

REVIEW

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## Microbiological safety aspects of mangoes (*Mangifera indica*) and papayas (*Carica papaya*): a mini-review

### Aspectos de segurança microbiológica de manga (*Mangifera indica*) e papaya (*Carica papaya*): mini revisão

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#### ABSTRACT

This review describes several aspects related to microbiological safety in mangoes and papayas, such as incidence, outbreaks, internalisation and growth/survival of bacterial pathogens. Mangoes and papayas are often served sliced in food establishments in fresh pieces at salad bars, deli counters and as pulp juice. In general, these products do not undergo any process to eliminate pathogenic microorganisms before consumption, and a long shelf life could theoretically provide time for these microorganisms to multiply without affecting the organoleptic qualities of the fruit, thereby increasing the risks of food-borne illness. The data presented in this review show that low temperatures can impede microbial growth, but not completely inhibit such growth in mangoes and papayas. Highest growth rates were observed in the range between 22 and 37°C. In the last 20 years, several outbreaks of salmonellosis caused by these fruits or by food made with these fruits have been reported. The control of the temperature in the fruit washing water is important to prevent the internalisation of *Salmonella* spp. The implementation of strategies such as Good Agricultural Practices, Good Manufacturing Practices and Hazard Analysis Critical is important, as these methods can eliminate or significantly reduce microbial contamination.

**KEYWORDS:** Mango; Papaya; Safety; Pathogens; bacterium

#### RESUMO

Esta revisão descreve diversos aspectos relacionados à segurança microbiológica em manga e mamão papaya como; incidência, surtos, internalização e crescimento/sobrevivência de patógenos bacterianos nestas frutas. Mangas e papayas são frequentemente servidas fatiadas em estabelecimentos alimentícios como pedaços frescos, em misturas para saladas, expostas em balcões e como polpas de frutas. No geral, estes produtos não passam por qualquer processo para eliminar microrganismos patogênicos antes do seu consumo e uma vida longa de prateleira poderia teoricamente fornecer tempo para que estes microrganismos se multipliquem sem afetar as qualidades organolépticas destas frutas e assim aumentar o risco de doenças de origem alimentar. Os dados apresentados nesta revisão mostram que baixas temperaturas podem diminuir o crescimento de microrganismos mas não inibi-los em mangas e papayas. Os melhores crescimentos foram observados na faixa de 22-37°C. Nos últimos 20 anos diversos surtos de salmonelose nestas frutas ou produtos feitos com as mesmas foram relatados. O controle da temperatura da água de lavagem de frutas é importante para prevenir a internalização de *Salmonella* spp. A implementação de estratégias como Boas Práticas Agrícolas, Boas Práticas de Produção e Análise Crítica de Pontos de Controle são importantes já que podem eliminar ou reduzir significativamente a contaminação microbiana.

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**PALAVRAS- CHAVE:** Manga; Papaya; Segurança; Patógenos; bactérias



## INTRODUCTION

The consumption of fresh produce, an important source of nutrients, vitamins and fibre for humans, is steadily increasing worldwide. The World Health Organisation (WHO) and the Food and Agricultural Organisation (FAO) recommend a minimum of 400 g of fruit and vegetables per day for the prevention of chronic diseases, such as heart disease, cancer, diabetes and obesity, and for the prevention and alleviation of several micronutrient deficiencies, especially in less developed countries<sup>1,2</sup>.

From 1980 to 2004, global fruit and vegetable production increased. This resulted in higher food industry profits and export rates, but also in more frequent disease outbreaks and spoilage problems<sup>2,3,4</sup>.

Mango (*Mangifera indica* Linn) and papaya (*Carica papaya*) are tropical fruits of great economic importance and some of the most commonly eaten fruits in tropical countries around the world<sup>5</sup>. According to the FAO in 2013, India leads the world production of papaya with 5,544,000,00 tonnes, followed by Brazil (1,582,638,000 tonnes), Nigeria (773,000,000 tonnes) and Mexico (764,514,000 tonnes). India also has the highest mango production (18,002,000,000 tonnes), followed by Indonesia (2,058,607,000 tonnes), Mexico (1,901,871,000 tonnes), Pakistan (1,658,562,000 tonnes) and Brazil (1,163,000,000 tonnes)<sup>6,7</sup>.

At all stages of production, harvesting and processing, fruits and vegetables can become contaminated with microorganisms capable of causing human diseases<sup>8</sup>.

Fresh produce, such as fruit and salad, is often consumed raw without undergoing processing, such as cooking, to inactivate harmful microorganisms. In addition, further cutting, slicing or peeling causes tissue damage, which releases nutrients and facilitates microbial growth, putting consumers at risk of infection by contaminating organisms<sup>9,10</sup>. Mangoes and papayas are often served sliced in food establishments at salad bars and deli counters and as raw pulp juice. Preventing fruit and vegetable contamination with pathogenic microorganisms is complex because pathogens are normally present in the soil and may therefore be present on the surfaces of fruits and vegetables at harvesting<sup>8</sup>.

### Strategies for limiting microbiological contamination of fruits

according to Kokkinakis and Fragkiadakis<sup>11</sup>, Strawn et al.<sup>12</sup>, and Estrada-Acosta et al.<sup>13</sup>, implementation of good agricultural practices (GAPs) and good manufacturing practices (GMPs) enhances the safety of fresh produce and its value throughout the food chain. This also facilitates the implementation of Hazard Analysis Critical Control Points (HACCP), which have been developed to identify specific risks related to food processing and as risk control measures. Generally, HACCP programs are a proactive way to limit food safety risks.

