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Listeria monocytogenes: A CONSIDERABLE PATHOGENIC MICROORGANISM OF CONCERN IN MINIMALLY PROCESSED FRUITS AND VEGETABLES

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ABSTRACT

The increasing demand of minimally processed fruits and vegetables signify a challenge to make them stable and safe to be consumed. The processing of this type of product may contribute higher risks of food borne illnesses. One of the foodborne pathogens in minimally processed fruits and vegetables is *Listeria monocytogenes*. The importance of understanding the characteristics of *L. monocytogenes* will help to determine how this microorganism occurs, grows, and survives in minimally processed fruits and vegetables. Proper and suitable methods to reduce *L. monocytogenes* so that it can not pose a significant risk to cause disease therefore are able to be determined according to the model of the growth and survival of *L. monocytogenes* in minimally processed fruits and vegetables.

Keywords: *Listeria monocytogenes*, minimally processed fruits and vegetables

INTRODUCTION

The popularity of minimally processed fruits and vegetables (MPFV) has been increased recently. Consumers are increasingly demanding convenient, ready to use, ready to eat fruits and vegetables with fresh like quality and containing only natural ingredients. Because of its convenience, freshness, and human health benefits, the marketing of fruit and vegetables with minimal processing is gaining forward motion. It has been the fastest growing portion of the food retail markets during the past 10 years. The MPFV industry was initially developed to supply hotels, restaurants, catering services and other institution. More recently, it was expanded to include food retailers for home consumption (Ahvenainen, 1996; Alzamora *et al.*, 2000; Leverentz *et al*., 2003; Chaudry *et al*., 2004).

MPFV are products that maintain their attributes and quality similar to those of fresh products. In some cases, a minimally processed product is raw food and the tissue cells are alive and can be used directly for consumption. However, it is not necessarily product for direct consumption but can be considered as preserved foods that maintain their freshness characteristics and can be later transformed into processed products using conventional methods (Huxsoll and Bolin, 1989). Minimal processing includes all operations such as washing, peeling, slicing or shredding of vegetables and fruits, that must be carried out before blanching in a conventional processing line and that keep the food as living tissue for sale within 7-8 days after preparation and storage at low temperature (Carlin *et al*., 1990). Therefore, sometimes MPFV can be regarded as ready to use, ready to eat, lightly processed, fresh-cut, or minimally processed refrigerated fruits and vegetables.

Despite improved methods of maintaining quality and shelf life of MPFV produces, a limiting factor to optimum quality is the role of microorganisms in the spoilage and safety of those produces. Microorganisms are important factor when one is dealing with MPFV. The presence of spoilage bacteria, yeasts, and molds and the occasional pathogens on fresh produce has been recognized for many years, and they can be present in minimally processed, too (Seymour *et al*., 2002).

Since plants and plants part used as vegetables and fruits usually play a key role in transmitting the pathogen from natural habitats to human food supply, and one of the pathogenic organisms which is widely distributed on plant vegetation is *Listeria monocytogenes*, then it can also be present in MPFV and may pose considerable risk to human health. *L.*

monocytogenes has been associated with a number of serious food borne outbreaks and recalls in some developed countries. Fresh produce has also been implicated in outbreaks and sporadic cases of listeriosis, a disease caused by *L. monocytogenes*. These pathogens are more likely to be present on vegetables including cabbage, cucumbers, mushrooms, potatoes, and radishes (Ryser and Marth, 1991). *L. monocytogenes* still can have a potential risk to human if they are occur at pathogen population greater than 10^3 CFU per gram or per mL food (Tompkin, 2002.

Food borne outbreaks and incidence of *L. monocytogenes* in MPFV illustrate the importance of understanding the fundamental mechanism of *L. monocytogenes*, the occurrence, survival, growth as they related to food production. Furthermore, it is not less important to understand how the bacterium establishes a niche and persists on food processing equipment, and what are the proper technologies to be applied in MPFV so that they can cause reduction of possible microbial risks by *L. monocytogenes* without quality losses. Therefore, this review is aimed to discuss the nature of *L. monocytogenes* in MPFV, the source of contamination, the occurrence, survival, and growth, as well as possible and suitable methods to reduce them from the products of MPFV.

