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Factors affecting the incidence of pathogenic micro-organisms in fresh-cut produce

Rosa M Raybaudi-Massilia* and Jonathan Mosqueda-Melgar
Institute of Food Science and Technology, Faculty of Science, Central University of Venezuela, Calle Suapure, Colinas de Bello Monte, Caracas, Venezuela

Abstract

Purpose of review: This review focuses on the main factors influencing the incidence of pathogenic micro-organisms in fresh-cut produce.

Findings: The increase in the *per capita* consumption of fresh-cut produce is expected to continue during the forthcoming years, therefore it is necessary to strengthen microbial safety issues related to fresh-cut produce. Different factors affecting the presence of pathogenic micro-organisms in fresh-cut produce during processing have been identified: the raw produce coming from field and process water quality; the hygiene and health of food handlers; the sanitation and cleanliness of facilities; processing equipment and utensils; and the presence of pests in processing plants. Raw produce quality is one of the most important factors that can affect the arrival of pathogenic micro-organisms on fresh-cut fruits and vegetables.

Limitations/implications: The high cost associated with implementing Good Agricultural Practices (GAPs), Good Manufacturing Practices (GMPs) and Hazard Analysis and Critical Control Points (HACCP) as food safety programs.

Directions for future research: Efforts should be made to develop feasible procedures for small- and medium-sized industries that ensure high microbiological safety and quality standards, through HACCP plans, for each fresh-cut produce. It is also necessary to explore new treatments that improve the safety and quality of fresh-cut produce in order to prevent the incidence, survival and growth of pathogenic micro-organisms, as well as to prolong the microbiological shelf-life.

Keywords: fresh-cut produce; food safety; food processing; foodborne pathogens

Abbreviations

GAP	Good Agricultural Practice
GMP	Good Manufacturing Practice
HACCP	Hazard Analysis and Critical Control Points

***Correspondence to:** Rosa M Raybaudi-Massilia, Institute of Food Science and Technology, Faculty of Science, Central University of Venezuela, Calle Suapure, Colinas de Bello Monte, 1041-A Caracas, Venezuela. Tel: +58 212 7534403; Fax: +58 212 7533871. Email: rosa.raybaudi@ciens.ucv.ve

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Introduction

Consumption of fresh-cut produce, mainly derived from fruits and vegetables, has increased markedly in the last decade; this has been due to their promotion as healthy foods that can help to prevent cardiovascular diseases and several cancers [1], in addition to their fresh-like characteristics, convenience, easy-to-use and ready-to eat features, and retained nutritional qualities [2]. However, with increased consumption and importation of these produce, the risk of foodborne illness associated with fresh or raw produce and fresh-cut produce has also increased [3]. The United States Food and Drug Administration [4] pointed out that from 1996 to 2006, 72 foodborne illness outbreaks were associated with consumption of fresh produce. Of these produce-related outbreaks, 25% (18 outbreaks) were linked to fresh-cut produce. Majority of these outbreaks associated with produce and fresh-cut produce may be caused by microbial hazards including pathogenic bacteria, viruses and parasites.

As is well known, raw produce is the major source of contamination from pathogenic micro-organisms for fresh-cut produce. For that reason, the quality of raw produce coming from field is one of the most important factors affecting the incidence of pathogenic micro-organisms in fresh-cut produce [5*]. Nonetheless, there is enough scientific evidence suggesting that human pathogens may also be transferred to fresh-cut produce through the different practices of postharvest including trimming, washing, peeling, cutting, handling and packing [5*–9], which are discussed in detail in later sections of this review.

Inadequate agricultural and manufacturing practices applied by the growers/farmers and plant processors, or the lack of effective intervention strategies for preventing microbial hazards associated with fresh-cut produce are the main causes of contamination. Therefore, processing of fresh produce into fresh-cut products without proper sanitation procedures can increase the risk of microbial diseases and spoilage [10, 11].

The purpose of this review is to discuss the factors affecting the incidence of pathogenic micro-organisms in fresh-cut produce during the chain of operations from field to consumer, as well as to highlight some alternatives for minimising the risk of microbial contamination for these produce.