On an international level, GAPs and GMPs are described in the Codex Alimentarius Commission's code of hygienic practice for fresh fruits and vegetables<sup>14</sup>. This code helps to control microbial,

chemical and physical hazards associated with all stages of fruit and vegetable production (i.e. environmental hygiene, agricultural input requirements, water for primary production, manure, biosolids and other natural fertilisers, soil, indoor facilities associated with growing and harvesting, personnel health, hygiene and sanitary facilities, equipment associated with growing and harvesting, handling, storage and transport, cleaning, maintenance and sanitation of premises and harvesting equipment).

Meanwhile, the HACCP is a tool to assess hazards and establish control systems that focus on prevention rather than rely mainly on end-product testing; it consists of seven principles as described by the Codex Alimentarius<sup>15</sup>.

To minimise the risk associated with microbial hazards of fruits, producers and processors have access to several detailed codes, guidelines and regulations, such as "The guide to minimise the microbial food safety hazards for fresh fruits and vegetables, Guidance for industry (FDA)<sup>16</sup>" "Microbiological hazards in fresh fruits and vegetables (FAO/WHO)<sup>17</sup>"; "Microbiological Risk Assessment (Codex)<sup>18</sup> and Microbial Risk Assessment Guideline (FSIS/USDA)<sup>19</sup>".

### Intervention methods to extend shelf-life and enhance safety

the best method to eliminate pathogens from produce is to prevent contamination in the first place. However, this is not always possible, and the need to wash and sanitise many types of produce remains of paramount importance to prevent disease outbreaks<sup>20</sup> and increase shelf life. As a result, different treatment methods can be applied to fresh produce, such as chemical, physical, controlled atmosphere storage and modified atmosphere packaging.

### Chemical methods

Calcium-based solutions. Calcium treatments have been used to extend the shelf life of fruits and vegetables. Calcium helps to maintain the vegetable cell wall integrity by interacting with pectin to form calcium pectate<sup>21</sup>. One of the most used compounds is calcium lactate, which has antibacterial properties due to its ability to uncouple microbial transport processes<sup>22</sup>.

Chlorine (hypochlorite) chemicals are often used to sanitise produce and surfaces in produce processing facilities as well as to reduce microbial populations in water used for cleaning and packaging operations. However, there are safety concerns about the production of chlorinated organic compounds and their impacts on human and environmental safety. Liquid chlorine and hypochlorites are generally used in the 50 to 200 ppm concentration range, with a contact time of 1 to 2 min to sanitise produce surfaces and processing equipment<sup>20</sup>.

Chlorine dioxide is a strong oxidising agent and a safe bactericide; it generates only a small amount of trihalomethanes (THMs) as a byproduct<sup>23</sup>. The Food and Drug Administration<sup>24</sup> has allowed the



use of aqueous chlorine dioxide in washing fruits and vegetables. A maximum of 3 ppm is allowed for contact with whole produce<sup>20</sup>.

**Electrolyzed water.** There are two types of electrolysed water with sanitising properties: acidic electrolysed water, or electrolysed oxidising water (AEW), and neutral electrolysed water (NEW). These solutions are conventionally generated by electrolysis of aqueous sodium chloride, and an electrolysed acidic solution (AEW) or an electrolysed basic solution (NEW) is produced at the anode and cathode, respectively<sup>22</sup>. Recently, the use of electrolysed water as a sanitising agent for fresh produce has received considerable attention in microbial load reduction<sup>25</sup>.

Hydrogen peroxide is a colourless gas at room temperature. Because of its high oxidation potential, it has high bacteriostatic and bactericidal properties and has gained interest as a potential disinfectant in the fresh produce industry because of its strong oxidising power. It does not react with the organic compounds present in perishables to produce carcinogenic compounds and breaks down into water and oxygen. It has gained the status of Generally Regarded as Safe (GRAS) in 1986 for some of the food commodities<sup>25</sup>.

Ozone is efficient in reducing pathogens on fresh produce because of its strong oxidising capacity. However, using ozone as a disinfectant has disadvantages, including its instability and reactivity with organic materials. Thus, the effective elimination of microorganisms may require high concentrations, which may, in turn, cause sensory defects in fresh produce<sup>2</sup>. The effectiveness of ozone treatment on microbial loads depends on several factors, such as product type, target microorganism, initial microbial load level, physiological state of the bacterial cells and zone physical state, which may explain the diversity of published results<sup>26</sup>.

Quaternary ammonium compounds, commonly called "QAC", are cationic surfactants that are able to penetrate food contact surfaces more readily than other sanitisers<sup>22</sup>. The mode of action of these compounds against bacterial cells involves a general perturbation of lipid bilayer membranes<sup>27</sup>. Although they are not approved for direct food contact, QAC may only partly be useful when applied to whole produce, since the product must be peeled prior to consumption<sup>20</sup>.

Organic acids (e.g. lactic, citric, acetic or tartaric acid). The antimicrobial action of organic acids is due to a pH reduction of the environment, disruption of membrane transport and/or permeability, anion accumulation or reduction in internal cellular pH<sup>20</sup>. Organic acids have a potential to reduce microbial populations on fresh vegetables<sup>27</sup>.

## Physical methods

### Irradiation

Gamma-ray, X-ray and electron beams are called ionizing radiations, because they are capable of producing ions, electronically charged atoms or molecules. They have the same mechanisms in terms of their effects on foods and microorganisms<sup>22</sup>. Irradiation is an alternative treatment which is effective in decreasing microbial counts on ready-to-eat vegetables<sup>28</sup>.

### Steam jet-injection

Application of heat treatment is the most used method for stabilising foods not involving any chemicals, based on its capacity to destroy microorganisms and inactivate enzymes. However, heat can impair many organoleptic properties of foods and reduce the contents or bioavailability of some nutrients<sup>29</sup>. Short-time steam processing can be used as an alternative to chlorine in sanitising fresh-cut lettuce; such treatment can significantly reduce antioxidant levels, especially ascorbic acid concentration, and, to a lower extent, carotenoid levels<sup>30</sup>. From a safety point of view, steam treatment can keep the mesophilic load as low as chlorine treatment<sup>30</sup>.

