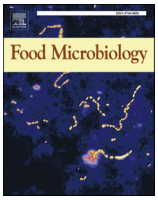
Food Microbiology 102 (2022) 103930

Contents lists available at ScienceDirect

Food Microbiology

journal homepage: www.elsevier.com/locate/fm



Growth behavior of low populations of *Listeria monocytogenes* on fresh-cut mango, melon and papaya under different storage temperatures

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ARTICLE INFO

Keywords:
L. monocytogenes
Food safety
Minimally processed fruit
Growth potential

ABSTRACT

The growth behavior of *Listeria monocytogenes* low population (1–4 cells/sample) on fresh-cut mango, melon, papaya and fruit mix stored at 4, 8, 12 and 16 °C was evaluated over 10 days. Mango showed the lowest counts for *L. monocytogenes* during 10 days regardless of storage temperature (<1.7 log cfu.g^{-1}). Melon supported high bacterial growth over 10 days, reaching 5 log cfu.g^{-1} at 16 °C. Both the fruit and storage temperature influenced the *Listeria* low population growth potential (δ). Cumulative frequency distribution of *L. monocytogenes* showed that after 10 days, 100% of fresh-cut fruits and fruit mix stored at 4 °C remained ≤2 log cfu.g^{-1}, while at 12 and 16 °C 100% of melon, papaya and fruit mix samples exceeded this limit. At 8 °C, 100% of mango and fruit mix samples remained below this limit after 10 days, whereas 100% of melon and papaya reached it after 7 days. Results indicate 4 °C as the ideal to store safely fresh-cut mango, melon, papaya and fruit mix for 10 days. Besides, 8 °C can also be an option, but not for melon and papaya. Findings highlight the ability of *L. monocytogenes* to survive and grow in fresh-cut fruits even at a very low initial population levels.

1. Introduction

Global tropical fruit production has increased by 6.4% from 2018 to 2019, reaching levels of around 7.7 million tons in 2018, accounting for a large part of the food market (FAO, 2020). It reflects the change in the population's lifestyle and eating habits, increasing the demand for fresh, healthy, nutritious foods that present convenience for consumption. In this scenario, ready-to-eat fresh-cut fruits stand out due to their practicality and high nutritional value (De Cesare et al., 2018; De Corato, 2020). Only in 2019, the minimally processed fruits market exceeded USD 260 billion globally and it is estimated to grow approximately 7% until 2026 (Global Market Insights, 2020).

Fresh-cut fruits are subjected to washing, rinsing, sanitized, peeling, and cutting. These products should be stored under refrigeration after packaging to maintain safety, besides the sensory and quality properties (FDA, 2008; De Corato and Cancellara, 2019). Injuries caused by minimal processing favor undesirable enzymatic reactions (e.g., browning, softening) and facilitating microbial contamination (Putnik et al., 2017). Therefore, fresh-cut fruits are highly perishable and generally stored for no longer than 10 days, but they can have an even shorter shelf-life if storage conditions are not appropriate (Amaro et al., 2018). Despite the recommendation to be kept in cold storage, fresh-cut fruit is often subjected to temperature changes during processing and distribution and by opening and closing the refrigerator doors in retails and supermarkets or domestic storage. Such exposures can facilitate the growth and proliferation of pathogens and compromise food safety (Huang et al., 2019).

Among the variety of tropical fruits currently available on the market as fresh-cut products, mango (*Mangifera indica* L.), melon (*Cucumis melo* L.), and papaya (*Carica papaya* L.) are the most popular (Amaro et al., 2018). The consumption of these fruits has been related to healthy habits primarily due to their bioactive compounds with a range of benefits to the human body (Wolfenden et al., 2021).

L. monocytogenes growth is of concern in fresh-cut mango, papaya, and melon because of its ability to grow at refrigeration temperatures in the presence or absence of oxygen (Zhang et al., 2020). Increasing reports of outbreaks caused by *L. monocytogenes* involving these products have been noticed (EFSA, 2016; FDA, 2021). This pathogen causes fever and diarrhea and, in severe cases, a systemic illness with high

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https://doi.org/10.1016/j.fm.2021.103930
Received 29 April 2021; Received in revised form 25 October 2021; Accepted 26 October 2021
Available online 28 October 2021

hospitalization rates and lethality in susceptible populations, such as pregnant women, elderly and immunocompromised individuals (Fana et al., 2019).

A range of studies has assessed the growth of *L. monocytogenes* on artificially contaminated fresh-cut fruits under varied environmental conditions in the last decade (Danyluk et al., 2014; Huang et al., 2019; Qi et al., 2020). However, they have approached an inoculum concentration that may not reproduce the microbial contamination in such matrices. The use of high initial inoculum may underestimate the microbial growth scenario and fail to predict the pathogen behavior during processing or storage conditions (Manios et al., 2013). Although these fruits' capacity to support the growth of *L. monocytogenes* has been reported, specific information on the growth behavior of pathogen at low-inoculum is lacking, especially considering the effects of temperature.

Single cell enumeration has been used as an approach to determine the kinetic parameters of individual cells under various environmental conditions (Francois et al., 2003). The method employs successive micro-dilution, and it estimates the needed dilutions to decrease the population to 1–4 cells per well using multiple columns, combining a high chance of having single cell per well (Francois et al., 2003, Manios et al., 2013).

Considering these aspects, this study assessed the growth behavior of low cell populations of *L. monocytogenes* on fresh-cut mango, melon, papaya stored solely or mixed (fruit mix) as a function of different temperatures (4, 8, 12, and 16 °C) over 10 days of storage.

2. Materials and methods

2.1. Test strain and 1–4 cells inoculum preparation

L. monocytogenes serotype 1/2a lineage II strain C1-387, isolated from food (genome available at GenBank: CP006591.1, BioSample: SAMN02203127) was used as a test strain in the present study. The stock cultures of *L. monocytogenes* were maintained in cryovials with Brain Heart Infusion (BHI) broth (HiMedia, Mumbai, India) containing glycerol 20% v/v (Sigma Aldrich, St, Louis, USA) at −80 °C. The strain was grown in BHI broth for 24 h at 37 °C (Ziegler et al., 2019). Cells were harvested through centrifugation (4500 g × 15 min at 4 °C), washed twice in sterile saline solution, and re-suspended in 10 mL of the same diluent (0.85% NaCl w/v) (Barbosa et al., 2019). Standard cell suspension with an optical density (OD) at 660 nm (OD_{660}) of 0.1 provided viable counts of 8.0 ± 0.1 log cfu.mL^{-1}.

The 1–4 cells inoculum was performed as previously described by Manios et al. (2013). The standardized inoculum was initially diluted up to 3 log cfu.mL^{-1}. Then, 200 μL of 3 log cfu.mL^{-1} suspension were transferred to the well of the first microplate column of a 96-well microplate prefilled with 200 μL of saline solution. Thereafter, two-fold serial dilutions were performed across the microplate to determining the target well (TW), which each one of the eight well containing at least one cell and maximum four cells. The full content of each TW in the target column (8 wells) was used as the inoculum in each independent sample. An independent microplate was used as a control to check the distribution of the cells in the wells of the target column on Tryptic Soya Agar (TSA; HiMedia, Mumbai, India) in each experimental trial. To check if all the samples were inoculated with 1–4 cells of *L. monocytogenes*, the pooled relative and cumulative frequency of the controls at each incubation temperature was calculated at the beginning of the experiments (time zero) (Fig. 1). Results indicated that all the samples were inoculated with 1–4 cells.

