Research Article

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Salmonella enterica serovar Enteritidis and *Listeria monocytogenes* in mango (*Mangifera indica* L.) pulp: growth, survival and cross-contamination

Ana L Penteado,[a,*] M Fernanda P M de Castro[b] and Ana C B Rezende[c]

Abstract

BACKGROUND: This study examined the ability of *Salmonella enterica* serovar Enteritidis and *Listeria monocytogenes* to grow or survive in mango pulp stored at −20°C, 4°C, 10°C and 25°C, as well as to cross-contaminate mangoes by means of a knife contaminated with different levels of these pathogens.

RESULTS: At 25°C lag phase durations of 19 h and 7.2 h and generation times of 0.66 and 1.44 were obtained, respectively, for *S.* Enteritidis and *L. monocytogenes*. At 10°C only the growth of *L. monocytogenes* was observed. At 4°C both bacteria survived for 8 days. At −20°C *S.* Enteritidis was able to survive for 5 months while *L. monocytogenes* survived for 8 months. Cross-contamination was observed for knives contaminated with 10^6, 10^5 and 10^4 CFU mL^{-1} of *S.* Enteritidis and 10^6 and 10^5 CFU mL^{-1} of *L. monocytogenes*.

CONCLUSION: Both microorganisms can grow well in mango pulp at 25°C, thus lower temperatures for the maintenance of the pulps are crucial to avoid growth of these microorganisms. A refrigeration temperature of 10°C will avoid only the growth of *S.* Enteritidis. Thus good handling practices should be rigidly enforced to avoid any contamination as even at refrigeration and freezing temperatures survival of these pathogens may occur.

Keywords: growth; survival; mango pulp; *Listeria monocytogenes*; *Salmonella* sv. Enteritidis; cross-contamination

INTRODUCTION

The demand for fresh cut fruits has increased due to their characteristics of freshness and convenience. In general, these products do not pass through any process to eliminate pathogen microorganisms before their consumption. A long shelf life could theoretically provide time for these microorganisms to multiply without affecting the organoleptic qualities of the fruit and so could increase the risks of food-borne illness.[1]

Mango is one of the most important tropical fruits, much appreciated for its attractiveness, flavor, aroma and color characteristics.[2] However, like other fruits that are consumed *in natura*, it can be a vehicle for pathogenic bacteria. In the last 6 years four outbreaks of salmonellosis in mangoes or foods made with this fruit have been reported.[3−6] In spite of the protection offered by the fruit peel, pathogenic bacteria can be internalized in the hot water treatment to which mangoes are submitted for elimination of fly larvae.[7] The potential for contamination also exists in the steps of fruit slicing, juice and pulp preparation. In the recent multi-state outbreak linked to whole cantaloupes in the USA 25 people died and fruit contamination was attributed to a lack of hygienic conditions and good agricultural practices.[8] In fruits of low acidity, in which mango is included, *Listeria monocytogenes* may also find the conditions to survive and multiply. Growth and survival of these bacteria was reported by Penteado and Leitão[9,10] in melon, watermelon and papaya pulp fruits. In the evaluation of potential risks associated with pathogenic bacteria in fruits it is important to know the behavior of the bacteria under different storage conditions. This study was undertaken to examine the ability of *Salmonella enterica* serovar Enteritidis (*S.* Enteritidis) and *L. monocytogenes* to grow or survive in mango pulp stored at −20°C, 4°C, 10°C and 25°C. Studies were also carried out on cross-contamination of mangoes by means of a knife contaminated with different levels of these pathogens.