### Temperature

Control of temperature is a key point in microbial growth control. Either refrigerating or heating can be applied to control or reduce microbial load, respectively. Furthermore, the air temperature can also be reduced to delay microbial proliferation. However, this method should be used as complementary technique as, on its own, it is not effective enough to ensure product safety<sup>31</sup>. The hygiene and temperature of water used during the handling of produce are of primary importance. Immersion of warm whole or fresh-cut produce in cool process solutions may induce infiltration of the solution (including contaminating microorganisms) into the product through openings in the peel, such as stem-end vascular tissue, lenticels, stomata, puncture wounds or other physical disruptions<sup>20</sup>.

Ultrasound is a nonthermal technology using sonic waves and requires the presence of a liquid medium for power transmission<sup>32,22</sup>. The inactivation of microorganisms through ultrasound is a complex process, and a number of factors influence its efficiency<sup>32</sup>.

Ultraviolet light is one alternative to decrease pathogenic bacterial levels on fresh produce; the maximum effect of the use of ultraviolet C (UV-C) light is obtained at a wavelength at 254 nm<sup>33,34</sup>. A dose in the range from 0.5 to 20 Jm<sup>-2</sup> leads to lethality by directly altering microbial DNA through dimer formation, thereby eliminating the risk of microbially induced disease<sup>33</sup>. Most commonly, UV-C is applied to fresh fruits and vegetables, since it acts directly or indirectly as an antimicrobial agent<sup>22</sup>.

Cold Atmospheric Plasma (CAP) is an emerging antimicrobial technology for decontaminating infected surfaces. The treatment uses non-thermal ionised gases (cold gas plasmas)<sup>22</sup> that are produced by the excitation of gas with electrical discharges at room temperature and atmospheric pressure<sup>35</sup>. This treatment shows significant potential for sanitation of fresh produce<sup>2</sup>.

Modified atmosphere packaging (MAP) involves the modification of the internal atmosphere composition of a package by reducing the amount of oxygen (O<sub>2</sub>) and replacing it by carbon dioxide (CO<sub>2</sub>) and/or nitrogen (N<sub>2</sub>)<sup>22</sup>. The MAP can be achieved passively (the package is sealed under normal air conditions) or actively (the package is flushed with a gas mixture before being closed)<sup>21</sup>. In combination with low temperatures, MAP could be used as a mild preservation technique to enhance the safety of minimally processed products<sup>36</sup>.



Active packaging has been defined as a packaging system actively changing the condition of the package to improve food safety, extend shelf life, enhance sensory properties and maintain the quality of the products<sup>37</sup>. There are different concepts of active food packaging, including oxygen scavengers, carbon dioxide absorbers and emitters, moisture absorbers, ethylene scavengers, ultraviolet (UV) barriers and other mechanisms delivering antioxidant, flavouring or antimicrobial activity<sup>38,39</sup>. Substances responsible for the active function can be obtained in a separate container, for instance in a small paper sachet, or be directly incorporated in the packaging material<sup>39</sup>. The substances that can be added are diverse ranging from organic acids, enzymes, bacteriocins, fungicides, natural extracts and ions to ethanol<sup>39,40</sup>. Currently, active packaging with ethylene scavengers, moisture and liquid absorbers as well as with antimicrobial effects is used in commercial fruit distribution<sup>41</sup>.

There are different technologies to reduce/eliminate the microorganisms present in fresh-cut fruits and vegetables. However, none of these sanitising methods can control all the parameters that maintain the quality and shelf-life of MPFVs. Therefore, the use of combined methods is crucial<sup>22</sup>.

In this review, the main focus was on the incidence, outbreaks, growth/survival and internalisation of bacterial pathogens in mangoes and papayas.

## METHOD

### Search strategy

The search for data on the incidence, outbreaks, growth/survival and internalisation of bacterial pathogens in mangoes and papayas was conducted from 1986 to 2016. Electronic searches were conducted using the following scientific bases: Web of Science, PubMed, Science Direct, Scopus and data from the "Centers for Disease Control-CDC-USA". The keywords used were incidence, isolation, prevalence, detection, growth, behaviour, survival, fruits, papayas, mangoes, internalisation, fruits, microbiological, quality, safety, outbreak.

## RESULTS

### Incidence

From farm to table, there are multiple opportunities for fresh produce to become contaminated by *Salmonella*, *Escherichia coli* O157:H7, *Campylobacter jejuni*, *Vibrio cholerae*, parasites and viruses that may contaminate raw manure or unpotable water as well as animals or potentially tainted surfaces, including human hands. In addition, pathogens such as *Listeria monocytogenes*, *Bacillus cereus* and *Clostridium botulinum* are naturally present in the soil<sup>42</sup> and may also be a problem.

An important consideration when addressing safety issues is the incidence of pathogens and outbreaks associated with particular food products<sup>9</sup>. The following studies show the incidence of bacterial pathogens in mangoes and papayas.

One hundred and fifty samples of fresh fruits and vegetables, collected over a period of 12 months from various localities in Karachi, Pakistan, were screened for *Listeria monocytogenes*. Two out of thirty samples of papaya were positive for this pathogen<sup>43</sup>.

The microbiological quality of street-sold fruits in San José, Costa Rica, was analysed over a two-year period from March 1990 to March 1993. Researchers evaluated the presence of *Salmonella* spp., *Shigella* spp., *Escherichia coli* and faecal coliforms in several foods. The results showed that *E. coli* was present in more than 10% of the mango and papaya samples, while *Salmonella* spp. and *Shigella* spp. could not be isolated from these fruits<sup>44</sup>.

