated two temperatures. Temperature monitors (TempTale4, Sensitech, Beverly, MA) were used to measure the temperature over the course of the study.

Sample collection and microbial enumeration. For wet-inoculated samples, subsamples were taken on the day of inoculation, after 48 h of drying (Day 0), Day 5, Day 15, Day 30, and then every 30 days after that for a total of 6 months. For sand-inoculated samples, subsamples were taken after the sand-inoculation were considered as the Day 0 samples. The rest of the sampling schedule was the same as the wet-inoculated ones. At each sampling point, three samples were taken for each type of inoculated fruit. To plate for enumeration, 25 g of each dried peaches were combined with 100 mL of PBS in a 24-oz filter bag. Each sample was then homogenized at fast speed for 1 min using the Smasher. Once mixed samples were serially diluted with PBS if necessary and 50 µL was spiral plated onto TSAR and selective agar with 50 µg/L of rifampicin and then incubated at 37 °C. The selective agars used were Xylose Lysine Tergitol 4 agar (XLT-4), MacConkey agar (MAC, BD), and Modified Oxford agar (MOX, BD) for *Salmonella* spp., *E. coli* O157:H7, and *L. monocytogenes* respectively. Plates were counted at 24 h, and final microbial counts were confirmed after 48 h. The limit of detection (LOD) for the dried fruits was calculated below:

$$LOD = \frac{1\ CFU * 20 * 100}{25\ g\ of\ dried\ fruit} = 80 \frac{CFU}{g} = \mathbf{1.9\ Log\ CFU/g}$$

To determine the pathogen levels in inoculated sand, 5 g of inoculated sand (right after inoculation or after 48 h of drying) was combined with 95 mL of PBS, stomached for 1 min in the Smasher, and then serially diluted with PBS. Samples (50 µL each) were spirally-plated in duplicate onto selective and non-selective agar with rifampicin, incubated at 37 °C, and then counted after 48 h. The limit of detection for the inoculated sand was calculated below:

$$LOD = \frac{1\ CFU * 20 * 100}{5\ grams\ of\ sand} = \ 400\frac{CFU}{g} = \ \mathbf{2.6\ Log\ CFU/g}$$

Enrichment of samples below LOD. An enrichment was performed for samples where no colonies were detected on plates at the lowest dilution. Samples were stored in the filter bag in which they were homogenized at 5 °C. One hundred milliliters of each double strength (2×) enrichment broth was added to every homogenized samples. Samples were incubated at 37 °C for 24 h before streaking onto selective agar and incubated at 37 °C for another 24 h. The presence of suspected colonies was recorded. For the first trial, lactose Broth was used for the enrichment of *Salmonella* while Frasier Broth was used for *E. coli* O157:H7 and *L. monocytogenes.* After reviewing the FDA Bacteriological Analysis Manual (BAM), lab protocols were changed (FDA, 2017; FDA 2020; FDA, 2021). For the second trial, 2X modified buffered peptone water pyruvate (mBPWp) and buffered *Listeria* enrichment broth (BLEB) was used for the enrichment of *E. coli* O157:H7 and *L. monocytogenes,* respectively.

Sulfur measurement. Dried peaches made with sulfur treatment (60 g) were weighed in a tared blender jar and combined with 140 g of distilled water. The mixture was blended for 60 s to form a thick and well homogenized puree. After blending, sample was brought back to the scale and additional distilled water was added to bring the total weight to 300 g. The mixture was blended again for additional 30 s. After blending, 60 g of the slurry was each of the four

250 mL narrow mouth Erlenmeyer flasks and distilled water was added to bring the volume to 100 mL. In each flask, there was 12 g of dried fruits. Approximately 5 mL of 1% soluble starch indicator was added to each flask. Five milliliters of sulfuric acid solution (750 mL of ACS grade concentrated sulfuric acid was mixed with 2,250 ml of distilled water; VWR, Radnor, PA) was added to the first flask and swirled to mix. After that, another 5 mL of 3% hydrogen peroxide was added into the flask, swirled again and stoppered. The mixture was held at ambient temperature for 2 min with occasional swirling, then titrated with 0.02 N iodine to a purple starch endpoint that was stable for 15-30 s. To the second flask, 5 mL of same sulfuric acid solution was added first, swirled to mix, and immediately titrated with 0.02 N iodine to a purple starch endpoint that was stable for 15-30 s. To the remaining two flasks, 5 mL of 10% sodium hydroxide was added into each flask, mixed thoroughly, stoppered, and allowed to stand for 20 min at ambient temperature to facilitate the release of bound sulfite to the free form. After 20 min, 5 mL of the sulfuric acid solution was added to one flask, swirled to mix, and immediately titrated with 0.02 N iodine to a purple starch endpoint stable for 15-30 s. The following equations were used to calculate the free SO_2 and total SO_2 in the samples:

Free SO_2-

$$\frac{\text{mg free SO2}}{\text{Kg of fruit}} = \frac{[(\text{mL Iodine}_{\text{free suflite+nonsulfite}} - \text{mL Iodine}_{\text{nonsulfite}}) \text{ x } 0.02 \text{ x } 32 \text{ x } 1000]}{12}$$

Total SO_2-

$$\frac{\text{mg total SO2}}{\text{Kg of fruit}} = \frac{[(\text{average mL Iodine}_{\text{free and bound suflite+nonsulfite}} - \text{mL Iodine}_{\text{nonsulfite}}) \text{ x } 0.02 \text{ x } 32 \text{ x } 1000]}{12}$$

Measurements in triplicate were taken at the same sampling points as the survival study on dried fruit that had PBS added to them to replicate the environment of the inoculated fruit. SO_2 was also measured before "inoculation" with PBS.

Statistical analysis. Two independent trials were conducted for every temperature, pathogen, and dried fruit type combination. At each sampling point, three samples were analyzed (n=6). Means comparisons were performed using Excel to determine whether sampling day and type of media had significant impact on pathogen survival as well as sampling day's impact on sulfur content in sulfured dried peaches. Analysis of variance (ANOVA), and Tukey multiple comparison test were performed using R studio (RSTudio, PBC, Boston, MA) to determine whether sampling day, storage temperature, media, presence of sulfur, or inoculation method significantly impact pathogen survival. Differences between mean values were considered significant at $P < 0.05$.

2.3 Results

Inoculum levels in wet carrier and dry carrier. The concentrations of the initial liquid inoculum were 11.07 ± 0.04 log CFU/mL for *Salmonella*, 10.91 ± 0.23 log CFU/mL for *E. coli* O157:H7, and 10.89 ± 0.20 log CFU/mL for *L. monocytogenes*. After inoculating and drying on the sand, the final inoculum levels on sand were 6.70 ± 0.45 log CFU/g for *Salmonella*, 5.47 ± 0.42 log CFU/g for *E. coli* O157:H7, and 6.45 ± 0.49 Log CFU/g for *L. monocytogenes*. Up to 3.3 Log reduction was observed during the preparation of sand inoculum.

Survival of *Salmonella* on dried peaches. Figure 2.1 and Table 2.3 show the behavior of *Salmonella* on the dried peaches. The *Salmonella* population on the wet-inoculated peaches and peaches with sulfur immediately after inoculation were 9.68 ± 0.03 and 9.61 ± 0.07 log

CFU/g respectively (TSAR). The *Salmonella* population on wet-inoculated peaches after the 2-day drying was 9.33 ± 0.37 log CFU/g (TSAR) on peaches made without sulfur treatment. For peaches made with sulfur treatment, 9.02 ± 0.40 log CFU/g (TSAR) of *Salmonella* survived after the 2-day drying period performed at the ambient temperature. No significant difference was observed between the surviving *Salmonella* populations recovered from the wet-inoculated peaches made with and without sulfur treatment.

After drying, inoculated samples were stored at refrigerated and ambient temperatures. At ambient temperature, the *Salmonella* population decreased to 8.76 ± 0.05 log CFU/g (0.69 log reduction) on Day 5 at 5 °C and further decreased to 7.59 ± 0.08 log CFU/g on Day 15 (additional 1.17 log reduction) on peaches made without sulfur. When comparing that with the samples stored at ambient temperature, a 2-log reduction was observed on Day 5 while no significant reduction was observed from Day 5 to Day 15. Similar trend was observed from the peaches made with sulfur treatment. During the first 5 days of storage, a 2-log reduction was observed from samples stored at 5 °C while 1.71 log reduction was observed from samples stored at 20 °C. However, on Day 15, while there were still 5.56 ± 0.05 log CFU/g of *Salmonella* surviving on peaches made with sulfur treatment when stored at 5 °C; *Salmonella* surviving on samples stored at 20 °C fell below the limit of detection (1.9 Log CFU/g). When looking at the dried peaches made without sulfur, *Salmonella* survived at higher numbers for longer periods of time when samples were stored at 5 °C for 180 days. When the same samples were stored at 20 °C, the level of surviving *Salmonella* cells fell below the limit of detection after 90 days of storage although they could still be detected via enrichment until Day 180. The presence of sulfur speeded up the die-off rates of *Salmonella* and this impact of sulfur was observed from

both storage temperatures. Inoculated *Salmonella* fell below the limit of detection on Day 60 when the storage temperature was 5 °C and fell below the limit of detection on Day 15 when the storage temperature was at 20 °C. Most importantly, no suspect *Salmonella* was detected even after enrichment, indicating the strong antimicrobial effect of sulfur treatment.

Dry-inoculation, due the cell loss during the drying of inoculated sand, had lower initial inoculation levels. On Day 0 (before storage), there were 7.15 ± 0.11 log CFU/g (TSAR) of *Salmonella* on dried peaches. Storage temperatures directly impacted the die-off pattern of *Salmonella*. Taking dried peaches made without sulfur as an example, *Salmonella* survived the entire 180 days of storage with the final level of 4.38 ± 0.08 log CFU/g when the storage temperature was 5 °C. When the storage temperature was 20 °C, *Salmonella* fell below the limit of detection after 90 days of storage although they could still be recovered by enrichment from two of six samples that were tested. The presence of sand (or the dry-inoculation method) together with the presence of sulfur speeded up the die-off of *Salmonella*. The impact of dry inoculation might be due the low initial inoculation level or the limit nutrient available when *Salmonella* cells were dried on the surface of the sand. As shown in Figure 2.1D and Table 2.3, *Salmonella* fell below the limit of detection after 30 days of storage at 5 °C and could no longer be detected even by enrichment. When the storage temperature was at 20 °C, *Salmonella* fell below the limit of detection and could not be detected by enrichment after 15 days of storage.

Injured cells were observed starting from Day 0. The difference between the counts obtained from TSAR and XLT-4R indicated the formation of injured cells. These differences were as big as 3.68 log CFU/g (sand-inoculated dried peaches made with sulfur stored at 5 °C on Day 5).

Survival of *E. coli* O157:H7 on dried peaches. Figure 2.2 and Table 2.4 show the behavior of *E. coli* O157:H7 survival on the dried peaches. The *E. coli* O157:H7 population on the wet-inoculated peaches and peaches with sulfur immediately after inoculation were 9.40 ± 0.32 and 9.43 ± 0.45 log CFU/g respectively (TSAR). The initial (Day 0) *E. coli* population on the wet-inoculated dried peaches was 8.70 ± 0.26 log CFU/g (TSAR). After 5 days of storage, approximately 1 log reduction was observed from the inoculated dried peaches made without sulfur and stored at 5 and 20 °C. From Day 5 to Day 15, greater reduction was observed from peaches made without sulfur that were stored at 20 °C. A 3.53 log reduction was observed from 20 °C, while 0.97 log reduction was observed from samples stored at 5 °C (both based on the TSAR counts). From Day 15 to 60, while *E. coli* O157:H7 further declined to 3.77 ± 0.40 log CFU/g on inoculated-dried peaches without sulfur stored at ambient temperature and maintained at similar levels from Day 15 to Day 60. The surviving *E. coli* O157:H7 on wet-inoculated dried peaches made without sulfur fell below the limit of detection after 90 days of storage at both refrigerated and ambient temperatures and could no longer be detected by enrichment after 150 days of storage. The addition of sulfur speeded up the die-off of *E. coli* O157:H7. When the storage temperature was at 5 °C, *E. coli* O157:H7 on dried peaches made with sulfur decreased to 5.74 ± 0.12 log CFU/g after 15 days of storage. After additional 15 days (Day 30), *E. coli* O157:H7 could not be detected by neither directly plating nor enrichment. When being stored at ambient temperature, *E. coli* O157:H7 fell below the limit of detection after 15 days of storage and could not be detected via enrichment after 30 days of storage.

Similar to what was observed from *Salmonella*, lower initial inoculation levels were seen from sand-inoculated samples. When the storage temperature was 5 °C, *E. coli* O157:H7

presence on dry-inoculated peaches made without sulfur gradually decreased from 6.52 ± 0.45 log CFU/g to 3.27 ± 0.06 log CFU/g (TSAR) on Day 120. No *E. coli* O157:H7 could be detected via plating or enrichment on Day 180. When the storage temperature was 20 °C*, E. coli* O157:H7 only decreased by approximately 1.3 log by Day 60. After Day 60, a sharp decrease was seen on Day 90 as no *E. coli* O157:H7 can be detected by plating. The pathogen can be detected by enrichment until Day 150. No pathogen can be detected on Day 180.

When looking at the dry-inoculated peach made with sulfur treatment, a 2.15 log reduction was observed in the first 5 days during the storage at 5 °C (TSAR, 6.15 log to 4.00 log). While *E. coli* O157:H7 could still be detected by enrichment on Day 15, it could not be detected after Day 30. Storing at ambient temperature increased the reduction see on Day 5. Greater than 4.25 log reduction was observed in the first 5 days*. E. coli* O157:H7 could not be detected after 15 days of storage. The differences between counts obtained from TSAR and MACR were also observed due to the formation of injured cells during inoculation and storage.

Survival of *L. monocytogenes* on dried peaches. Figure 2.3 and Table 2.5 show the behavior of *L. monocytogenes* survival on the dried peaches. The *L. monocytogenes* population on the wet-inoculated peaches and peaches with sulfur immediately after inoculation were 9.52 ± 0.70 and 9.54 ± 0.34 log CFU/g respectively (TSAR). The initial (Day 0) *L. monocytogenes* population on the wet-inoculated dried peaches was 8.57 ± 0.04 and 8.20 ± 0.23 log CFU/g as determined on TSAR and MOXR respectively. When the storage temperature was 5 °C, *L. monocytogenes* decreased to 7.92 ± 0.06 (TSAR) and maintained at similar levels for the between Day 5 to Day 90. When the storage temperature was 20 °C, a 2-log reduction (TSAR) was observed during the first 5-day of storage. Another sharp decreasing of survival *L.*

monocytogenes numbers was observed between Day 30 and Day 60. An approximately 2.4 log reduction was seen from TSAR. Starting from Day 90, *L. monocytogene*s could not be recovered by either direct plating nor enrichment on peaches made without sulfur. The pre-drying sulfur treatment sped up the die-off of *L. monocytogenes*. Starting from Day 15, *L. monocytogenes* could not be recovered from any dried peaches made with sulfur treatment stored at both temperatures.

Dry inoculation yielded lower initial inoculation levels compared with wet inoculation. There were 6.56 ± 0.09 log CFU/g (TSAR) of *L. monocytogenes* on peaches made without sulfur treatment and 5.81 ± 0.03 log CFU/g (TSAR) of *L. monocytogenes* on peaches made with sulfur treatment. This lower inoculation level together with the presence of sulfur led to a significant reduction of *L. monocytogenes* (greater than 3.91 log CFU/g) during the first 5 days of storage regardless of the storage temperatures. Starting from Day 5*, L. monocytogenes* could only be detected from dry-inoculated dried peaches made with sulfur treatment by enrichment. With dried peaches made without sulfur treatment, the surviving *L. monocytogenes* gradually decreased from Day 0 to Day 120 when the storage temperature was at 5 °C. Starting from Day 150, *L. monocytogenes* could no longer be recovered from dry-inoculated dried peaches without sulfur treatment. When the storage temperature was at 20 °C, *L. monocytogenes* could not be detected by either plating nor enrichment starting from Day 120.

Sulfur measurement. The amount of total sulfur dioxide and free SO_2 was measured during the first 30 days of ambient storage (Table 2.6). The measurement was suspended after Day 30 due to the “shelter-in-place” order placed in March 2020. As shown in Table 2.6, the initial level of free SO_2 and total SO_2 in the dried peaches made with sulfur treatment were 830

± 32 mg/Kg and 2,108 ± 32 mg/Kg respectively. The wet inoculation led to a loss of approximately 122 mg/kg of free SO_2 and approximately 73 mg/kg of total SO_2. The 2-day drying after inoculation only impacted the total SO_2 level and didn't impact the free SO_2 level (comparing Day 0 vs. Day -2). No significant change was observed in free SO_2 from Day 0 to Day 5. A significant loss of free SO_2 was seen from Day 5 to Day 15 (620 mg/kg vs. 373 mg/kg). On Day 30, there were 393 ± 46 mg/kg of free SO_2 and 1,544 ± 12 mg/kg of total SO_2 present in these dried peaches.

2.4 Discussion

The survival of common bacterial pathogens, *Salmonella, E. coli* O157:H7, and *L. monocytogenes,* was monitored on dried peaches made without and with sulfur pre-drying treatment. Two inoculation carriers were applied, and the two storage temperatures were tested.