Pathogenic micro-organisms associated with fresh-cut produce

The majority of fresh fruits and vegetables may become contaminated with human pathogens while growing in fields, vineyards or orchards, or during harvesting, postharvest handling, processing and distribution [10**]. This contamination can occur because these micro-organisms including bacteria, viruses and parasites may to be present in the water used for irrigation, in soil where produce is grown, air (dust), manure used as a soil fertiliser, faeces from animals, food handlers, equipment, packages, etc [3]. In such sense, the United States Food and Drug Administration [4] reported that processing of fresh produce into fresh-cut products may increase the risk of microbial growth and contamination by breaking the natural exterior barrier of the produce. The release of plant cellular fluids when produce is peeled, cut, sliced or shredded provides a nutritive medium in which pathogens can survive or grow. Thus, if pathogens are present when the surface integrity of the fruit or vegetable is broken, then microbial growth can occur and contamination may spread [4].

Different bacteria, viruses and parasites have been isolated from or associated with fresh-cut produce (Table 1). However, bacteria have had a higher impact than viruses and parasites in terms of illness severity and numbers of foodborne cases and outbreaks [3].

In addition, changes in food consumption patterns and advances in agronomic practices, processing, preservation methods, packaging, storage, shipping and marketing technologies on a global scale have also added new problems related to increased risk for human illnesses associated with emerging pathogenic micro-organisms [6, 13].

Factors affecting the incidence of pathogenic micro-organisms in fresh-cut produce

To minimise the risk of microbial infection or intoxication associated with fresh-cut fruits and vegetables it is necessary to indentify the potential sources of contamination in all aspects of the production chain within processing plants (Figure 1). For these plants key factors in minimal processing from fresh to fresh-cut produce that can influence the incidence of pathogenic micro-organisms will be discussed in the following paragraphs:

The quality of fresh produce coming from the field

This is one of the most influential factors affecting the arrival or transfer of pathogens to fresh-cut produce, which can become an established source of contamination inside the processing plant, infecting other products that enter the process line [5*, 14]. The presence of pathogenic micro-organisms in fresh or raw produce coming from the field presents a risk for the fresh-cut produce industry, if these are not effectively eliminated before processing, because, during washing, handling, peeling, cutting, packing, etc, these pathogens may be carried or spread to and contaminate internal parts of produce [9, 15]. A better way to prevent the presence of pathogens in fresh-cut produce is to ensure good raw produce quality by applying Good Agricultural Practices (GAPs) during preharvest and harvesting. These practices include:

Manure quality

Manure used as a soil fertiliser can be a potential source of pathogens that contaminate fresh fruits and vegetables regardless of whether it is adequately treated [3]. Animal manure, typically from cattle, horses or sheep, can contain enteric pathogenic micro-organisms as *Escherichia coli* O157:H7 and *Salmonella* spp., and if they are not completely killed, then pathogens may survive and persist for long periods of time (up to 3 months) in those soils fertilised [16].

Table 1. Major pathogenic microorganisms isolated from or associated with fresh-cut produce.

Pathogenic microorganism		Fresh-cut produce	Main reservoirs	Diseases
Bacteria	*Aeromonas hydrophila*	Cut lettuce, cut vegetable salads (mix of chicory, lettuce, watercress or endive)	Water	Gastroenteritis
	Bacillus cereus	Cut lettuce salad, shredded cabbage	Soil, decaying plant matters	Emetic and diarrheic syndrome
	Campylobacter spp.	Cut lettuce, cut potato salad	Animals including poultry, livestock, wild birds and wild mammals	Gastrointestinal tract infection
	Clostridium botulinum	Shredded cabbage, chopped garlic, squash tomato	Soil, decaying plant matters	Botulism
	Escherichia coli O157:H7	Sliced cantaloupe melon, shredded carrots, cut parsley, cut vegetable salads (mix of lettuce, endive, carrots or cucumbers), pressed apples	Intestinal tract of all animals including humans	Nonbloody diarrhoea, hemorrhagic colitis, haemolytic uremic syndrome, or thrombotic thrombocytopenic purpura
	Listeria monocytogenes	Shredded cabbage and lettuce, sliced cucumbers, cut vegetable salads (mix of carrots, celery, leeks, cucumbers, watercress, onions, cabbage, fennel or lettuce)	Soil, water, decaying plant matters, animals, humans and raw produce	Listeriosis
	Salmonella multiserotypes	Sliced cantaloupe, honeydew and musk melons, watermelons, mangoes, and tomatoes, cut potato salad	Intestinal tract of all animals including humans	Salmonellosis
	Shigella spp.	Shredded lettuce, chopped parsley, sliced melon and watermelon, cut potato salad	Intestinal tract of humans	Shigellosis
	Yersinia enterocolitica	Cut lettuce, grated carrots, cut vegetable salads (mix of lettuce, spinach, watercress or chicory)	Animals, particularly swine	Yersiniosis
Viruses	Hepatitis A	Sliced green onion and scallions, shredded lettuce, sliced tomatoes	Intestinal tract of all animals including humans	Hepatitis
	Norovirus ("Norwalk")	Fresh-cut tomato, sliced onions and cucumber, shredded cabbage, cut vegetable salads (mix of broccoli, lettuce, cucumbers, potatoes or carrots), fresh-cut fruit salads (mix of papaya, pineapple, strawberries, grapes, banana, watermelon, and cantaloupe or honeydew melons), sliced pineapple and melons	Intestinal tract of all animals including humans	Acute gastroenteritis
Parasites	*Cryptosporidium* spp.	Pressing apples, shredded cabbage	Water, pesticide and human	Cyclosporiasis
	Giardia lamblia.	Shredded lettuce and onions	Intestinal tract of humans	Giardiasis