Thirty samples of ripe papaya (*Carica papaya*) slices were collected in Calcutta, India, from itinerant roadside vendors over a three-month period. *Salmonella* and *Vibrio cholerae* results were positive in one sample each, and low levels of coagulase-positive *Staphylococcus aureus* were detected in 17% of the samples<sup>45</sup>.

Bordini et al.<sup>46</sup> analysed 100 mango samples produced in the Northeastern region of Brazil from September 2001 to May 2002 and marketed in the state of São Paulo. The authors did not observe the presence of *Salmonella* in any of the 33 samples of mangoes destined for export. However, *Salmonella* was detected in 2 out of 67 samples destined for the internal market.

The prevalence and quantity of *Salmonella* spp., *Salmonella* Typhi and *Salmonella* Typhimurium were identified in sliced fruits from hawker stalls and hypermarkets in Kuala Lumpur, Malaysia. *Salmonella* spp. and *Salmonella* Typhi were found, respectively, in six and three out of twenty samples of papaya and in two and one out of twenty samples of mango from hawker stalls<sup>47</sup>.

A total of 125 samples of fresh produce were collected from major supermarkets and local markets across Singapore and characterised with respect to microbiological quality. *Salmonella* and *E. coli* O 157:H7 were absent in only ten mango samples<sup>48</sup>.

### Outbreaks

Foodborne illness is a major public health concern worldwide in terms of the number of persons affected and the entailed economic costs<sup>2</sup>. According to the WHO<sup>49</sup>, in the year 2010, 31 foodborne global hazards caused 600 million foodborne illnesses and 420,000 deaths worldwide. Foodborne diarrhoeal disease agents caused 230,000 deaths, particularly non-typhoidal *Salmonella enterica*.

In the USA, in 2013, the Centers for Disease Control and Prevention (CDC) reported 818 foodborne disease outbreaks, resulting in 13,360 illnesses, 1,062 hospitalisations, 16 deaths and 14 food recalls (CDC, 2013)<sup>50</sup>. In the developing world, epidemiological data on foodborne diseases remain scarce. Even the most visible foodborne outbreaks often go unrecognised, uninvestigated or unreported and may only be visible if connected to major public health or economic impacts<sup>49</sup>.

According to the Brazilian Ministry of Health<sup>51</sup>, from 2007 to 2016, 6.848 outbreaks were related to the consumption of



contaminated food, with 121.283 illnesses, 17.517 hospitalisations and 111 deaths. Fruits and food were responsible for 0.6% of the outbreaks, while the number of non-identified foods related to the total of the outbreaks is still as high as 66.9%.

The following reported outbreaks are related to the consumption of mango and papaya contaminated by bacteria. A large outbreak of food poisoning occurred in September 1996 and involved at least 116 workers at a shipyard in Jurong, Singapore. Four samples of cut watermelon, pineapple, papaya and honeydew melon were tested positive for *Salmonella weltevreden*<sup>52</sup>. In 1998, an outbreak caused by *S. Oranienburg*, with nine cases and three hospitalisations, occurred in a private home in Washington state and was associated with the consumption of fresh imported mangoes purchased from a particular grocery chain<sup>53</sup>. In December 1999, the first reported outbreak of salmonellosis with mangoes in the United States occurred. Seventy-eight patients from 13 states were infected with *Salmonella* Newport. Fifteen patients were hospitalised and two died. The mangoes had been imported from a single farm in Brazil<sup>54</sup>. Another outbreak in 2001 of *S. enterica*, associated with the consumption of imported mangoes from Peru, occurred in the United States. The serotype was *Saintpaul*; 26 cases were reported<sup>55</sup>. In 2003, an outbreak due to the consumption of mangoes contaminated with *S. saintpaul* in a restaurant/delicatessen occurred in California, US, with 17 cases<sup>56</sup>. During the period from October 2006 to January 2007, an outbreak with 26 cases of *Salmonella* Litchfield infection occurred in Australia. This was the first Australian *Salmonella* outbreak associated with the consumption of papaya<sup>57</sup>. A total of 106 individuals were infected with *Salmonella* Agona in 25 states in the US from January 1 to August 25, 2011; no deaths were reported. This outbreak was related to eating fresh, whole imported papayas from Mexico<sup>58</sup>.

During August 2012, a multistate outbreak of *Salmonella* Braenderup in the USA occurred due to the consumption of imported mangoes from Mexico. A total of 127 persons were infected; 33 were hospitalised, but no deaths were reported. From July to August 2012, a similar strain of *S. braederup* caused 21 illnesses in Canada; the infection was linked to mangoes from Mexico<sup>59</sup>. In 2013, a multistate (4) outbreak occurred in the USA due to the consumption of papaya contaminated with *S. Thomson*, resulting in 13 cases, 6 hospitalisations and 1 death<sup>60</sup>. In 2014, two outbreaks due to the consumption of mango contaminated with *Salmonella* were reported in the USA, one multistate and the other in the state of Connecticut, each with four illnesses and one hospitalisation<sup>61</sup>.

All cited outbreaks were caused by *Salmonella* spp. Other pathogens, such as *L. monocytogenes*, are not sufficiently established as relevant fruit juice-borne pathogens in the scientific literature, as compared to *Salmonella* and *E. coli* O157:H7. However, this pathogen is of concern in fresh fruits and fruit juices, due to its ability to survive under a variety of adverse conditions. The reason why there are no reports of listerioses linked to the consumption of fruit or fresh juices, in contrast to the variety of outbreaks related to other enteropathogens, is unclear<sup>3</sup>.

