Food Control 28 (2012) 258–264

Contents lists available at SciVerse ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Microbiological quality and safety of minimally processed vegetables marketed in Campinas, SP – Brazil, as assessed by traditional and alternative methods

Liliane Corrêa Maistro [a], Norma Teruko Nago Miya [a], Anderson S. Sant'Ana [b], José Luiz Pereira [a,*]

[a] Department of Food Science, Faculty of Food Engineering (FEA), University of Campinas (UNICAMP), Rua Monteiro Lobato, 80, Caixa Postal: 6121, CEP: 13081-970, Campinas, SP, Brazil
[b] Department of Food and Experimental Nutrition, Faculty of Pharmaceutical Sciences (FCF), University of São Paulo (USP), São Paulo, SP, Brazil

ARTICLE INFO

Article history:
Received 12 December 2011
Received in revised form
2 May 2012
Accepted 9 May 2012

Keywords:
Ready-to-eat vegetables
Rapid methods
Salmonella
Listeria monocytogenes
Microbiological quality
Food safety

ABSTRACT

In this study, a total of 172 samples of minimally processed vegetables (MPV) were collected from supermarkets in the city of Campinas, Brazil. The MPV were analyzed using traditional and/or alternative methods for total aerobic mesophilic bacteria, total coliforms, *Escherichia coli*, coagulase positive staphylococci, *Salmonella* and *Listeria monocytogenes*. All the MPV analyzed presented populations of aerobic mesophilic microorganisms and total coliforms were >4 $\log_{10}$ CFU/g and 1.0–3.4 $\log_{10}$ CFU/g, respectively. *E. coli* was enumerated in only 10 samples out of 172 collected, while none of the 172 samples of MPV presented contamination by coagulase positive *Staphylococcus* ($<10^1$ CFU/g). Among the four methods used for detection of *Salmonella* in MPV (Vidas, 1,2 Test, Reveal, and Traditional), when Reveal was used a total of 29 positive samples were reported. For *L. monocytogenes*, the four methods tested (Vidas, Vip, Reveal, and traditional) performed similarly. The presence of *Salmonella* and *L. monocytogenes* in MPV was confirmed in one (watercress) and two samples (watercress and escarole), respectively. In conclusion, it has been observed that the microbiological quality of MPV commercialized in Campinas is generally satisfactory. Besides, the choice of microbiological method should be based not only on resource and time issues, but also on parameters such as sensitivity and specificity for the specific foods under analysis.

1. Introduction

The microbiological quality and safety of minimally processed vegetables (MPV) has been cause of concern in the last two decades due to their bigger association with foodborne disease outbreaks (Elviss et al., 2009; Little & Gillespie, 2008; Sivapalasingam, Friedman, Cohen, & Tauxe, 2004). Several studies have reported on the occurrence of foodborne pathogens in vegetables, particularly in MPV, such as *Salmonella* spp. (Bhagwat, 2004; Bohaychuk et al., 2009; Elviss et al., 2009; Islam et al., 2004; Khoo et al., 2009; Salleh et al., 2003; Sant'Ana, Landgraf, Destro, & Franco, 2011), pathogenic *Escherichia coli* (Balagué et al., 2006), and *Listeria monocytogenes* (Aguado, Vitas, & García-Jalón, 2004; Cordano & Jacquet, 2009; Little et al., 2007; Oliveira, Ribeiro, Bergamini, & Martinis, 2010; Ponniah et al., 2010; Vitas & Garcia-Jalon, 2004). Studies have also been focused on the MPV shelf-life and hygiene indicators microorganisms, such as mesophilic and psychrotrophic microorganisms, *Pseudomonas* spp., coliforms, and *E. coli* (Abadias, Usall, Anguera, Solsona, & Viñas, 2008; Fröder et al., 2007; Ilic, Odomeru, & LeJeune, 2008; Meldrum et al., 2009). These facts have led to the development of rapid, sensitive, specific, and cost-effective methods to be used by food microbiologists and public health authorities in order to assess the microbiological quality and safety of foods (Jasson, Jacxsens, Luning, Rajkovic, & Uyttendaele, 2010).

Nowadays, several rapid methods are available for detection of foodborne pathogens in foods and include from modifications and automation of conventional methods to molecular-based assays (De Boer & Beumer, 1999; Jasson et al., 2010). Among the rapid methods, modified or automated plating techniques reduce the amount of material and time for their preparation and are easy to interpret. Furthermore, immunological-based techniques are ease of use, fast, and very effective as a screening tool. These advantages make modified or automated plating and immunological-based techniques very acceptable by food microbiologists. Although several studies have been carried out to compare the rapid methods for enumeration and/or detection of indicator microorganisms and foodborne pathogens in several foods (Feldsine, Kerr,

* Corresponding author. Tel.: +55 19 3521 2171.
E-mail address: pereira@fea.unicamp.br (J.L. Pereira).