The pre-drying sulfur treatment had significant impact on the survival of these pathogens. This impact is applicable to all pathogens tested. For example, *Salmonella* could not be recovered by enrichment from wet-inoculated dried peaches made with sulfur treatment after 60 days of storage at 5 °C and 15 days of storage at 20 °C. When they were inoculated onto dried peaches made without sulfur treatment, there were 4.95 ± 0.07 log CFU/g of *Salmonella* survived on these samples by the end of 180 days of storage at 5 °C. Even when the storage temperature was at 20 °C, *Salmonella* was still detected via enrichment from dried peaches made without sulfur. The impact of sulfur treatment on the survival of pathogens was also reported by Liu et al. (2021). Similarly, no *Salmonella* cell was recovered from sulfur-treated apricots (initial inoculation level 6.5 log CFU/g) via enrichment after 90 days of storage

at 22 °C, while ~2.5 log CFU/g of *Salmonella* was recovered from dried apricots made without sulfur dioxide (Liu et al., 2021). Although sulfur dioxide treatment facilitates bacterial die-off, it has the potential to induce asthmatic reactions in some people (Alp & Bulantekin, 2021). The free SO_2 levels detected in dried peaches made with sulfur used in this study were higher than previously reported numbers. Although the processors did label the packages with "made with sulfur treatment", additional studies may be needed to gain insight into sulfur levels present in samples sold at the farmers markets and the changes of free and total SO_2 during storage.

Storage temperature is another factor that generates a significant impact on pathogen survival. In general, pathogens survived at a higher level for longer period of time at low temperatures (5 °C) than ambient temperature. For example, *Salmonella* survived on dried peaches made without sulfur at 5 °C for up to 180 days with a final level of 4.59 log CFU/g on wet-inoculated ones and 4.38 ± 0.08 log CFU/g on dry-inoculated ones. On the same samples stored at ambient temperature, *Salmonella* could only be detected via enrichment after 90 days of storage, indicating the surviving level was below 1.9 Log CFU/g. Cuzzi, et al. (2021) found similar results. In their study, *L. monocytogenes* was inoculated onto dried applies, strawberries and raisins with sand and stored at 4 °C (25-81% relative humidity) and 23 °C (30-35% RH). Since *L. monocytogenes* could not be recovered from inoculated dried apples at Day 0 (inoculation day), only the survival in dried strawberries and raisins were monitored in this study (Cuzzi et al., 2021). When the storage temperature was at 23 °C, *L. monocytogenes* decreased rapidly by greater than 4 and 3.6 log CFU/g after 14 and 7 days of storage on raisins and dried strawberries. However, when the storage temperature was at 4 °C, *L. monocytogenes* only decreased by approximately 0.1 and 0.2 log CFU/g/month. After 336 days of storage, *L.*

monocytogenes only decreased by 1.4 and 3.1 log CFU/g on raisins and strawberries, respectively.

The impact of inoculation carriers on the survival of pathogen was completed by the fact that different carriers led to, sometimes, different initial inoculation levels before storage. For example, the wet inoculation brought 9.45 ± 0.06 log CFU/g (TSAR) of *Salmonella* on dried peaches made without sulfur on Day 0, while the dry inoculation had an initial inoculation level of 7.26 ± 0.14 Log CFU/g (TSAR) to dried peaches. Lower initial inoculation levels from sand inoculated samples were also seen for *E. coli* O157:H7. In this case, the impact of carriers can not be fully studied. In the study conducted by L. R. Beuchat and Mann (2014), two inoculation methods were used to inoculate the dried fruits. One was misting dried fruits with an aqueous suspension of a 5-serotype cocktail of *Salmonella*, and the other was mixing the dried fruits with sand on which a 5-serotype cocktail had been dried. Authors found that the survival of *Salmonella* on dried cranberries, raisins, strawberries, and date paste inoculated using the dry carrier (sand) and wet carrier (mist) followed similar trends. In the study conducted by Feng et al. (2018), plate-grown *E. coli* O157:H7 were inoculated onto in-shell hazelnuts via wet or dry carriers (buffered peptone water vs. sand). After that, samples were stored at 24 ± 1 °C for 12 months. Their results showed that *E. coli* O157:H7 reduced rapidly on sand-inoculated hazelnut than wet-inoculated ones, although the initial inoculation levels before storage were similar (~6.5 log CFU/nut). In the study conducted by Liu et al. (2021), *Salmonella* was inoculated onto dried apricots made with or without sulfur. The liquid inoculum was diluted for wet inoculation so that the same initial inoculation levels were achieved for both wet- and dry-inoculated dried apricots. Based on their results, *Salmonella* survived at higher levels for longer period of time

on sand-inoculated dried apricots. This indicated that after exposing to the drying stress during sand inoculum preparation, this pre-storage stress response enhanced the survivability of *Salmonella* during storage. This also indicated the impact of inoculation carriers on the survival of pathogens.

It is important to point out that the types of media determined the pathogen counts. The differences observed between selective and non-selective agar indicate the formation of stressed or injured cells during storage. For example, in the wet-inoculated peaches made with sulfur treatment, after 5 days of storage at 5 °C, there were 7.21 log CFU/g of *L. monocytogenes* recovered from TSAR while only 5.32 log CFU/g of *L. monocytogenes* recovered from MOXR, indicating the formation of approximately 1.89 log CFU/g of stressed or injured cells. The difference between selective and non-selective agar could show up as early as Day 0. For example, when looking into Table 2.4, when *E. coli* O157:H7 was wet-inoculated onto dried peaches made without sulfur treatment, the differences between TSAR and MACR were up to 1.47 log CFU/g (5 °C) and up to 1.76 log CFU/g (~22 °C). Sometimes, differences between the selective agar counts and the non-selective agar counts increased as the storage length increased. For example, in the same table (Table 2.4), on dry-inoculated peaches made without sulfur treatment, when the storage temperature was at 20 °C, the differences between selective and non-selective agar were 1.26 log CFU/g on Day 0 (6.52 vs. 5.26 log CFU/g) and 2.65 on Day 60 (5.28 vs. 2.63 log CFU/g). The difference between selective and non-selective agar counts were seen in the studies conducted by Blessington et al. (2013). Microorganisms exist in different metabolic states and growth phases under various environmental conditions. Active replication of cells is not always included in all phases (Davis, 2014). The formation of the

viable but non-culturable (VBNC) state and the sub-lethally injured cells pose distinct threat to food safety as the conventional culturing methods might under-estimate the pathogen levels and injured cells might regain their ability to be cultured and cause infections in humans (Schottroff et al., 2018; Espina et al., 2016). Although the use of both selective agar and non-selective agar help us minimize the chance of underestimating surviving cells in this study, other detection methods, such as molecular-based detection protocols, may also needed in future studies to better evaluate the surviving levels of various pathogens.

Table 2.1. Pathogen parent strains used for inoculation of dried fruits. All isolates used for this study were resistant to > 50ug/L rifampicin.

Organism	Strain	Source	Date Received
E. coli O157:H7	Odwalla strain#223	Odwalla outbreak (apple juice)	November 11th 2018
E. coli O157:H7	EC4042	Clinical from spinach outbreak	November 11th 2018
E. coli O157:H7	EC1738: lot 550659	Cookie dough	November 11th 2018
E. coli O157:H7	H1730	Clinical isolate from lettuce outbreak	November 11th 2018
E. coli O157:H7	F658	Clinical from cantaloupe outbreak	November 11th 2018
L. monocytogenes 4b or 1/2c Confirmed Serotype	LCDC81-861	Raw cabbage associated outbreak	November 11th 2018
L. monocytogenes Scott A 4b Confirmed Serotype	LJH1223	Clinical from milk associated outbreak	November 11th 2018
L. monocytogenes V7 1/2a Confirmed Serotype	LJH1224	Milk associated outbreak	November 11th 2018
L. monocytogenes 101M 4b Confirmed Serotype	LJH1225	Beef associated outbreak	November 11th 2018
L. monocytogenes	LJH1229	Tomatoes	November 11th 2018
Salmonella Enteritidis PT30	ATCCBAA-1045	Raw almonds associated with an outbreak	November 11th 2018
Salmonella Tennessee	K4643	Clinical isolate associated with peanut butter outbreak	November 11th 2018
Salmonella Montevideo	FDA-2010-149-pistachios2	Pistachios from FDA	November 11th 2018
Salmonella Saintpaul	LJH1375	DFA walnut Salmonella survey	November 11th 2018
Salmonella Gaminara	F2712	Orange juice outbreak 1995	November 11th 2018

Table 2.2. Bacterial populations in inoculated sand (log CFU/g).

	Salmonella				*E. coli* O157:H7				*L. monocytogenes*			
	Right after inoculation before drying		Final inoculation level		Right after inoculation before drying		Final inoculation level		Right after inoculation before drying		Final inoculation level	
	TSAR	XLT-4R	TSAR	XLT-4R	TSAR	MACR	TSAR	MACR	TSAR	MOXR	TSAR	MOXR
Sand	10.08 ± 0.12	8.71 ± 0.19	6.70 ± 0.45	6.41 ± 0.23	9.62 ± 0.54	8.46 ± 0.22	5.47 ± 0.42	4.30 ± 0.73	8.76 ± 0.26	8.66 ± 0.05	6.45 ± 0.49	6.10 ± 0.47

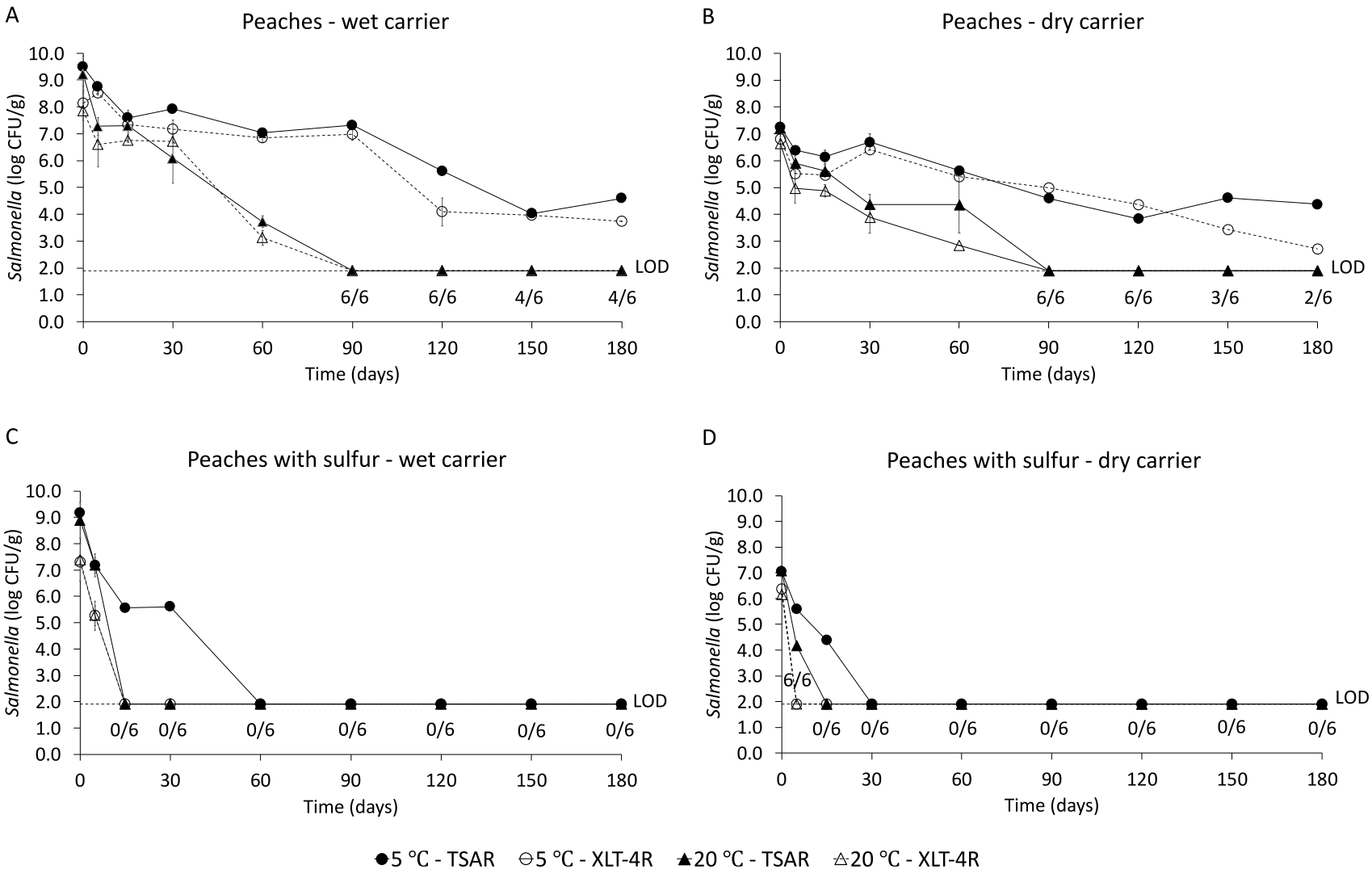

Figure 2.1. Survival of *Salmonella* in dried peaches. TSAR: tryptic soy agar with rifampicin. XLT-4R: Xylose lysine tergitol 4 agar with rifampicin. LOD: limit of detection of 1.9 log CFU/g.

Table 2.3. Survival of *Salmonella* in dried peaches. These data are also shown in Figure 2.1.

		***Salmonella* spp. (log CFU/g)**							
		wet inoculated				**dry inoculated**			
		dried peaches		**dried peaches with sulfur**		**dried peaches**		**dried peaches with sulfur**	
temp	**time (days)**	**TSAR**	**XLT-4R**	**TSAR**	**XLT-4R**	**TSAR**	**XLT-4R**	**TSAR**	**XLT-4R**
5°C	0	9.45 ± 0.06^{Aa}	8.13 ± 0.65^{Ab}	9.16 ± 0.12^{Aa}	7.30 ± 0.15^{Ab}	7.26 ± 0.14^{Aa}	6.81 ± 0.26^{Ab}	7.05 ± 0.04^{Aa}	6.37 ± 0.09^{b}
	5	8.76 ± 0.05^{Ba}	8.51 ± 0.07^{Bb}	7.18 ± 0.30^{Ba}	5.27 ± 0.38^{Bb}	6.39 ± 0.04^{Ba}	5.52 ± 0.04^{Bb}	5.58 ± 0.12^{B}	< 1.9
	15	7.59 ± 0.08^{Ca}	7.25 ± 0.35^{Ca}	5.56 ± 0.05^{C}	< 1.9	6.15 ± 0.25^{Ca}	5.46 ± 0.04^{Bb}	4.38 ± 0.14^{C}	< 1.9
	30	7.90 ± 0.13^{Da}	7.09 ± 0.33^{CDb}	5.61 ± 0.13^{C}	< 1.9	6.70 ± 0.30^{Ba}	6.41 ± 0.16^{Ca}	< 1.9	< 1.9 [0/6]
	60	7.03 ± 0.04^{Ea}	6.83 ± 0.17^{Db}	< 1.9	< 1.9 [0/6]	5.63 ± 0.10^{Da}	5.41 ± 0.15^{Bb}	< 1.9	< 1.9 [0/6]
	90	7.31 ± 0.02^{Fa}	6.93 ± 0.22^{CDb}	< 1.9	< 1.9 [0/6]	4.60 ± 0.07^{Ea}	4.99 ± 0.13^{Db}	< 1.9	< 1.9 [0/6]
	120	5.61 ± 0.09^{Ga}	4.09 ± 0.52^{Eb}	< 1.9	< 1.9 [0/6]	3.84 ± 0.13^{Fa}	4.37 ± 0.12^{Eb}	< 1.9	< 1.9 [0/6]
	150	4.01 ± 0.15^{Ha}	3.96 ± 0.03^{Ea}	< 1.9	< 1.9 [0/6]	4.62 ± 0.08^{Ea}	3.44 ± 0.05^{Fb}	< 1.9	< 1.9 [0/6]
	180	4.59 ± 0.07^{Ia}	3.73 ± 0.09^{Fb}	< 1.9	< 1.9 [0/6]	4.38 ± 0.08^{Ga}	2.72 ± 0.02^{Gb}	< 1.9	< 1.9 [0/6]
20°C	0	9.21 ± 0.69^{Aa}	7.85 ± 0.77^{Ab}	8.89 ± 0.68^{Aa}	7.39 ± 0.71^{Ab}	7.23 ± 0.07^{Aa}	6.64 ± 0.27^{Ab}	7.07 ± 0.06^{Aa}	6.15 ± 0.38^{b}
	5	7.28 ± 0.33^{Ba}	6.60 ± 0.83^{ABa}	7.18 ± 0.44^{Ba}	5.27 ± 0.55^{Bb}	5.91 ± 0.34^{Ba}	4.97 ± 0.54^{Bb}	4.18 ± 0.03^{B}	< 1.9
	15	7.30 ± 0.57^{Ba}	6.74 ± 0.07^{Bb}	< 1.9	< 1.9 [0/6]	5.61 ± 0.23^{Ba}	4.87 ± 0.23^{Bb}	< 1.9	< 1.9 [6/6]
	30	6.08 ± 0.93^{Ca}	6.72 ± 0.06^{Ba}	< 1.9	< 1.9 [0/6]	4.37 ± 0.37^{Ca}	3.90 ± 0.59^{Cb}	< 1.9	< 1.9 [0/6]
	60	3.73 ± 0.21^{Da}	3.12 ± 0.28^{Cb}	< 1.9	< 1.9 [0/6]	4.36 ± 1.06^{Ca}	2.85 ± 0.17^{Db}	< 1.9	< 1.9 [0/6]
	90	< 1.9	< 1.9 [6/6]	< 1.9	< 1.9 [0/6]	< 1.9	< 1.9 [6/6]	< 1.9	< 1.9 [0/6]
	120	< 1.9	< 1.9 [6/6]	< 1.9	< 1.9 [0/6]	< 1.9	< 1.9 [6/6]	< 1.9	< 1.9 [0/6]
	150	< 1.9	< 1.9 [4/6]	< 1.9	< 1.9 [0/6]	< 1.9	< 1.9 [3/6]	< 1.9	< 1.9 [0/6]
	180	< 1.9	< 1.9 [4/6]	< 1.9	< 1.9 [0/6]	< 1.9	< 1.9 [2/6]	< 1.9	< 1.9 [0/6]

Values are means ± standard deviation. Within each type of dried peach, storage temperature, inoculation method, and plating media (TSAR or XLT-4R), different uppercase letters denote significantly different values ($P < 0.05$) among days of storage. Within each type of dried peach, storage temperature, inoculation method, and storage time, different lowercase letters denote significantly different values between media. [#/6] is number of positive samples out of 6 after enrichment and plating on selective agar.