### Internalisation

Physical barriers, such as skin or rind, do not necessarily prevent the contamination of produce, because cutting and slicing eliminate this protection and microbes can invade the internal tissue. In addition, bacterial microorganisms from contaminated washing water can enter fruits and vegetables under certain conditions<sup>45,62</sup>. Mangoes and papayas are tree fruits with similar processing procedures on the farm<sup>57</sup>. For example, at least three salmonellosis outbreaks may have been caused by the same mechanism through the immersion of warm papaya/mango in cooler water, resulting in a pressure difference between the produce core and the surrounding water, which allowed *Salmonella* present in the water to enter the fruit, generally through the area around the stem<sup>54,55,57</sup>.

### Growth/survival

The survival and/or growth of pathogens on fresh produce is influenced by the organism, produce item and environmental conditions in the field and thereafter, including storage conditions. In general, pathogens will survive, but not grow on the uninjured outer surface of fresh fruits or vegetables, partly due to the protective character of the plant's natural barriers (for example, cell walls and wax layers). In some cases, pathogen levels will decline on the outer surface<sup>9</sup>. One exception is the study conducted by Bordini et al.<sup>46</sup>, which reported that the number of *Salmonella* present on mango rind surfaces depended on the storage temperature; at 22°C, an increase up to 2.30 logs was observed, while at 8°C, no significant variation occurred.

Microorganisms can grow and survive on mangoes and papayas, as shown in the following studies. The ability of five strains of enteropathogenic bacteria (*Shigella sonnei*, *S. flexneri*, *S. dysenteriae*, *Salmonella derby* and *S. typhi*) to survive and grow on sliced jicama, papaya and watermelon was investigated. Small increases in the numbers of *Shigella* species occurred on inoculated papaya after storage for only 2 h at 25-27°C, and an increase of about 1.4 in 6 hours at room temperature was observed for *S. typhi* inoculated on this fruit. Both microorganisms could grow on papaya stored at temperatures of 22-27°C<sup>63</sup>.

Castillo and Escartin<sup>64</sup> studied the survival of *Campylobacter jejuni* on sliced watermelon and papaya. The populations on papaya cubes inoculated with this microorganism survived for at least 6 h. The percentage of survivors at 6 hours of storage ranged from 7.7 to 9.4. Decreases in count were substantial at 2 h of storage.

Yegeremu et al.<sup>65</sup> studied the fate of *Salmonella* species and *E. coli* in fresh-prepared orange, avocado, papaya and pineapple juices. They observed that *S. typhimurium* and *S. choleraesuis* could proliferate in papaya juice when stored at ambient temperatures. *Salmonella typhimurium* reached counts as high as 10<sup>9</sup> CFU/mL in 24 h, steadily increasing until 48 h. *Salmonella choleraesuis* reached its maximum count (10<sup>8</sup> CFU/ml) at 24 h, with a slight decrease thereafter. Counts of both *Salmonella* species increased by one log unit in 24 h at 4°C, but did not exceed 10<sup>5</sup> CFU/ml throughout the storage time.



Penteado and Leitão<sup>66,67</sup> investigated the growth of *L. monocytogenes* and *S. enteritidis* in papaya pulp. For *L. monocytogenes*, maximum populations of about 5, 4 and 7 log units were reached at temperatures of 10, 20 and 30°C, respectively, at the end of the incubation periods. Generation times (g) of 15.05, 6.42 and 1.16 were obtained and decreased as the temperatures increased. The same authors observed maximum populations of 10<sup>8</sup> CFU/g for 24- and 48-hr incubation periods and generation times of 16.61, 1.74, and 0.66 hrs at incubation temperatures of 10, 20 and 30°C, respectively.

Mutaku et al.<sup>68</sup> evaluated the growth potential of *E. coli* O 157:H7 in fresh juices of papaya, pineapple and avocado. In papaya juice, counts of the test strains increased at varying rates at both storage temperatures, ambient (20-25°C) and refrigeration (4°C).

Bordini et al.<sup>46</sup> studied *Salmonella enterica* behaviour in mangoes stored at temperatures of 8 and 22°C for 24 and 144 h and observed that mean population numbers increased in the rind, stem, middle and blossom end of the fruits at 22°C, over a period of 24 h, with values of 0.53, 1.16, 1.47 and 1.36 logs, respectively. With an incubation period of 144 h, the values were 1.84, 1.74, 2.30 and 2.30, respectively. At an incubation temperature of 8°C, the increase in the number of bacteria was smaller: 0.59 log in the stem end, 0.82 log in the middle side and 0.80 log in the blossom end after 24 h of incubation and 0.21, 0.22 and 0.47 log, respectively, after 144 h. At the rind surface, there was a decrease in the number of bacteria: 0.41 log MPN/g after 144 h.

Strawn and Danyluk<sup>69</sup> reported growth of *Salmonella* on cut mangoes stored at 23 ± 2°C and survival at 4 ± 2°C, regardless of initial inoculum concentrations. Population level was a factor at 12 ± 2°C, with *Salmonella* growth only at the high (5 log CFU/g) and medium (3 log CFU/g) inoculum levels. *Escherichia coli* O157:H7 grew rapidly on fresh-cut papayas at 23 ± 2°C and 12 ± 2°C and survived throughout the shelf life of cut, refrigerated papayas. Similarly, *Salmonella* grew rapidly on fresh-cut papayas at 23 ± 2°C and 12 ± 2°C and survived throughout the shelf life of refrigerated fresh-cut papayas (4 ± 2°C). Inoculum levels had no effect on *Salmonella* behaviour in cut papaya. Both microorganisms can survive on frozen cut mangoes and papayas for at least 180 days.

Barbosa et al.<sup>70</sup> inoculated mango slices with *S. aureus* and *L. monocytogenes* (10<sup>7</sup> CFU/g), and the viable cell numbers exhibited a reduction of only one log unit after six days of storage for *S. aureus*, while being constant at 10<sup>7</sup> CFU/g for *L. monocytogenes* over the same period.