2.2. Fruits and minimal processing to prepare fresh-cut samples

Mango, melon and papaya fruits in the commercial maturation stage (≥10 °Brix) were acquired from Supplies and Services Company of Paraíba (EMPASA, João Pessoa, Paraíba, Brazil). Fruits were selected and standardized based on uniformity of size and shape, firmness, absence of spots, depressions or cracks, and homogeneous color. Fruits were randomly selected and checked regarding the total soluble solids in a digital refractometer Brix/RI Chek Model (Reichert Analytical

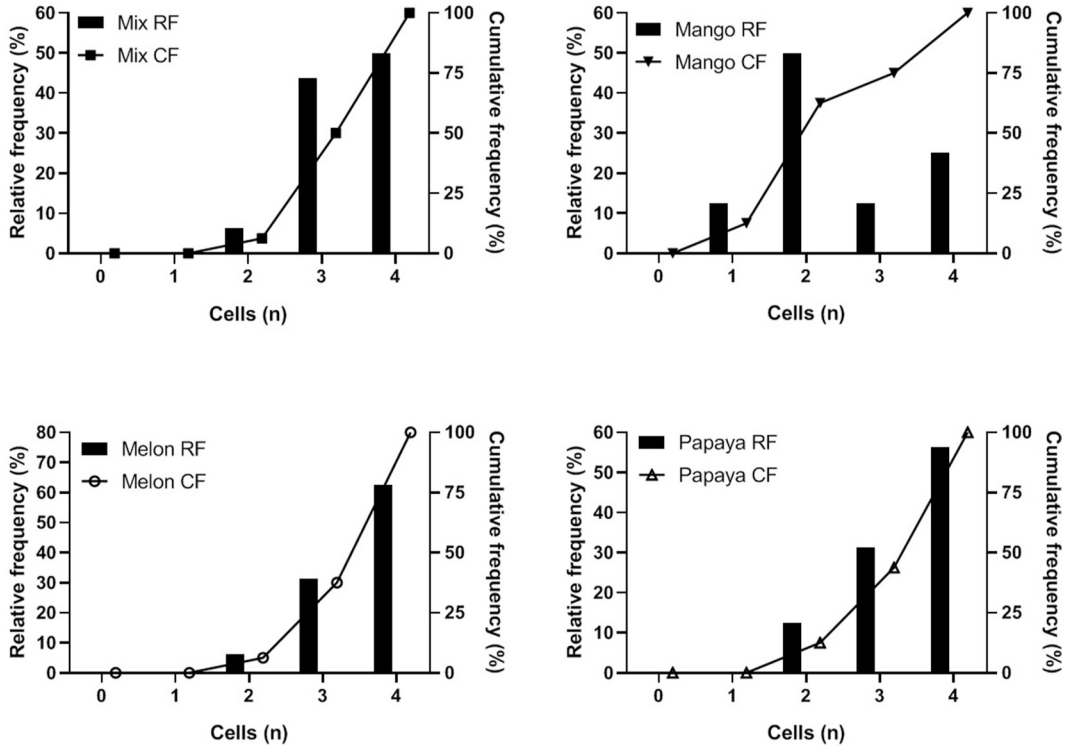

Fig. 1. Relative frequency (%: bars) and cumulative probability (line) of *Listeria monocytogenes* cells contained in a microplate well and used to inoculate samples of fresh-cut mango, melon, papaya, and fruit mix.

Instruments, Depew, NY, USA), expressed as °Brix (Method 932.12; AOAC, 2016) to verify the maturation stage. The intact fruits were stored under refrigeration and maintained 1 h at room temperature (25 ± 1 °C) before minimal processing.

Before the experiments, whole fruits (10 fruits of each fruit) were washed in running water and immersed in sodium hypochlorite solution (150 mg.L^{-1}) for 5 min, rinsed in abundant sterile distilled water for 15 min, and left to dry in a biosafety cabinet for 15 min (Camelo, 2004; FDA, 2020). Each fruit was individually and aseptically peeled and sliced in cubes of approximately 1 cm^3. Samples were then collected and analyzed to ensure no contamination with *L. monocytogenes*.

2.3. *L. monocytogenes* inoculation on fresh-cut fruits and storage conditions

Mango, melon and papaya were analyzed separately and mixed (fruit mix) (1:1:1) because these fruits are widely marketed as fruit mix in many countries. Furthermore, when fresh-cut fruit are mixed, a different environment may be created, which may influence in the growth behavior of *Listeria* (Ziegler et al., 2018).

Ten grams of each fresh-cut fruit, or fruit mix were transferred to sterile plastic bags (120 mL, size 178 × 76 mm, 0.07 mm thickness), spot inoculated with 1–4 cells of *L. monocytogenes* (the full content of one TW) per sample. Samples were gently hand-shaked for 10 s to spreads the inoculum across the surface of the fruits, and stored at 4, 8, 12 and 16 °C. These temperatures were selected to simulate the refrigerated storage (4 °C), cold chain temperatures (8 °C) and temperature abuse (12 and 16 °C) for ready-to-eat fresh-cut fruit (Huang et al., 2019). Enumeration of L. *monocytogenes* was performed after 1, 3, 5, 7 and 10 days, considering the shelf-life of this type of product (Amaro et al., 2018).

2.4. Enumeration of *L. monocytogenes* and indigenous microflora

At each pre-established storage time period, 10 mL of saline solution were added to each sample, hand-shaken for 30 s, and plated to assess *L. monocytogenes* and the indigenous microflora, respectively. For the detection of 1–4 cells of *L. monocytogenes*, specifically, 1 mL-aliquots of the fruit/fruit mix saline solution homogenized were distributed onto the surface of *Listeria* Selective Agar with supplement *Listeria* II (HiMedia, Mumbai, India) in a standard plate followed by incubation at 37 °C for 48 h (Manios et al., 2013).

Following the same procedures, the homogenized samples of fruits or fruit mix were serially diluted (10^{-1}–10^{-5}) and spread out on Tryptic Soy Agar (TSA), de Man, Rogosa and Sharpe (MRS) agar (HiMedia, Mumbai, India), Eosyne-Metilen-Blue agar (HiMedia, Mumbai, India), and Potato Dextrose agar (HiMedia, Mumbai, India) for enumeration of total viable counts (TVC), lactic acid bacteria, enterobacteria and, molds and yeasts, respectively. Plates were inoculated at proper conditions considering the microorganism monitored. Non-inoculated fresh-cut mango, melon, papaya, and fruit mix samples were similarly assayed and used as control.

The pH of fresh-cut fruits and fruit mix was measured on day 0 (just after the minimal processing) and day 10 of storage, using a digital pH meter (Quimis, São Paulo, Brazil), following standard procedures (AOAC, 2016).