* *Correspondence to: Ana L Penteado, Centro Nacional de Pesquisa de Tecnologia Agroindustrial de Alimentos (CTAA – EMBRAPA), Av. das Américas, 29501, Guaratiba – R.J., Brazil, E-mail: analucia.penteado@embrapa.br*

a *Centro Nacional de Pesquisa de Tecnologia Agroindustrial de Alimentos (CTAA – EMBRAPA), Av. das Américas, 29501, Guaratiba, – R.J., Brazil*

b *Grupo de Engenharia e Pós-Colheita, Instituto de Tecnologia de Alimentos (ITAL), Av. Brasil 2880, Jd. Brasil, Campinas, – S.P., CEP 13070-178, CP 139, Brazil*

c *Faculdade de Ciências Farmacêuticas, Universidade de São Paulo (FCF/USP), Av. Prof. Lineu Prestes, 580, 13B, Cidade Universitária, CEP 05508-000, São Paulo, SP, Brazil*

MATERIAL AND METHODS

Survival and growth of *S.* Enteritidis and *L. monocytogenes* in mango pulp

Fruit

The fruits used in this study were ripe, damage-free mangoes (*Mangifera indica* L., variety Palmer) obtained from a wholesaler in Rio de Janeiro, Brazil.

Sample pulp preparation

The external surfaces of the fruit were cotton scrubbed with an alcoholic iodine solution (2%),[11] and allowed to air dry inside a laminar airflow cabinet (VLFS-12; VECO, Campinas, São Paulo, Brazil). The fruits were opened aseptically in a laminar flow cabinet (VECO), removing portions (approximately 300 g) of pulp which were homogenized in sterile 'stomacher' bags. After mixing, 50 g portions of the pulp were carefully removed with a spoon and transferred to sterilized Erlenmeyer flasks (250 mL) and pasteurized at 80°C for 1 min to eliminate background microflora. Due to the viscosity, sterilized magnets were placed inside the Erlenmeyer flasks to allow proper homogenization in a vortex mixer (MS1 Minishaker IKA; Wilmington, North Carolina, USA) at the sampling time. Before the inoculation tests, the pulp was checked for sterility and then frozen.

Bacteria culture

A strain of *S.* Enteritidis isolated from poultry and *L. monocytogenes* Scott A (serotype 4b), from the culture collection of the Hygiene Laboratory, Food Engineering College, Campinas State Univesity, Brazil, was used in this study. The cultures of *S.* Enteritidis and *L. monocytogenes* were maintained, respectively, in tryptone soy agar slants (TSA; Oxoid, Basingstoke, UK) and TSA + yeast extract (YE; Oxoid) at 5°C. The identity of the strains was previously confirmed by biochemical and serological tests.[12]

Inocula preparation

Prior to each experiment the *S.* Enteritidis and *L. monocytogenes* cultures were streaked onto TSA and TSA + YE and incubated at 35°C and 30°C, respectively, for 24 h. Inocula transfer to the respective culture media was carried out for each bacterium after three consecutive 24 h intervals immediately before their use in the experiment. Cells of each strain were collected from the medium and transferred to 5 mL saline solution (0.85% NaCl) to adjust the suspension to a concentration of 10^8 CFU mL^{-1} according to the MacFarland turbidity scale using a Densimat (BioMerieux, Marcy L'Etoile Rhône-ALpes, France). The bacterial suspensions were serially diluted (1:10) in 0.1% peptone water, and 1-mL aliquots of each dilution were pour plated in TSA agar, followed by incubation at 35°C for 24 h for *S.* Enteritidis, and in TSA-YE agar, followed by incubation at 30°C for 24 h, for *L. monocytogenes*, to determine the viable cell concentration. Suspensions of the testing organism yielding final populations of approximately 7×10^2 CFU g^{-1} were used.

Fruit inoculation

Each triplicate test portion of the pulp (50 g) was inoculated with 1 mL of a 10^4 CFU mL^{-1} suspension of *S.* Enteritidis and of *L. monocytogenes*. Mango samples were placed into sterile stomacher bags (Nasco, Modesto, CA, USA). Each bag was folded over and not sealed to allow air movement in order to prevent creation of an anaerobic environment due to fruit respiration. Mango samples were stored at -20 ± 2°C, 4°C ± 2°C, 10 ± 2°C and 25 ± 2°C. Three replications were performed.

