Review

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Microbiological safety of ready-to-eat minimally processed vegetables in Brazil: an overview

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Abstract

The market of ready-to-eat minimally processed vegetables (RTE-MPV) is increasing in Brazil and many other countries. During processing, these vegetables go through several steps that modify their natural structure while maintaining the same nutritional and sensory attributes as the fresh produce. One of the most important steps is washing-disinfection, which aims to reduce the microbial load, prevent cross-contamination and inactivate pathogenic microorganisms that may be present. Nonetheless, the presence of pathogens and occurrence of foodborne illnesses associated with consumption of RTE-MPV concern consumers, governments and the food industry. This review brings an overview on the microbiological safety of RTE-MPV, focusing on Brazilian findings. Most of the published data are on detection of *Salmonella* spp. and *Listeria monocytogenes*, indicating that their prevalence may range from 0.4% to 12.5% and from 0.6% to 3.1%, respectively. The presence of these pathogens in fresh produce is unacceptable and risky, mainly in RTE-MPV, because consumers expect them to be clean and sanitized and consequently safe for consumption without any additional care. Therefore, proper control during the production of RTE-MPV is mandatory to guarantee products with quality and safety to consumers.

Keywords: ready-to-eat vegetables; foodborne illness; microbiological safety; minimum processing; pathogenic microorganisms

INTRODUCTION

The market of ready-to-eat minimally processed vegetables (RTE-MPV) has increased in Brazil and many other countries, due to the current lifestyle characterized by a reduced time for food preparation and growing consumer demand for fresh and healthy products. Ready-to-eat products (e.g. fresh-cut lettuce, pre-packaged vegetable mixes, pre-washed bagged salads, etc.) provide convenience to consumers and have gained ground in restaurants, convenience stores, fast food chains and other establishments that prepare, serve or sell fresh produce.[1,2]

RTE-MPV go through several steps during their processing, which can modify their natural structure but maintain the same nutritional value and sensory attributes as the fresh produce.[3] One of these steps is washing, which aims to remove soil and debris and reduce microbial contamination, mainly when sanitizers are added to the wash water.[4] Consequently, consumers believe that these products are clean, healthy and do not require additional treatment (i.e. additional washing step) before consumption.

Nonetheless, an increased number of foodborne illnesses caused by pathogenic microorganisms, such as *Salmonella* spp., *Listeria monocytogenes* and pathogenic *Escherichia coli*, have been associated with the consumption of fresh and fresh-cut (i.e. RTE-MPV) produce over the past decades.[5–12] The occurrence of these pathogens in fresh produce has been often reported in many studies worldwide, highlighting these products as potential sources of human pathogens.

This review brings an overview on the microbiological safety of RTE-MPV, focusing on Brazilian findings, providing insights on the occurrence of bacterial pathogens in these products. Data on the occurrence of foodborne pathogens in RTE-MPV found in other countries are also compiled for comparison.

READY-TO-EAT MINIMALLY PROCESSED VEGETABLES (RTE-MPV)

RTE-MPV may be defined as fresh vegetables that have been subjected to one or more-unit operations, such as selecting, peeling, cutting, slicing, shredding, washing-disinfection, drying and

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packaging.[3,13] The greatest advantages of these products are the convenience in terms of easy and quick preparation of meals, reduction of waste caused by discarding undesirable parts, maintenance of freshness and nutritional value, and extended shelf-life depending on the type of preservation.[3]

The market of RTE-MPV in Brazil started in the late 1970s, with the arrival of fast-food restaurants in the south-eastern region of the country, and it has been growing quickly over the past years.[14] Although there is no official data on the market for these products in Brazil, the increase in their consumption and consumer attitudes toward RTE-MPV have been reported in some studies. Furthermore, these products are increasingly present in supermarkets and grocery stores across the country.

Perez *et al.*[15] conducted a survey with 246 individuals in supermarkets of the city of Belo Horizonte, Minas Gerais State, and observed that 56 (23%) of them consumed these products regularly, mainly due to convenience (46%), short preparation time (21%) and hygiene (11%). The main reasons for non-consumption were high price (31.9%), preference for selecting and preparing fresh vegetables (23%) and distrust in these products (17.9%). Sato *et al.*[16] conducted a similar survey in the city of Sao Paulo, Sao Paulo State, interviewing 42 individuals, of which 27 (64.3%) reported buying RTE-MPV on a regular basis. The main reasons for purchase were convenience (88.9%) and hygiene (29.6%), while the main reason for not purchasing was high price (52%).

Another survey conducted by Amorim and Nascimento[17] in supermarkets and grocery stores of two cities in Rio de Janeiro State, found similar results. Among 180 interviewed individuals, 120 (66.7%) reported buying RTE-MPV regularly and the main reasons were convenience (58.3%) and quality (25%). The reasons for not buying RTE-MPV were not addressed, but most interviewed individuals considered them expensive (29.2%) or very expensive (30%). These data point out that, despite being considered high-cost products, RTE-MPV are consumed regularly by a significant portion of the Brazilian population, mainly due to their convenience.