2.5. Growth potential (δ) of *L. monocytogenes*

Growth potential (δ) of *L. monocytogenes* on fresh-cut mango, melon, papaya, and fruit mix at each storage temperature was determined by calculating the differences between counts (expressed as log cfu.g^{-1}) at the end of storage time (10 days) and the beginning of the experiments (time zero). $\delta > 0.5$ log cfu.g^{-1} was considered "able to sustain *L. monocytogenes* growth" at the corresponding storage temperature (Ziegler et al., 2019). The limit of detection was defined was the lowest.

2.6. Statistical analysis

All experiments were carried out in three biological replicates in triplicate, using an independent batch of fruits for each replicate. In each experiment, 24 samples were assessed (8 wells in triplicate) and a total of 72 data were obtained (24 in each experiment). Populations of the pathogen were enumerated, log-transformed, and were expressed as mean log ± standard deviation or cumulative frequency. The limit of detection was considered the lowest count achieved in our set of experiments. Analysis was done using Graphpad prism 8.0.1. The significant differences in pathogen cell count for each temperature over storage time were determined based on ANOVA followed by the Tukey test, using the software Sigma Stat 3.5 (Jandel Scientific Software, San Jose, California).

3. Results

3.1. Growth behavior of *L. monocytogenes* low population on fresh-cut fruits during storage

Growth curves of *L. monocytogenes* low population on fresh-cut fruits and fruit mix during refrigerated storage and temperature abuse are shown in Fig. 2. On fresh-cut mango stored at 4 °C, *L. monocytogenes* population was close to 0.8-log cfu.g^{-1} until 7 days of storage, while after 10 days it was below the detection limit (0.3 log cfu.g^{-1}). In contrast, after 10 days of storage at 8 °C, *L. monocytogenes* the population was about 1.2 log cfu.g^{-1}, reaching this same level after 5 days at 12 °C. After 10 days of storage at 16 °C, *L. monocytogenes* population was about 1.7 log cfu.g^{-1} in fresh-cut mango.

Among fruit samples, melon supported faster bacterial growth and higher pathogen count over storage time and, as the temperature increased, a shorter time was required to reach the same population. *L. monocytogenes* population on fresh-cut melon reached approximately 2 log cfu.g^{-1} after 10 days at 4 °C, while this population level was observed after 5 days of storage at 8 °C. When fresh-cut melon was stored at 12 °C, *L. monocytogenes* population was around 2 log cfu.g^{-1} after 3 days reaching close to 4 log cfu.g^{-1} after 10 days of storage. On the other hand, at 16 °C, the population of the pathogen was approximately 5 log cfu.g^{-1} after 10 days of storage.

Similar to the behavior observed on melon, *L. monocytogenes* needed a larger time to increase in the fresh-cut papaya at 4 and 8 °C compared to 12 and 16 °C. On fresh-cut papaya stored at 4 °C, *L. monocytogenes* population was around 2 log cfu.g^{-1} after 10 days, and this population level was observed after 7 days at 8 °C. On the other hand, *L. monocytogenes* population was already at a level of 3.7 log cfu.g^{-1} after 10 and 3 days of storage at 12 and 16 °C, respectively.

L. monocytogenes low population showed a different behavior on fruit mix in comparison to fresh-cut fruits solely. No difference in counts was observed between samples stored at 4 and 8 °C. At 12 °C, the *L. monocytogenes* population was about 3.4 log cfu.g^{-1} after 10 days of storage, while at 16 °C it reached close to 4.5 log cfu.g^{-1}.

3.2. Growth potential (δ) of *L. monocytogenes*

Fresh-cut melon, papaya and fruit mix supported the *L. monocytogenes* low population growth at 4 and 8 °C (δ between 0.78 and 1.19 log cfu.g^{-1}). At these temperatures, no growth of *L. monocytogenes* was observed on fresh-cut mango (Table 1). Otherwise, at 12 °C all fruits were able to support the growth of *L. monocytogenes* with δ values higher than 3 log cfu.g^{-1} on melon and papaya, and 2 log cfu.g^{-1} on fruit mix. (Table 1). At 16 °C, the highest δ value of *L. monocytogenes* was observed on melon followed by papaya and fruit mix, respectively. The lowest δ of *L. monocytogenes* was observed on mango, regardless of storage temperature (Table 1).

Temperatures 12 and 16 °C favored the *L. monocytogenes* low population growth on all fresh-cut fruits and fruit mix, while 4 °C inhibited

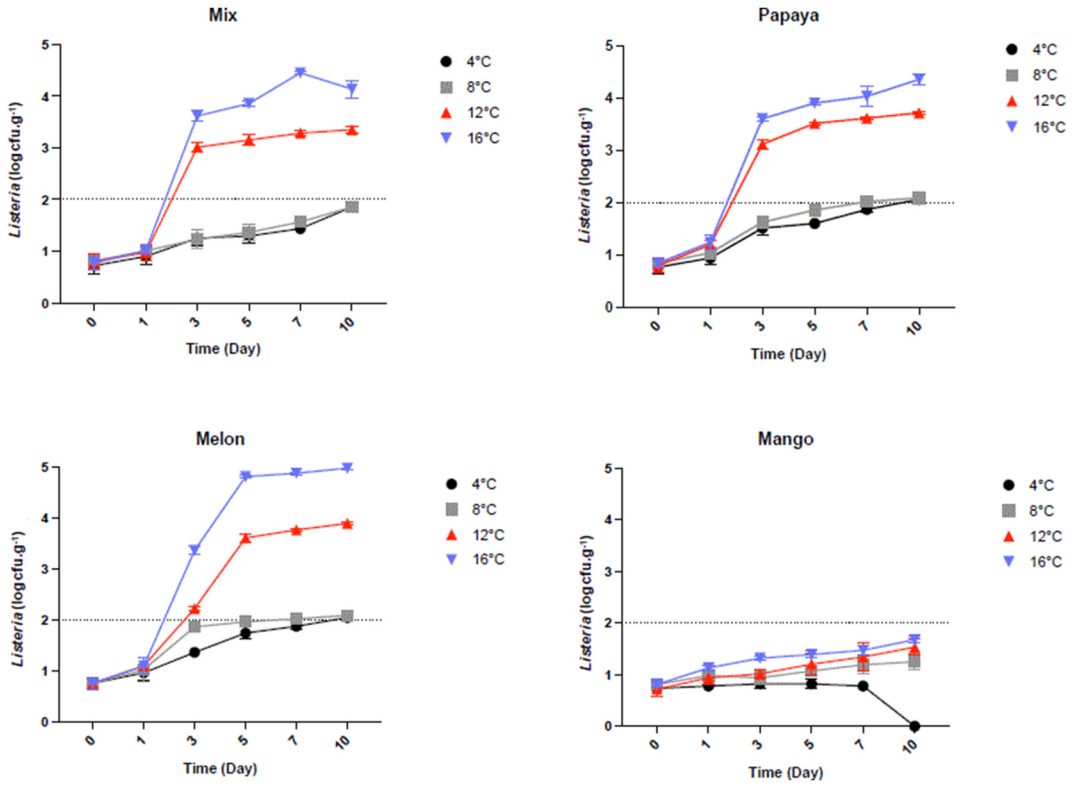

Fig. 2. Growth curves of *Listeria monocytogenes* inoculated at low-population (1–4 cells/sample) on fresh-cut fruit mix, papaya, melon, and mango stored at 4, 8, 12 and 16 °C ± 1 °C for 10 days.