THE CHARACTERISTICS OF *Listeria monocytogenes*

L. monocytogenes is one of the six species in the genus *Listeria*. It is a gram-positive, non-spore forming, non-acid fast, pleomorphic rod-shaped bacterium with rounded ends. In addition, it is motile with flagella and exhibits a characteristic tumbling motility which can aid identifying the organism (Ryser and Marth, 1991; Swaminathan, 2001).

Optimum growth temperature for *L. monocytogenes* is between 30 and 37°C, but the organism is capable to initiate grow in the temperature range of 0-45°C. It is also a psychrotolerant organism with the ability to grow at refrigerated temperature and thus to resist the traditional food preservation technique of chilling. When contaminated food is stored for extended period at low temperatures, *L. monocytogenes* is able to grow, leading to significant public health threat (Cole *et al*., 1990; Ryser and Marth, 1991; Swaminathan, 2001; Liu *et al*., 2002). According to Junttila *et al*. (1988) *L. monocytogenes* has a minimum growth temperature estimated just below 2°C.

The pH range for the growth of *Listeria monocytogenes* was thought to be 5.6 – 9.6 and can not grow at pH below 4.5 – 4.6 (McClure *et al*., 1989; Cole *et al*., 1990), however, due to a phenomenon called stress hardening i.e. increased tolerance after adaptation to stressful environment, the organism may become highly resistant to even extremely acidic conditions, in addition, investigation indicate that the organism can initiate growth in the laboratory media at pH as low as 4.4 (Lou and Yousef, 1997).

L. monocytogenes grows optimally at water activity (a_w) ≥ 0.97. For most strains, the minimum a_w for growth is 0.93 (Lou and Yousef,1997). It is also able to grow in the presence of 10-12% sodium chloride (Cole *et al*., 1990). It grows to high population in moderate salt concentrations (6.5%). The bacterium survives for long periods in high salt concentrations; the survival in high salt environment is significantly increased by lowering temperature.

THE SOURCE OF CONTAMINATION FOR *Listeria monocytogenes* IN MPFV

Microbial contamination of fruits and vegetables in general is reported to arise during growth, from the soil, organic matter, organic fertilizer, irrigation processes, insects, animals and human contacts, and from post-harvest techniques, including washing, trimming, and peeling (Beuchat, 1996). Bacterial contamination of MPFV also begins from the field. Despite post-harvest trimming and decontamination processes, this contamination persists during commercial preparation, and the products can contain in excess of 10^6 viable bacteria at the time of packaging. The MPFV maybe further contaminated during transport from processing steps and during packaging.

L. monocytogenes is widely distributed on plant vegetation. Plants and plant parts used as vegetable play a key role in disseminating the pathogens from natural habitats to human food supply (Beuchat *et al*., 1990). A handful of laboratory studies have addressed the fertilization with manure as a source of *L. monocytogenes* produce contamination. According to Al-Ghazali and Al-azawi (1990) when sewage cake contaminated with *L. monocytogenes* (3- 15 cells/g) was added to soil, 10% of the alfalfa crop was

positive for pathogen, although levels were low (< 5 cells/g). Similarly, some of the parsley samples growing in pot with same fertilizer was positive for pathogen. Beuchat *et al*. (1998) said it is also a saprophyte and can survive on decaying plant material, and it is disseminated on farms by animals grazing on decaying plants, spreading their feces onto fresh fields.

MPFV products are particularly susceptible to microbial attack because of changes occurring to the tissue during processing. Processing operations such as cutting, shredding, and slicing provide opportunities for contamination of *L. monocytogenes*. If it contaminates the processing environment, it may colonize processing surfaces, surviving in drains, cracks in floors and walls and in cervices in equipments. Vatanyoopaisarn *et al*. (2000) reported that *L. monocytogenes* can attach itself to the stainless-steel processing surfaces, and the flagella, independent of cell motility do indeed act as an adhesive structure during early stages of attachment under statistic conditions. In addition, changes in surface structure other than the presence or absence of the flagella also favour the attachment of cells to stainless steel at low temperatures.

MPFV with cut surfaces during processing made it suitable for *L. monocytogenes* to multiply throughout storage of products. Colonization of cut surface is two stages processes: initial adhesions that occurs rapidly and is followed by the production of extra cellular polysaccharides by the adhered bacteria (Brocklehurst, 1994). Garrood *et al*. (2004) reported that the number of *L. monocytogenes* organisms attached to the surface of potato disks showed expected increase with time of contact and increase in inoculum concentration would result in directly proportional increase in attachment. *L. monocytogenes* also has the capacity to attach sufficiently well such that they can not be removed during washing of radish slices (Gorski *et al*., 2003).