None of these Brazilian studies addressed the consumer´s perception of microbiological hazards associated with the consumption of RTE-MPV. However, a study conducted in the United States (US) regarding consumers' risk perceptions toward fresh-cut produce showed that most of them are very concerned about foodborne illnesses associated with fresh produce. However, they rarely perceive that fresh-cut produce may contains foodborne pathogens.[18]

MAIN SOURCES OF PRODUCE CONTAMINATION BY FOODBORNE PATHOGENS

Contamination of fresh produce with pathogenic microorganisms may occur during preharvest and postharvest stages. Several reviews point out that contaminated soil, irrigation water, raw (or poorly composted) animal manure used as fertilizer, and animals (domestic and wild) are the main sources of contamination at the preharvest stage. In contrast, postharvest sources may include human handling, contaminated harvesting equipment, containers and vehicles, wash and rinse water, improper storage and packaging.[5,19–22]

Studies conducted in Brazil on the occurrence of pathogenic microorganisms in primary production of leafy vegetables have highlighted that organic fertilizers, manured soils and water are the most important sources of contamination. Ceuppens *et al.*[23] submitted 260 samples, including lettuce, composted manure, soil, water and contact surfaces (swab of hands and transport boxes) collected in organic farms located in southern Brazil, to enumeration of hygiene indicator microorganisms and detection of *Salmonella* and *E. coli* O157:H7. They detected *Salmonella* in lettuce (one out of 75 samples), composted manure (one out of 18 samples), manured soil (two out of 78 samples) and water (one out of 53 samples). However, *E. coli* O157:H7 was only detected in water samples (two out of 53 samples).

Rodrigues *et al.*[24] conducted a similar study evaluating the occurrence of hygiene indicators and pathogens (*Salmonella* and *E. coli* O157:H7) in 132 samples from organic farms, including manure, soil, water, workers' hands and equipment, lettuce seedlings, and crops. *Escherichia coli* O157:H7 was detected in two out of 27 water samples. *Salmonella* was detected in manure (one out of nine samples), manured soil (two out of 36 samples), lettuce (one out of 36 samples) and irrigation water (one out of 27 samples).

Decol *et al.*[20] evaluated the occurrence of generic *E. coli* and *E. coli* O157:H7 in 219 water samples used for irrigation of lettuces. The prevalence of generic *E. coli* was 84.8%, with counts ranging from 2.1 to 5.4 log CFU $(100\ \text{mL})^{-1}$, which were above the limits of the Brazilian regulation of CONAMA that sets a maximum of 200 thermotolerant coliforms per 100 mL of water used for irrigation.[25] Using quantitative polymerase chain reaction (qPCR) for investigation of *E. coli* O157:H7 in water, the authors found positive results in 13 out of 62 samples, but only four were confirmed by isolation in culture media.

Occurrence of enteric pathogens in raw vegetables is expected as they can survive in the environment for long periods. Studies conducted by Islam *et al.*[26,27] showed that *Salmonella* Typhimurium and *E. coli* O157:H7 can survive in soil and vegetables experimentally contaminated via manure or irrigation water. *Salmonella* persisted in soils amended with contaminated composts for 161 and 231 days where lettuce and parsley were grown, respectively, and the pathogen was detected on the 63rd and 231th days on lettuce and parsley, respectively. *Escherichia coli* O157:H7 also persisted for long periods in soils amended with contaminated composts (154 and 217 days for lettuce and parsley, respectively) being detected on lettuce and parsley for up to 77 and 177 days, respectively, after seedlings were planted.

A study conducted by Kisluk and Yaron[28] investigated the short- and long-term (from 1 h to 28 days) persistence of *Salmonella* Typhimurium in the phyllosphere and the rhizosphere of parsley, following spray irrigation with contaminated water. They observed that irrigation with water containing 8.5 log CFU mL^{-1} resulted in persistence of bacteria in the phyllosphere and the rhizosphere for at least 4 weeks. However, irrigation with water containing as little as ~300 CFU mL^{-1} resulted in the persistence of *Salmonella* in plants for 48 h, detected through the application of quantitative real-time PCR after enrichment.

As a result of the various sources of microbiological contamination to which the vegetables may be exposed in the field, some pathogens can end up contaminating the processing plants. Moreover, contamination may also occur during minimal processing, due to numerous opportunities for cross-contamination.