Enumeration of pathogens

Bacterial populations were enumerated from fruit pulp samples at different storage temperatures and periods of time:

- *Salmonella*: −20°C (zero time, 1, 2 and 3 months), 4°C and 10°C (0, 24, 48, 72, 96, 120, 144 and 168 h), and 25°C (0, 2, 4, 6, 8, 24, 25, 26, 28, 29, 30, 31, 32 and 48 h)
- *L. monocytogenes*: −20°C (zero time, 1, 2, 3, 4, 5, 6, 7 and 8 months), 4°C and 10°C (0, 24, 48,120, 144, 168,192 and 216 h), and 25°C (0, 2, 4, 8,19, 20, 21, 23, 24, 25, 26, 27, 29,30, 31, 43 and 48 h).

At each sampling time, 1 mL of fruit pulp was collected, serially diluted (1:10) in peptone water (0.1%) and pour plate dispersed in TSA and TSA-YE (45°C) for *Salmonella* and *L. monocytogenes*, respectively. The plates were incubated at 35°C for 24 h for *Salmonella* and at 30°C for 24 h for *L. monocytogenes* followed by counting, using a colony counter (Phoenix CP 600 Plus; Araraquara, São Paulo, Brazil), with the results being expressed in CFU g^{-1}.

Physical–chemical analyses

Soluble solids were determined by using a hand refractometer (model N-1α; Atago, Tokyo, Japan) with a scale of 0 to 32°Brix. Titratable acidity was determined by titrating 10 g of the homogenized pulp with 0.01 mol L^{-1} NaOH to an end point of pH 8.1 with the results expressed as g citric acid 100 g^{-1}.[13] The pH value was obtained by the potentiometer method, with a pH meter, blend Tecnopon, model mPA 210/mPA – 210P (Piracicaba, São Paulo, Brazil). The pH was not monitored during the incubation period.

Growth modeling

Data for growth modeling were compiled in Excel spreadsheets (Microsoft, Redmond, WA, USA). Statistical Program version 8.0, 2000 (Statistical Analyses System Institute, Cary, NC, USA,) was used to fit observed growth data to the Gompertz function: $y = A \times \exp[-\exp(B - Cx)]$, where y is $\text{Log}(N/N_0)$, $A = \text{Log}(N_{max}/N_0$, $B = 1 + (\mu_m \times \lambda/A)$, x = time (h) and $C = (B - 1)/\lambda$. N is the microbial population at a specific time (log CFU g^{-1}), N_0 is the initial microbial population (log g^{-1}), N_{max} is the maximum population, μ_m is the maximum growth rate, λ is the lag time and G is the generation time. Kinetic values, including the exponential growth rate (μ), generation time (G) and lag-phase duration (λ) were calculated from the generated Gompertz parameters as follow: $\mu = AC/\exp(1)$; $\lambda = (B - 1)/C$ and $G = \log(2)/\mu$.[14]

Statistical analyses

The growth parameters were compared using Tukey's paired comparison procedure.[15]

Cross-contamination of *S.* Enteritidis and *L. monocytogenes* into mango by means of contaminated cutting knives

Cross-contamination procedure

Fruits without any defects (ruptures to the peel, bruised areas) were used in the experiment. Surfaces of the mangoes were disinfected aseptically with an alcoholic solution of iodine[11] and left for 30 min in an air flow cabinet. Each knife (duplicated)

was previously contaminated on one side with a final bacterial population of 10^4, 10^5 and 10^6 CFU in 100 µL of peptone water (0.1%), and left to dry inside the air cabinet. These contaminated knives were used to cut the mango. The cut-contaminated mango pieces and the knife were separately put inside a plastic bag with peptone water, homogenized/washed and 1 mL portions were taken and serially diluted and pour plated in TSA (*S.* Enteritidis) and TSA-YE (*L. monocytogenes*). Three repetitions for each assay were carried out. The plates were incubated at 35°C and 30°C/24h, respectively for *S.* Enteritidis and *L. monocytogenes* and followed by counting, with the results being expressed in CFU g^{-1}

Recovery of bacteria from the contaminated cutting knife

Salmonella Enteritidis populations of 10^4, 10^5 and 10^6 CFU were separately used to contaminate (0.1 mL in one side of the knife) previously sterilized knives, two knives for each contamination level. The knives were left inside the laminar flow cabinet until completely dried and were then washed individually with 100 mL of peptone water (0.1%). The same procedure was conducted with *L. monocytogenes* with populations of 10^4, 10^5 and 10^6 CFU. Aliquots of 1 mL was removed from the washing water and pour plated in TSA (*S.* Enteritidis) and TSA-YE (*L. monocytogenes*) for counting.