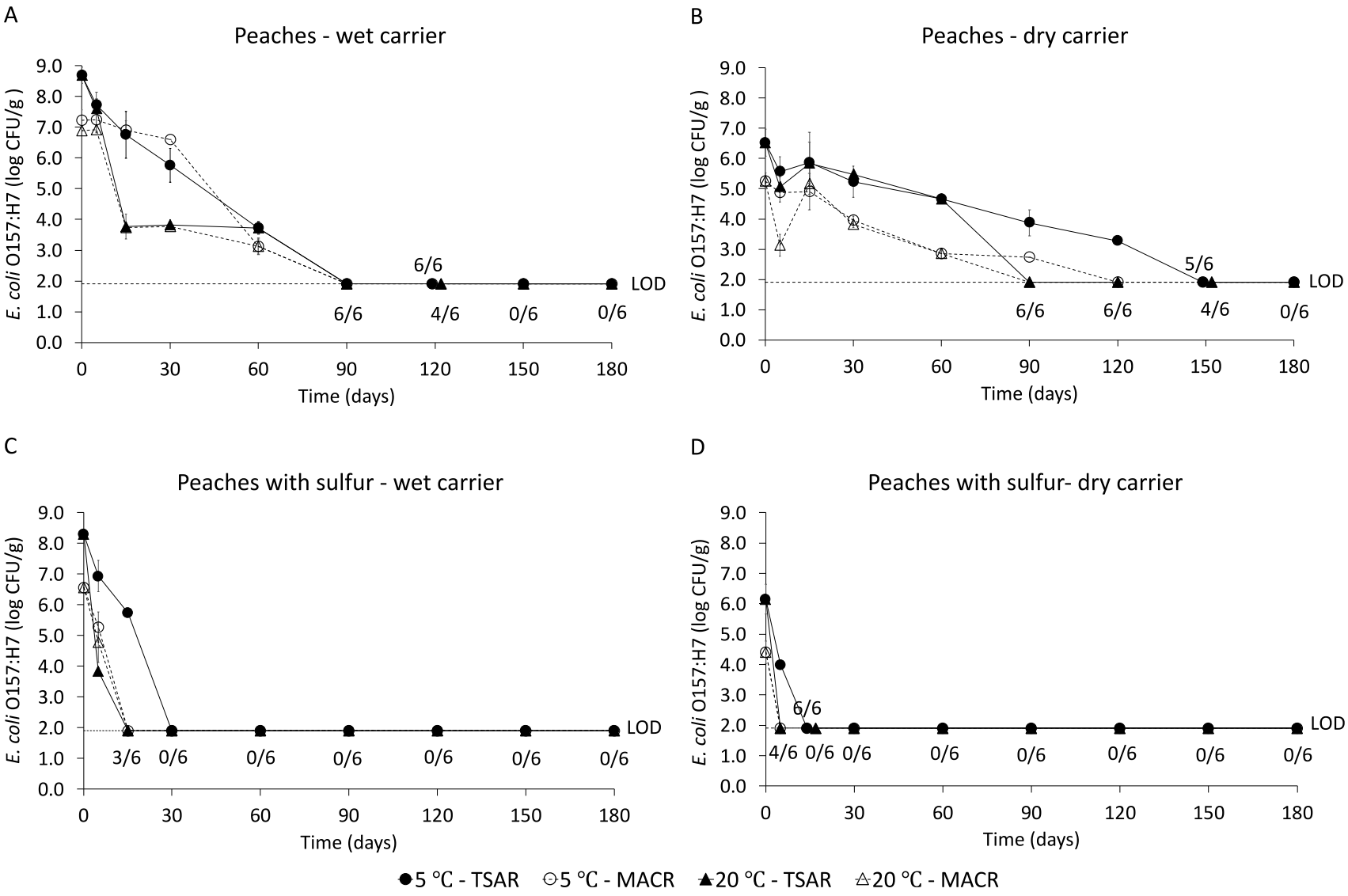

Figure 2.2. Survival of *E. coli* O157:H7 in dried peaches. TSAR: tryptic soy agar with rifampicin. MACR: MacConkey agar with rifampicin. LOD: limit of detection of 1.9 log CFU/g.

Table 2.4. Survival of *E. coli* O157:H7 in dried peaches. These data are also shown in Figure 2.2.

		***E. coli* O157:H7 (log CFU/g)**							
		wet inoculated				**dry inoculated**			
		dried peaches		**dried peaches with sulfur**		**dried peaches**		**dried peaches with sulfur**	
temp	**time (days)**	**TSAR**	**MACR**	**TSAR**	**MACR**	**TSAR**	**MACR**	**TSAR**	**MACR**
5°C	0	8.70 ± 0.26^{Aa}	7.23 ± 0.34^{Ab}	8.29 ± 0.17^{Aa}	7.03 ± 0.34^{Ab}	6.52 ± 0.45^{Aa}	5.26 ± 0.27^{Ab}	6.15 ± 0.49^{Aa}	4.40 ± 0.37^{b}
	5	7.73 ± 0.19^{Ba}	7.24 ± 0.38^{Ab}	6.93 ± 0.52^{Ba}	5.27 ± 0.49^{Bb}	5.57 ± 0.49^{Ba}	3.88 ± 0.32^{Bb}	4.00 ± 0.07^{B}	< 1.9
	15	6.76 ± 0.76^{Ca}	6.91 ± 0.31^{ABa}	5.74 ± 0.12^{C}	< 1.9	5.86 ± 0.67^{Ba}	4.90 ± 0.60^{Cb}	< 1.9	< 1.9 [6/6]
	30	5.76 ± 0.55^{Da}	6.59 ± 0.18^{Bb}	< 1.9	< 1.9 [0/6]	5.23 ± 0.51^{Ca}	3.95 ± 0.15^{BCb}	< 1.9	< 1.9 [0/6]
	60	3.73 ± 0.19^{Ea}	3.12 ± 0.25^{Cb}	< 1.9	< 1.9 [0/6]	4.66 ± 0.05^{CDa}	2.85 ± 0.16^{Db}	< 1.9	< 1.9 [0/6]
	90	< 1.9	< 1.9 [6/6]	< 1.9	< 1.9 [0/6]	3.88 ± 0.43^{DEa}	2.73 ± 0.00^{Eb}	< 1.9	< 1.9 [0/6]
	120	< 1.9	< 1.9 [6/6]	< 1.9	< 1.9 [0/6]	3.27 ± 0.06^{E}	< 1.9	< 1.9	< 1.9 [0/6]
	150	< 1.9	< 1.9 [0/6]	< 1.9	< 1.9 [0/6]	< 1.9	<1.9 [5/6]	< 1.9	< 1.9 [0/6]
	180	< 1.9	< 1.9 [0/6]	< 1.9	< 1.9 [0/6]	< 1.9	<1.9 [0/6]	< 1.9	< 1.9 [0/6]
20°C	0	8.70 ± 0.26^{Aa}	7.23 ± 0.34^{Ab}	8.29 ± 0.17^{Aa}	6.53 ± 0.02^{Ab}	6.52 ± 0.45^{Aa}	5.26 ± 0.27^{Ab}	6.15 ± 0.49^{a}	4.40 ± 0.37^{b}
	5	7.60 ± 0.54^{Ba}	6.93 ± 0.10^{Bb}	3.83 ± 0.13^{Ba}	4.77 ± 0.65^{Bb}	5.07 ± 0.32^{Ba}	3.12 ± 0.36^{Bb}	< 1.9	< 1.9 [4/6]
	15	3.77 ± 0.40^{Ca}	3.73 ± 0.01^{Cb}	< 1.9	< 1.9 [3/6]	5.84 ± 1.03^{ABa}	5.16 ± 0.07^{Aa}	< 1.9	< 1.9 [0/6]
	30	3.82 ± 0.12^{Ca}	3.77 ± 0.08^{Da}	< 1.9	< 1.9 [0/6]	5.52 ± 0.12^{Ba}	3.82 ± 0.11^{Cb}	< 1.9	< 1.9 [0/6]
	60	3.75 ± 0.25^{Ca}	3.24 ± 0.41^{Eb}	< 1.9	< 1.9 [0/6]	5.28 ± 0.60^{Ba}	2.63 ± 0.22^{Bb}	< 1.9	< 1.9 [0/6]
	90	< 1.9	< 1.9 [6/6]	< 1.9	< 1.9 [0/6]	< 1.9	< 1.9 [6/6]	< 1.9	< 1.9 [0/6]
	120	< 1.9	< 1.9 [4/6]	< 1.9	< 1.9 [0/6]	< 1.9	< 1.9 [6/6]	< 1.9	< 1.9 [0/6]
	150	< 1.9	< 1.9 [0/6]	< 1.9	< 1.9 [0/6]	< 1.9	< 1.9 [4/6]	< 1.9	< 1.9 [0/6]
	180	< 1.9	< 1.9 [0/6]	< 1.9	< 1.9 [0/6]	< 1.9	< 1.9 [0/6]	< 1.9	< 1.9 [0/6]

Values are means ± standard deviation. Within each type of dried peach, storage temperature, inoculation method, and plating media (TSAR or MACR), different uppercase letters denote significantly different values ($P < 0.05$) among days of storage. Within each type of dried peach, storage temperature, inoculation method, and storage time, different lowercase letters denote significantly different values between media. [#/6] is number of positive samples out of 6 after enrichment and plating on selective agar.

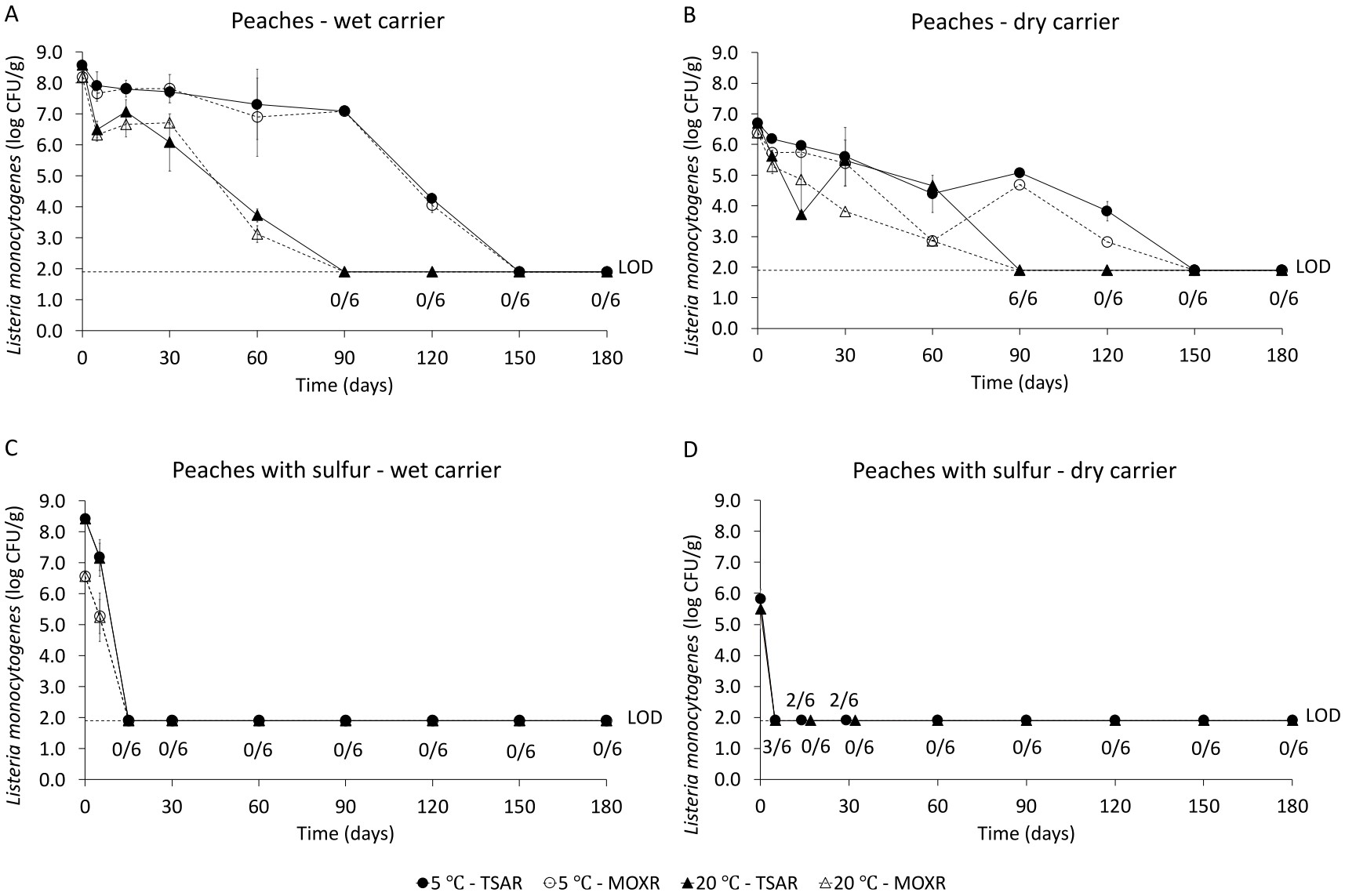

Figure 2.3. Survival of *L. monocytogenes* in dried peaches. TSAR: tryptic soy agar with rifampicin. MOXR: Modified Oxford agar with rifampicin. LOD: limit of detection of 1.9 log CFU/g.

Table 2.5. Survival of *L. monocytogenes* in dried peaches. These data are also shown in Figure 2.3

		***Listeria monocytogenes* (log CFU/g)**							
		wet inoculated				**dry inoculated**			
		dried peaches		**dried peaches with sulfur**		**dried peaches**		**dried peaches with sulfur**	
temp	**time (days)**	**TSAR**	**MOXR**	**TSAR**	**MOXR**	**TSAR**	**MOXR**	**TSAR**	**MOXR**
5°C	0	8.57 ± 0.04^{Aa}	8.20 ± 0.23^{Ab}	8.42 ± 0.06^{Aa}	6.56 ± 0.09^{Ab}	6.71 ± 0.09^{Aa}	6.39 ± 0.20^{Ab}	5.81 ± 0.03^{a}	5.50 ± 0.64^{a}
	5	7.92 ± 0.44^{Ba}	7.67 ± 0.27^{Ba}	7.21 ± 0.46^{Ba}	5.32 ± 0.68^{Bb}	6.19 ± 0.04^{Ba}	5.74 ± 0.08^{Bb}	< 1.9	< 1.9 [3/6]
	15	7.81 ± 0.13^{Ba}	7.82 ± 0.27^{ABa}	< 1.9	< 1.9 [0/6]	5.96 ± 0.07^{Ca}	5.75 ± 0.08^{Bb}	< 1.9	< 1.9 [2/6]
	30	7.71 ± 0.18^{Ba}	7.82 ± 0.45^{ABCa}	< 1.9	< 1.9 [0/6]	5.61 ± 0.97^{Ca}	5.39 ± 0.76^{ABa}	< 1.9	< 1.9 [2/6]
	60	7.31 ± 1.14^{ABCa}	6.89 ± 1.27^{ABCa}	< 1.9	< 1.9 [0/6]	4.39 ± 0.61^{Da}	2.85 ± 0.17^{Cb}	< 1.9	< 1.9 [0/6]
	90	7.09 ± 0.06^{Ca}	7.09 ± 0.02^{Ca}	< 1.9	< 1.9 [0/6]	5.08 ± 0.03^{Ea}	4.70 ± 0.09^{Db}	< 1.9	< 1.9 [0/6]
	120	4.26 ± 0.18^{Da}	4.05 ± 0.23^{Da}	< 1.9	< 1.9 [0/6]	3.82 ± 0.31^{Da}	2.82 ± 0.12^{Cb}	< 1.9	< 1.9 [0/6]
	150	< 1.9	< 1.9 [0/6]	< 1.9	< 1.9 [0/6]	< 1.9	<1.9 [0/6]	< 1.9	< 1.9 [0/6]
	180	< 1.9	< 1.9 [0/6]	< 1.9	< 1.9 [0/6]	< 1.9	<1.9 [0/6]	< 1.9	< 1.9 [0/6]
20°C	0	8.57 ± 0.04^{Aa}	8.20 ± 0.23^{Ab}	8.42 ± 0.06^{Aa}	6.56 ± 0.09^{Ab}	6.71 ± 0.09^{Aa}	6.39 ± 0.20^{Ab}	5.81 ± 0.03^{a}	5.50 ± 0.64^{a}
	5	6.50 ± 0.28^{Ba}	6.32 ± 0.20^{Ba}	7.14 ± 0.59^{Ba}	5.23 ± 0.78^{Bb}	5.63 ± 0.11^{Ba}	5.29 ± 0.24^{Bb}	< 1.9	< 1.9 [3/6]
	15	7.08 ± 0.38^{Ca}	6.66 ± 0.40^{Ba}	< 1.9	< 1.9 [0/6]	3.72 ± 0.12^{Ca}	4.87 ± 1.09^{Ca}	< 1.9	< 1.9 [0/6]
	30	6.11 ± 0.94^{BCa}	6.72 ± 0.08^{Ba}	< 1.9	< 1.9 [0/6]	5.48 ± 0.02^{Ba}	3.82 ± 0.11^{Cb}	< 1.9	< 1.9 [0/6]
	60	3.75 ± 0.18^{Da}	3.24 ± 0.34^{Ca}	< 1.9	< 1.9 [0/6]	4.66 ± 0.05^{Da}	3.02 ± 0.32^{Cb}	< 1.9	< 1.9 [0/6]
	90	< 1.9	< 1.9 [0/6]	< 1.9	< 1.9 [0/6]	< 1.9	<1.9 [6/6]	< 1.9	< 1.9 [0/6]
	120	< 1.9	< 1.9 [0/6]	< 1.9	< 1.9 [0/6]	< 1.9	<1.9 [0/6]	< 1.9	< 1.9 [0/6]
	150	< 1.9	< 1.9 [0/6]	< 1.9	< 1.9 [0/6]	< 1.9	<1.9 [0/6]	< 1.9	< 1.9 [0/6]
	180	< 1.9	< 1.9 [0/6]	< 1.9	< 1.9 [0/6]	< 1.9	<1.9 [0/6]	< 1.9	< 1.9 [0/6]

Values are means ± standard deviation. Within each type of dried peach, storage temperature, inoculation method, and plating media (TSAR or MOXR), different uppercase letters denote significantly different values ($P < 0.05$) among days of storage. Within each type of dried peach, storage temperature, inoculation method, and storage time, different lowercase letters denote significantly different values between media. [#/6] is number of positive samples out of 6 after enrichment and plating on selective agar.