During processing of RTE-MPV, washing plays an important role in the quality and safety of the final product. At this step, the application of sanitizers to the wash water is common, mainly chlorine-based compounds, in order to reduce microbial populations and eliminate pathogenic microorganisms that may be present. Wash water can however become a source of cross-contamination and pathogenic microorganisms can be spread from a contaminated batch of product to the next batch. In fact, several studies published over the past years have evidenced that wash water can be a source of cross-contamination if not properly sanitized.[29–38]

Maffei *et al.*[39] developed a quantitative microbial risk assessment (QMRA) model to estimate the impact of cross-contamination during washing of RTE leafy greens on the risk of illness caused by *Salmonella*, based on the most common practices in Brazilian RTE-MPV processing plants. Their QMRA model indicated quantitatively that higher chlorine concentrations resulted in lower risk of illness, since simulations performed with less than 5 mg L^{-1} of chlorine showed that most (> 96%) of the predicted illnesses arose from cross-contamination. However, when a triangular distribution with 10, 120 and 250 mg L^{-1} of chlorine was simulated, no illnesses arising from cross-contamination were predicted. They concluded that the concentration of chlorine should be kept above 10 mg L^{-1} in order to minimize the risk of illnesses due to the consumption of these products.

OCCURRENCE OF PATHOGENIC MICROORGANISMS AND FOODBORNE OUTBREAKS LINKED TO FRESH PRODUCE AND RTE-MPV

The most recent studies on the occurrence of foodborne pathogens in RTE-MPV in Brazil are summarized in Table 1, indicating that prevalence of *Salmonella* and *L. monocytogenes* ranges from 0.4% to 12.5% and from 0.6% to 3.1%, respectively. Except for the data of Cruz *et al.*,[40] the results do not differ from those found in other countries (Table 2), where the reported prevalence of these pathogens range from 0.8% to 5% and from 0.6 to 4.3%, respectively.

Over the past decades, several foodborne outbreaks have been attributed to the consumption of contaminated fresh produce. From 2000 to 2017, a total of 12 660 foodborne disease outbreaks were reported by the Brazilian Ministry of Health, resulting in 239 164 illnesses and 186 deaths. Among these outbreaks, 138 (1.09%) were associated with the consumption of fresh produce. However, it is not known whether these outbreaks were associated with the consumption of fresh vegetables or RTE-MPV. The etiologic agent was not determined in most (31%) of the outbreaks linked to fresh produce but *Salmonella* was detected in 25% of the tested samples, followed by *E. coli* (23%), *Bacillus cereus* (16%) and *Staphylococcus aureus* (13%).[41]

Callejón *et al.*[6] conducted a review study addressing foodborne outbreaks associated with the consumption of fresh vegetables, sprouts and fruits in the US and the European Union (EU) between 2004 and 2012, reporting occurrence of 377 and 198 produce-associated outbreaks in the US and EU, respectively. Norovirus was responsible for most of these outbreaks (59% in the US and 53% in the EU), followed by *Salmonella*, the most common bacterial pathogen (18% in the US and 20% in the EU).

A recent outbreak involving *E. coli* O157:H7 linked to romaine lettuce was reported in the US, affecting 210 people from 36 states, of which 96 were hospitalized and 27 developed hemolytic uremic syndrome, and five deaths were reported.[42] Consumption of contaminated RTE-MPV has also been pointed as a cause of foodborne illnesses in the US. In 2013, an outbreak of Shiga toxin-producing *E. coli* O157:H7 infections linked to RTE salads affected 33 individuals from four states: seven were hospitalized, two developed hemolytic uremic syndrome and no deaths were reported.[43]

CONTROL MEASURES

Current strategies to control bacterial contamination during the production of RTE-MPV are based on the implementation of Good Agricultural Practices in primary production and Good Handling Practices during postharvest stages and processing. It is well known that processing may cause mechanical injury in vegetable tissues, leading to loss of water, color changes and formation of exudates rich in nutrients that may support microbial growth.[44] This highlights the need for control measures throughout the whole production chain.

Several intervention measures, including chemical and physical treatments, are used to reduce contamination, extend shelf-life and enhance the quality and safety of these products. Among these, a washing-disinfection step, modified atmosphere packaging and the use of refrigeration can inhibit or slow down bacterial growth.[13,45]

Table 1 Occurrence of pathogenic microorganisms in ready-to-eat minimally processed vegetables (RTE-MPV) in Brazil

	Number of samples		
Pathogen	Total *N*	Positive *n* (%)	Reference
Cronobacter spp.	30	13 (43.4)	Vasconcellos *et al.*[57]
Listeria monocytogenes	181	1 (0.6)	Froder *et al.*[58]
Salmonella	133	4 (3.0)	
Listeria monocytogenes	172	2 (1.2)	Maistro *et al.*[53]
Salmonella		1 (0.6)	
Listeria monocytogenes	162	2 (1.2)	Oliveira *et al.*[44]
Salmonella		2 (1.2)	
Listeria monocytogenes	512	16 (3.1)	Sant'Ana *et al.*[59]
Salmonella		2 (0.4)	Sant'Ana *et al.*[60]
Staphylococcus aureus	32	14 (43.8)	Cruz *et al.*[40]
Salmonella		4 (12.5)	

Table 2 Occurrence of pathogenic microorganisms in ready-to-eat minimally processed vegetables (RTE-MPV) around the world