RESULTS AND DISCUSSION

S. Enteritidis and *L. monocytogenes* in mango pulp

The physical–chemical results show that the fruit allows the growth of *S.* Enteritidis and *L. monocytogenes*. The average pH was 5.16, the value for total soluble solids was 10.9°Brix and total titratable acidity was 0.230 g citric acid 100 g^{-1}.

The procedure applied for superficial disinfectation of the peel and aseptic removal of pulp fruit was adequate. All the analyses of uninoculated samples performed initially and during the incubation period revealed the absence of *Salmonella* or other endogenous microbiota on the surface and in the internal tissues of the fruits. Samish *et al.*[16] mentioned that in healthy fruits the bacterial flora is assumed to be limited to the surface, while the inner tissue remains sterile.

The results presented in Fig. 1A and B confirm the adequacy of the pulp as a substrate to the growth of *S.* Enteritidis and *L. monocytogenes* in mango pulp when stored at 25°C for 48 h. At this temperature there was an increase of about 4.8 cycles log^{-1} after 48 h and the maximum population achieved was 7.6 log units for *S.* Enteritidis (Fig. 1A), while *L. monocytogenes* there was an increase of about 6 cycles log^{-1} with a maximum population of 8.6 log (Fig. 1B). At 10°C no growth occurred for *S.* Enteritidis, while for *L. monocytogenes* (Fig. 2) an increase of about 4 cycles log^{-1} was observed and the maximum population of 7 log units after 200 h. At 4°C both bacteria survived for 8 days. At −20°C *S.* Enteritidis was able to survive for 5 months while *L. monocytogenes* could still be recovered after 8 months.

The ability of these bacteria to grow in the pulp of fruits has been demonstrated by some authors. Escartin *et al.*[17] studied the growth of *S.* Typhi in a watermelon suspension in distilled water (up to 20% weight/vol, pH 5.03) at 22°C and reported a maximum population of 7.53 log CFU mL^{-1}. Golden *et al.*[18] inoculated watermelon with a suspension containing five species of *Salmonella* (*S.* Anatum, *S.* Chester, *S.* Havana, *S.* Poona and *S.* Senftenberg) and observed an increase of 6.0 log cycles and a maximum population of 8.6 log CFU g^{-1} after 24 h at 23°C.

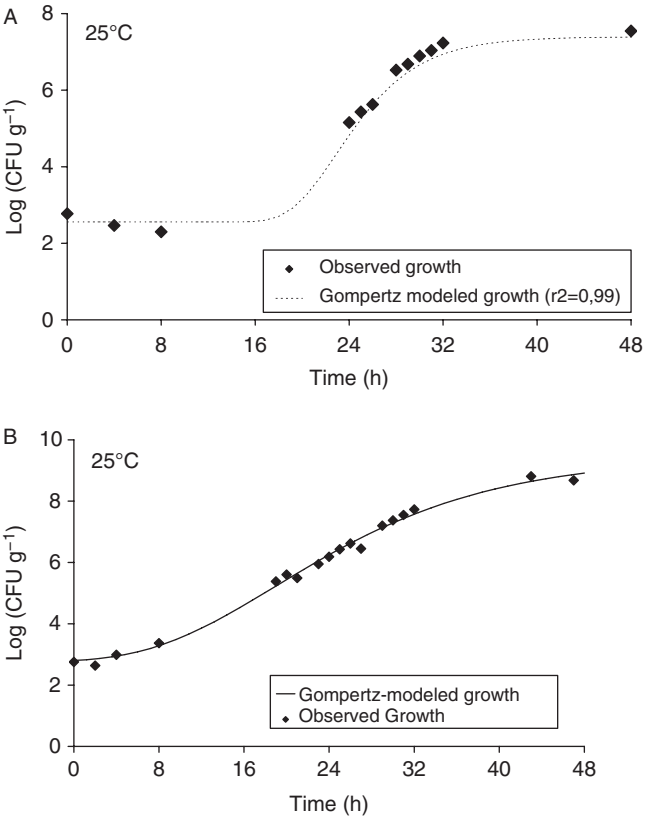

Figure 1. Observed and Gompertz-modeled growth curves for (A) *Salmonella enterica* serovar Enteritidis and (B) *Listeria monocytogenes* inoculated in mango pulp at 25°C.