Hence, raw produce generally grown in the ground such as root crops (eg, carrots, potatoes, onions, radishes, beetroots, etc), crops near to the ground (eg, lettuce, endive, cabbage, parsley, melons, etc) [5*] or raw produce fallen to the ground (eg, apples, mangoes, oranges, etc) may more easily be contaminated. To prevent this microbial contamination fresh or improperly aged manure must not be used on arable land.

Animal presence

Wild and domestic animals, including mammals, birds, reptiles and insects, can act as vehicles for pathogenic microorganisms to contaminate soil and raw produce that may come into contact with soil [17]. Pathogens such as *E. coli* O157:H7 and *Salmonella* spp. have been isolated from the intestinal tracts of most warm-blooded (eg, cattle, sheep, pigs, horses, rodents, rabbits, dogs, cats, etc) and many cold-blooded (eg, lizards, chameleons and snakes) animals, as well as from their feet. Birds such as gulls, pigeons and chicken have been recognised as important vectors of *L. monocytogenes* and enteric pathogens in sewage through their faeces. Insects such as fruit and domestic flies, and cockroaches are other potential vectors that could transport pathogens from sites of sewage or animal manure accumulation to produce production areas [7]. Therefore, animal waste products that may come into contact with sewage, soil, irrigation water or raw produce could be a microbiological hazard regardless of whether they are properly controlled.