		Number of samples		
Country	Pathogen	Total *N*	Positive *n* (%)	Reference
Australia	*Aeromonas hydrophila or A. caviae*	120	66 (55.0)	Szabo *et al.*[61]
	Listeria monocytogenes		3 (2.5)	
	Yersinia enterocolitica		71 (59.2)	
Croatia	*Listeria monocytogenes*	100	1 (1.0)	Kovačević *et al.*[62]
France	*Clostridium difficile*	104	3 (2.9)	Eckert *et al.*[63]
Greece	*Aeromonas hydrophila*	26	12 (46.1)	Xanthopoulos *et al.*[64]
	Yersinia enterocolitica		2 (7.7)	
Iran	*Salmonella* spp.	20	1 (5.0)	Jeddi *et al.*[65]
Korea	*Clostridium perfrigens*	129	5 (3.9)	Seo *et al.*[66]
	Salmonella spp.		1 (0.8)	
Finland	*Escherichia coli* (STEC)	100	7 (7.0)	Nousiainen *et al.*[67]
	Listeria monocytogenes		2 (2.0)	
	Salmonella spp.		2 (2.0)	
	Yersinia enterocolitica		2 (2.0)	
Mexico	*Salmonella*	220	9 (4.1)	Bautista-De León *et al.*[68]
Poland	*Cronobacter* spp.	20	6 (30)	Berthold-Pluta *et al.*[69]
Portugal	*Listeria monocytogenes*	151	1 (0.7)	Santos *et al.*[70]
	Aeromonas hydrophila		11 (7.3)	
	Bacillus cereus	66	15 (22.7)	
Spain	*Listeria monocytogenes*	236	2 (0.8)	Abadias *et al.*[71]
	Salmonella spp.		4 (1.7)	
Spain	*Listeria monocytogenes*	70	3 (4.3)	Moreno *et al.*[72]
Switzerland	*Cronobacter* spp.	142	2 (1.4)	Althaus *et al.*[73]
	Escherichia coli (EPEC)		11 (7.7)	
	Escherichia coli (STEC)		1 (0.7)	
	Listeria monocytogenes		5 (3.5)	

Appropriate washing is one of the most important procedures, aiming to remove soil, dirt and debris, apart from reducing the microbial load of fresh produce. The addition of sanitizers to the washing water is important to eliminate pathogenic microorganisms, and especially to avoid cross-contamination between contaminated and uncontaminated products.[4] Chlorine has been widely used in washing procedures due to its low cost and efficacy against a broad spectrum of microorganisms. However, the use of chlorine-based sanitizers also comes with other health concerns, mainly the potential to form by-products that are harmful to human health.[46] Consequently, other methods for disinfection of fresh produce have been considered over the past decades, including the use of chlorine dioxide, electrolyzed water, hydrogen peroxide, ozone, organic acids, irradiation, filtration, ultrasounds, ultraviolet light, cold plasma, etc.[47–49]

In Brazil, the use of chlorine, mainly sodium hypochlorite (200–-250 mg L^{-1}), is recommended for processors to disinfect fresh vegetables.[50] Despite this, other chemical treatments have been used in the country. Maffei *et al.*[51] conducted a study in ten selected processing plants located in the State of Sao Paulo, aiming to gather information on the practices employed during the production of RTE-MPV. They observed that in seven out of the ten visited plants, sodium dichloroisocyanurate, ranging from 75.5 to 155 mg L^{-1}, was used. In the other plants, chlorine dioxide (240 mg L^{-1}, two farms) and sodium hypochlorite (50 mg L^{-1}, one plant) were used in the washing water, with contact time ranging from 2 to 20 min. In another Brazilian study, Silveira *et al.*[52] investigated the processing characteristics of five RTE-MPV processing plants located in the State of Rio Grande do Sul and observed that sodium hypochlorite (200 mg L^{-1}) was used for disinfection in all of them, with contact time ranging from 1 to 15 min.

Cool chain management is essential to preserve the quality and safety of fresh vegetables and RTE-MPV, for maintenance of organoleptic properties, reduction of microbial growth during storage and extension of the shelf life. It is recommended that vegetables are stored at 1 to 4 °C during the entire production chain, up to consumption.[13] Maistro *et al.*[53] recorded the temperature of the displays in which packages of RTE-MPV were exposed for commercialization in a large supermarket chain in the city of Campinas, Sao Paulo State, Brazil. They observed that for RTE-MPV packaged with perforated films, the temperature varied from 5 to 15 °C, while for RTE-MPV packaged under modified atmosphere it varied from 7 to 12 °C. In both cases, the temperature was above the one recommended in labels (≤ 7 °C).