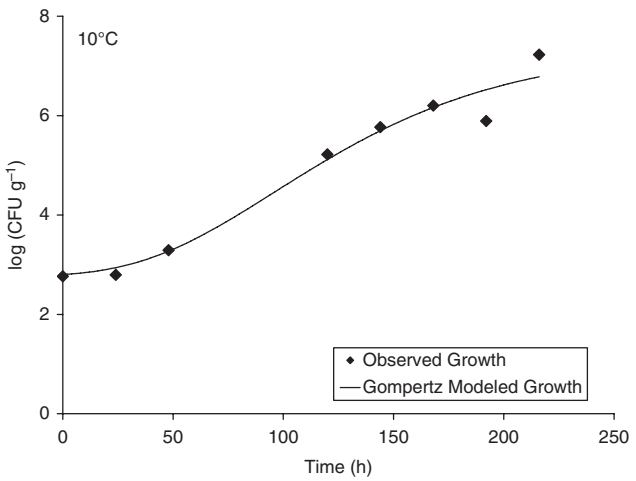

Figure 2. Observed and Gompertz-modeled growth curves for *Listeria monocytogenes* inoculated in mango pulp at 10°C.

Penteado and Leitão[10] verified an increase of 3.5 log cycles of *S.* Enteritidis in watermelon, melon and papaya pulp after the same period (24 h) at 20°C and maximum populations of 8, 9 and 8.5 log CFU g^{-1}, respectively. These same authors – working with *L. monocytogenes* inoculated in watermelon, melon and papaya pulp – verified a maximum population of 7.3, 9.2 and 4.43 log CFU g^{-1}, respectively, after 24 h at 20°C, and around of 8.9 CFU g^{-1} for melon, 6.10 log CFU g^{-1} for watermelon and 4.8 log CFU for papaya pulp after 168 h incubated at 10°C.[9]

Table 1. Generation time (*G*), exponential growth rate (μ), lag time (*λ*) for *Salmonella enterica* serovar Enteritidis and *Listeria monocytogenes* in different pulp fruit incubated at 10 and 20

Microorganism	Fruit	Temperature (°C)	*G* (h)	μ log [(CFU g^{-1}) h^{-1}]	*λ* (h)	Reference
S. Enteritidis	Melon pulp	20	1.54	0.20	8.00	24
L. monocytogenes	Melon pulp	20	1.74	0.17	6.00	24
L. monocytogenes	Melon pulp	10	7.16	0.04	24	24
L. monocytogenes	Persimmon pulp (Fuyu)	10	10.7	0.03	47.5	19
L. monocytogenes	Persimmon pulp (Rama Forte)	10	15.6	0.02	40.7	19
L. monocytogenes	Persimmon pulp (Fuyu)	20	1.5	0.20	5.3	19
L. monocytogenes	Persimmon pulp (Rama Forte)	20	1.6	0.18	7.5	19
S. Enteritidis	Persimmon pulp (Fuyu)	20	1.75	0.17	8.70	20
S. Enteritidis	Persimmon pulp (Rama Forte)	20	1.57	0.19	11.03	20