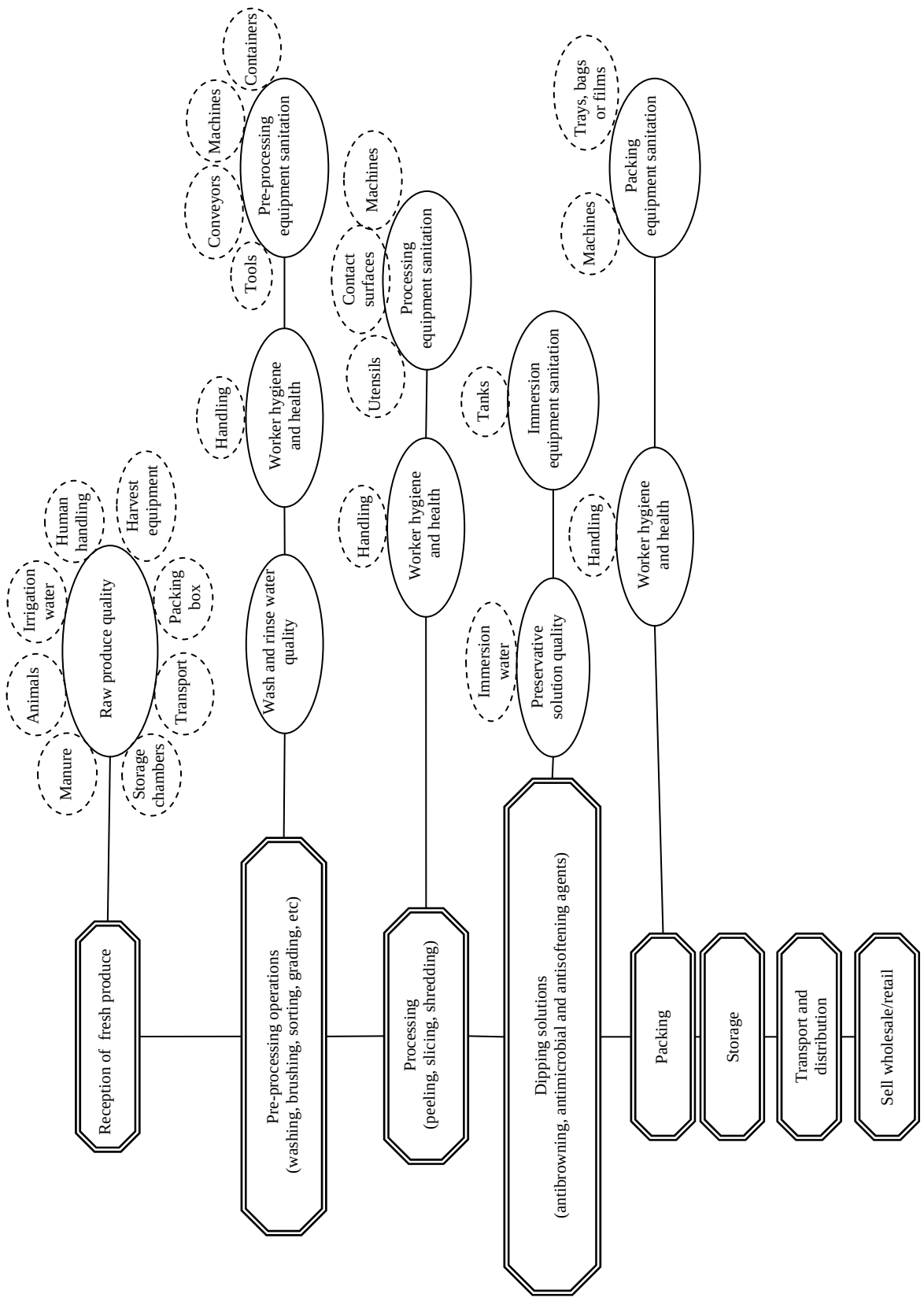

Figure 1. Factors affecting the incidence of pathogenic micro-organisms during processing of fresh fruits and vegetables

Irrigation water quality

The microbiological quality of irrigation water depends largely on the source of the water such as groundwater, surface water or human wastewater [18**]. Groundwater is generally of good microbial quality unless it is contaminated with surface runoff, urban solid waste or sewage. Surface water such as ponds, lakes, rivers and streams are of variable microbial quality, but much more susceptible than groundwater to contamination with pathogenic micro-organisms; nevertheless, sewage, septic tank, storm drains, manure piles runoff, birds or industrial effluents, can all have an effect on the quality of the irrigation water. Human wastewater is usually of very poor microbial quality and requires extensive treatment before it can be used safely for irrigation of crops. In addition, the methods of irrigation may affect the transmission of pathogens to the plant surface irrespective of whether the water is polluted. Drip irrigation is preferred to spray irrigation, because the former minimises contact of crops with contaminants present in irrigation water [19]. Pathogenic micro-organisms such as *Campylobacter* spp., *Salmonella* spp., *Vibrio cholerae*, *Shigella* spp., *Listeria* spp., *E. coli* O157:H7, *Cryptosporidium parvum*, *Giardia* spp., Noroviruses and Hepatitis A virus have been responsible for several foodborne outbreaks linked to fresh produce irrigated with contaminated waters [18**]. Therefore, irrigation water of good quality could be achieved by keeping irrigation sources away from livestock and ensuring that manure applied to fields does not run into irrigation sources.

Hygiene and health of agricultural workers

Food pickers and handlers may be vectors of foodborne diseases if they are carriers of pathogens and practise poor personal hygiene [20]. In this sense, Brackett [14] highlighted that several foodborne illnesses associated with fresh produce are transmitted by humans during handling (eg, harvesting, sorting or packing). Hence, workers involved in fielding or farming can have an important impact on the microbiological safety of the produce that they handle. The United States Food and Drug Administration [21] stated that 93% of fresh fruits and 89% of fresh vegetables grown in fields or farms are exclusively harvested by hands. Of them, only about 50% of fresh fruit and vegetable packers require their employees to wear gloves. In general, several micro-organisms such as Hepatitis A virus, Noroviruses, *Cyclospora cayetanensis* and most enteric pathogens may be transmitted from the hands of agricultural workers to fresh produce. Therefore, proper sanitary procedures at the time harvesting raw produce can significantly reduce the risk of contamination prior to processing. Among these sanitary procedures, periodically washing hands with antibacterial or antimicrobial soap and after using toilet facilities, in addition to wearing gloves during harvesting are the most important practices that workers should apply. Moreover, workers with bacterial or viral infections should avoid coming in contact with fresh fruits and vegetables, because these micro-organisms can be transferred to fresh produce during harvesting, sorting and packing.