Modified atmospheric packaging (MAP) is also important and has been widely used in combination with refrigeration to maintain the safety and extend the shelf life of perishable food products. It consists in altering the gas composition inside the package, replacing the atmospheric air by protective gas mix: oxygen (O_2), carbon dioxide (CO_2) and nitrogen (N_2) being the most frequently used gases. This technique extends the shelf life of leafy greens and helps to inhibit or retard the growth of spoilage and some pathogenic microorganisms.[13,54,55] Oliveira *et al.*[56]

conducted a review of the effect of MAP on the survival and growth of foodborne pathogens on fresh-cut fruits and vegetables. They concluded that the effect of MAP can vary, depending mainly on the storage conditions and the type of packaged product, although it is not an effective controller when used as a single preventive strategy. Hence, the combination of more than one technology may be the most effective approach to enhance the quality and safety of RTE-MPV.

CONCLUDING REMARKS

Data on the occurrence of foodborne pathogens in RTE-MPV sold in Brazil were compiled in the present study, and compared to data from other countries, based on the published literature. This enable us to conclude that most studies focus on the detection of *Salmonella* and *L. monocytogenes*, and that the prevalence of these pathogens is similar among those studies.

The occurrence of pathogenic microorganisms in fresh produce is risky, mainly in RTE-MPV, because most of these products are marketed as sanitized and do not need any additional care before consumption. Therefore, this remains a concern for consumers, governments and the food industry. Since the growth of the market for RTE-MPV is a trend worldwide, continuous efforts are necessary to ensure the quality and safety of these products.

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CONFLICT OF INTEREST

No conflict of interest declared.