Penteado et al.<sup>71</sup> studied the growth of *S. enteritidis* and *L. monocytogenes* in mangoes pulp at different temperatures and incubation times. At 25°C, the authors observed an increase of about 4.8 cycles log<sup>-1</sup> after 48 h of incubation and a maximum population of 7.6 log units for *S. enteritidis*, while *L. monocytogenes* exhibited an increase of about 6 cycles log<sup>-1</sup>, with a maximum population of 8.6 log for the same temperature and period of incubation. At 10°C, no growth could be observed for *S. enteritidis*. For *L. monocytogenes*, an increase of about 4 cycles log<sup>-1</sup> was observed, with a maximum population of 7 log units after 200 h. At 4°C, both

bacterial populations survived for eight days. At -20°C, *S. enteritidis* was able to survive for five months, while *L. monocytogenes* could still be recovered after eight months.

Ma et al.<sup>72</sup> studied the behaviour of *Salmonella* spp. on fresh-cut tropical fruits, such as dragon fruit, banana, starfruit, mango, pineapple, guava and wax apple, at 28 and 4°C at four inoculum levels: 0.1, 1.0, 2.0 and 3.0 log CFU/g. The population of *Salmonella* in mango remained equal to the initial inoculum level after six days of storage at 4°C for all fruits tested. At 28 ± 2°C/two days, there were increases of 0.11, 0.51 and 0.56 for inoculation levels of 0.1, 2.0 and 3.0, respectively, and a decrease of -0.39 for the inoculation level of 1.0 log CFU/g.

Table 1 shows the important factors to consider when conducting a bacterial growth study in fresh mango and papaya, including inoculation, storage conditions, incubation temperature and time, type of microorganism and pH. Along with storage temperature, pH is cited as the principal determinant of bacterial growth on fresh fruits. Many acidic fruits do not support the growth of human pathogens and even inactivate them<sup>73,74</sup>.

The chemical and biochemical composition of mango varies with cultivation, variety and stage of maturity. The major constituents of the pulp are water, carbohydrates, organic acids, fats, minerals, pigments, tannins, vitamins and flavour compounds<sup>76,77</sup>.

Fruits can be divided in two groups: those with pH ≤ 4 (high-acid fruits), where the growth of microbial pathogens is unlikely to occur, and those with a pH above 4 (low-acid fruits), where microbial growth is more likely (e.g. mango and papaya)<sup>8</sup>. Variation in pH exists among varieties, growing conditions and processing methods<sup>77</sup>, as in the case of the Dashehari mango cultivar. During ripening, the pH rose from 3.0 to 5.2<sup>78</sup>, demonstrating the importance of determining pH when conducting a growth study.

Papaya has low acidity when compared to other tropical fruits, which is a nutritional advantage, as it allows its consumption by people sensitive to fruit acids; however, this low acidity is a problem faced by processors, because high pH values favour enzymatic activity and microbial growth<sup>79</sup>.

As shown in Table 2, variation in pH occurs in both fruits, depending on the variables mentioned, which is of paramount importance when conducting studies related to the behaviour of microorganisms in this food.

## DISCUSSION

Mangoes and papayas are good substrates for pathogen growth and survival when stored in a variety of temperatures. Considering they are frequently manipulated, sliced and served in restaurants, hotels and at home (alone, mixed with other foods and as pulp juice) and remain exposed for hours on restaurant tables, normally at room temperature, these fruits could be considered as potential vehicles for foodborne diseases.

Possible microbiological contamination could be reduced if mangoes and papayas were cooked before consumption. This process is



Table 1. Growth and survival of pathogenic bacteria on mango and papaya.

Pathogen	Fruit type	pHo-pHf	Method of inoculation	Storage conditions	Temp. (°C)	Initial counts	Incub. Time	Final counts	Unit	Comments	Reference
<i>Salmonella</i> Typhi	Papaya cubes	5.69	Spot inoculation, cells suspended in saline solution, 1 drop	12 cm <sup>2</sup> cubes, inoculated stored in covered glass trays	25-27	2.9	6 h	4.3	CFU/cube	nd	63
<i>Shigella</i> (three species)	Papaya cubes	5.69	Spot inoculation, cells suspended in saline solution, 1 drop	12 cm <sup>2</sup> cubes, inoculated, stored in covered glass trays	25-27	1.9-2.2	6 h	3.8-4.4	CFU/cube	<i>S. sonnei</i> <i>S. flexneri</i> <i>S. dysenteriae</i>	63
						2.8		1.7			
<i>Campylobacter jejuni</i>	Papaya cubes	5.6-5.0	Spot inoculation cells suspended in saline solution, 0.02 ml inoculated per cube	24 cm <sup>2</sup> cubes. Stored in sterile stainless-steel trays with cover	25-29	2.8	6 h	1.7	CFU/cube	Serotype Penner 50	64
<i>Salmonella typhimurium</i>	Papaya juice	5.7-4.69	Inoculated with overnight broth culture	Inside screw cap bottles	4	3.99	24 h	5.2	CFU/mL	100 ml water/400 ml papaya. Steamed before inoculation 100°C/10 min/ papaya fresh ripened	65
						2.70	48 h	8.18			
<i>Salmonella choleraesuis</i>	Papaya juice	5.7-4.69	Inoculated with overnight broth culture	Inside screw cap bottles	37	3.52	24 h	4.45	CFU/mL	100 ml water/400 ml papaya. Steamed before inoculation 100°C/10 min/ papaya fresh ripened	65
						2.78	48 h	8.18			
<i>E. coli</i> (25922)	Papaya juice	5.7-4.69	Inoculated with overnight broth culture	Inside screw cap bottles	4	3.52	24 h	4.45	CFU/mL	100 ml water/400 ml papaya. Steamed before inoculation 100°C/10 min/ papaya fresh ripened	65
						2.70	48 h	9.40			
<i>E. coli</i> (9637)	Papaya juice	5.7-4.69	Inoculated with overnight broth culture	Inside screw cap bottles	4	2.78	24 h	2.54	CFU/mL	100 ml water/400 ml papaya. Steamed before inoculation 100°C/10 min/ papaya fresh ripened	65
						2.78	48 h	9.4			
<i>Salmonella</i> Enteritidis	Papaya pulp	4.87	Bacterial suspension diluted in 0.1% peptone water	50 g pulp, inoculated and stored in Erlenmeyer flasks	10	2.58	168 h	4.46	CFU/g	Pulps pasteurised (80°C/1 min) before inoculation tests	66
						2.42	48 h	8.68			
<i>Listeria monocytogenes</i>	Papaya pulp	4.87	Bacterial suspension diluted in 0.1% peptone water	50g pulp, inoculated and stored in Erlenmeyer flasks	30	2.54	24 h	8.81	CFU/g	Pulps pasteurised (80°C/1min) before inoculation tests	67
						2.45	168 h	4.84			
<i>E. coli</i> O 157:H7	Papaya fresh juice	5.17	Bacterial suspension at TSB + 0.6% yeast extract	250 ml juice inoculated and stored in screw-capped bottles	25	-3.3	96	9	CFU/ml	Four strains of <i>E. coli</i> O 157:H7 pH 4.5 after 120 hr/ juices steamed at 100°C/10min. before inoculation	68
						2.61	24 h	7.36			