Table 1
Growth potential (δ) of *L. monocytogenes* on fresh-cut fruits stored at different temperatures.

Fruits	Temperature			
	4 °C	8 °C	12 °C	16 °C
Mango	-0.34 ± 0.09^{Aa}	0.31 ± 0.16^{Ba}	0.59 ± 0.24^{BCa}	0.74 ± 0.11^{Ca}
Melon	1.11 ± 0.08^{Ab}	1.12 ± 0.12^{Ab}	3.48 ± 0.18^{Bb}	4.52 ± 0.13^{Cb}
Papaya	1.16 ± 0.23^{Ab}	1.19 ± 0.10^{Ab}	3.30 ± 0.16^{Bb}	3.94 ± 0.23^{Cc}
Mix	0.78 ± 0.19^{Ac}	0.91 ± 0.12^{Ac}	2.86 ± 0.10^{Bc}	3.70 ± 0.20^{Cc}

$^{a-c}$: different superscript small letters in the same column for different fruits at same storage temperature denote difference ($p < 0.05$) based on Tukey test; $^{A–C}$: different superscript capital letters in the same row for the same fresh-cut fruit at different storage temperature denote difference ($p < 0.05$) based on Tukey test.

or slowed the growth in all samples evaluated. Results showed that the pathogen grew faster on melon than on the other fruits evaluated.

3.3. Cumulative frequency of L. monocytogenes in fresh-cut fruits

Cumulative frequency distribution of *L. monocytogenes* cells on mango, melon, papaya and fruit mix varied during 10 days of storage at different temperatures (Supplementary Fig. 1). According our results, the frequency of listeria counts that exceed the 100 cfu g^{-1} increase with increase of the temperature. Considering the criteria established by European Union regulation N 2073/2005 for ready-to-eat products, *L. monocytogenes* counts must be < 100 cfu g^{-1} over the shelf life (EU, 2005). Therefore, the temperature of 4 °C is ideal to control *Listeria* levels under the EU law for 10 days. The temperature of 8 °C, may also be an option for mango and fruit mix since 100% of samples remained below the limit after 10 days, whereas melon and papaya reached the limit after 7 days. Overall, in 20–100% fresh-cut melon, papaya and fruit mix samples stored at 12 and 16 °C, and 35–100% of fresh-cut melon samples stored at 16 °C *L. monocytogenes* reached counts ≥2 log cfu.g^{-1} (Supplementary Fig. 1). In contrast, mango appears to be the most microbiologically safe product when stored after 10 days at all temperatures.

3.4. Enumeration of indigenous microflora

The TVC increased on all fresh-cut samples after 10 days of storage, except on fresh-cut melon stored at 4 °C. TVC was similar on fresh-cut mango, papaya and fruit mix stored at 4 and 8 °C, while on fresh-cut melon, TVC was lower in samples stored at 4 °C than at 8 °C. On fresh-cut melon, papaya and fruit mix, TVC increased after 3 days at 12 °C, while on fresh-cut mango TVC increased after 5 days. The highest TVC were observed in samples stored at 16 °C, except for fruit mix that showed similar counts at 12 and 16 °C (Fig. 3).

Enterobacteriaceae showed an increase since the beginning of the storage on fresh-cut mango, and after 10 days on fruit mix stored at 4 °C, but not on fresh-cut melon and papaya. Otherwise, at 8 °C, *Enterobacteriaceae* increased on all samples, especially on fresh-cut papaya. At 12 °C, *Enterobacteriaceae* increased after 3 days on fresh-cut papaya and fruit mix, and after 5 days on fresh-cut mango and melon. On the other hand, at 16 °C, increases were observed on fresh-cut mango, melon and fruit mix after 3 days but on fresh-cut papaya only after 5 days (Fig. 4).

The LAB counts increased on fruit mix after 10 days of storage but not on fruits assayed solely. Otherwise, at 8 °C, LAB increased on fresh-cut melon and fruit mix after 5 days, whereas on fresh-cut mango and papaya, the growth only occurred after 10 days. On fresh-cut melon, LAB behavior at 8 and 12 °C was similar. LAB increased on all fresh-cut fruits and fruit mix after 5 days at 12 °C. At 16 °C, the growth of LAB was more accelerated in the first 3 days in melon and fruit mix, while on mango and papaya, the growth of these bacteria was more gradual over storage time (Fig. 5).

Molds and yeast were able to grow at 4 and 8 °C only on melon and fruit mix. On fruit mix stored at 8 °C the growth of molds and yeasts was greater than on melon stored at the same temperature. At 12 °C, mold

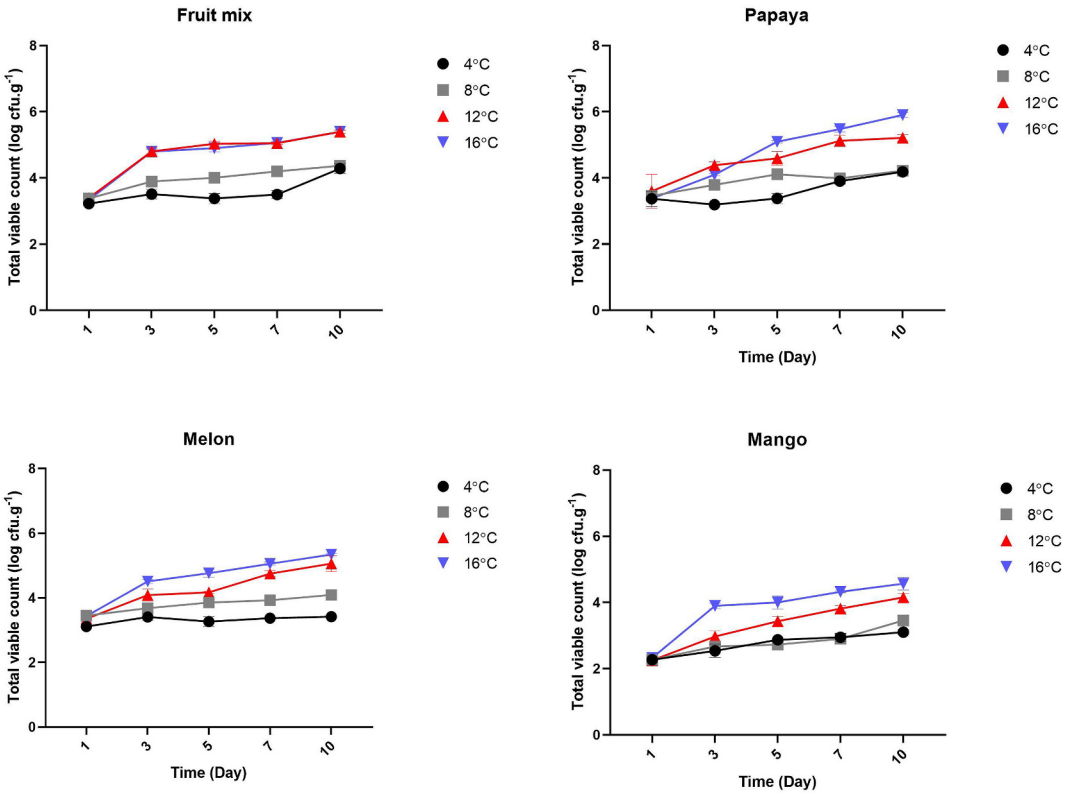

Fig. 3. Total viable counts bacteria on fresh-cut fruit mix, papaya, melon, and mango inoculated with low-population of *Listeria monocytogenes* (1–4 cells/sample) and stored at 4, 8, 12 and 16 °C ± 1 °C for 10 days.