MPFV products are also packaged under modified atmosphere conditions and stored refrigerated for up to 10-15 days. This creates a favourable environment and time for proliferation of *L. monocytogenes*, due to its nature as psychotropic bacteria that can grow in relatively low temperature (Ahvenainen, 1996; Francis *et al*., 1999) and a number of studies have indicated that modified atmosphere packaging may select for microaerophilic organisms that may be psychotropic (Bracket, 1994).

THE INCIDENCE, GROWTH AND SURVIVAL OF *Listeria monocytogenes* IN MPFV

An important consideration when addressing safety issue is the incidence, the growth, and the survival of pathogens, which in turn will lead to the outbreaks associated with particular food products. One of particular concern of *L. monocytogenes* is that it is psychotropic, capable of growing at refrigeration temperatures. It is also facultative anaerobic, capable of survival of growth under low O_2 concentrations (Francis and O'Beirne, 1997). In addition, Sizmur and Walker (1988) evaluated the incidence of *L. monocytogenes* on salads and found out that the population increased two-fold when salads were held at 4°C for 4 days.

Beuchat *et al*. (1986) determined the behaviour of *L. monocytogenes* on inoculated samples of shredded, raw, autoclaved cabbage and found out that *L. monocytogenes* presented similar behaviour patterns with the reduction of 3 log CFU/g during 42 days of refrigerated storage.

The incidence of *L. monocytogenes* in minimally processed salads with or without dressing was studied and was found that in levels of 10^4 CFU/g with pH damples from 4.47–5.12. Salads with dressing had lower levels. The survival of *L. monocytogenes* in both types of salads was also studied. In salads with dressing, the level of *L. monocytogenes* did not change during storage. The same fate was observed in salads without dressing. A significant decrease of pH during storage was observed in both cases. These results suggest that minimally processed salads could be a potential risk for public health (Martinez *et al*., 2000).

The ability of *L. monocytogenes* to survive and grow on ready to eat fresh salad vegetables has also been demonstrated in cabbage, celery, raisins, onions, carrot salads, lettuce, cucumber, radish, leek salad, asparagus, broccoli, cauliflower, and vacuum packaged pre-peeled potatoes (Martinez *et al*., 2000).

Berrang *et al*. (1989) found that *L. monocytogenes* grew to populations of more than 10^6 on asparagus, broccoli and cauliflower at an abuse temperature of 15°C. It was able to maintain original population for up to two weeks of storage at 10 or 21°C. Conner *et al*. (1986) more closely examined the influence of temperature, NaCl, and pH on the growth of *L. monocytogenes* in cabbage juice. Salt free juice was an excellent growth medium for it with population increasing from

10^4/mL to 10^9/mL after 8 days of incubation at 30°C. Although more acidic environment proven to be lethal, complete inactivation of *L. monocytogenes* did not occur until pH was reduced to 4.1. Lower temperatures help protect *L. monocytogenes* from harmful effects of low pH.

THE CONTROLLING OF *Listeria monocytogenes* IN MPFV

Control of *L. monocytogenes* in MPFV is needed to make sure that the product is free from or at least has minimum *L. monocytogenes* which are considered to be safe for human consumption. The control can be done prior to the processing, since the contamination of the products may be occurred in the field. Environmental conditions such as temperature and rain fall, farm practices and the standard of hygiene on the farm are known as factors affecting microbial quality. Therefore, good agricultural practices (GAP) are required and the use of contaminated sources such as contaminated water for irrigation should be avoided. The choice of fertilizer should be taken into consideration to minimize the possibility of the product is contaminated with *L. monocytogenes*. The use of inorganic fertilizers or composted, treated manure can reduce the risk of contamination.

Control of *L. monocytogenes* in a processing plant requires reducing number of these bacteria on the products and equipment surfaces via physical means and preventing general growth and proliferation of *L. monocytogenes,* by managing the environment. There are very few hurdles in packaged MPFV products to prevent the growth of microorganism. The products are washed to remove excessive contamination, but after processing, the main controls used are storage at refrigeration temperature and packaging in modified atmosphere.