Continue



Continuation

<i>Salmonella enterica</i>	Mangoes	4.49	Immersion in water with the inoculum	Inside sterile plastic bags	8	1.91 S <sup>1</sup> 0.76 M <sup>2</sup> 0.5 B <sup>3</sup> 1.7 R <sup>4*</sup>	24	2.5 S <sup>1</sup> 1.58 M <sup>2</sup> 1.3 B <sup>3</sup> 1.29* R <sup>4</sup> 2.29 S <sup>1</sup> 0.98 M <sup>2</sup> 0.97 B <sup>3</sup> 0.7* R <sup>4</sup> 3.07	CFU/g *MPN/g	Different portions of the mango (Stem-S, Middle-M, Blossom-B and Rind-R)	46	
					22	1.91 S 0.76 M 0.5 B 1.7 R*	24	2.23 1.86 2.23				
								3.65 3.06 2.8 3.5*				
						12	4.5	24 hr	5.9		Cocktail of <i>S. serovars. S. Michigan, S. Montevideo, S. Munchen, S. Newport, S. Saintpaul</i>	69
						23	2.9	7 d	2.4		<i>Mangoes cv Tommy Atkins</i> (ripe)	
						12	4.6	24 hr	4.5			
						23	2.9	28 d	1.5		Cocktail of four strains <i>Mangoes cv Tommy Atkins</i> (ripe)	69
						12	3.9	24 h	5.0			
						23	2.6	28 d	4.3		Cocktail of four strains <i>Papayas cv Red Lady</i> (ripe)	69
						23	2.6	24 h	6.0			
<i>E. coli</i> O157:H7					12	4.1	24 h	5.2		Cocktail of <i>S. serovars. S. Michigan, S. Montevideo, S. Munchen, S. Newport, S. Saintpaul</i>	69	
					23	2.6	7 d	6.3		<i>Papayas cv Red Lady</i> (ripe)		
					12	4.1	28 d	3.5				
					23	2.6	24 h	6.2				
<i>Salmonella</i>					5	7.0	12 d	5.0		<i>Mangoes at mature-green stage</i>	70	
					5	7.0	12 d	6.0		<i>Mangoes at mature-green stage</i>	70	
<i>S. aureus</i>					5	7.0	12 d	6.0			70	
					5	7.0	12 d	6.0			70	
<i>L. monocytogene</i>					5	7.0	12 d	6.0			70	
					5	7.0	12 d	6.0			70	
Continue												



Continuation																				
<i>Salmonella</i> Enteritidis	Mango pulp	5.16	Bacterial suspension diluted in 0.1% peptone water	50 g pulp, inoculated and stored in Erlenmeyer flasks	25	2.8	24 h	4.94	CFU/g	Pulps pasteurised (80°C/1min) before inoculation tests	71									
	Mango pulp	5.16	Bacterial suspension diluted in 0.1% peptone water	50 g pulp, inoculated and stored in Erlenmeyer flasks	25	2.79	48 h	6.14	CFU/g	Pulps pasteurised (80°C/1min) before inoculation tests	71									
<i>Listeria monocytogenes</i>	Mango slices	4.19	Bacterial suspension diluted in 0.1% peptone water	10g, inoculated, packed in polystyrene trays covered with PVC film	5	2.3	1 d	-4.0	CFU/g	Cocktail of six strains	75									
	Papaya slices	5.99	Bacterial suspension diluted in 0.1% peptone water	10g, inoculated, packed in polystyrene trays covered with PVC film	13	2.2	6 d	-4.0	CFU/g	Cocktail of six strains	75									
<i>S. aureus</i>	Mango slices	4.19	Bacterial suspension diluted in 0.1% peptone water	10g, inoculated, packed in polystyrene trays covered with PVC film	5	3.5	1 d	-3.5	CFU/g	Cocktail of four strains	75									
	Papaya slices	5.99	Bacterial suspension diluted in 0.1% peptone water	10g, inoculated, packed in polystyrene trays covered with PVC film	13	2.5	6 d	4.4	CFU/g	Cocktail of four strains	75									
	Mango (cv Palmer)	nd	Spot inoculated with 1 cell suspension	Fruit cubes packaged in sterilised bags	28	1.0	2 d	0.21	CFU/g	Cocktail 3 serotypes ( <i>S. Newport</i> , <i>S. Saintpaul</i> <i>S. enteritidis</i> )	72									
						2.0			0.61											
					3.0			2.51												
								3.56												