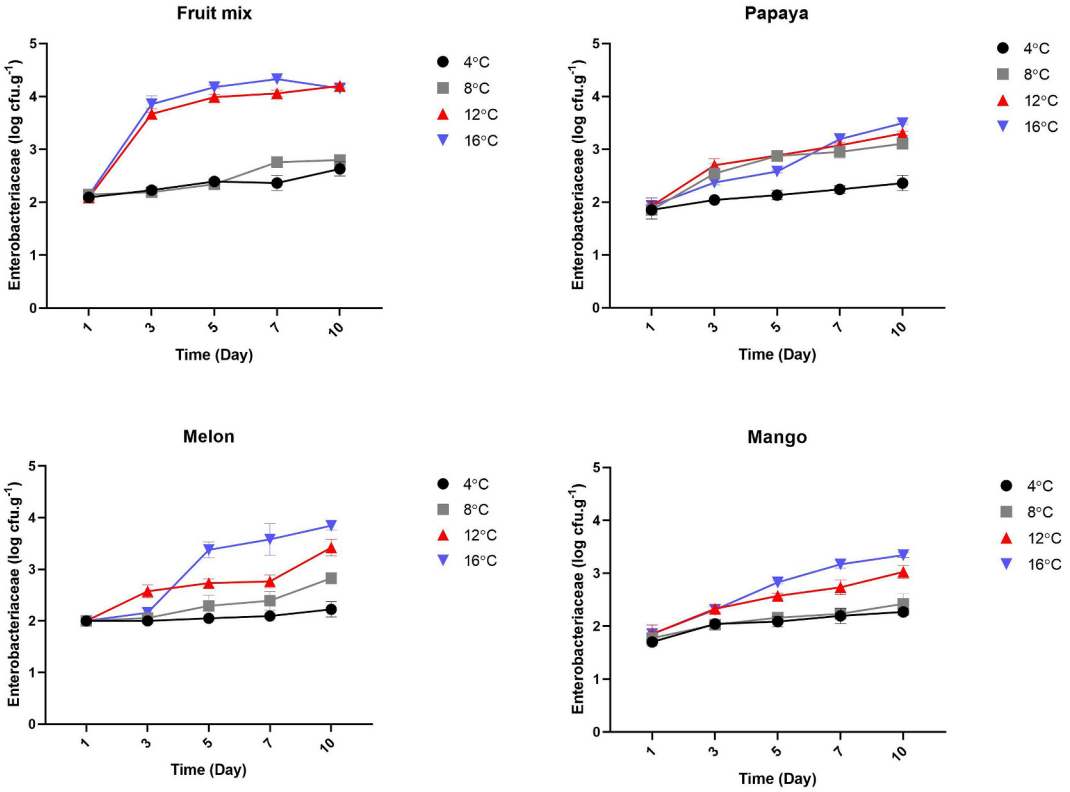

Fig. 4. *Enterobacteriaceae* counts on fresh-cut fruit mix, papaya, melon, and mango inoculated with low-population of *Listeria monocytogenes* (1–4 cells/sample) and stored at 4, 8, 12 and 16 °C ± 1 °C for 10 days.

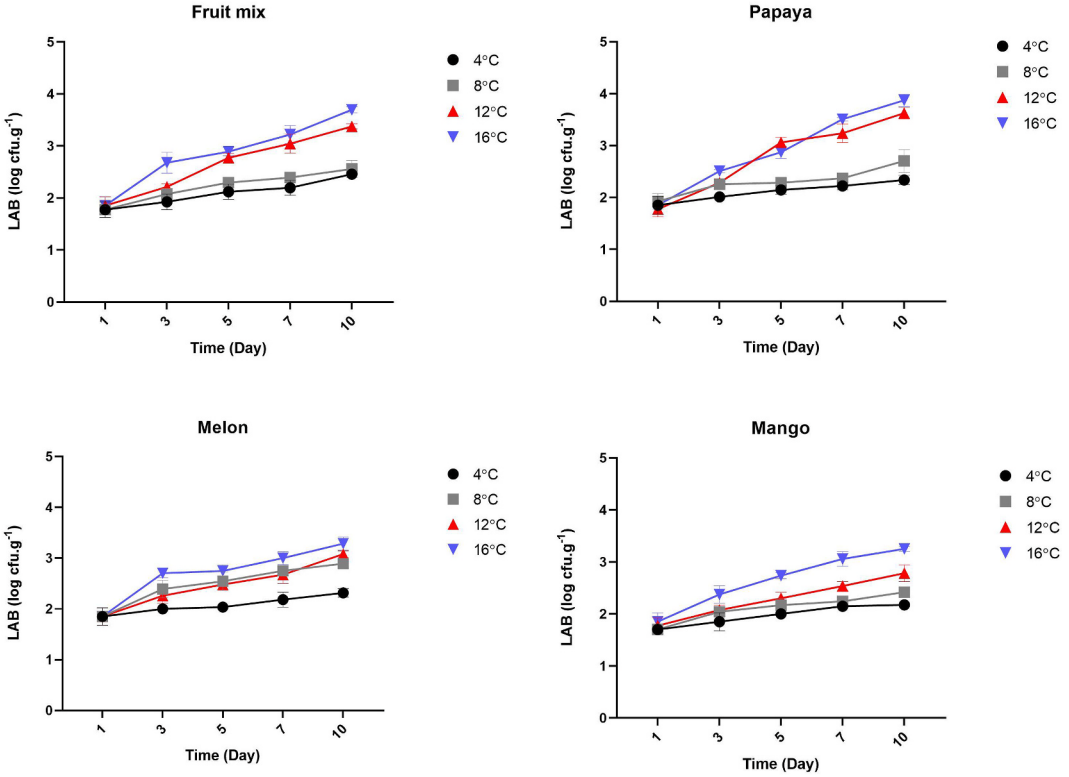

Fig. 5. Lactic acid bacteria counts on fresh-cut fruit mix, papaya, melon, and mango inoculated with low-population of *Listeria monocytogenes* (1–4 cells/sample) and stored at 4, 8, 12 and 16 °C ± 1 °C for 10 days.

and yeast counts increased after 3 days on melon and fruit mix and after 5 days on mango and papaya. Molds and yeasts grew on all samples after 3 days at 16 °C, with the highest counts observed on melon (Supplementary Fig. 2).

The pH values of fresh-cut fruits and fruit mix decreased after 10 days of storage at 12 and 16 °C, while no changes were observed on fresh-cut fruits or fruit mix stored at 4 and 8 °C (Supplementary Table 1).