Table 2.6. Levels of sulfur dioxide in dried peaches processed with sulfur. Difference in uppercase letters means significant difference between free SO_2 ($P < 0.05$). Difference in lowercase letters means significant difference between total SO_2 ($P < 0.05$).

Day	Free SO_2 (mg/Kg)	Total SO_2 (mg/Kg)
before inoculation	830 ± 32 A	2,108 ± 32 a
-2	688 ± 15 B	2,035 ± 26 b
0	625 ± 77 B	1,748 ± 11 c
5	620 ± 75 B	1,556 ± 97 d
15	373 ± 15 C	1,735 ± 25 c
30	393 ± 46 D	1,544 ± 12 d

Chapter 3: Pathogen survival on dates, dried pluots and sundried tomatoes

Note: Parts of this chapter have been submitted to the journal of "LWT-Food Science and Technology" and is currently under review.

The global production of dried fruits increased from 2,246,739 metric tons in 2009/2010 to 3,222,767 metric tons in 2019/2020 (International Nut & Dried Fruit Council, 2020). In California, dried fruits are economically valuable specialty crops and 1,174,000 tons of raisins, 325,500 tons of dried prunes, 11,000 tons of dried apricots, and 6,900 tons of dried freestone peaches were produced in 2017 (California Department of Food and Agriculture, 2020). Among different dried fruits, table dates, which accounted for 35% of world dried fruit production, showed the most significant increase over the last decade (International Nut & Dried Fruit Council, 2020). The consumption of dates also has the most significant increase among all dried fruits in the past 10 years (International Nut & Dried Fruit Council, 2020). Medjool dates are one of the major varieties in the United States (Vayalil, 2012). Medjool dates have also been involved in a recent recall due to possible contamination with hepatitis A (Food Standards Agency, 2021). Two types of Medjool dates prepared with different drying methods were used in this study. Low-moisture dates are dates allowed to continue drying in nets after falling off the tree; high-moisture dates are harvested directly from the tree and have softer texture compared with the low-moisture ones.

In addition to dates, the other two dried fruits, dried pluots made with sulfur dioxide treatment and sundried tomatoes were also used in this study. California is the major domestic supplier of sundried tomatoes in the United States (And & Barrett, 2006). Unfortunately, sundried tomatoes were involved in a foodborne outbreak of hepatitis A (Carvalho et al., 2012).

Sulfur dioxide treated dried pluots were also chosen in this study due to its lower pH compared with dates and sundried tomatoes.

Dried fruits can get contaminated throughout the production and supply chain from various sources, such as raw materials, processing equipment, and personnel handling (Nerín et al., 2016; Soon at al., 2020). Pathogens such as *S*. Typhimurium and *Listeria monocytogenes* have been detected on freshly harvested pears (Duvenage et al., 2017). In a 2014-2015 listeriosis outbreak associated with caramel apples, outbreak isolates of L. monocytogenes were recovered from whole apples (Angelo et al., 2017). *Listeria* spp. including *L. monocytogenes* have been repeatedly detected from fruits or other produce processing facilities (Ruiz et al., 2021; Simonetti et al., 2021; Sullivan & Wiedmann, 2020). Cross-contamination from fruit surfaces to the edible portions can occur during peeling, cutting, and slicing (Erickson et al., 2015; Jung et al., 2017; Qi et al., 2020).

3.1 Objective

The objective of this chapter was to evaluate the survival of three foodborne pathogens, including *Salmonella, E. coli* O157:H7, and *L. monocytogenes* on dates, dried pluots and sundried tomatoes.

3.2 Materials and Methods

Inoculation and samples collection of dried fruits with wet and dry carriers. Sundried tomatoes, dried pluots processed with sulfur, high- and low-moisture Medjool dates were inoculated and sampled in the same manner as the dried peaches in Chapter 2 (including enrichment). Dried pluots processed with sulfur and sundried tomatoes were inoculated using the wet carrier. High-moisture Medjool dates and low-moisture Medjool dates were inoculated

using the dry carrier. Triplicate samples for microbial enumeration were collected on the day of inoculation, after 48 h of drying (Day 0), Day 5, Day 15, Day 30, and then every 30 days after that for a total of 6 months. Two trials of the survival study were performed (n=6).

Water activity and pH measurement. pH and aw were measured following protocols described in Liu et al. (2021). Briefly, for aw measurement, dried fruits were first cut into slices and the a_w was measured with the water activity meter. To measure the pH of the dried fruits, 10 g of each dried fruit were homogenized with 4 mL of MilliQ water in a 24-oz filter bag using the Smasher at fast speed for 1 min. The pH was then measured using the pH meter. All measurements were repeated three times at each sampling or testing point.

Microbiological analysis. At each sampling point, three 25-g subsamples were taken from each pathogen × dried fruit combination. Each subsample was combined with 100 mL of PBS in a 24-oz filter bag. The mixture was then homogenized in the Smasher for 1 min, serially diluted with PBS, and spirally plated onto TSAR and selective agar. The same sampling, homogenizing and plating methods were followed as described in Chapter 2. Xylose Lysine Tergitol 4 agar with 50 µg/mL of rifampicin (XLT-4R), MacConkey agar with 50 µg/mL of rifampicin (MACR), and Modified Oxford agar with 50 µg/ml of rifampicin (MOXR) for *Salmonella* spp., *E. coli* O157:H7, and *L. monocytogenes*, respectively.

Statistical analysis. Triplicate samples were analyzed in each of the two trials of the survival study (n=6). Means comparisons were performed using Excel to determine whether sampling day and type of media had significant impact on pathogen survival, pH, and a_w. Analysis of variance (ANOVA), and Tukey multiple comparison test were performed using R studio to determine whether sampling day, storage temperature, or media significantly impact

pathogen survival, as well as sampling day, type of dried fruit, and storage temperature significantly impact pH and a_w. Differences between mean values were considered significant at $P < 0.05$.

3.3 Results

Survival of *Salmonella* on various dried fruits. Figure 3.1 and Table 3.1 summarize the survival of *Salmonella* on two types of dates, dried pluots, and sundried tomatoes. *Salmonella* was dry inoculated onto dates. The initial inoculation levels were 6.92 ± 0.03 and 6.43 ± 0.07 log CFU/g (TSAR) for low- and high-moisture dates respectively. S*almonella* gradually decreased on both low- and high-moisture dates at 5 °C. At the end of storage (Day 180), 5.31 ± 0.16 log CFU/g (TSAR) of *Salmonella* were still recovered from low-moisture dates. When the storage temperature increased to 20 °C, a greater than 2.5 log reduction was seen from Day 60 to Day 90. By Day 150, no *Salmonella* could be recovered from inoculated high- or low-moisture dates by enrichment.

Salmonella decreased much more rapidly when inoculated onto dried pluots and sundried tomatoes. The *Salmonella* population on the wet-inoculated dried pluots and sundried tomatoes immediately after inoculation were 9.39 ± 0.32 and 9.73 ± 0.14 log CFU/g respectively (TSAR). The initial (Day 0) wet-inoculation levels on dried pluots and sundried tomatoes were 8.09 ± 0.07 and 7.95 ± 0.10 log CFU/g respectively (TSAR). When looking at the dried pluots, a greater than 3 log reduction (TSAR) was observed during the first 5-day of storage. That reduction was even higher if we compare the XLT-4R data (6.79 log CFU/g on Day 0 and <1.9 log CFU/g on Day 5). From Day 5 to Day 15, the population of *Salmonella* further decreased and another sharp decrease in TSAR count was seen on Day 30. On Day 30, *Salmonella* could only be

detected by enrichment. Starting from Day 60, no *Salmonella* could be detected by neither direct plating nor enrichment from dried pluots. The decreasing *Salmonella* was more rapidly when the storage temperature was at 20 °C. Starting from Day 30, no *Salmonella* could be recovered from inoculated dried pluots stored at 20 °C.

The survival of *Salmonella* on sundried tomatoes was better than dried pluots when the storage temperature was at 20 °C. *Salmonella* gradually decreased from Day 0 to Day 30. After that, a greater 4.92 log reduction of *Salmonella* was seen between Day 30 and Day 60. Starting from Day 60, *Salmonella* could no longer be detected even by enrichment from inoculated sundried tomatoes. Another interesting observation made from inoculated sundried tomatoes was the more rapid die-off rate of *Salmonella* when the storage temperature was at 5 °C than at 20 °C. At 5 °C, *Salmonella* can only be counted by using TSAR plate on Day 5. Greater than 4 log of injured cells were formed from Day 0 to Day 5 by showing no colony on XLT-4R and 6.13 log CFU/g from TSAR. Starting from Day 15, *Salmonella* could not be detected by neither direct plating nor enrichment from inoculated sundried tomatoes stored at 5 °C.

Survival *of E. coli* O157:H7. Figure 3.2 and Table 3.2 summarize the survival of *E.* coli O157:H7 on the dried fruits. The initial dry-inoculation level of *E. coli* O157:H7 were 5.46 ± 0.75 and 6.04 ± 0.24 log CFU/g on low- and high-moisture dates respectively (TSAR). The number of surviving *E. coli* O157:H7 on low-moisture dates gradually decreased when the storage temperature was at 5 °C. A 2.61 Log reduction was seen between Day 150 and Day 180. When looking at the selective agar, surviving cells could not be counted on MACR since Day 120, indicating the formation of injured cells during storage. When the storage temperature was at 20 °C, the grater than 2.61 log reduction happened between Day 60 and Day 90, earlier than

samples stored at 5 °C. On Day 180, *E. coli* O157:H7 on low-moisture dates could no longer be recovered by even enrichment.

When comparing the survival of *E. coli* O157:H7 on low-moisture dates than that on high-moisture dates, a great reduction of cell counts obtained from MACR was seen on Day 60 on high-moisture dates while the similar reduction was observed on Day 120 on low-moisture dates (storage temperature 5 °C). At 20 °C, that reduction was seen on Day 30 and Day 90 respectively for high- and low-moisture dates.

The *E. coli* O157:H7 population on the wet-inoculated pluots and sundried tomatoes immediately after inoculation were 9.18 ± 0.37 and 9.40 ± 0.20 log CFU/g respectively (TSAR). On Day 0, a higher initial wet-inoculation level was seen from sundried tomatoes than dried pluots. Based on TSAR, 8.70 ± 0.71 log CFU/g (TSAR) of *E. coli* O157:H7 was inoculated onto sundried tomatoes while 6.28 ± 0.95 log CFU/g of *E. coli* O157:H7 was inoculated onto dried pluots. During storage, *E. coli* O157:H7 decreased rapidly on dried pluots. *E. coli* O157:H7 could no longer be detected from dried pluots starting from Day 30 and Day 15 respectively for storage temperatures of 5 and 20 °C. For sundried tomatoes, a greater than 4.92 log CFU/g reduction was seen on Day 60 (TSAR) at 5 °C. A similar reduction was seen on Day 30 when the storage temperature was at 20 °C. No *E. coli* O157:H7 could be detected from sundried tomatoes after 60 days of storage at 5 °C and 30 days of storage at 20 °C.

Survival of *L. monocytogenes*. Figure 3.3 and Table 3.3 summarize the survival of *L. monocytogenes* on the dried fruits. The initial dry inoculation levels on low- and high-moisture dates were 6.19 ± 0.15 and 6.57 ± 0.46 log CFU/g respectively. *L. monocytogenes* decreased gradually on both types of dates. On Day 150, *L. monocytogenes* could only be detected by

enrichment from high-moisture dates stored at 5 °C. On Day 180, *L. monocytogenes* could no longer be detected from either high- or low-moisture dates. *L. monocytogenes* decreased faster at 20 °C. Starting from Day 120, *L. monocytogenes* could not be detected from either type of date at 20 °C.

The *L. monocytogenes* population on the wet-inoculated pluots and sundried tomatoes immediately after inoculation were 9.35 ± 1.07 and 9.59 ± 0.25 log CFU/g respectively (TSAR). On Day 0, higher inoculation levels were seen from sundried tomatoes (7.85 ± 1.31 log CFU/g TSAR). Approximately 5.35 log CFU/g of *L. monocytogenes* were inoculated onto dried pluots. After inoculation and during storage, *L. monocytogenes* decreased rapidly on dried pluots. On Day 5, a greater than 2.84 log CFU/g of injured cells were observed at 5 °C (comparing MOXR with TSAR). Similar significant difference between TSAR and MOXR was seen on Day 5 at 20 °C. Starting from Day 15, no *L. monocytogenes* could be detected from dried pluots regardless of the storage temperature. Sharp reductions of survival *L. monocytogenes* cells on sundried tomatoes were seen on Day 60 for both storage temperatures. The reductions were greater than 2.15 log and 4.83 log respectively for 5 and 20 °C. Since Day 60, no *L. monocytogenes* could be detected from sundried tomatoes even by enrichment.

pH and water activity. The initial pH of low-moisture dates, high-moisture dates, dried pluots and sundried tomatoes were 5.04 ± 0.23, 4.65 ± 0.06, 3.17 ± 0.12, and 3.50 ± 0.05 respectively. The initial water activities of low-moisture dates, high-moisture dates, dried pluots and sundried tomatoes were 0.63, 0.65, 0.77, and 0.82 respectively. In general, the water activity and the pH did not change significantly during storage, except the water activity of sundried tomatoes.

3.4. Discussion

Three types of dried fruits were selected in this study including Medjool dates, sundried tomatoes, and dried pluots processed with sulfur dioxide. The order of water activity was sundried tomatoes > dried pluots > high-moisture dates > low-moisture dates. The order of pH was high-moisture dates > low-moisture dates > sundried tomatoes > dried pluots. The selection of inoculation carriers was based on two criteria: (1) it has the lowest impact on the visual and physiochemical properties of dried fruits, and (2) it's relevance to the commercial processing of dried fruits and ability mimic or represent contamination that happens during various processing or storage stages. Given wet inoculation caused detachment of date skin, dry inoculation was used to mimic the contamination during growing. In addition, dates are typically grown in sandy regions where sand storms are common and some dates are cleaned by air pressure without any contact with water before packaging (Glasner, Botes, Zaid, & Emmens, 2002). Although not big, dry inoculation reduced the pH of dried pluots and a_w of sundried tomatoes. Additionally, due to seasonal production of fresh fruits, some dried fruits such as sundried tomatoes and dried pluots are processed in large quantities, stored at frozen temperatures, and then washed and dried before packaging (based on survey and interview information obtained from regional producers). Therefore, wet inoculation was used for sundried tomatoes and dried pluots to mimic the contamination during washing.

When comparing Figures 3.1-3.3 the survival of pathogens on dry-inoculated dates was determined by bacteria species, storage temperature, and date type. Among the three tested pathogens, *Salmonella* survived better than *E. coli* O157:H7 and *L. monocytogenes* regardless of the date type or storage temperature. Juneja et al. (2021) also found that *Salmonella* and *E. coli*

O157:H7 wet-inoculated dates at ~8 log CFU/g increased by 0.2-0.4 log CFU/g after 31 days of storage (4 °C), while *L. monocytogenes* population decreased by 1.32 log CFU/g. L. R. Beuchat and Mann (2014) evaluated the survival of *Salmonella* on dry (sand)-inoculated date paste and they found that at a low inoculation level of 3.18 log CFU/g, all samples remained positive of *Salmonella* by enrichment after 242 days of storage at 4 °C. Liu et al. (2021) found that when *Salmonella* and *L. monocytogenes* were dry-inoculated on dried apricots made without sulfur dioxide treatment at ~6 log CFU/g, *Salmonella* survived for the entire storage period at 22 °C with the final level of ~2.5 log CFU/g after 90 days, while *L. monocytogenes* fell below the limit of enumeration (0.7 log CFU/g) at the end of storage.