Similar results were obtained by Uchima *et al.*[19] for *L. monocytogenes* inoculated in two persimmon pulp varieties, with increases of 3.5 and 5.1 cycles log for 'Fuyu' and of 4 and 6.1 cycles log for 'Rama Forte' after 216 h at 10°C and 41h at 20°C, respectively. Working with *S.* Enteritidis in the same persimmons varieties, Rezende *et al.*,[20] observed an increase of 5.3 cycles log with a maximum populations of 7.9 log for 'Fuyu' and an increase of 5.5 cycles log with maximum population of 7.6 log for 'Rama Forte' after 48h at 20°C, and different from our research, these same authors observed the growth of *S.* Enteritidis in the pulp of these two persimmon varieties incubated at 10°C, with increases of 1.4 and 2 cycles log after 168h. Alegre *et al.*[21] observed an increase of 2 log at the *Salmonella* and *L. innocua* populations on minimally processed peaches when stored at 20 and 25°C after 24 h. At 10°C only *L. innocua* grew more than 1 log and it was the only pathogen able to grow at 5°C. In a similar study to ours, Strawn and Danyluk[22] verified an increase of 3.1 and 3.6 log for *Salmonella* spp. on cut mango and papaya, respectively, stored at 23°C after 24 h, and when these fruits were stored at 4, 12 and −20°C they observed only growth of *Salmonella* spp.

The observed data were fitted to the Gompertz function to obtain the growth parameters (generation time, exponential growth rate and lag phase duration). Duh and Schaffner[23] used the r^2 values as an indication of the model trustworthiness. In the present study the determination coeficient (r^2) was 0.90 indicating an excellent adjustment of the Gompertz function to the observed data.

The generation time and lag phase duration observed for *S.* Enteritidis in mango pulp at 25°C obtained from the Gompertz equation were 0.66 h and 19.02 h, respectively, while for *L. monocytogenes* the values were 1.44 (generation time) and 10.77 h (lag phase). At 10°C the generation time increased to 7.2 h while the lag phase was 35.42 h. The *G* and *λ* values were about seven and five times shorter when *L. monocytogenes* was incubated at 25°C than at 10°C; this shows that *L. monocytogenes* is able to grow in mango even at low temperature, because the rate of growth is reduced but not inhibited. The growth parameters obtained by Uchima *et al.*,[19] Rezende *et al.*[20] and Castro *et al.*[24] in a similar study carried out for some fruits are shown in Table 1.

The data presented in Table 1 show longer *G*, and smaller μ and *λ* than the obtained for *S.* Enteritidis in the present study at 25°C. For *L. monocytogenes*, the *G* values presented in Table 1 by Uchima *et al.*[19] are similar to those obtained in our research at 25°C, while the *G* obtained by Castro *et al.*[24] was 1.50 times shorter than in our research. The *λ* value was 1.47 and 1.2 times shorter than in our research, for Castro *et al.*,[24] respectively, at 10 and 20°C, and 1.3 times shorter for 'Fuyu' variety at 20°C,[19] 1.15 and 1.34 times longer for 'Rama Forte' and 'Fuyu' persimmons, respectively,[19] at 10°C. This shows that mango is a good substrate for the growth of both microorganisms studied.

Table 2. Recovery of *Salmonella enterica* serovar Enteritidis from the inoculated cutting knife

Inoculum concentration (CFU mL^{-1})	Inoculated on each knife (CFU mL^{-1})	Recovery from the knife (CFU mL^{-1})
2.6×10^7	2.6×10^6	1.4×10^4 (0.5%)
2.6×10^6	2.6×10^5	1.0×10^4 (3.8%)
2.6×10^5	2.6×10^4	8.75×10^2 (3.3%)

Table 3. Recovery of *Listeria monocytogenes* from the inoculated cutting knife

Inoculum concentration (CFU mL^{-1})	Inoculated on each knife (CFU mL^{-1})	Recovery from the knife (CFU mL^{-1})
8.3×10^7	8.3×10^6	1.02×10^4 (0.1%)
8.3×10^6	8.3×10^5	5.0×10 (0.006%)
8.3×10^5	8.3×10^4	0

Cross-contamination

Contamination of the fruit may occur through the transfer of bacteria from the external fruit surfaces to the edible portions during cutting and dividing, contaminating fresh-cut products. In addition, fresh-cut products may be re-contaminated through cross-contamination due to deficient hygiene practices, contaminated equipment, contamination via food handlers, processing or inadequate storage. Table 2 and Table 3 show the recovery of *S.* Enteritidis and *L. monocytogenes* respectively, from the cutting knife. The recovery of *S.* Enteritidis was between 0.5 and 3.8%, while the recovery of *L. monocytogenes* was between 0 and 0.1%.