4. Discussion

In this study, we assessed the behavior of *L. monocytogenes* inoculated in low populations in fresh-cut tropical fruits stored at distinct temperatures. Previous researchers have described the survival and growth of *L. monocytogenes* inoculated in high-inoculum sizes (i.e., 4–6 log cfu.g^{-1}) in a range of fruits that were stored at different temperatures (Amaro et al., 2018; Danyluk et al., 2014; Huang et al., 2019; Rangel-Vargas et al., 2018, Ziegler et al., 2019). However, it is unlikely that this phenomenon represents the real contamination in fresh-cut fruits. Moreover, high inoculum sizes increases the probability of cells to grow in stressful conditions (Manios et al., 2013). When cells in a large population are exposed to varied conditions of pH, a_w and temperature, the probability that one or more cells grow close to the boundary conditions increase compared to a low population scenario. Consequently, a large inoculum allows growth initiation under conditions where growth of low inoculum is usually inhibited before it gets the ability to out-compete endogenous microflora (Koutsoumanis and Sofos, 2005). Therefore, the evaluation of stressful conditions using low populations can represent a more real safety scenario for *L. monocytogenes* contamination in fruits and fresh produce.

The results of the present study indicated that even when inoculated in a very low concentration, *L. monocytogenes* cells were able to grow on fresh-cut melon, papaya, and fruit mix regardless of storage temperature. Interestingly, *L. monocytogenes* survived on fresh-cut mango at 8 °C and grew at higher tested temperatures. These results show that the intrinsic characteristics of the fruit influence the behavior of *L. monocytogenes* during storage. Fruits are a source of different bioactive compounds, such as phenolics and organic acids that may affect bacteria's survival by acting as antimicrobials (Kawacka et al., 2021; Dantas et al., 2019).

Notably, there was a clear difference between the dynamics of the *L. monocytogenes* low population on fresh-cut mango. One of the factors related to this difference is the lower pH of mango (3.9–4.1) compared to the other fruits. Furthermore, melon and papaya showed pH values > 5.4, which provide a more favorable environment for the pathogen survival and growth (Colás-Medà et al., 2017). Supporting our findings, Zhang et al. (2020) reported a higher occurrence *of L. monocytogenes* on fresh-cut fruit with pH > 5 (melon, honeydew, papaya, and mixed melons) compared to fruits with more acid pH, such as mango, pineapple, and strawberry.

Huang et al. (2019) evaluated the growth and survival of *L. monocytogenes* artificially inoculated (3 log cfu.g^{-1}) in cantaloupes, honeydews, watermelons and pineapples stored at 4, 8 and 12 °C for 7 days, and found different growth behavior in pineapple, which has more acid pH (3.3). While L. *monocytogenes* increased after 2 days on fresh-cut cantaloupe, honeydew and watermelon stored at 4 °C, on pineapple stored at the same condition, the pathogen was below the detection limit (3 log cfu.g^{-1}). Furthermore, on fresh-cut melon stored at 8 and 12 °C, *L. monocytogenes* increased around 4 and 5 log cfu.g^{-1}, respectively after 7 days, while after this storage time the pathogen was not detected on pineapple. Colás-Medà et al. (2016) also observed higher growth of *L. monocytogenes* on fresh-cut melon compared to pear. The pathogen population increased around 2 and 4 cfu g^{-1} in pear and melon over 7 days of storage at 10 °C. According the authors, the lower *L. monocytogenes* population on pear was probably due to its lower pH (4.99) compared to the pH of melon (6.13).

The results found by Danyluk et al. (2014) showed the ability of *L. monocytogenes* to grow in melon cubes stored for 7 days in temperatures ranging from 4 to 25 °C, with increased growth at abusive

temperatures, similar to observed in our study, despite the higher inoculum used (3 log cfu.g^{-1}) compared to our study. Together, these results suggest that melon has higher probability to cause listerioses than other fruits even if contaminated with very low population of *L. monocytogenes* before packaging, probably due in part to its pH.

It is worth noticing that *L. monocytogenes* low population showed $\delta \geq$ 0.8 log cfu.g^{-1} on fresh-cut melon, papaya, and fruit mix regardless of storage temperature. These findings indicate that the bacteria can grow on these fruits, even when contaminated with a low microbial load and kept in cold storage. However, when stored at 4 and 8 °C, despite the δ values, the time (days) needed for *L. monocytogenes* low population to exceed the microbiological criterion of 100 cfu g^{-1} (i.e., 2 log cfu.g^{-1}) was longer than when stored at 12 and 16 °C. These findings challenge the common practice of storing melon and papaya in retail for more than 7 days of storage. At 12 °C, the situation is even worse since all fruits and fruit mix supported the growth of *L. monocytogenes* low-population. Considering that this temperature has been reported storage in retailers and could also be easily reached in domestic refrigerators, this raises a safety concerns in the cold chain (Huang et al., 2019).

Considering the cumulative distribution of *L. monocytogenes* calculated on mango, melon, papaya and fruit mix, the storage at 4 °C should be the option concerning safety during 10 days, since 100% of the fruits and fruit mix remained below the recommended limit of 2 log cfu.g^{-1} (EU, 2005). Storage at 8 °C may also be an option. However, at this temperature, *L. monocytogenes* counts reached the limit allowed in 100% of melon and papaya samples after 7 days of storage. In contrast, storage at 12 and 16 °C are concern on melon, papaya and fruit mix with 100% of samples exceeding the *L. monocytogenes* safety limits.

It is known that temperature is one of the most important factors that affect cellular metabolic reactions and is a critical factor that determines the survival and growth of pathogens on various food matrices, including fresh-cut fruits (Ziegler et al., 2019). Based on the findings of this study, the significant growth of low populations of *L. monocytogenes* observed in fresh-cut fruits and fruit mix during storage at abusive temperatures and the survival of this pathogen in refrigerated temperature further confirms the critical importance of temperature control to ensure food safety. Overall, regulatory agencies recommend that fresh-cut produce, including fruits, to be stored at 4 °C to prevent potential growth of the pathogen. This recommendation should be strictly followed since the increase in temperature may not prevent *L. monocytogenes* growth in all fresh-cut fruits over the 10-days of storage generally applied for these products.

The most natural microbial load of fresh-cut fruits comprises microorganisms related to whole fruit microbiota and surface mainly from soil particles, airborne spores, and irrigation water, further to those from cross-contamination during the handing, cutting, shredding, and storage of the fresh-cut fruits (Barth et al., 2009). In the current study, *Enterobacteriaceae* was the predominant microbial group among those evaluated, followed by LAB.

The antagonism between the natural competitive microbiota and *L. monocytogenes* in minimally processed fresh-produce has an important role on dynamics population of this pathogen (Carlin et al., 1996; Francis and O'Beirne, 1998). This ecological relation is primarily based on availability of nutrients. A high population of natural competitive microbial groups can delay the exponential phase and reduce the growth of *L. monocytogenes* showing anti-listerial effects (Koseki, 2015; Francis and O'Beirne, 1998).