First, cleaning and sanitizing the product as well as equipment's, walls, and drains should be adequate to destroy or remove *L. monocytogenes* (Suslow and Harris, 2002). The use of disinfectant chemical in wash water provides a barrier to contamination of products. Research reported by Nguyen-the and Carlin (1994) suggests that inactivation of *L. monocytogenes* on vegetables by chlorine is limited. Zhang and Farber (1996) showed that treatment of shredded lettuce and cabbage with 200 ppm chlorine for 10 minutes reduced the population of *L. monocytogenes* by 1.7 and 1.2 log CFU/g, respectively. Reductions were only marginally greater when exposure time was increased from 1-10 minutes. Since chlorine reacts with organic matter, components leaching from tissues of cut product surfaces may neutralize some of the chlorine before it reaches the microbial cells, thereby reducing its effectiveness. Additionally, crevices, cracks, and small fissures in product, along with hydrophobic nature of the waxy cuticule on the surface of many fruits and vegetables, may prevent the chlorine and other sanitizers from reaching the microorganism (Zhang and Farber, 1996). Exposure of *L. monocytogenes* cells to alkaline stress in food processing facilities may occur repeatedly through the use of alkaline detergents and disinfectants used to remove food residues from the equipment and floors which also happen to favour the survival and the growth of *L. monocytogenes* (Taormina and Beuchat, 2001).

The fresh nature of MPFV prevents the use of traditional processing such as cooking or heating, and consumers are demanding that food contain no chemical preservatives. Therefore, application of biocontrol concepts maybe used to create preservation hurdles for MPFV. Francis and O'Beirne (1998) found that mixed population of bacteria isolated from shredded lettuce generally diminished the growth of *L. monocytogenes*. They suggested that natural background microflora could be an important influence on growth of *L. monocytogenes* on lettuce. In addition, Leverentz (2003) examined that the phage mixtures reduced *L. monocytogenes* populations by 2.0 -4.6 log units on honeydew melons and 0.4 units on apples. The population was much reduced when nisin (bacteriocin) was applied in combination with phage. However, the effectiveness of phage treatment also depended on the initial concentration of *L. monocytogenes*.

The final stage in the production of MPFV is packaging. The package provides protection for the product from damage and further contamination with microorganism. The use of controlled and modified atmosphere packaging also provides to some extent, a hurdle against the growth of remaining foodborne pathogens (Philips, 1996). Early studies showed that *L. monocytogenes* inoculated onto broccoli, asparagus, and cauliflower was unaffected by a MAP of 3% CO_2, 18% O_2, and 79% N_2 for 10 days at 10°C (Berrang *et al*., 1989). Further studies by Beuchat and Brackett (1990) clearly demonstrated that *L. monocytogenes* increased significantly in number on lettuce stored in MA 3% CO_2 and 97% N_2. In addition, increasing CO_2 levels from 10 to 20% has

been reported to stimulate growth of *L. monocytogenes* in a surface model system (Amanatidou *et al*., 1999). It can be seen that packaging under MA generally does not inhibit the growth of *L. monocytogenes*, although other factors influence its survival. Therefore, more research needs to be done to examine the influence of different atmosphere, background microflora and storage temperature on the survival and growth of *L. monocytogenes* on MAP of MPFV.

CONCLUSION

The increasing popularity and demand of MPFV products have led to the increasing of how important is the safety of those products to be consumed. Regarding the safety of the MPFV products, *L. monocytogenes* is one of the foodborne pathogens of concern which can cause disease in human such as listeriosis. *L. monocytogenes* once has contaminated the product from the beginning, will have the capability to survive in the processing environment due to its characteristics which are benefited by the process of the production. Physically damaged MPFV can promote multiplication of *L. monocytogenes*. One important characteristic of *L. monocytogenes* which is able to grow in low temperature (psychotropic), will become a serious problem since most of the MPFV products are kept at refrigerated temperature to extend shelf life. According to the source of the contamination, the incidence, the growth, and the survival of *L. monocytogenes*, the possible method to reduce or to keep their number in MPFV as low as possible so that it can not act as disease causing agent, still are the primary method of good agricultural practices and good manufacturing practices. Type of packaging such MAP with the proper amount of gasses used is still needed to be taken into serious consideration, because it usually did not inhibit the growth of *L. monocytogenes*. Therefore, more research is needed to examine the influence of different atmosphere and storage temperatures on the survival and growth of *L. monocytogenes* on MAP of MPFV.

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