These results showed that the *S.* Enteritidis population is able to adhere more to the cutting surface of the knife than can *L. monocytogenes*. This occurs because, for some bacteria, attachment to a surface is their predominant form of survival in nature and in man-made ecosystems.[25]

Table 4. *Salmonella enterica* serovar enteritidis on different sets of mangoes after cutting with a knife contaminated with different inoculum doses

Inoculum dose before cutting (CFU mL^{-1})	*Salmonella* in cutting mango (CFU mL^{-1})	In the knife after cutting (CFU mL^{-1})
2.6×10^6	5.1×10^2 (0.1%)	2.6×10^6 (99.9%)
2.6×10^5	3.3×10^2 (0.3%)	2.5×10^5 (99.7%)
2.6×10^4	4.8×10 (0.4%)	2.5×10^4 (99.6%)

Table 5. *Listeria monocytogenes* on different sets of mangoes after cutting with a knife contaminated with different inoculum doses

Inoculum dose before cutting (CFU mL^{-1})	*L. monocytogenes* in cutting mango (CFU mL^{-1})	In the knife after cutting (CFU mL^{-1})
8.3×10^6	5.3×10^2 (0.1%)	8.29×10^6 (99.9%)
8.3×10^5	1.5×10 (0.1%)	8.29×10^5 (99.9%)
8.3×10^4	0	8.3×10^4 (100%)

In the cross-contamination study it was observed that when cutting with a contaminated cutting knife, the mango had been contaminated as well. Table 4 and Table 5 show the counts of the *S.* Enteritidis and *L. monocytogenes* populations, respectively, that were found on the mango after it had been cut with the contaminated knife, as well as recovery of microorganisms on the knife after cutting the fruit.

When mangos were cut by knives inoculated with 10^6 and 10^5 there was a transfer of 10^2 CFU mL^{-1} (0.1 and 0.3%, respectively) of *S.* Enteritidis and 10^2 CFU mL^{-1} and 1.5×10 CFU mL^{-1}, respectively, for *L. monocytogenes*. As transfer rates can vary according to the organism, the type of surface and food product, cross-contamination should be assessed on a case-by-case basis. Different results were observed by Ravishankar *et al.*[26] when they evaluated cross-contamination and transfer rates of *Salmonella* from chicken to lettuce under three different food-handling scenarios, where they verified that *Salmonella* was easily transferred from cutting boards and knives to lettuce if utensils were not carefully sanitized after cutting chicken and before cutting lettuce. The transfer rate obtained was 45% (3 log CFU g^{-1}) to the lettuce and 35% (2 log CFU cm^{-2}) to the cutting board and knife, when they were unwashed after cutting the chicken. In a study by Moore *et al.*[27] *S.* Typhimurium showed transfer rates of 36% to 66% from stainless steel surfaces to lettuce.

After cutting the fruits, it was possible to recover both microorganisms studied from the surface of knives; the recovery of *S.* Enteritidis (Table 4) and *L. monocytogenes* (Table 5) was about 100%.

The study indicates that the inside of a mango could be contaminated with *S.* Enteritidis and *L. monocytogenes* during slicing by using a contaminated knife. Precautions should be taken in handling mango to minimize such contamination from the surface to the interior of the foods by using a cutting knife. Since *S.* Enteritidis can survive on the mango pulp even at lower temperatures good manufacturing practices should be applied for manipulation of this fruit.

CONCLUSION

The results confirmed that fruits such as mango are good substrates for the survival and growth of *Salmonella enterica* serovar Enteritidis and *Listeria monocytogenes*, and that low temperature (10°C) retards but does not stop the growth of *L. monocytogenes*. Mangoes can be considered as a potential risk as vehicles for food-borne diseases, considering that cross-contamination can occur if this fruit is cut with equipment, such as knives, that is contaminated by these microorganisms.

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