S<sup>1</sup> = Stem end; M<sup>2</sup> = Middle; B<sup>3</sup> = Blossom end; R<sup>4</sup> = Rind

usually effective in the elimination of any potentially pathogenic organisms, rendering these fruits safe to be consumed. However, mangoes and papayas are commonly eaten in a raw state, and the possible presence of pathogens on their surface or inside the fruits can be problematic during the manipulation process or even in the case of internalisation, which would allow the growth/survival of foodborne pathogens in these foods and pose a problem for the consumers. Pathogen internalisation into those fruits is a process that should be controlled with attention to the quality and temperature of the water applied in washing these fruits.

The first step to prevent contamination is to respect the preventive measures included in the GAPs, GMPs and HACCP.

Studies have shown that the application of preventive measures, such as washing hands, good personal hygiene, appropriate use of sanitary facilities, treated manure (fertilisers), quality of the irrigation water, avoiding flooding events, cleaning and sanitising of equipment, can reduce microbial contamination on fresh produce.

Himathongkham and Riemann<sup>86</sup> showed that treatment of dry chicken manure with ammonia results in a significant reduction of *Salmonella typhimurium*, *E. coli* O 157:H7 and *Listeria monocytogenes*.

In cooling and storage facilities, contamination can be reduced with the use of ozone; treatment of cold rooms has been reported to be effective in significantly reducing *Listeria monocytogenes*<sup>87</sup>.

Zhou et al.<sup>88</sup> studied the effect of ultrasound in combination with chlorine on the reduction of *Escherichia coli* O 157:H7 populations on lettuce coring knives; the results of these treatments with redesigned coring knives may provide practical options for minimising microbial safety hazards of lettuce processed by core-in-field operations.

According to Park et al.<sup>89</sup>, microbial contamination of produce is influenced by farm management and environmental factors. Specifically, contamination seems to be strongly influenced by the time since last irrigation, worker personal hygiene and field use prior to planting.

Rodrigues et al.<sup>90</sup> mentioned that preventive measures on lettuce farms, such as microbial quality and method of composting manure as well the source and quality of irrigation waters and washing waters, are of utmost importance in accordance with the obtained microbiological results. The authors also demonstrated that the fertiliser control program and the water used for irrigation and washing were important factors to be controlled in the production chain of organic lettuce in order to ensure food safety and a high hygiene status. With regards to irrigation and rinsing water, the results showed the importance of using water from safe sources.

Monaghan and Hutchison<sup>91</sup> showed that the numbers of generic *Escherichia coli* isolated from workers' hands declined with increasing thoroughness of hand-washing treatments. As reported by Park et al.<sup>89</sup>, contamination with generic *E. coli* was significantly reduced with an irrigation lapse time of > five days as well as by several factors related to field workers, including the use



Table 2. pH values of mangoes and papayas.

Fruit	pH	Comments	References
Mangoes var. Tommy Atkins	4.49	Central portion of the mango	46
Mango pulp (variety Palmer)	5.16	Ripe	66
Manga "Ubá"	3.90-4.29	-	80
Manga cv. Haden	4.28	Pulp	81
Mangoes cv "Tommy Atkins"	4.30	Mature-green stage	38
Mangoes cv Golden	5.39-6.14	Pulp, homogenised in distilled water, different maturity stages	82
Mango cv. Haden	2.4-4.5	Mix of pulps of the same maturity stage; pH increase with maturity	44
Mangoes	3.9-4.6	-	83
Papaya cv. "Maradol" red	5.5	Partially ripe	84
Papaya ( <i>Carica papaya</i> )	6.4-6.8	Ripe	15
Ripe papaya	5.69	Surface pH	32
Papaya pulp	4.87	Ripe	35
Papaya Formosa cv. Tainung 01	5.06-5.10	Stage 4 (51-75% yellow colour)/fruit juice	47
	4.1	75% ripe/fruit	85
Papaya	5.17	Fresh/juice	37
	5.2-5.7	-	83

of portable toilets, training to use portable toilets and the use of hand-washing stations.

Increased microbial load and pathogen prevalence in lettuce production was revealed for high temperature, flooding of lettuce fields, application of contaminated organic fertiliser, irrigation with water of inferior quality and large distances between the field and toilets, showing the importance of controlling the composting process of organic manure and the quality of the irrigation water, to improve and/or maintain the safety of lettuce during primary production<sup>92</sup>.

As stated by Bracket<sup>93</sup>, it should be remembered that a systems approach in maintaining sanitation and quality should be taken. All steps, from production through consumption, will affect the microflora. Applying proper sanitary procedures and insisting on utmost hygiene are indispensable. However, employing a good

HACCP program is also necessary to assure safety, as the use of HACCP helps to minimise the potential hazards that may be associated with fresh-cut produce processing<sup>93,94</sup>.

The application of HACCP to control enteric pathogens in processed crops was reviewed by Leifter et al.<sup>95</sup>. As mentioned by Hurst<sup>96</sup>, HACCP is the most comprehensive, science-based program for reducing pathogen contamination in fruit and vegetable products.

## CONCLUSION

Therefore, the implementation of strategies such as Good Agricultural Practices, Good Manufacturing Practices and Hazard Analysis Critical can eliminate or significantly reduce microbial contamination on fresh mangoes and papayas.

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#### Conflict of Interest

Authors have no potential conflict of interest to declare, related to this study's political or financial peers and institutions.



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