Salmonella is a microorganism that has been mostly commonly involved in outbreaks associated with low water activity food (Larry R. Beuchat et al., 2013). The exact mechanisms used by *Salmonella* to survive in low moisture conditions remain to be fully elucidated. Finn et al (2013) summarized the potential responses of *Salmonella* upon transition into a low moisture environment; these responses include uptake of potassium ions, transportation of osmoprotectants, synthesis of glutamate and trehalose, up-regulation of sigma factors *Rpo*E and *Rpo*S, increased fatty acid catabolism, and filament formation. These responses facilitate the survival of *Salmonella* in low moisture conditions.

The survival of *L. monocytogenes*, *E. coli* O157:H7, and *Salmonella* also significantly correlated with storage temperatures in low a_w food. As the temperature increases microorganisms die more rapidly (Igo & Schaffner, 2021). In this study, a faster die-off was also observed at 20 °C than 5 °C for all three pathogens tested in both low- and high-moisture dates. Similarly, when *Salmonella* was inoculated in date paste at 6.57 log CFU/g, bacterial populations

were reduced by 0.77 and 4.25 log CFU/g at 4 °C and 25 °C, respectively, after 21 days of storage (L. R. Beuchat & Mann, 2014). Populations of *L. monocytogenes* on dried raisins fell below the limit of detection (0.4 log CFU/g) after 14 days of storage at 23 °C while only decreased by 1.4 log after 336 days of storage at 4 °C (Cuzzi et al., 2021).

The overall survivability of all three pathogens was better on the low-moisture dates than the high-moisture dates. Although the initial water activity of the low-moisture dates was lower than that of the high-moisture dates (Table 3.5), it increased to similar levels with that of the high-moisture dates within 5 days of storage at both temperatures (Figure 3.4B). Since both dates were inoculated with the dry carrier and they have comparable pH and a_w , the overall better survival of pathogens in low-moisture dates might be due to other intrinsic factors. For example, naturally dried Medjool dates (high-moisture dates which were dried on the tree) at a water activity of 0.55 contained significantly higher levels of phenolic acids (coumarin, vanillic acid, vernolic acid, caffeic acid, gallic acid, and p-hydroxylbenzoic acid) and sugars (fructose and sucrose) compared with sundried Medjool dates (low-moisture dates dried in the baskets after fallen off the tree) at a water activity of 0.49 (Alsmairat et al., 2019). Phenolic acids have antimicrobial effects against foodborne pathogens (Cueva et al., 2010) while sugars such as sucrose might improve pathogen survival (Flessa et al., 2005).

PBS was used as the carrier for sundried tomatoes and dried plouts made with sulfur treatment. Although drying the inoculum on sand also led to significant reductions of each pathogen, once inoculated onto the final dried fruits, no more immediate change in bacterial counts was observed (Tables 3.1-3.3). When using the liquid carrier, the drying took place on dried fruit surfaces. Various intrinsic factors associated with dried fruits may impact the

reduction of pathogens during drying, such as the presence of free sulfur dioxide. This might be one of the reasons why there *E. coli* O157:H7 populations of 8.70 ± 0.71 log CFU/g survived on dried tomatoes after 48 h of drying while *E. coli* O157:H7 populations of 6.28 ± 0.15 log CFU/g were recovered on dried plouts (which were processed with sulfur) in the same time. The differences can be more significant when looking into selective agar (e.g. XLT-4R). This observation indicates a drawback associated with the use of liquid carrier. Since the drying processing took place on dried fruits, such inoculation method can lead to uneven initial inoculation levels on various products, thus impacting pathogen behavior during storage.

Once being stored at designated conditions, drastic decrease, and die-off of all three pathogens were immediately observed in dried plouts (within 5 to 15 days). After 15 days of storage at both temperatures, no *E. coli* O157:H7 or *L. monocytogenes* was recovered from dried plouts after enrichment. *Salmonella* was not recovered from inoculated dried plouts after 30 days of storage at 20 °C and 60 days at 5 °C. The presence of free sulfur should be one of the factors contributing to this rapid die-off during storage. In the study conducted by Liu et al. (2021), *Salmonella* was dry-inoculated on dried apricots using sand at ~6.5 log CFU/g, no cell was recovered from sulfur-treated apricots (initial total sulfur dioxide 1001 mg/kg) after enrichment during 90 days of storage at 22 °C, while ~2.5 log CFU/g of *Salmonella* was recovered from dried apricots made without sulfur dioxide (Liu et al., 2021). However, although sulfur dioxide treatment facilitates bacterial die-off, it has the potential to induce asthmatic reactions in some people (Alp & Bulantekin, 2021).

Besides inoculation method and the presence of sulfur dioxide, the rapid decrease of bacterial populations on sundried tomatoes might also be due to having the lowest pH and

highest water activity of the dried fruits tested. In the study by Cuzzi et al., (2021) the die-off rate of *L. monocytogenes* in dried apples (pH 3.7) and dried strawberries (pH 3.8-3.9) was faster than that in raisins (pH 4.2-4.3) during storage at 4 °C and 23 °C. In a recent study in which the authors analyzed 67 publications about survival of foodborne pathogens in low water activity food held at temperatures less than 37 °C, the data showed that a_w could significantly impact the survival of both pathogenic and generic *E. coli* (Igo & Schaffner, 2021). Another interesting observation made from the sundried tomato is that, unlike the faster die-off at 20 °C for all pathogens on dry-inoculated dates or wet-inoculated dried pluots, *L. monocytogenes* and *Salmonella* population decreased more rapidly at 5 °C than 20 °C on sundried tomatoes. This might be due to the increased a_w of sundried tomatoes during the storage at 20 °C (Fig. 3.4B). Similar results were reported by Farakos et al. (2017). In this study, when *Salmonella* was inoculated on hazelnuts and stored at 25 °C with different RH (34% or 57%), corresponding to nut a_w of 0.37 or 0.54 respectively, the time for the first log reduction of *Salmonella* was estimated at 24 and 9 weeks, respectively (Farakos, Pouillot, & Keller, 2017). *Salmonella* died faster on higher water activity nuts.

Overall, our results showed that common foodborne pathogens can survive on dried fruits, emphasizing the importance of implementing additional control strategies to improve their microbial safety from every aspect. Alp and Bulantekin (2021) reviewed methods to effectively inactivate microorganisms on dried foods at various stages of production including pre-drying treatments, novel drying methods, and post-drying treatments. For example, dipping Gala apple slices in 0.5% ascorbic acid, lactic acid, citric acid, and sodium bisulfate for 2 min following dehydration for 5 h at 60 °C caused an additional 2.29, 2.69, 2.75, and 5.58 log CFU/g

reduction of *Salmonella* compared with the untreated dehydrated control (Gurtler et al., 2020). Phungamngoen et al. (2013) found that vacuum drying and low-pressure superheated steam drying (LPSSD) had superior antimicrobial effect against *S*. Anatum on cabbages compared with hot air drying. The drying time to obtain a 3-log reduction of *Salmonella* on cabbages was 270, 94, and 58 min for hot air drying, vacuum drying, and LPSSD, respectively (Phungamngoen et al., 2013). At the post-drying stage, advanced ozonation process, which simultaneously applied UV-C, ozone, and hydrogen peroxide, reduced *Salmonella* inoculated on raisins, dried strawberries, and dried apples at 5.55-6.59 log CFU/g to undetectable level even after enrichment (Hasani et al., 2020). Active packaging that can generate gaseous chlorine dioxide reduced background fungi and bacterial populations of semi-dry longan pulp by ~3.4 and 2.0 log CFU/g compared with the untreated controls after 28 and 180 days of storage at ambient temperature (~25 °C), respectively (Lin et al., 2021). For the dried fruits that are stored at freezing temperature and rewashed and dried before packaging, sanitizers can be used to reduce potential contamination. For instance, 75 ppm peroxyacetic acid wash for 1 min reduced *Salmonella, E. coli* O157:H7, and *L. monocytogenes* inoculated on dried Deglet Noor dates at ~7 log CFU/g by 4.80, 4.08, and 4.96 log CFU/g, respectively (Juneja et al., 2021).

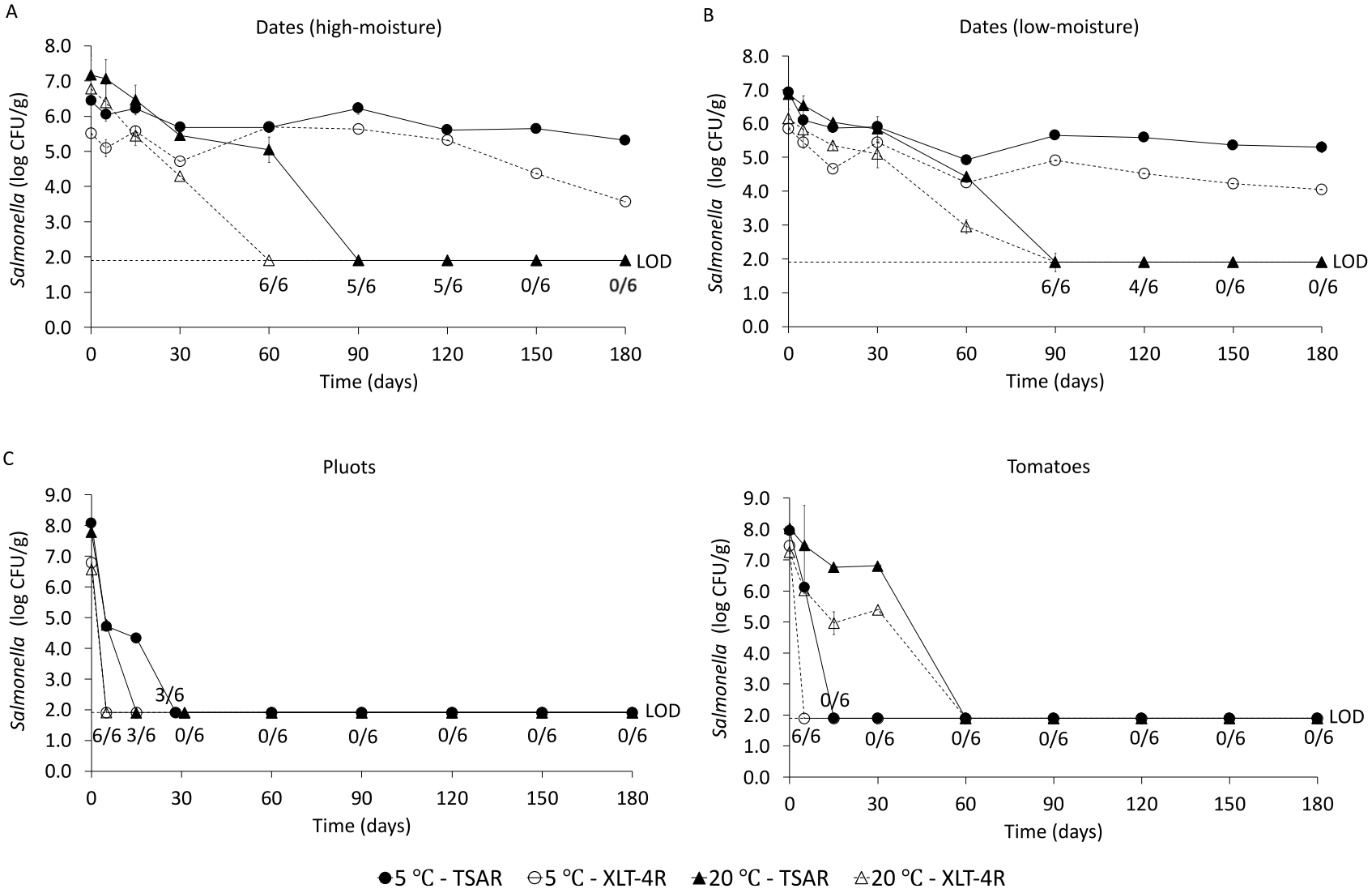

Figure 3.1. Survival of *Salmonella* on dates, dried pluots, and sundried tomatoes. TSAR: tryptic soy agar with rifampicin. XLT-4R: Xylose lysine tergitol 4 agar with rifampicin. LOD: limit of detection of 1.9 log CFU/g.

Table 3.1. Survival of *Salmonella* on dates, dried pluots, and sundried tomatoes. These data are also shown in Figure 3.1.

		***Salmonella* spp. (log CFU/g)**							
		low-moisture dates		**high-moisture dates**		**dried pluots**		**sundried tomatoes**	
temp	**time (days)**	**TSAR**	**XLT-4R**	**TSAR**	**XLT-4R**	**TSAR**	**XLT-4R**	**TSAR**	**XLT-4R**
5°C	0	6.92 ± 0.03^{Aa}	5.85 ± 0.03^{Ab}	6.43 ± 0.07^{Aa}	5.50 ± 0.06^{Ab}	8.09 ± 0.07^{Aa}	6.79 ± 0.06^{b}	7.95 ± 0.10^{Aa}	7.46 ± 0.05^{b}
	5	6.10 ± 0.04^{Ba}	5.45 ± 0.18^{Bb}	6.05 ± 0.07^{Ba}	5.09 ± 0.24^{Bb}	4.72 ± 0.09^{B}	< 1.9	6.13 ± 0.13^{B}	< 1.9
	15	5.88 ± 0.07^{Ca}	4.66 ± 0.04^{Cb}	6.21 ± 0.09^{Ca}	5.57 ± 0.07^{ACb}	4.33 ± 0.08^{C}	< 1.9	< 1.9	< 1.9 [0/6]
	30	5.91 ± 0.06^{Ca}	5.45 ± 0.10^{Bb}	5.68 ± 0.09^{Da}	4.71 ± 0.05^{Db}	< 1.9	< 1.9 [3/6]	< 1.9	< 1.9 [0/6]
	60	4.93 ± 0.06^{Da}	4.25 ± 0.12^{Db}	5.67 ± 0.03^{DEa}	5.69 ± 0.11^{Ea}	< 1.9	< 1.9 [0/6]	< 1.9	< 1.9 [0/6]
	90	5.65 ± 0.10^{Ea}	4.91 ± 0.10^{Eb}	6.22 ± 0.16^{Ba}	5.64 ± 0.06^{CEb}	< 1.9	< 1.9 [0/6]	< 1.9	< 1.9 [0/6]
	120	5.59 ± 0.06^{Fa}	4.53 ± 0.08^{Fb}	5.61 ± 0.07^{DEa}	5.32 ± 0.07^{Fb}	< 1.9	< 1.9 [0/6]	< 1.9	< 1.9 [0/6]
	150	5.36 ± 0.03^{Ga}	4.23 ± 0.06^{Db}	5.65 ± 0.02^{DFa}	4.36 ± 0.02^{Gb}	< 1.9	< 1.9 [0/6]	< 1.9	< 1.9 [0/6]
	180	5.30 ± 0.16^{Ga}	4.05 ± 0.09^{Gb}	5.31 ± 0.06^{Ga}	3.56 ± 0.08^{Hb}	< 1.9	< 1.9 [0/6]	< 1.9	< 1.9 [0/6]
20°C	0	6.87 ± 0.07^{Aa}	6.15 ± 0.26^{Ab}	7.17 ± 0.55^{Aa}	6.77 ± 0.82^{Ab}	7.79 ± 1.17^{Aa}	6.57 ± 0.39^{a}	8.03 ± 0.13^{Aa}	7.26 ± 0.46^{Ab}
	5	6.54 ± 0.28^{Ba}	5.81 ± 0.16^{Bb}	7.07 ± 0.54^{Ba}	6.38 ± 0.51^{Ab}	4.76 ± 0.11^{Ba}	< 1.9	7.47 ± 1.30^{Ba}	6.04 ± 0.10^{Bb}
	15	6.04 ± 0.07^{Ca}	5.35 ± 0.12^{Cb}	6.45 ± 0.42^{Ca}	5.44 ± 0.28^{Bb}	< 1.9	< 1.9 [3/6]	6.78 ± 0.03^{Ca}	4.96 ± 0.38^{Cb}
	30	5.84 ± 0.37^{Ca}	5.11 ± 0.42^{Cb}	5.46 ± 0.11^{Da}	4.29 ± 0.05^{Cb}	< 1.9	< 1.9 [0/6]	6.82 ± 0.04^{Ca}	5.40 ± 0.10^{Db}
	60	4.43 ± 0.09^{Da}	2.96 ± 0.20^{Db}	5.05 ± 0.36^{E}	< 1.9	< 1.9	< 1.9 [0/6]	< 1.9	< 1.9 [0/6]
	90	< 1.9	< 1.9 [6/6]	< 1.9	< 1.9 [5/6]	< 1.9	< 1.9 [0/6]	< 1.9	< 1.9 [0/6]
	120	< 1.9	< 1.9 [4/6]	< 1.9	< 1.9 [5/6]	< 1.9	< 1.9 [0/6]	< 1.9	< 1.9 [0/6]
	150	< 1.9	< 1.9 [0/6]	< 1.9	< 1.9 [0/6]	< 1.9	< 1.9 [0/6]	< 1.9	< 1.9 [0/6]
	180	< 1.9	< 1.9 [0/6]	< 1.9	< 1.9 [0/6]	< 1.9	< 1.9 [0/6]	< 1.9	< 1.9 [0/6]

Values are means ± standard deviation. Within each type of dried fruit, storage temperature, and plating media (TSAR or XLT-4R), different uppercase letters denote significantly different values ($P < 0.05$) among days of storage. Within each type of dried fruit, storage temperature, and storage time, different lowercase letters denote significantly different values between media. [#/6] is number of positive samples out of 6 after enrichment and plating on selective agar.