Harvesting equipment and containers sanitation

Raw produce usually comes into contact with harvesting equipment (eg, knives, machetes and scissors) and containers (eg, bags, bins, boxes, trucks and storage chambers). So, if fresh fruits and vegetables, soil and air (dust) were previously contaminated with pathogenic micro-organisms, then pathogens may be transferred to harvesting equipment and containers and remain in them until sanitisation. The United States Food and Drug Administration [21] indicated that some agricultural commodities may be exposed to only a container or to a few pieces of equipment, whereas, most agricultural commodities are exposed to about 5–12 different containers or pieces of equipment. Therefore, if equipment and containers are not adequately and frequently sanitised, then they may be harbours of pathogenic micro-organisms, and consequently pose a risk to public health.

Washing, cooling, rinsing and preserving water quality

Water is used extensively in the fresh-cut produce industry in almost all aspects of processing, including during washing, cooling, rinsing, conveying and preserving of produce [4]. Raw produce coming from the field are usually washed to remove the undesirable physical, chemical and biological materials from the surface (eg, soil, insects, pesticide residues, plant debris, micro-organisms, etc) before they enter the processing line [9]. However, the high cost of potable tap water and waste water treatment used for washing and rinsing has driven the industry to wash large quantities of raw fruit and vegetables several times in a limited volume of water; thus making the reuse or recirculation of wash water into an industry common practice [2, 22*]. Successive use of such wash and rinse water would then increase the microbial contamination level, including human pathogens (if they are present), on the raw produce instead of reducing it, thus representing a public health risk irrespective of whether it is adequately treated with sanitisers [2, 4]. For this reason, sanitation techniques capable of inactivating micro-organisms in process water are needed to reduce water cost through process water reuse.

Sanitisers, mainly chlorine, are commonly used in fruit and vegetable processing water for maintaining the microbiological quality of the water rather than the produce [13]. This fact is supported in a study by Brackett [23], where populations of *L. monocytogenes* were reduced by more than 8 logs in chlorinated water (200 ppm of sodium hypochlorite) but only 2 logs were achieved on Brussels sprouts. Nonetheless, the antimicrobial activity of chlorine-based disinfectants depend on the amount of hypochlorous acid (also called “free chlorine”) present in the water; which in turn, depend on the pH of the water, the amount of organic material in the water, and, to some extent, the temperature of the water [4, 24*]. In addition, Allende *et al.* [22*] indicated that wash water quality can rapidly deteriorate during produce washing due to the accumulation of organic matter from injury tissue fluids, solids and others foreign materials. This is due to the fact that

pathogens such as *E. coli* O157:H7, *Salmonella* spp., and *L. monocytogenes* can be enmeshed in organic matter, which may serve as a shield against several lethal agents, and allow them to survive for a relatively long time in water. Other sanitisers such as trisodium phosphate, peroxyacetic acid, hydrogen peroxide, electrolysed water, ozonated water, citric and ascorbic acids, etc, have been evaluated for their potential use in disinfecting produce surface without leaving residues, but none of them have assured total elimination of pathogens on produce surface [7, 25**].

The temperature of water used during the handling of produce is another factor of primary importance, because a negative temperature differential (from warm to cool) among washing, rinsing and cooling water may induce infiltration of pathogenic micro-organisms into the product through openings in the peel [26*]. The issue of infiltration is of special concern during hydrocooling where water is used to cool the product. Therefore, it is important that water used during these processes is clean and free of human pathogens to prevent this problem. Examples of the infiltration of pathogenic micro-organisms into fruits and vegetables are well documented in a review by Parish *et al*. [26*].

Once produce is cut, sliced, cored, chopped or shredded, it is then conveyed toward wash systems for reducing the number of micro-organisms and rinsing tissue fluids of cut produce [27]. Those wash systems, such as flume and spray, work by saturating (dipping or spraying) the fresh-cut produce in sanitised water or with preservative for a certain minimum time as required by food safety standards. This step is of great importance during fresh-cut processing because the occurrence of pathogenic micro-organisms may be eliminated from products that have been cut with contaminated processing equipment or utensils.