REFERENCES

1 Bezerra IN, Moreira TMV, Cavalcante JB, Souza AM and Sichieri R, Food consumed outside the home in Brazil according to places of purchase. *Rev Saúde Públ* **51**:15 (2017).
2 Rocha A and Morais AMMB, Role of minimally processed fruit and vegetables on the diet of the consumers in the XXI century. *Acta Hortic* **746**:265–272 (2007).
3 Bansal V, Siddiqui MW and Rahman MS, Minimally processed foods: overview. In: Siddiqui M., Rahman M. (eds) *Minimally processed foods: technologies for safety, quality, and convenience*. Springer: Berlin; p.306. (Food engineering series).
4 Gil MI, Selma MV, López-Gálvez F and Allende A, Fresh-cut product sanitation and wash water disinfection: problems and solutions. *Int J Food Microbiol* **134**:37–45 (2009).
5 Berger CN, Sodha SV, Shaw RK, Griffin PM, Pink D, Hand P *et al*., Fresh fruits and vegetables as vehicles for the transmission of human pathogens. *Environ Microbiol* **12**:2385–2397 (2010).
6 Callejón RM, Rodríguez-Naranjo MI, Ubeda C, Hornedo-Ortega R, Garcia-Parrilla MC and Troncoso AM, Reported foodborne outbreaks due to fresh produce in the United States and European Union: trends and causes. *Foodborne Pathog Dis* **12**:32–38 (2015).
7 Elias SO, Decol LT and Tondo EC, Foodborne outbreaks in Brazil associated with fruits and vegetables: 2008 through 2014. *Food Qual Saf* **2**: 173–181 (2018).
8 Garner D and Kathariou S, Fresh produce-associated listeriosis outbreaks, sources of concern, teachable moments, and insights. *J Food Prot* **79**:337–344 (2016).
9 Jung Y, Jang H and Matthews KR, Effect of the food production chain from farm practices to vegetable processing on outbreak incidence. *J Microbial Biotechnol* **7**:517–527 (2014).
10 Sagoo SK, Little CL, Ward L, Gillespie IA and Mitchell RT, Microbiological study of ready-to-eat salad vegetables from retail establishments uncovers a national outbreak of salmonellosis. *J Food Prot* **66**: 403–409 (2003).
11 Sewell AM and Farber JM, Foodborne outbreaks in Canada linked to produce. *J Food Prot* **64**:1863–1877 (2001).
12 Zhu Q, Gooneratne R and Hussain MA, *Listeria monocytogenes* in fresh produce: outbreaks, prevalence and contamination levels. *Foods* **6**: 1–11 (2017).
13 Castro-Ibáñez I, Gil MI and Allende A, Ready-to-eat vegetables: current problems and potential solutions to reduce microbial risk in the production chain. *LWT-Food Sci Technol* **85**:284–292 (2017).
14 Moretti CL, Manual de processamento mínimo de frutas e hortaliças. Brasília: Embrapa Hortaliças. p.531, (2007).
15 Perez R, Ramos AM, Binoti ML, Sousa PHM, Machado GM and Cruz IB, Perfil dos consumidores de hortaliças minimamente processadas de Belo Horizonte. *Hortic Bras* **26**:441–446 (2008).
16 Sato GS, Martins VA and Bueno CRF, Análise exploratória do perfil do consumidor de produtos minimamente processados na cidade de São Paulo. *Inform Econ* **37**:62–71 (2007).
17 Amorim AM and Nascimento KO, Caracterização do perfil de consumidores de alimentos minimamente processados. *Nutr Brasil* **10**: 347–353 (2011).
18 Yu H, Neal JA and Sirsat SA, Consumers' food safety risk perceptions and willingness to pay for fresh-cut produce with lower risk of foodborne illness. *Food Control* **86**:83–89 (2018).
19 Alegbeleye OO, Singleton I and Sant'Ana AS, Sources and contamination routes of microbial pathogens to fresh produce during field cultivation: a review. *Food Microbiol* **73**:177–208 (2018).
20 Decol LT, Casarin LS, Hessel CT, Batista ACF, Allende A and Tondo EC, Microbial quality of irrigation water used in leafy green production in southern Brazil and its relationship with produce safety. *Food Microbiol* **65**:105–113 (2017).
21 Maffei DF, Batalha EY, Landgraf M, Schaffner DW and Franco BDGM, Microbiology of organic and conventionally grown fresh produce. *Braz J Microbiol* **47S**:99–105 (2016).
22 Sant'Ana AS, Silva FFP, Maffei DF and Franco BDGM, Fruits and vegetables: introduction, in *Encyclopedia of food microbiology*, 2nd edn, ed. by Batt CA and Tortorello ML. Academic Press, Amsterdam, pp. 972–982 (2014).
23 Ceuppens S, Hessel CT, Rodrigues RQ, Bartz S, Tondo EC and Uyttendaele M, Microbiological quality and safety assessment of lettuce production in Brazil. *Int J Food Microbiol* **181**:67–76 (2014).
24 Rodrigues RQ, Loiko MR, de Paula CMD, Hessel CT, Jacxsens L, Uyttendaele M *et al*., Microbiological contamination linked to implementation of good agricultural practices in the production of organic lettuce in southern Brazil. *Food Control* **42**:152–164 (2014).
25 Brazil. Ministério do Meio Ambiente. Resolução Conama n° 357, de 17 de março de 2005. Dispõe sobre a classificação dos corpos de água e diretrizes ambientais para o seu enquadramento, bem como estabelece as condições e padrões de lançamento de efluentes, e dá outras providências. Diário Oficial da União, Brasília, 18 de março de 2005, pp. 58–63. (2005).
26 Islam M, Doyle MP, Phatak SC, Millner P and Jiang X, Persistence of enterohemorrhagic *Escherichia coli* O157:H7 in soil and on leaf lettuce and parsley grown in fields treated with contaminated manure composts or irrigation water. *J Food Prot* **67**:1365–1370 (2004).
27 Islam M, Morgan J, Doyle MP, Phatak SC, Millner P and Jiang X, Persistence of *Salmonella enterica* serovar Typhimurium on lettuce and parsley and in soils on which they were grown in fields treated with contaminated manure composts or irrigation water. *Foodborne Pathog Dis* **1**:27–35 (2004).
28 Kisluk G and Yaron S, Presence and persistence of *Salmonella enterica* serotype Typhimurium in the phyllosphere and rhizosphere of spray-irrigated parsley. *Appl Environ Microbiol* **78**:4030–4036 (2012).
29 Allende A, Selma MV, Lopez-Galvez F, Villaescura R and Gil M, Impact of wash water quality on sensory and microbial quality, including *Escherichia coli* cross-contamination, of fresh-cut escarole. *J Food Prot* **71**:2514–2518 (2008).
30 Danyluk MD and Schaffner DW, Quantitative assessment of the microbial risk of leafy greens from farm to consumption: preliminary framework, data, and risk estimates. *J Food Prot* **74**:700–708 (2011).
31 Holvoet K, de Keuckelaere A, Sampers I, van Haute S, Stals A and Uyttendaele A, Quantitative study of cross-contamination with *Escherichia coli*, *E. coli* O157, MS2 phage and murine norovirus in a simulated fresh-cut lettuce wash process. *Food Control* **37**:218–227 (2014).