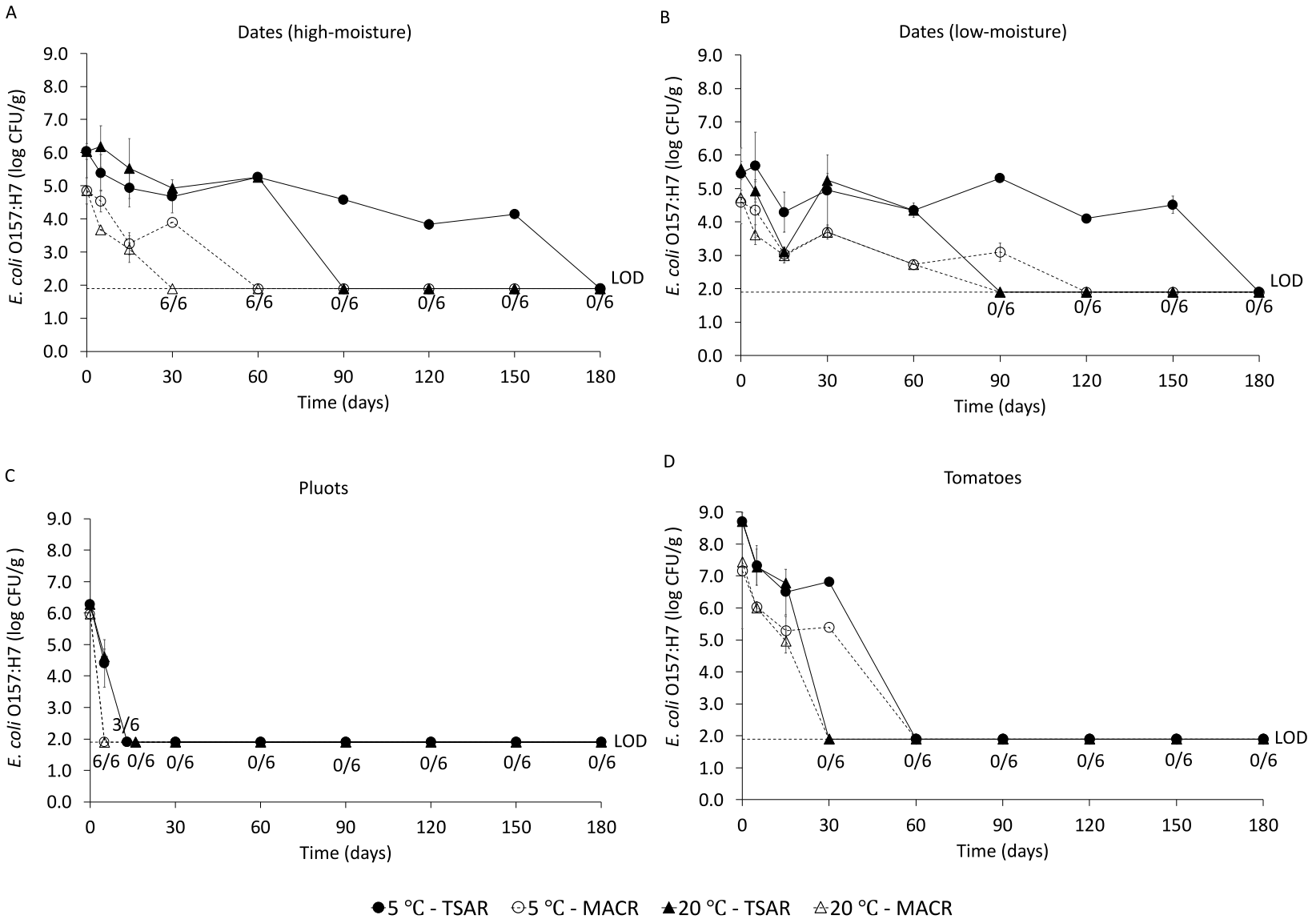

Figure 3.2. Survival of *E. coli* O157:H7 on dates, dried pluots, and sundried tomatoes. TSAR: tryptic soy agar with rifampicin. MACR: MacConkey agar with rifampicin. LOD: limit of detection of 1.9 log CFU/g.

Table 3.2. Survival of *E. coli* O157:H7 on dates, dried pluots, and sundried tomatoes. These data are also shown in 3.2.

		***E. coli* O157:H7 (log CFU/g)**							
		low-moisture dates		**high-moisture dates**		**dried pluots**		**sundried tomatoes**	
temp	**time (days)**	**TSAR**	**MACR**	**TSAR**	**MACR**	**TSAR**	**MACR**	**TSAR**	**MACR**
5°C	0	5.46 ± 0.75^{Aa}	4.60 ± 0.66^{Ab}	6.04 ± 0.24^{Aa}	4.85 ± 0.39^{Ab}	6.28 ± 0.95^{Aa}	5.97 ± 0.10^{a}	8.70 ± 0.71^{Aa}	7.22 ± 1.83^{Ab}
	5	5.68 ± 1.00^{Aba}	4.37 ± 0.83^{Ab}	5.39 ± 0.25^{Ba}	4.54 ± 0.33^{Aba}	4.40 ± 0.76^{B}	< 1.9	7.33 ± 0.62^{Ba}	6.04 ± 0.16^{Ab}
	15	4.30 ± 0.60^{CDa}	3.05 ± 0.20^{Bb}	4.93 ± 0.26^{Ca}	3.25 ± 0.34^{Bb}	< 1.9	< 1.9 [3/6]	6.50 ± 0.71^{Ca}	5.29 ± 0.44^{Bb}
	30	4.95 ± 1.06^{Ca}	3.70 ± 0.13^{Ca}	4.68 ± 0.27^{Ca}	3.89 ± 0.14^{Bb}	< 1.9	< 1.9 [0/6]	6.82 ± 0.04^{BCa}	5.40 ± 0.09^{Bb}
	60	4.35 ± 0.05^{Ca}	2.73 ± 0.00^{Db}	5.26 ± 0.28^{B}	< 1.9	< 1.9	< 1.9 [0/6]	< 1.9	< 1.9 [0/6]
	90	5.31 ± 0.01^{Ba}	3.09 ± 0.28^{Bb}	4.58 ± 0.29^{C}	< 1.9	< 1.9	< 1.9 [0/6]	< 1.9	< 1.9 [0/6]
	120	4.10 ± 0.06^{D}	< 1.9	3.84 ± 0.30^{D}	< 1.9	< 1.9	< 1.9 [0/6]	< 1.9	< 1.9 [0/6]
	150	4.51 ± 0.26^{C}	< 1.9	4.14 ± 0.31^{E}	< 1.9	< 1.9	< 1.9 [0/6]	< 1.9	< 1.9 [0/6]
	180	< 1.9	< 1.9 [0/6]	< 1.9	< 1.9 [0/6]	< 1.9	< 1.9 [0/6]	< 1.9	< 1.9 [0/6]
20°C	0	5.46 ± 0.75^{Aa}	4.60 ± 0.66^{Ab}	6.04 ± 0.24^{Aa}	4.85 ± 0.39^{Ab}	6.28 ± 0.95^{Aa}	5.97 ± 0.10^{a}	8.70 ± 0.71^{Aba}	7.22 ± 1.83^{Ab}
	5	4.93 ± 0.34^{BCa}	3.62 ± 0.29^{BCb}	6.18 ± 0.63^{Aa}	3.67 ± 0.13^{Bb}	4.62 ± 0.25^{B}	< 1.9	7.28 ± 0.56^{BCa}	5.99 ± 0.08^{Bb}
	15	3.11 ± 0.13^{Da}	2.99 ± 0.07^{Ba}	5.52 ± 0.91^{Ba}	3.01 ± 0.40^{Cb}	< 1.9	< 1.9 [0/6]	6.78 ± 0.12^{Ca}	5.03 ± 0.44^{Bb}
	30	5.24 ± 0.02^{Ba}	3.70 ± 0.14^{Cb}	4.93 ± 0.04^{C}	< 1.9	< 1.9	< 1.9 [0/6]	< 1.9	< 1.9 [0/6]
	60	4.38 ± 0.17^{Ca}	2.72 ± 0.01^{Bb}	5.26 ± 0.08^{B}	< 1.9	< 1.9	< 1.9 [0/6]	< 1.9	< 1.9 [0/6]
	90	< 1.9	< 1.9 [0/6]	< 1.9	< 1.9 [0/6]	< 1.9	< 1.9 [0/6]	< 1.9	< 1.9 [0/6]
	120	< 1.9	< 1.9 [0/6]	< 1.9	< 1.9 [0/6]	< 1.9	< 1.9 [0/6]	< 1.9	< 1.9 [0/6]
	150	< 1.9	< 1.9 [0/6]	< 1.9	< 1.9 [0/6]	< 1.9	< 1.9 [0/6]	< 1.9	< 1.9 [0/6]
	180	< 1.9	< 1.9 [0/6]	< 1.9	< 1.9 [0/6]	< 1.9	< 1.9 [0/6]	< 1.9	< 1.9 [0/6]

Values are means ± standard deviation. Within each type of dried fruit, storage temperature, and plating media (TSAR or MACR), different uppercase letters denote significantly different values ($P < 0.05$) among days of storage. Within each type of dried fruit, storage temperature, and storage time, different lowercase letters denote significantly different values between media. [#/6] is number of positive samples out of 6 after enrichment and plating on selective agar.

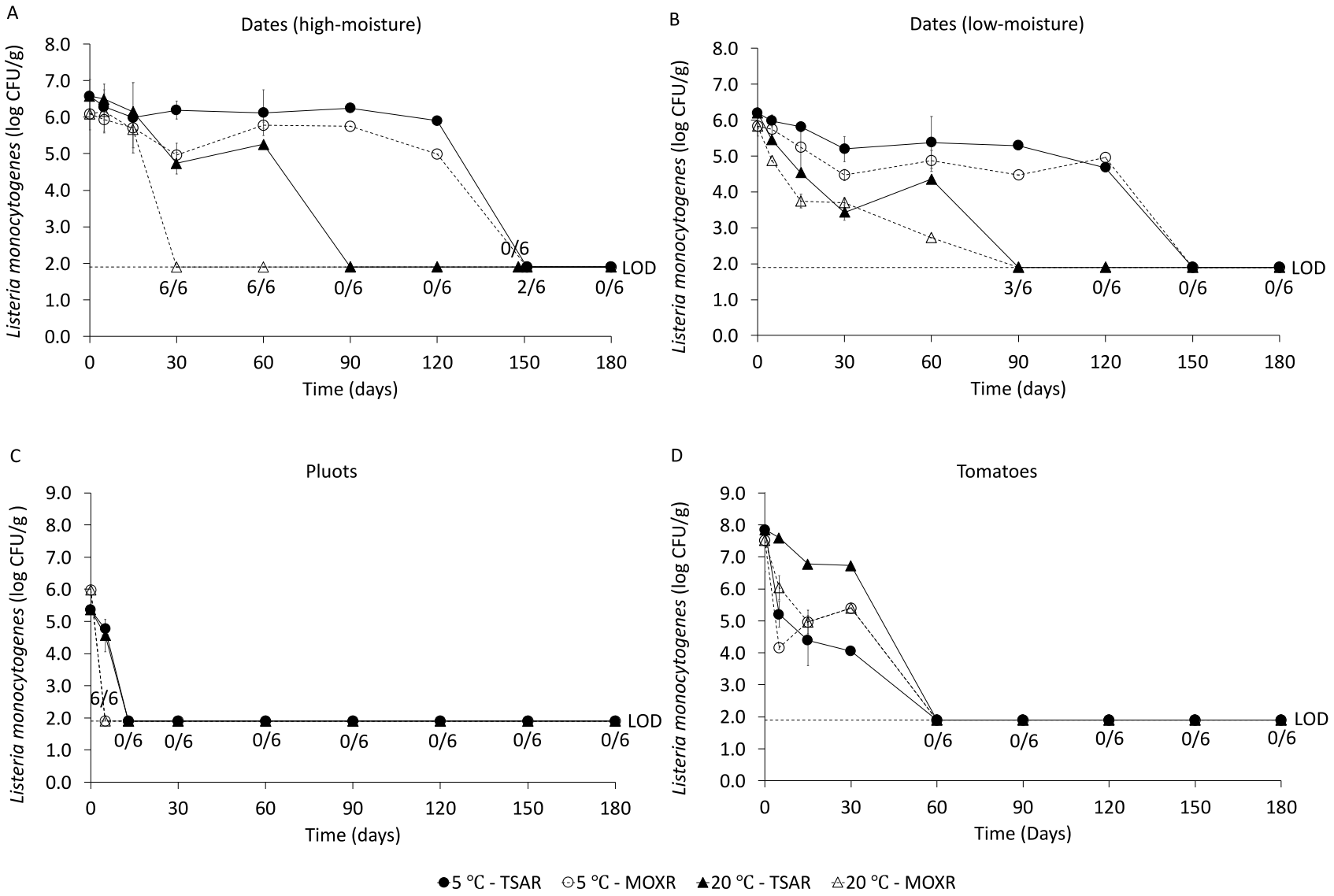

Figure 3.3. Survival of *L. monocytogenes* on dates, dried pluots, and sundried tomatoes. TSAR: tryptic soy agar with rifampicin. MOXR: Modified Oxford agar with rifampicin. LOD: limit of detection of 1.9 log CFU/g.

Table 3.3. Survival of *L. monocytogenes* on dates, dried pluots, and sundried tomatoes. These data are also shown in 3.3.

		***Listeria monocytogenes* (log CFU/g)**							
		low-moisture dates		**high-moisture dates**		**dried pluots**		**sundried tomatoes**	
temp	**time (days)**	**TSAR**	**MOXR**	**TSAR**	**MOXR**	**TSAR**	**MOXR**	**TSAR**	**MOXR**
5°C	0	6.19 ± 0.15^{Aa}	5.83 ± 0.27^{Ab}	6.57 ± 0.46^{Aa}	6.10 ± 0.44^{Ab}	5.35 ± 0.11^{Aa}	5.98 ± 0.15^{b}	7.85 ± 1.31^{Aa}	7.51 ± 0.07^{Aa}
	5	5.97 ± 0.16^{Ba}	5.74 ± 0.36^{Bb}	6.28 ± 0.33^{Ba}	5.94 ± 0.33^{Ab}	4.74 ± 0.28^{Ba}	< 1.9	5.20 ± 0.40^{Aa}	4.17 ± 0.06^{Bb}
	15	5.81 ± 0.13^{Ca}	5.25 ± 0.60^{ABCb}	5.99 ± 0.96^{CEa}	5.71 ± 0.13^{Aa}	< 1.9	< 1.9 [0/6]	4.39 ± 0.79^{Ba}	4.88 ± 0.41^{BCa}
	30	5.19 ± 0.35^{Da}	4.47 ± 0.16^{Cb}	6.19 ± 0.25^{BCDa}	4.97 ± 0.32^{Bb}	< 1.9	< 1.9 [0/6]	4.05 ± 0.06^{Ba}	5.42 ± 0.16^{Cb}
	60	5.37 ± 0.71^{CEa}	4.86 ± 0.29^{ABCa}	6.12 ± 0.63^{Da}	5.78 ± 0.08^{BCa}	< 1.9	< 1.9 [0/6]	< 1.9	< 1.9 [0/6]
	90	5.29 ± 0.12^{Ca}	4.47 ± 0.05^{Cb}	6.25 ± 0.03^{CDa}	5.76 ± 0.04^{Cb}	< 1.9	< 1.9 [0/6]	< 1.9	< 1.9 [0/6]
	120	4.67 ± 0.02^{Ea}	4.96 ± 0.04^{Db}	5.90 ± 0.07^{Ea}	5.00 ± 0.07^{Db}	< 1.9	< 1.9 [0/6]	< 1.9	< 1.9 [0/6]
	150	< 1.9	< 1.9 [0/6]	< 1.9	< 1.9 [2/6]	< 1.9	< 1.9 [0/6]	< 1.9	< 1.9 [0/6]
	180	< 1.9	< 1.9 [0/6]	< 1.9	< 1.9 [0/6]	< 1.9	< 1.9 [0/6]	< 1.9	< 1.9 [0/6]
20°C	0	6.19 ± 0.15^{Aa}	5.83 ± 0.27^{Ab}	6.57 ± 0.46^{Aa}	6.10 ± 0.44^{Ab}	5.35 ± 0.11^{Aa}	5.98 ± 0.15^{b}	7.85 ± 1.31^{Aa}	7.51 ± 0.07^{Aa}
	5	5.44 ± 0.09^{Ba}	4.87 ± 0.12^{Bb}	6.50 ± 0.42^{Aa}	6.16 ± 0.59^{Ab}	4.57 ± 0.36^{B}	< 1.9	7.60 ± 0.04^{Aa}	6.36 ± 0.20^{Bb}
	15	4.53 ± 0.10^{Ca}	3.74 ± 0.19^{Cb}	6.15 ± 1.05^{ABCa}	5.67 ± 0.51^{Bb}	< 1.9	< 1.9 [0/6]	6.77 ± 0.03^{Ba}	5.10 ± 0.42^{Ca}
	30	3.43 ± 0.22^{Da}	3.70 ± 0.16^{Ca}	4.74 ± 0.30^{B}	< 1.9	< 1.9	< 1.9 [0/6]	6.73 ± 0.09^{Ba}	5.45 ± 0.10^{Cb}
	60	4.35 ± 0.05^{Ea}	2.72 ± 0.08^{Db}	5.29 ± 0.08^{C}	< 1.9	< 1.9	< 1.9 [0/6]	< 1.9	< 1.9 [0/6]
	90	< 1.9	< 1.9 [3/6]	< 1.9	< 1.9 [0/6]	< 1.9	< 1.9 [0/6]	< 1.9	< 1.9 [0/6]
	120	< 1.9	< 1.9 [0/6]	< 1.9	< 1.9 [0/6]	< 1.9	< 1.9 [0/6]	< 1.9	< 1.9 [0/6]
	150	< 1.9	< 1.9 [0/6]	< 1.9	< 1.9 [0/6]	< 1.9	< 1.9 [0/6]	< 1.9	< 1.9 [0/6]
	180	< 1.9	< 1.9 [0/6]	< 1.9	< 1.9 [0/6]	< 1.9	< 1.9 [0/6]	< 1.9	< 1.9 [0/6]

Values are means ± standard deviation. Within each type of dried fruit, storage temperature, and plating media (TSAR or MOXR), different uppercase letters denote significantly different values ($P < 0.05$) among days of storage. Within each type of dried fruit, storage temperature, and storage time, different lowercase letters denote significantly different values between media. [#/6] is number of positive samples out of 6 after enrichment and plating on selective agar.