Interestingly, *Enterobacteriaceae* generally competes with pathogenic microorganisms, causing inhibition of *L. monocytogenes* (Campo et al., 2001). However, in our study, *Enterobacteriaceae* did not inhibit *L. monocytogenes*. It is likely, the high availability of nutrients in fresh-cut fruits with a low population of *L. monocytogenes* reduced the competition, or even the production of anti-listeria compounds by *Enterobacteriaceae*.

LAB, the second group more abundant in fresh-cut fruits, may antagonize pathogenic *L. monocytogenes* through antimicrobial metabolites such as lactic acid, ethanol, and bacteriocins (Liner-es-Morales et al., 2018). However, in our research, *L. monocytogenes* grew in all fresh-cut fruits and fruit mix even when LAB reached the highest counts. Tissue injuries caused by cut processing may have created different microenvironments for *L. monocytogenes* more favorable for growth by offering greater resistance against the action of LAB and their metabolites (De Cesare et al., 2018). In addition, one of the adaptive mechanisms in *L. monocytogenes* is its ability to develop resistance to acids, including lactic acid that is the main product of BAL metabolism (Liu et al., 2020).

Molds and yeasts, generally reported as the majority in fresh-cut fruits microorganisms (Raybaudi-Massilia et al., 2009), were found in lower counts than the other groups. This fact may be due to the good hygienic practices during minimal processing. Most yeasts and molds hardly penetrate the skin of fruits without injuries due to the lack of enzymes to break the fruit peel. Thus, the contamination of these microorganisms in fresh-cut fruits is mainly due to inadequate hygiene conditions during processing, unsatisfactory cleaning of the tools, utensils, equipment, and building surfaces (Piano and Castillo-Israel, 2019). Since the present study was carried out in a biosafety cabinet and sterilized utensils, this justifies the low mold and yeast load.

The impact of pH of fresh-cut fruits and fruit mix on *L. monocytogenes* growth was evidenced in the results since in the fresh-cut mango (lower pH compared to other fruits), the pathogen only grew at 12 and 16 °C. In contrast, the fruit pH did not interfere in the growth of the indigenous bacteria, as noted by the TVC and *Enterobacteriaceae* counts. However, these bacterial counts comprise many species, some of which may include some better adapted to the fruit microenvironment (Ziegler et al., 2019).

5. Conclusion

This study evaluated the growth of *L. monocytogenes* low populations on fresh-cute mango, melon, papaya, and fruit mix under four storage temperatures. Results indicated that even at low levels, *L. monocytogenes* cells could survive and grow in fresh-cut fruits and fruit mix during refrigerated storage. Furthermore, even though temperature alone did not limit *L. monocytogenes* growth, the combination of storage conditions and the low pH seems to slow growth and, consequently, ensure microbial safety. The findings highlight the ability of *L. monocytogenes* low-population to survive and grow in fresh-cut fruits and the importance of specific risk-based food safety measures concerning temperature control in the food retail.

Declaration of competing interest

The authors declare no conflict of interest.

Acknowledgments

Authors would like to thank the "*Conselho Nacional de Desenvolvimento Científico e Tecnológico*" (Grants # CNPq, Grants #303437/2017-0 and #405644/2018-3) and "*Coordenação de Aconselhamento de Pessoal de Nível Superior*" (CAPES-Brazil; UFPB/CAPES/PrInt 88881.311776/2018-01 Sandwich Doctorate Grant 88887.370630/2019-00).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fm.2021.103930.

References

Amaro, A.L., Spadafora, N.D., Pereira, M.J., Dhorajiwala, R., Herbert, R.J., Muller, C.T., Rogers, H.J., Pintado, M., 2018. Multitrait analysis of fresh-cut cantaloupe melon enables discrimination between storage times and temperatures and identifies potential markers for quality assessments. Food Chem. 241, 222–231.

AOAC (Ed.), 2016. Official Methods of Analysis, twentieth ed. AOAC Intl, Gaithersburg, MD.

Barbosa, I.M., Almeida, E.T.C., Castellano, L.R.C., de Souza, E.L., 2019. Influence of stressing conditions caused by organic acids salts on tolerance of *Listeria monocytogenes* to *Origanum vulgare* L. and *Rosmarinus officinalis* L. essential oils and damage in bacterial physiological functions. Food Microbiol. 84, 103240.

Barth, M., Hankinson, T.R., Zhuang, H., Breidt, F., 2009. Microbiological spoilage of fruits and vegetables. In: Sperber, W., Doyle, M. (Eds.), *Compendium of the Microbiological Spoilage of Foods and Beverages.* Food Microbiol. Food Safety, pp. 135–183.

Camelo, A.F.L., 2004. Manual for the Preparation and Sale of Fruits and Vegetables: from Field to Market, vol. 151. FAO Agricultural Services Bulletin, Rome.

Campo, J., Carlin, F., Nguyen-The, C., 2001. Effects of epiphytic enterobacteriaceae and pseudomonads on the growth of *Listeria monocytogenes* in model media. J. Food Protect. 64, 721–724.

Carlin, F., Nguyen-The, C., Morris, C.E., 1996. Influence of background microflora on Listeria monocytogenes on minimally processed fresh broad-leaved endive (Cichorium endicia var. latifolia. J. Food Protect. 59, 698–703.

Colás-Medà, Pilar, Abadias, Maribel, Oliveira, Marcia, Usall, Josep, Vinas, Immaculada, 2016. Influence of fruit matrix and storage temperature on the survival of Listeria monocytogenes in a gastrointestinal simulation. Food Contr. 73. https://doi.org/10.1016/j.foodcont.2016.10.018.

Colás-Medà, P., Viñas, I., Oliveira, M., Anguera, M., Serrano, J.C.E., Abadias, M., 2017. Exposure to minimally processed pear and melon during shelf life could modify the pathogenic potential of *Listeria monocytogenes*. Food Microbiol. 62, 275–281.

Dantas, A.M., Mafaldo, I.M., Oliveira, P.M.L., Lima, M.S., Magnani, M., Borges, G.S.C., 2019. Bioaccessibility of phenolic compounds in native and exotic frozen pulps explored in Brazil using a digestion model coupled with a simulated intestinal barrier. Food Chem. 274, 202–221.

Danyluk, M.D., Friedrich, L.M., Schaffner, D.W., 2014. Modeling the growth of *Listeria monocytogenes* on cut cantaloupe, honeydew and watermelon. Food Microbiol. 38, 52–55.

De Cesare, A., Vitali, S., Tessema, G.T., Trevisani, M., Fagereng, T.M., Beaufort, A., Manfreda, G., Skjerdal, T., 2018. Modeling the growth kinetics of *Listeria monocytogenes* in pasta salads at different storage temperatures and packaging conditions. Food Microbiol. 76, 154–163.

De Corato, U., 2020. Improving the shelf-live and quality of fresh and minimally-processed fruits and vegetables for a modern food industry. A comprehensive critical review from the traditional technologies into the most promising advancements. Crit. Rev. Food Sci. Nutr. 60, 940–975.

De Corato, U., Cancellara, F.A., 2019. Measures, technologies, and incentives for cleaning the minimally processed fruits and vegetables supply chain in the Italian food industry. J. Clean. Prod. 237, 117735.