32 Jensen DA, Friedrich LM, Harris LJ, Danyluk MD and Schaffner DW, Cross contamination of *Escherichia coli* O157:H7 between lettuce and wash water during home-scale washing. *Food Microbiol* **46**: 428–433 (2015).
33 López-Gálvez F, Allende A, Selma MV and Gil MI, Prevention of *Escherichia coli* cross contamination by different commercial sanitizers during washing of fresh-cut lettuce. *Int J Food Microbiol* **133**: 167–171 (2009).
34 Luo Y, Nou X, Yang Y, Alegre I, Turner E, Feng H *et al*., Determination of free chlorine concentration needed to prevent *Escherichia coli* O157: H7 cross-contamination during fresh-cut produce wash. *J Food Prot* **74**:352–358 (2011).
35 Maffei DF, Sant'Ana AS, Monteiro G, Schaffner DW and Franco BDGM, Assessing the effect of chlorine concentration on transfer of *Salmonella enterica* serotype Typhimurium in wash water for production of minimally processed iceberg lettuce (*Lactuca sativa* L). *Lett Appl Microbiol* **62**:444–451 (2016).
36 Perez-Rodriguez F, Saiz-Abajo MJ, Garcia-Gimeno RM, Moreno A, Gonzalez D and Vitas AI, Quantitative assessment of the *Salmonella* distribution on fresh-cut leafy vegetables due to cross-contamination occurred in an industrial process simulated at laboratory scale. *Int J Food Microbiol* **184**:86–91 (2014).
37 Tomás-Callejas A, López-Gálvez F, Sbodio A, Artés F, Artés-Hernández F and Suslow TV, Chlorine dioxide and chlorine effectiveness to prevent *Escherichia coli* O157:H7 and *Salmonella* cross-contamination on fresh-cut red chard. *Food Control* **23**:325–332 (2012).
38 Zhang G, Ma L, Phelan VH and Doyle MP, Efficacy of antimicrobial agents in lettuce leaf processing water for control of *Escherichia coli* O157:H7. *J Food Prot* **72**:1392–1397 (2009).
39 Maffei DF, Sant'Ana AS, Franco BDGM and Schaffner DW, Quantitative assessment of the impact of cross-contamination during the washing step of ready-to-eat leafy greens on the risk of illness caused by *Salmonella*. *Food Res Int* **92**:106–112 (2017).
40 Cruz MRG, Leite YJBS, Marques JL, Pavelquesi SLS, Oliveira LRA, Silva ICR *et al*., Microbiological quality of minimally processed vegetables commercialized in Brasilia, DF, Brazil. *Food Sci Technol* **16**:2019 (2019).
41 Brazil, Ministério da Saúde. Secretaria de Vigilância em Saúde, Departamento de Vigilância das Doenças Transmissíveis. Surtos de Doenças Transmitidas por Alimentos no Brasil. (2018). http://portalarquivos2.saude.gov.br/images/pdf/2018/julho/02/Apresentacao-Surtos-DTA-Junho-2018.pdf [accessed January 30, 2020].
42 CDC (Centers for Disease Control and Prevention), Multistate outbreak of *E. coli* O157:H7 infections linked to romaine lettuce (final update). (2018). https://www.cdc.gov/ecoli/2018/o157h7-04-18/index.html [accessed May 1, 2019].
43 CDC (Centers for Disease Control and Prevention), Multistate outbreak of Shiga toxin-producing *Escherichia coli* O157:H7 infections linked to ready-to-eat salads (final update). (2013). https://www.cdc.gov/ecoli/2013/O157H7-11-13/index.html [accessed May 1, 2019.
44 Oliveira MA, de Souza VM, Bergamini AMM and de Martinis ECP, Microbiological quality of ready-to-eat minimally processed vegetables consumed in Brazil. *Food Control* **22**:1400–1403 (2011).
45 Gil MI, Selma MV, Suslow T, Jacxsens L, Uyttendaele M and Allende A, Pre- and postharvest preventive measures and intervention strategies to control microbial food safety hazards of fresh leafy vegetables. *Crit Rev Food Sci* **55**:453–468 (2015).
46 Lee WN, Huang CH and Zhu G, Analytical methods for conventional and emerging disinfection by-products in fresh-cut produce. *Food Chem* **291**:30–37 (2019).
47 De Corato U, Improving the shelf-life 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* **7**: 1–36 (2019).
48 Meireles A, Giaouris E and Simões M, Alternative disinfection methods to chlorine for use in the fresh-cut industry. *Food Res Int* **82**:71–85 (2016).
49 Siddiqui MW, *Postharvest Disinfection of Fruits and Vegetables*. Academic Press, London, p. 322 (2018).
50 Brazil. Portaria CVS 5 de 09 de abril de 2013. Aprova o regulamento técnico sobre boas práticas para estabelecimentos comerciais de alimentos e para serviços de alimentação, e o roteiro de inspeção, anexo. Diário Oficial do Estado. Sec. 1, 32–35. (2013).
51 Maffei DF, Alvarenga VO, Sant'Ana AS and Franco BDGM, Assessing the effect of washing practices employed in Brazilian processing plants on the quality of ready-to-eat vegetables. *LWT – Food Sci Technol* **69**: 474–481 (2016).
52 Silveira JB, Hessel CT and Tondo EC, Inactivation of *Salmonella* Enteritidis on lettuces used by minimally processed vegetable industries. *J Infect Dev Ctries* **11**:34–41 (2017).