Hygiene and health of plant-processing handlers

Human contact during fresh-cut produce processing is one the most important factors involved in the transfer of pathogenic micro-organisms caused by the poor hygiene practices of workers. Food handlers can carry pathogens on their skin, hair, hands and digestive systems or respiratory tracts. Thus, if operators do not wear gloves, caps and masks then contamination can be transferred to fresh-cut produce, food contact surfaces, and water supplies during unit operations such as sorting, trimming, grading, washing, peeling, cutting, slicing, shredding, draining and packing; thus increasing, the probability of causing foodborne illness [4].

Peeling and cutting cause injuries to fruit and vegetable tissues, and exposes produce flesh or internal parts to the environment. In addition, these operations cause leakage of nutrients, enzymes, and others compounds which can favour the growth of pathogenic micro-organisms, if they are present [28].

Faithfulness to Good Manufacturing Practices (GMPs) is strongly advised in the processing plant for such commodities in order to avoid contamination. In this way, all workers, maintenance personnel, and visitors should be required to wear gloves, caps and masks. Moreover, the operators should hand wash before entering to the plant, renew the gloves several times during a working shift and apply good hygienic practices [4, 28].

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Sanitation of facilities, processing equipment and utensils

Facilities, processing equipment and utensils of the fresh-cut processing plants including the floors, walls, ceiling, drains, wash water tanks, blades and inside surfaces of mechanical shredders, cutters, or slicers, belt and roller conveyors, equipment for sorting, grading, drainage, spinning and packaging, and knives, may be excellent sources of pathogenic micro-organisms. This fact, reinforces the importance of facilities, equipment, and utensils surfaces as points of microbial contamination regardless of whether sanitation practices are adequately carried out [4]. Pathogenic micro-organisms from faecal origin such as *Salmonella* spp. and *E. coli* O157:H7 or from ubiquitous origin as *L. monocytogenes* may easily be transferred to processing facilities, equipment and utensils through food handlers or improperly washed raw produce.

For example, *L. monocytogenes* is a bacterium commonly found in the food industry that can easily enter into food-processing plants through soil on transport equipment, workers' shoes and clothing, raw plant tissues, among others. This pathogen is most often detected in moist areas such floors drains, condensed and stagnant water, floors, residues and processing equipment [29]. Therefore, sanitation of facilities, processing equipment and utensils are critical points for preventing microbial contamination of the product.

Pest presence

Pests such as rodents, birds, reptiles, amphibians and insects have been recognised as important carriers or vectors for a variety of pathogens within processing plants [30]. *Salmonella* spp. has been isolated from amphibians, house- and fruit-flies. Such pests are able to enter the processing facility, contaminate fresh-cut produce and cause outbreaks of foodborne diseases [24, 31, 32]. On the other hand, *E. coli* was isolated from houseflies obtained from electrocuting insect traps [33]. These studies revealed that pests may enter the processing plant, contaminate the fresh-cut produce and cause diseases, regardless of whether they are properly controlled.

The United States Food and Drug Administration [4] recommends implementing a pest control program throughout the entire processing facility to eliminate/prevent such pests. The use of window screens, screen doors, pesticides, traps, bait, chemicals, etc, may be helpful for its control.

Conclusions

Several factors that influence the presence of pathogenic micro-organisms in fresh-cut produce during its processing were identified. The raw produce quality coming from field

is one of the most sources of pathogenic micro-organisms that may be transferred to fresh-cut products. Maintaining the microbiological safety of fresh-cut fruits and vegetables requires a system approach that covers all aspects of processing. Therefore, it is essential that effectiveness intervention strategies be made to prevent contamination of fresh-cut produce. In such sense, it is indispensable adherence to GAPs, GMPs and application of a HACCP program in the processing plants to guarantee microbiological safety of the produce. However, the high cost associated with its implementation is still one limitation; because costs of audit, personnel training, development of documentation, and design of facilities, among others, are required [34].

Future directions

The increase in the *per capita* consumption of fresh-cut produce is expected to continue during the forthcoming years; therefore the fresh-cut produce industry will have to make efforts for developing procedures that ensure high safety and quality standards for fresh-cut produce. Research and development of feasible strategies for pathogenic micro-organism prevention/control, especially in the case of small- and medium-sized industries with limited financial resources, are necessary.

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