EFSA – European Food Safety Authority, 2016. The European Union summary report on trends and sources of zoonoses, zoonotic agents and foodborne outbreaks in. Available in: https://www.efsa.europa.eu/en/efsajournal/pub/5077. Acesse in: september 20th 2020.

EU - European Commission, 2005. Consolidated text: commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs (Text with EEA relevance). https://ec.europa.eu/food/safety/biosafety/food_hygiene/microbiological_criteria_en. Acesse in: september 10th 2020.

Fana, Z., Xiea, J., Lib, Y., Wanga, H., 2019. Listeriosis in mainland China: a systematic review. Int. J. Infect. Dis. 81, 17–24.

FAO – Food and Agriculture Organization, 2020. Minor tropical fruits - mainstreaming a niche market. http://www.fao.org/3/a-I8080e.pdf. Acesse in: december 20th 2020.

FDA – Food and Drug Administration, 2008. Guide to minimize microbial food safety hazards of fresh-cut fruits and vegetables. Available in: http://www.fds.gov/food/GuidanceComplianceRegulatoryInformation/GuidanceDocuments. Acesse in: september 09th 2020.

FDA – Food and Drug Administration, 2020. Department of health and human Services. Code of Federal Regulations (CRF). Chemicals used in washing or to assist in the peeling of fruits and vegetables. Code of Federal Regulations 21 (Part 173. 315). Available at: https://www.govinfo.gov/app/collection/cfr. Accessed June 20th 2021.

FDA - US Food and Drug Administration, 2021. Outbreaks of foodborne illness. Available in: https://www.fda.gov/food/recalls-outbreaks-emergencies/outbreaks-foodborne-illness. Acesse in: september 09th 2020.

Francis, G.A., O'Beirne, D., 1998. Effects of the indigenous microflora of minimally processed lettuce on the survival and growth of *Listeria innocua*. Int. J. Food Sci. Technol. 33, 477–488.

Francois, K., Devlieghere, F., Standaert, A.R., Geeraerd, A.H., Van Impe, J.F., Debevere, J., 2003. Modelling the individual cell lag phase. Isolating single cells: protocol development. Lett. Appl. Microbiol. 37, 26–30.

Global Market Insights, 2020. Insights to Innovation. In: https://www.gminsights.com/industry-analysis/processed-fruits-and-vegetables-market. Acesse in: december 20th 2020.

Huang, J., Luo, Y., Zhou, B., Zheng, J., Nou, X., 2019. Growth and survival of *Salmonela* enterica and *Listeria monocytogenes* on fresh-cut produce and their juice extracts: impacts and interactions of food matrices and temperature abuse conditions. Food Control 100, 300–304.

Kawacka, I., Olejnik-Schmidt, A., Schmidt, M., Sip, A., 2021. Natural plant-derived chemical compounds as *Listeria monocytogenes* inhibitors *in vitro* and in food model systems. Pathogens 10, 12.

Koseki, A., 2015. Modelling the spread of pathogen contamination in fresh produce. In: Sofos, J. (Ed.), Advances in Microbial Food Safety. Woodhead Publishing Series in Food Science, Technology and Nutrition. Woodhead Publishing, pp. 220–237.

Koutsoumanis, K.P., Sofos, J.N., 2005. Effect of inoculum size on the combined temperature, pH and a_w limits for growth of *Listeria monocytogenes*. Int. J. Food Microbiol. 104, 83–91.

Lineres-Morales, J.R., Gutiérrez-Méndez, N., Rivera-Chavira, B.E., Pérez-Veja, S.B., Nevárez-Moorillón, G.V., 2018. Biocontrol processes in fruits and fresh produce, the use of lactic acid bacteria as sustainable option. Front. Sustain. Food Syst. 2, 50–63.

Liu, Y., Zhu, L., Dong, P., Liang, R., Mao, Y., Yang, X., Zhang, Y., Luo, X., 2020. Acid tolerance response of *Listeria monocytogenes* in various external pHs with different concentrations of lactic acid. Foodb. Pathog. Dis. 17 (4), 253–261.

Manios, S.G., Konstantinidis, N., Gounadaki, A.S., Skandamis, P.N., 2013. Dynamics of low (1-4 cells) *vs* high populations of *Listeria monocytogenes* and *Salmonella* Typhimurium in fresh-cut salads and their sterile liquid or solidified extracts. Food Control 29, 318–327.

Piano, A.M.P., Castillo-Israel, K.A.T., 2019. Physico-chemical quality and microbial safety evaluation of ready-to-eat fresh-cut watermelon and pineapple sold in Imus, Cavite, Philippines. Food Res. 3 (6), 684–692.

Putnik, P., Kovacevic, D.B., Herceg, K., Roohinejad, S., Greiner, R., Bekhit, A.E., Levaj, B., 2017. Modelling the shelf-life of minimally-processed fresh-cut apples packaged in a modified atmosphere using food quality parameters. Food Control 81, 55–64.

Qi, Y., He, Y., Beuchat, L.R., Zhang, W., Deng, X., 2020. Glove-mediated transfer of *Listeria monocytogenes* on fresh-cut cantaloupe. Food Microbiol. 88, 103396.

Rangel-Vargas, E., Luna-Rojo, A.M., Cadena-Ramírez, A., Torres-Vitela, Ma, R., Gomez-Aldapa, C.A., Villarruel-López, A., Tellez-Jurado, A., Villagómez-Ibarra, J.R., Reynoso-Camacho, R., Castro-Rosas, J., 2018. Behavior of 11 foodborne bacteria on whole and cut mangoes var. Ataulfo and Kent and antibacterial activities of *Hibiscus sabdariffa* extracts and chemical sanitizers directly onto mangoes contaminated with foodborne bacteria. J. Food Protect. 81, 743–753.

Raybaudi-Massilia, R.M., Mosqueda-Melgar, J., Soliva-Fortuny, R., Martín-Belloso, O., 2009. Pathogenic and spoilage microorganisms in fresh-cut fruits and fruit juices by traditional and alternative natural antimicrobials. Compr. Rev. Food Sci. Food Saf. 8, 157–180.

Wolfenden, L., Barnes, C., Lane, C., McCrabb, S., Brown, H.M., Gerritsen, S., Barquera, S., Véjar, L.S., Munguía, A., Yoong, S.L., 2021. Consolidating evidence on the effectiveness of interventions promoting fruit and vegetable consumption: an umbrella review. Int. J. Behav. Nutr. Phys. Activ. 18, 11.

Zhang, H., Yamamoto, E., Murphy, J., Locas, A., 2020. Microbiological safety of ready-to-eat fresh-cut fruits and vegetables sold on the Canadian retail market. Int. J. Food Microbiol. 335, 108855.

Ziegler, M., Kent, D., Stephan, R., Guldímann, C., 2019. Growth potential of *Listeria monocytogenes* in twelve different types of RTE salads: impact of food matrix, storage temperature and storage time. Int. J. Food Microbiol. 296, 83–92.

Ziegler, M., Ruegg, S., Stephan, R., Guldimann, C., 2018. Growth potential of *Listeria monocytogenes* in six different RTE fruit products: impact of food matrix, storage temperature and shelf life. Italian J. Food Sfety. 7, 7581.