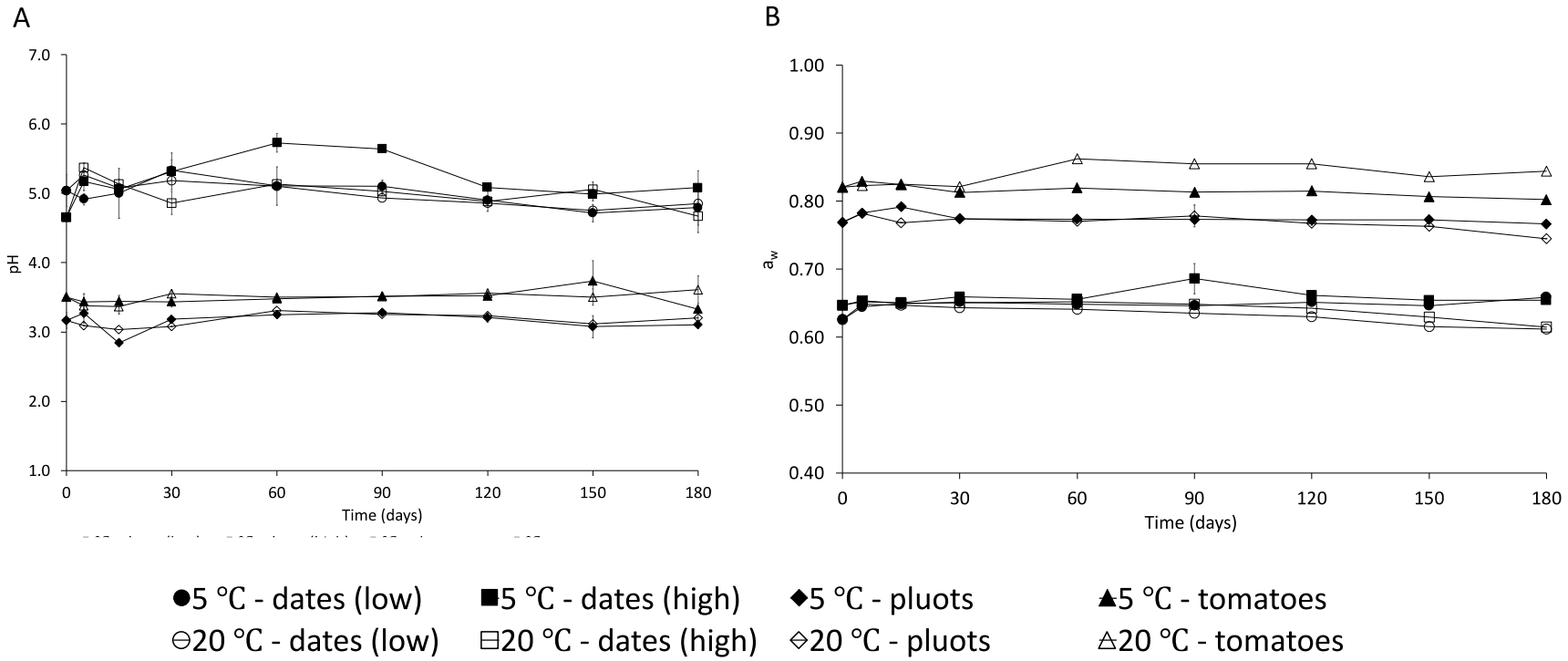

Figure 3.4. pH and water activity (a_w) changes of inoculated dates, dried pluots, and sundried tomatoes during storage.

Table 3.4. pH of inoculated dates, dried pluots, and sundried tomatoes during storage.

	pH							
	low-moisture dates		high-moisture dates		dried pluots		sundried tomatoes	
time (days)	5℃	20℃	5℃	20℃	5℃	20℃	5℃	20℃
0	5.04 ± 0.23^{ABCa}	5.04 ± 0.23^{ABCa}	4.65 ± 0.06^{Aa}	4.65 ± 0.06^{Aa}	3.17 ± 0.12^{ABCa}	3.17±0.12^{ABCDa}	3.50 ± 0.05^{ABa}	3.50 ± 0.05^{ABa}
5	4.92 ± 0.08^{ACa}	5.26 ± 0.12^{ACa}	5.14 ± 0.14^{BCa}	5.37 ± 0.07^{Ba}	3.27 ± 0.14^{ABa}	3.09 ± 0.03^{AEa}	3.44 ± 0.12^{ABa}	3.38 ± 0.03^{ACa}
15	5.00 ± 0.36^{ABCa}	5.08 ± 0.14^{ABCa}	5.06 ± 0.06^{BCa}	5.14 ± 0.07^{CDa}	2.84 ± 0.05^{Ca}	3.04 ± 0.01^{Ab}	3.44 ± 0.09^{ABa}	3.36 ± 0.11^{ADa}
30	5.33 ± 0.15^{BEa}	5.19 ± 0.11^{ACa}	5.32 ± 0.27^{CDa}	4.86 ± 0.17^{ACDa}	3.18 ± 0.03^{ADa}	3.08 ± 0.04^{ACb}	3.44 ± 0.08^{ABa}	3.55 ± 0.05^{BDa}
60	5.11 ± 0.28^{ABa}	5.1 ± 0.28^{ACDa}	5.73 ± 0.13^{DEa}	5.13 ± 0.08^{Cb}	3.25 ± 0.03^{Ba}	3.31 ± 0.03^{Bb}	3.48 ± 0.00^{Aa}	3.50 ± 0.04^{BDa}
90	5.10 ± 0.09^{BCa}	4.93 ± 0.06^{ABa}	5.64 ± 0.04^{DEa}	5.03 ± 0.06^{CEb}	3.28 ± 0.02^{BDa}	3.25 ± 0.04^{BFa}	3.52 ± 0.05^{Aa}	3.51 ± 0.02^{BDa}
120	4.90 ± 0.07^{ADa}	4.86 ± 0.05^{CEa}	5.09 ± 0.06^{CFa}	4.89 ± 0.15^{DEa}	3.21 ± 0.06^{BDa}	3.23 ± 0.02^{DFa}	3.52 ± 0.03^{Aa}	3.56 ± 0.06^{ABDa}
150	4.72 ± 0.13^{ADa}	4.75 ± 0.17^{BEa}	4.99 ± 0.10^{CFa}	5.06 ± 0.10^{Ca}	3.08±0.16^{ABCDa}	3.11 ± 0.01^{CEa}	3.74 ± 0.29^{ABa}	3.50 ± 0.12^{BCEa}
180	4.79 ± 0.25^{CDEa}	4.85 ± 0.12^{BDEa}	5.08 ±0.25^{ABEFa}	4.67 ± 0.24^{ACDb}	3.10 ± 0.06^{Aa}	3.20 ± 0.07^{DEFb}	3.33 ± 0.04^{Ba}	3.61 ± 0.20^{ADEa}

Values are means ± standard deviation. Within each type of dried fruit and storage temperature, different uppercase letters denote significantly different pH values ($P < 0.05$) among days of storage. Within each type of dried fruit and storage time, different lowercase letters denote significantly different values between storage temperatures.

Table 3.5. Water activity of inoculated dates, dried pluots, and sundried tomatoes during storage.

	water activity							
	low-moisture dates		high-moisture dates		dried pluots		sundried tomatoes	
time (days)	5℃	20℃	5℃	20℃	5℃	20℃	5℃	20℃
0	0.63 ± 0.00^{Aa}	0.63 ± 0.00^{Aa}	0.65 ± 0.00^{Aa}	0.65 ± 0.00^{Aa}	0.77 ± 0.00^{Aa}	0.77 ± 0.00^{Aa}	0.82 ± 0.00^{Aa}	0.82 ± 0.00^{Aa}
5	0.64 ± 0.00^{Ba}	0.65 ± 0.00^{Bb}	0.65 ± 0.00^{BCa}	0.65 ± 0.00^{Ba}	0.78 ± 0.00^{Ba}	0.78 ± 0.00^{BCa}	0.83 ± 0.00^{Ba}	0.82 ± 0.01^{Ab}
15	0.65 ± 0.00^{Ca}	0.65 ± 0.00^{Ba}	0.65 ± 0.00^{Ba}	0.65 ± 0.00^{Ca}	0.79 ± 0.00^{Ca}	0.77 ± 0.00^{Ab}	0.82 ± 0.00^{Ca}	0.83 ± 0.02^{Aa}
30	0.65 ± 0.00^{DEa}	0.64 ± 0.00^{Cb}	0.66 ± 0.00^{DEa}	0.65 ± 0.00^{Deb}	0.77 ± 0.00^{Da}	0.77 ± 0.00^{DEa}	0.81 ± 0.00^{Da}	0.82 ± 0.03^{Ab}
60	0.65 ± 0.00^{CDFa}	0.64 ± 0.00^{CDb}	0.66 ± 0.00^{CDa}	0.65 ± 0.00^{BDb}	0.77 ± 0.00^{Ada}	0.77 ± 0.00^{AEFa}	0.82 ± 0.00^{Ca}	0.86 ± 0.04^{Bb}
90	0.65 ± 0.00^{BFa}	0.64 ± 0.00^{Eb}	0.69 ± 0.02^{ABDa}	0.65 ± 0.00^{CEa}	0.77 ± 0.00^{Da}	0.78 ± 0.02^{ACEa}	0.81 ± 0.00^{Da}	0.85 ± 0.05^{BCb}
120	0.65 ± 0.00^{Ea}	0.63 ± 0.01^{ADEb}	0.66 ± 0.00^{Ea}	0.64 ± 0.00^{Fb}	0.77 ± 0.00^{Da}	0.77 ± 0.00^{AEFb}	0.81 ± 0.00^{Da}	0.85 ± 0.06^{Cb}
150	0.65 ± 0.00^{BCDa}	0.62 ± 0.01^{AFb}	0.65 ± 0.00^{BDFa}	0.63 ± 0.00^{Gb}	0.77 ± 0.00^{Da}	0.76 ± 0.00^{Fb}	0.81 ± 0.00^{Ea}	0.84 ± 0.07^{Db}
180	0.66 ± 0.00^{Ga}	0.61 ± 0.00^{Fb}	0.65 ± 0.00^{Fa}	0.61 ± 0.00^{Hb}	0.77 ± 0.00^{Fa}	0.74 ± 0.00^{Gb}	0.80 ± 0.00^{Fa}	0.84 ± 0.08^{Eb}

Values are means ± standard deviation. Within each type of dried fruit and storage temperature, different uppercase letters denote significantly different water activity ($P < 0.05$) among days of storage. Within each type of dried fruit and storage time, different lowercase letters denote significantly different values between storage temperatures.

Chapter 4: Mathematical modelling of pathogen behavior on dried fruits

Microbial behavior is closely related to the different intrinsic and extrinsic factors associated with food (Stavropoulou and Bezirtzogou, 2019). Being able to estimate and predict microbial behavior in various food is the foundation for risk assessments and a key in preventing foodborne illnesses (Koseki, 2016). There are two main types of mathematical models for predictive microbiology as reviewed by Stavropoulou and Bezirtzogou (2019). They are kinetic models and probability models. Kinetic models are used to calculate the rate of growth or death responses and predict the concentration or levels of a given microorganism in given conditions (Smith and Schaffner, 2004). Probability models are used to predict the production of microbial toxins. These models suggest the probability of bacterial growth and their toxins but not the growth or die-off rates (Gakruddin et al., 2011; Baker and Genigeorgis, 1990).

There are different levels of kinetic models that can be established based on the data obtained from challenge studies. Primary predictive models are typically developed to describe population dynamics of pathogenic and spoilage bacteria under different environmental conditions (Fernandez-Piquer et al. 2011). Based on the primary models, the secondary predictive models are constructed to evaluate the effect of temperature on growth rates (GRs) or inactivation rates (IRs) of bacteria (Parveen et al., 2013). Tertiary models are established based on primary and secondary models and use predicted values of growth parameters from secondary models to predict changes in pathogen density at times and levels of independent variables that have not been tested or used in the model development (Oscar, 2005; Buchanan, 1997).

4.1 Objective

The objective of this chapter is to establish primary predictive models to better describe and compare the behavior of three tested pathogens in various dried fruits.

4.2 Material and Methods

Program. The USDA Integrated Pathogen Modeling Program (IPMP) was used to create primary models to describe the pathogens' behavior in various dried fruit (Huang, 2013). Since the challenge studies were done at constant temperatures, the models could be kept relatively simple. IPMP can be used to make many different types of models, but for this experiment the group 3 Models were utilized: Survival Models (Huang, 2013).

Modelling. Linear models were produced for every pathogen/dried fruit/temperature combination that had countable microbe data for at least three sampling points. The linear equation produced by IPMP for each model was:

$$y(t) = y_0 - \frac{1}{D}t$$

where y is bacterial count in log CFU/g, D is the decimal reduction time at a constant temperature, and t is time in days.

This equation was used for the modelling of microbial survival at a specific temperature. Since this program requires at least three data points to produce an equation, only microbial counts that survived at least three sampling dates were used. The lower the decimal reduction time is, the more quickly the bacteria dies off. A preliminary test was conducted to find which model fit the data better and the linear model was chosen in the IPMP for the whole dataset. An r^2 value was produced for each model to show how well the linear model fit the data.

4.3 Results

Primary testing of the Linear, Weibull, and two-phase Linear models. When deciding which model to use, we compared the Linear, the Weibull, and the two-phase Linear models available in the IPMP. The survival datasets of L. *monocytogenes* on wet-inoculated peaches made without sulfur were used as the sample data. Figure 4.1 shows the curve fitting and the Root Mean Square Error (RMSE) values obtained from these three models. The smaller the RMSE value, the better fit of the models. As shown in Figure 1, the RMSE values for Linear, Weibull, and Two-phase linear were 0.828, 0.871, and 0.906 respectively. Although the difference was not significant, the RMSE value of the linear model was the lowest among all three models. In this case, linear was chosen as the model for the following part of the study.

Linear models for all the data collected. Table 4.1 shows the D values of the pathogen survival in dried fruits calculated by the UDSA IPMP. For low-moisture dates, the D values when stored at 5 °C were 35.13, 54.77, and 241.97 days/log reduction for *L. monocytogenes*, *E. coli*, and *Salmonella* respectively. With increased storage temperature, D values were decreased; at 20 °C, the D values were 18.24, 21.72, and 14.5 days/log reduction. Temperature had the same effect on high-moisture dates: D values when stored at 5 °C were 36.94, 46.55, and 176.24 days/log reduction for *L. monocytogenes*, *E. coli*, and *Salmonella* respectively. At 20 °C, the D values for the high-moisture dates were 15.79, 17.47, and 14.02 days/log reduction.

For all three pathogens, dates showed the highest D values of all the dried fruits. However, the r^2 for the low-moisture and high-moisture dates for *Salmonella* at 5 °C were 0.4367 and 0.5505 respectively. This r^2 was lower compared to the r^2 for *Salmonella* in the other dried fruits and the other two pathogens in dates. The r^2 is a measure of how dependent

the variation in the bacterial population is on the variation in time, in a sense how well the model describes pathogen survival. The higher the absolute value of the r^2 value is (out of 1), the more dependent the variation is.

For dried pluots, the D value for *Salmonella* was 4.18 with an r^2 of 0.91 when stored at 5 °C (Table 4.1). No other D value were produced for dried pluots, as there were not enough data points to produce the model in IPMP. For sundried tomatoes, the D values when stored at 5 °C were 8.98 and 7.59, and 241.97 days/log reduction for *L. monocytogenes* and *E. coli* respectively. Not enough data points were present to produce a D value for *Salmonella* spp. At 20 °C, the D values were 7.89, 3.59, and 7.86 days/log reduction for *L. monocytogenes*, *E. coli*, and *Salmonella* spp. respectively. For wet-inoculated dried peaches, the D values when stored at 5 °C were 22.13, 11.19, and 38.19 days/log reduction for *L. monocytogenes*, *E. coli*, and *Salmonella* spp. respectively. At 20°C, the D values were 11.88, 12.68, and 10.89 days/log reduction with the latter having the highest r^2 value of all the samples of 0.97. For dry-inoculated dried peaches, the D values when stored at 5 °C were 29.52, 29.06, and 62.75 days/log reduction for *L. monocytogenes*, *E. coli*, and *Salmonella* spp. respectively. At 20 °C, the D values were 17.91, 17.08, and 15.10 days/log reduction. For wet-inoculated dried peaches with sulfur, the D value for *Salmonella* spp. was 7.33 days/log reduction with an r^2 of 0.93 when stored at 5 °C. No other D value were produced for wet-inoculated dried peaches with sulfur, as there were not enough data points to produce the model in IPMP. There were not enough data points for any samples of dry-inoculated dried peaches with sulfur to produce a D value.