53 Maistro LC, Miya NTN, Sant'Ana AS and Pereira JL, Microbiological quality and safety of minimally processed vegetables marketed in Campinas, SP - Brazil, as assessed by traditional and alternative methods. *Food Control* **28**:258–264 (2012).
54 Ma L, Zhang M, Bhandari B and Gao Z, Recent developments in novel shelf life extension technologies of fresh-cut fruits and vegetables. *Trends Food Sci Technol* **64**:23–38 (2017).
55 Mir SA, Shah MA, Mir MM, Dar BN, Greiner R and Roohinejad S, Microbiological contamination of ready-to-eat vegetable salads in developing countries and potential solutions in the supply chain to control microbial pathogens. *Food Control* **85**:235–244 (2018).
56 Oliveira M, Abadias M, Usall J, Torres R, Teixidó N and Viñas I, Application of modified atmosphere packaging as a safety approach to fresh-cut fruits and vegetables – a review. *Trends Food Sci Technol* **46**:13–26 (2015).
57 Vasconcellos L, Carvalho CT, Tavares RO, de Mello MV, de Oliveira RC, Silva JN *et al*., Isolation, molecular and phenotypic characterization of *Cronobacter* spp. in ready-to-eat salads and foods from Japanese cuisine commercialized in Brazil. *Food Res Int* **107**:353–359 (2018).
58 Froder H, Martins CG, de Souza KL, Landgraf M, Franco BDGM and Destro MT, Minimally processed vegetable salads: microbial quality evaluation. *J Food Prot* **70**:1277–1280 (2007).
59 Sant'Ana AS, Igarashi MC, Landgraf M, Destro MT and Franco BDGM, Prevalence, populations and pheno- and genotypic characteristics of *Listeria monocytogenes* isolated from ready-to-eat vegetables marketed in Sao Paulo, Brazil. *Int J Food Microbiol* **155**:1–9 (2012).
60 Sant'Ana AS, Landgraf M, Destro MT and Franco BDGM, Prevalence and counts of *Salmonella* spp. in minimally processed vegetables in Sao Paulo, Brazil. *Food Microbiol* **28**:235–1237 (2011).
61 Szabo EA, Scurrah KJ and Burrows JM, Survey for psychrotrophic bacterial pathogens in minimally processed lettuce. *Lett Appl Microbiol* **30**: 456–460 (2000).
62 Kovačević M, Burazin J, Pavlović H, Kopjar M and Piližota V, Prevalence and level of *Listeria monocytogenes* and other *Listeria* sp. in ready-to-eat minimally processed and refrigerated vegetables. *World J Microbiol Biotechnol* **29**:707–712 (2013).
63 Eckert C, Burghoffer B and Barbut F, Contamination of ready-to-eat raw vegetables with *Clostridium difficile* in France. *J Med Microbiol* **62**: 1435–1438 (2013).
64 Xanthopoulos V, Tzanetakis N and Litopoulou-Tzanetak E, Occurrence and characterization of *Aeromonas hydrophila* and *Yersinia enterocolitica* in minimally processed fresh vegetable salads. *Food Control* **21**: 393–398 (2010).
65 Jeddi MZ, Yunesian M, Gorji ME, Noori N, Pourmand MR and Khaniki GRJ, Microbial evaluation of fresh, minimally-processed vegetables and bagged sprouts from chain supermarkets. *J Health Popul Nutr* **32**:391–399 (2014).
66 Seo YH, Jang JH and Moon KD, Microbial evaluation of minimally processed vegetables and sprouts produced in Seoul, Korea. *Food Sci Biotechnol* **19**:1283–1288 (2010).
67 Nousiainen LL, Joutsen S, Lunden J, Hänninen ML and Fredriksson-Ahomaa M, Bacterial quality and safety of packaged fresh leafy vegetables at the retail level in Finland. *Int J Food Microbiol* **232**:73–79 (2016).
68 Bautista-De León H, Gómez-Aldapa CA, Rangel-Vargas E, Vázquez-Barrios E and Castro-Rosas J, Frequency of indicator bacteria *Salmonella* and diarrhoeagenic *Escherichia coli* pathotypes on ready-to-eat cooked vegetable salads from Mexican restaurants. *Lett Appl Microbiol* **56**:414–420 (2013).
69 Berthold-Pluta A, Garbowska M, Stefańska I and Pluta A, Microbiological quality of selected ready-to-eat leaf vegetables, sprouts and non-pasteurized fresh fruit-vegetable juices including the presence of *Cronobacter* spp. *Food Microbiol* **65**:221–230 (2017).
70 Santos MI, Cavaco A, Gouveia J, Novais MR, Nogueira PJ, Pedroso L *et al*., Evaluation of minimally processed salads commercialized in Portugal. *Food Control* **23**:275–281 (2012).
71 Abadias M, Usall J, Anguera M, Solsona C and Viñas I, Microbiological quality of fresh, minimally processed fruit and vegetables, and

sprouts from retail establishments. *Int J Food Microbiol* **123**:121–129 (2008).
72 Moreno Y, Sánchez-Contreras J, Montes RM, García-Hernández J, Ballesteros L and Ferrús MA, Detection and enumeration of viable *Listeria monocytogenes* cells from ready-to-eat and processed vegetable foods by culture and DVC-FISH. *Food Control* **27**: 374–379 (2012).
73 Althaus D, Hofer E, Corti S, Julmi A and Stephan R, Bacteriological survey of ready-to-eat lettuce, fresh-cut fruit, and sprouts collected from the Swiss market. *J Food Prot* **75**:1338–1341 (2012).