4.4 Discussion

The survival of all three pathogens was longest in high-moisture and low-moisture dates at refrigerated temperature. The combination of their high pH values and low a_w compared to the other dried fruits may be reasons why the dates have larger D-value than the other dried fruits. Juneja et al (2021) found that *L. monocytogenes*, *E. coli* O157:H7, and *Salmonella* spp. were able to survive on dates for 32 days when stored at 4 °C. Furthermore they found that there was no significant difference in any of the pathogens when the dates were treated with antimicrobial washes of peracetic acid or with ethanol (Jejuna et al, 2021). Because of this strong ability for pathogens to survive, Medjool dates should be further explored from a microbial safety viewpoint.

The storage temperature had the biggest influence on decimal reduction time in *Salmonella*. While having the highest D values of the three pathogens in refrigerated dried fruit, *Salmonella* had the lowest D values in the dried fruit stored at ambient temperature (Table 4.1). This shows that temperature has a large influence on the survival time of *Salmonella*. While thermal death time does increase for all three pathogens when put in colder conditions, the difference in *Salmonella* is the starkest. For example, according to the models made, the time is would take to reduce *Salmonella* by 90% in high moisture dates at refrigerated temperature would be 396 days, whereas at ambient temperature it would take 21 days (Table 4.1). These results suggest that *Salmonella* has particularly increased survival at lower temperatures compared to other pathogens. While the specific mechanisms that allow for this survival in low-moisture environments are not completely clear, temperature most likely has an influence on those mechanisms. Andino and Hanning (2015) suggest one possible mechanism that

Salmonella spp. may use to enhance its survival at lowered temperatures is cold shock proteins (CSPs). Upregulation of these proteins allow *Salmonella* to adapt to colder environments as temperatures drop, leading to better survival of the pathogen.

Looking at the dried peaches (with no sulfur), the decimal reduction time of the pathogens were higher when the peaches were inoculated with the dry carrier versus the wet carrier. This suggests that pathogens are more persistent when using a dry carrier to simulate a dry environment. However, a factor that might have influenced the lower D value in the wet inoculated peach is that the initial inoculation strength is several logs higher with a wet carrier than a dry carrier. Due to the higher initial microbial load in the wet inoculated peaches compared to the dry inoculated, there is difficulty in comparing the true impact that the wet and dry carrier had on the decimal reduction time.

Table 4.1. D-values (days/log reduction) of pathogen survival in dried fruits calculated by the USDA Integrated Pathogen Modelling Program

	L. monocytogenes				*E. coli* O157:H7				*Salmonella*			
	5 °C		20 °C		5 °C		20 °C		5 °C		20 °C	
Dried fruits	D	R^2	D	R^2	D	R^2	D	R^2	D	R^2	D	R^2
Dates (low-moisture)	35.13	0.64	18.24	0.79	54.77	0.50	21.72	0.60	241.97	0.44	14.50	0.89
Dates (high-moisture)	36.94	0.48	15.79	0.80	46.55	0.66	17.47	0.75	176.24	0.55	14.02	0.87
Pluots	NA		NA		NA		NA		4.18	0.91	NA	
Tomatoes	8.98	0.91	7.89	0.87	7.59	0.88	3.59	0.90	NA		7.86	0.87
Peaches (wet)	22.13	0.79	11.88	0.94	11.19	0.98	12.68	0.79	38.19	0.89	10.89	0.97
Peaches (dry)	29.52	0.80	17.91	0.69	29.06	0.88	17.08	0.77	62.75	0.79	15.1	0.88
Peaches with sulfur (wet)	NA		NA		3.71	0.95	NA		7.33	0.93	NA	
Peaches with sulfur (dry)	NA		NA		NA		NA		NA		NA	

NA: not available. Rapid die-off of pathogens in the dried fruits, not enough countable data for modeling.

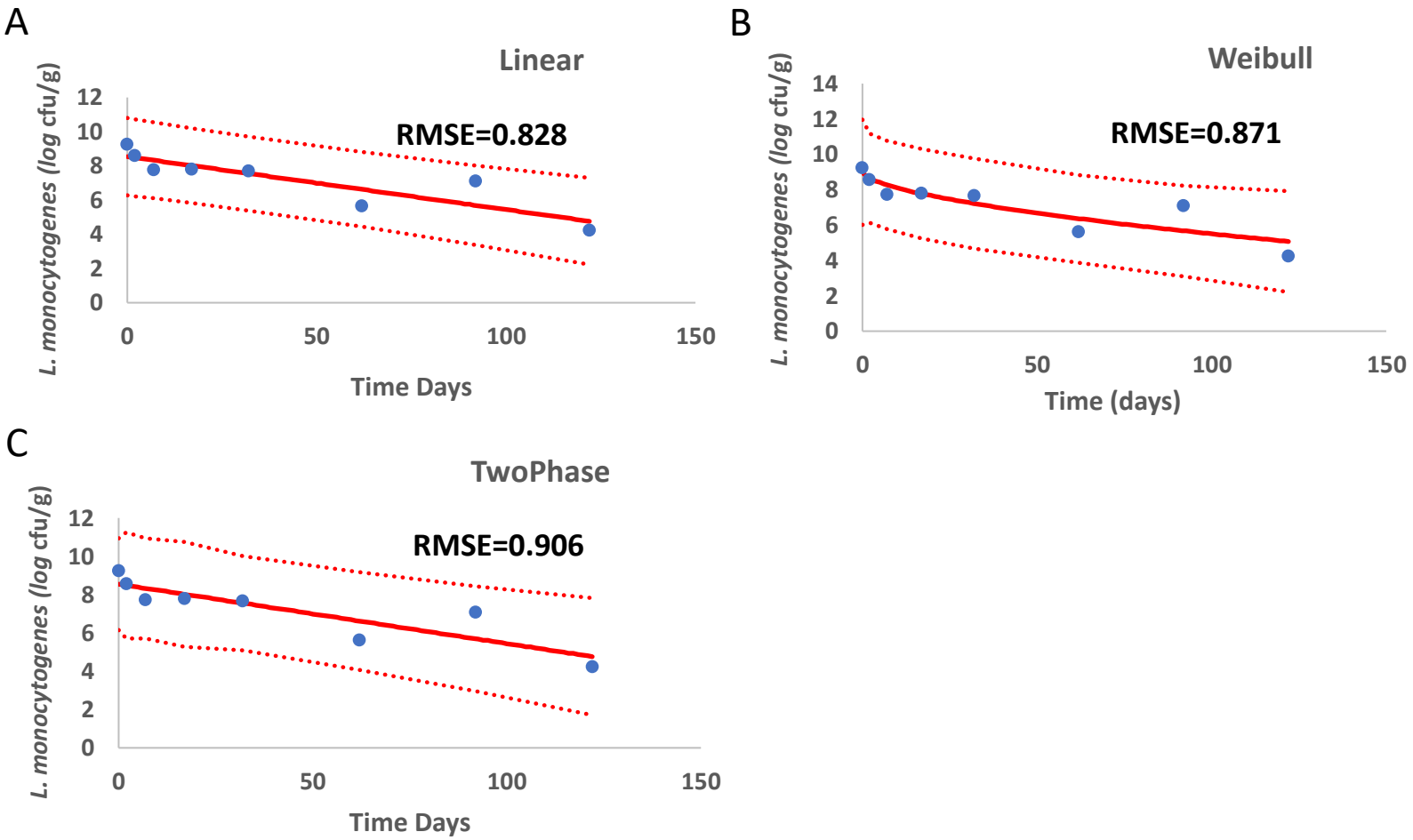

Figure 4.1. Curve fitting and RMSE values of Linear, Weibull, and Two-phase models available in IPMP. Data used was the *L. monocytogenes* survival data in the wet-inoculated dried peaches made without sulfur treatment.

Conclusions and Future Work

Dried fruit were inoculated with *Salmonell*a spp., *Escherichia* coli, or *L. monocytogenes* to determine how they would survive in two storage conditions. All three pathogens were able to survive in dried fruits and should be taken into consideration when looking at the safety of dried fruit processing. *Salmonella* had the longest survival potential among all three tested pathogens. This observation is expected as *Salmonella* is known for its ability to survive in low-moisture conditions.

The condition that allowed for longer pathogens survival was storage at refrigerated temperature rather than ambient temperature. This is important because many dried fruit processors store their dried fruits at refrigerated temperatures if not being sold immediately. This allows for a longer shelf life of the dried fruit compared to ambient storage, but increases the ability for bacterial survival.

The survival of the pathogens varied amongst the different types of dried fruits. This may be due to the intrinsic factors of the dried fruit: pH, a_w, and available nutrient. Based on the current data, survival was the longest in the dried fruits that had the highest relative pH and the lowest relative a_w. *Salmonella* inoculated in Medjool dates survived to the very end of the 180-day survival study. The Medjool dates (both high-moisture and low-moisture) had the highest pH of all the dried fruits and had some of the lowest a_w of the dried fruits. Other intrinsic factors that were not measured could have also played a role in the long survival time in the dates. For instance, dates are known to have a high sugar content, which may have played a role in pathogen survival. Measuring various compounds in the dried fruits might give more insight on why certain dried fruits allowed for longer survival than others. Regardless the

reason, pathogen survival was long in Medjool dates, and should be something that those who produce dates consider. Since dates are not dried the same way other dried fruits are, the steps in the date harvesting process should be looked at carefully.

While conducting this research, a new potential outbreak associated with Medjool dates was reported (Food Safety News, 2021a). Twenty-eight people in England were infected with Hepatitis A in 2021 and is suspected to be from Medjool dates (Food Safety News, 2021a). The dates have since been recalled due to their possible contamination (Food Safety News, 2021a). In 2018 there was another outbreak of Hepatitis A in Denmark and was believed to be from dates from Iran (Food Safety News, 2021b). There has been no evidence to show that those dates were contaminated with the virus (Food Safety News, 2021b). That potentially makes this 2021 outbreak the first to be associated with a dried fruit not part of a mixed product. With the occurrence of this outbreak, it makes it all the more important to understand when and where potential contamination of pathogens in dried fruits can occur. Although the data generated from our study is based on bacteria, we did see that the survival of pathogens in Medjool dates is longer compared to other dried fruits. Additional research will be necessary to better understand the survival of foodborne virus on dried fruits.

As discussed earlier in this thesis, there are many pre- and post-drying treatments that can be applied to fresh or dried fruits. When looking into the available literature, the efficacy of these treatments has not been systematically evaluated. One on-going project in the lab is to summarize the current knowledge about these treatments and their efficacies and develop a study that fills in the knowledge gaps. In the meantime, identifying a surrogate for testing the different pre- and post-drying treatments as well as different drying methods (dehydrator,

oven, and sun-drying) is needed. *Enterococcus faecium* NRRL B-2354 has been validated and approved for being used as a surrogate for almond thermal processing validation (Almond Board of California, 2014). However, whether this strain can be as a surrogate for dried fruit-related studies or not still needs further evaluation. One on-going test project in the lab is go evaluate the survival of *E. faecium* NRRL B-2354 in dried peaches and apricots. In this first test trial, *E. faecium* was inoculated onto two types of dried fruits and its survival at ambient and refrigerated temperatures is being monitored. In addition, the highest temperatures (fruit temperatures) that can be achieved by various dry methods are being monitored and recorded. The efficacy of different pre-drying treatments (e.g. dipping in hot water, washing fruits with lemon juices and other sanitizers) is also being tested in the lab by using *Salmonella*-inoculated peaches and *E. faecium*-inoculated peaches.

In summary, the microbial safety of dried fruits is important and needs more research attention. The survivability of common foodborne pathogens on different types of dried fruits and the recalls and outbreaks associated with dried fruits highlight the importance of the validation of pre- and post-drying treatments as well as different drying methods. The findings of this study, along with future work, hopes to provide the foundation needed for the development of food safety plans for dried fruits.

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Appendix 1. Survey for Dried Fruit Processors

We want to hear from California dried fruit processors. California is a major producer of dried fruits and we want to support the production of high quality dried products. A team of researchers in the Food Science and Technology Department at UC Davis is interested in developing guidance on the best practices to ensure the safety and quality of dried California specialty crops.

To support our California dried fruit producers we need information on the most common industry practices currently being employed for dehydrating. Do you sun dry or use a mechanical dehydrator? We want to know. Do you apply a chemical treatment to reduce browning and increase shelf life? We want to know. The information gathered will be used to inform research priorities and will result in guidance that can be used by you and others to improve processes and satisfy regulatory requirements.

Q1 When is the major harvesting season of the commodity(ies) that you dry?

- ☐ May-June (1)
- ☐ June-July (2)
- ☐ July-August (3)
- ☐ August-September (4)
- ☐ September-October (5)
- ☐ other (6) _____

Q2 What types of fruit (that you grow or buy) do you dry?

- ☐ plum (1)
- ☐ apricot (2)
- ☐ grape (3)
- ☐ berries (strawberry, raspberry, blueberry, and/or blackberry) (4)
- ☐ tomato (5)
- ☐ citrus (6)
- ☐ apple (7)
- ☐ peach (8)
- ☐ dates (9)
- ☐ pluot (10)
- ☐ other (11) _____

Q3 Do you harvest your own fruits or buy from a supplier?

- ○ I harvest my own fruit (1)
- ○ I buy from a supplier (2)

Q4 Do you import any dried fruits?

- ○ yes (1)
- ○ no (2)

Q5 How are the fruit handled before you use them for dried fruit preparation (please select all that apply)?

- [ ] fruit is rinsed with potable water (1)
- [ ] fruit is submerged in potable water (2)
- [ ] fruit is submerged in potable water containing a sanitizer (3)
- [ ] fruit is "pre-dried" using towels (4)
- [ ] fruit is "pre-dried" using forced air (5)
- [ ] fruit is stored at room temperature (6)
- [ ] fruit is stored under refrigeration (7)
- [ ] fruit is stored frozen (8)
- [ ] fruit is not stored prior to drying (9)
- [ ] fruit is stored for a few days (1-3) prior to drying (10)
- [ ] fruit is stored for a week prior to drying (7 days or less) (11)
- [ ] fruit is stored for several weeks (7-28 days) prior to drying (12)
- [ ] Please list any other procedures used for handling fruit prior to drying not listed above (13) _____

Q6 During the preparation of the dried fruit, do you apply/add preservatives (e.g. sulfur, citric acid, etc.) to your dried products?

- ○ yes (1)
- ○ no (2)

Q7 If you answered "yes" to the previous question, what is/are the preservative(s) and when and how are they added or applied?

_____

Q8 How do you dry your products (e.g. sun-drying or other thermal drying method)?

- ☐ sun dried (1)
- ☐ dryer or dehydrator (2)
- ☐ other (3) _____

Q9 If you use dehydrator or dryer to dry your fruit, what kind do you use?

- ○ cabinet dryer (1)
- ○ tunnel dryer (2)
- ○ drum dryer (3)
- ○ other (please specify) (4) _____
- ○ unsure (5)

Q10 Is there standard time and temperature you use to dry your product that you monitor?

- ○ yes (1)
- ○ no (2)

Q11 If you answered "yes" to the previous question, how do you monitor the temperature and time?

Q12 What is the desired water activity of your dried product?

- ○ 0.92 - 0.99 (1)
- ○ 0.86 - 0.91 (2)
- ○ 0.85 (3)
- ○ 0.80 - 0.85 (4)
- ○ 0.70 - 0.79 (5)
- ○ 0.60 - 0.69 (6)
- ○ below 0.60 (7)
- ○ unknown (8)
- ○ If you know the exact water activity target please specify (9)

_____

Q13 How do you decide if your products are dried properly (e.g. water activity or pH or temperature/time)?

- [ ] process for a specific temperature and time (1)
- [ ] measure pH (2)
- [ ] measure water activity (3)
- [ ] visual appearance (4)
- [ ] measure water/moisture content (5)
- [ ] measure brix (6)
- [ ] other (please specify) (7)

_____

Q14 Do you have any non-thermal treatments you apply to your products (e.g. washing or ozone treatment)?

- ○ yes (1)
- ○ no (2)

Q15 If you answered "yes" to the previous question, what non-thermal treatment(s) are applied? When and how are they applied?

_____

Q16 How do you store your dried fruits (i.e. length of storage time, storage temperature, different temperatures for different products, any products added [e.g. dry ice] to the fruit during storage)?

_____

Q17 Are your products stored in their retail bags or in bulk

- retail bags (1)
- bulk (2)
- other (3) _____

Q18 What method do you use to package your products?

- packaged by hand (1)
- mechanically packed (2)
- other (3) _____

Q19 How do you decide the shelf-life of your products (select all the apply)?

- based on visual appearance (e.g. color degradation, mold growth) (1)
- based on flavor (sensory) attributes (2)
- based on microbial test results (3)
- based on other analytical test results (e.g. vitamin content) (4)
- based on results from a formal shelf life study (5)
- based on experience with the product (6)
- other (please specify) (7)

_____

Q20 Do you have a flowchart of the post-harvest process that you can share with us

- yes (if so, please email us at sscanakapalli@ucdavis.edu) (1)
- no (2)