0956-7135/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodcont.2012.05.021

Shen, & Lienau, 2009; Oktay & Heperkan, 2006; Reiter, López, Jordano, & Medina, 2010; Silbernagel, Jechorek, Carver, Barbour, & Mrozinski, 2003; Silbernagel, Jechorek, Carver, Horter, & Lindberg, 2003; Yeh, Tsai, Chen, & Liao, 2002), research focusing on the use of rapid methods in vegetables are rare. Due to its background microbiota (Shi et al., 2009), naturally occurring inhibitors such as proteins presenting antimicrobial activity (Noriega et al., 2010; Sitohy & Osman, 2010), residuals of pre and/or post-harvest treatments such as pesticides (Cengiz, Certel, Karakaş, & Göçmen, 2007; Zhang, Liu, Yu, Zhang, & Hong, 2007), these studies are very relevant. Thus, in this study, alternative methods were compared to conventional methods in the study of the microbiological quality and safety of MPV.

2. Material and methods

2.1. Samples

A total of 172 samples of MPV were taken intermittently during 24 months from a large supermarket chain in the city of Campinas, Brazil. Among the 172 samples collected, 100 were packaged in perforated bags, while the remaining bags were packaged under modified atmosphere. MPV packaged in perforated bags were: chard (*Beta vulgaris var. cicla*) ($n = 13$), watercress (*Nasturtium officinale*) ($n = 15$), lettuce (*Lactuca sativa L.*) ($n = 13$), chicory (*Cichorium intybus*) ($n = 14$), collard greens (*Brassica oleracea L. var. acephala D.C.*) ($n = 15$), cabbage (*Brassica oleracea Linne*) ($n = 15$), and arugula (*Eruca sativa L.*) ($n = 15$). All these vegetables were sliced or shredded, excepting watercress and arugula that were available as whole leaves. MPV packaged under modified atmosphere were: watercress (*N. officinale*) ($n = 18$), leaf lettuce (*Lactuca sativa* var. *crispa*) ($n = 18$), escarole (*Cichorium endivia*) ($n = 18$), and arugula (*Eruca sativa L.*) ($n = 18$). The collection of vegetables was based on their availability in the supermarkets when random samplings were done. At the moment of purchasing, the temperature of the displays in which the packages were exposed for commercialization was recorded. Besides, the expiration date and the time the packages were available at the displays until the time of collection, were registered. Samples were transported to the laboratory in styrofoam boxes and immediately analyzed.

2.2. Determination of gaseous atmosphere in the MPV packages

Analyzes of O_2 and CO_2 contents in MPV packaged under modified atmosphere were performed using a Mocon Pack Check™, Model 650 – Dual Head Space Analyzer (MN, USA).

2.3. Microbiological analysis

All the 172 samples of MPV were analyzed for each of the following microorganisms or microbial groups: total aerobic mesophilic bacteria, total coliforms, *E. coli*, coagulase positive staphylococci, *Salmonella* spp., and *L. monocytogenes*.

2.3.1. Conventional methods

An amount of 25 g of each MPV sample was diluted in 225 mL of diluents and further stomached for 1 min in medium speed. For enumeration of total aerobic mesophilic counts, samples were serially diluted in buffered peptone water (BPW) and then, inoculated in Plate Count Agar (PCA), following incubation at 37 °C/48 h (Downes & Ito, 2001).

The enumeration of coagulase positive staphylococci was performed using Baird-Parker Agar plus egg yolk (BPA) after serial dilution in BPW. BPA plates were incubated at 37 °C/48 h and checked for typical/atypical colonies (black, shiny, convex, and surrounded or not by clear zones, 2–5 mm). Between 5 and 10 typical and atypical colonies were purified in BHI agar plates and further sub-cultured in BHI broth. Confirmation was carried out through the tests of catalase, coagulase, and TNase (Downes & Ito, 2001). Results were expressed based on the number of coagulase positive staphylococci in BPA plates, number of colonies taken for confirmation, and number of colonies confirmed based on biochemical tests.

The detection of *Salmonella* was carried out with pre-enrichment in Lactose broth and incubation at 37 °C/24 h. Subsequently, 1 mL of this medium was inoculated in Tetrathionate and Selenite cystine broths that were incubated at 37 °C/24 h. Further, hektoen enteric agar, bismuth sulfite, and xylose lysine desoxycholate agars were streaked with sterile loops carrying inoculums took from Tetrathionate and Selenite cystine broths. The plates were incubated at 37 °C/24 h in order to isolate characteristic colonies of *Salmonella* that were sub-cultured in NA. Triple sugar iron (TSI) was used for presumptive confirmation of colonies that were further confirmed through biochemical miniaturized tests (API20E) and serology (polyvalent antiserum) (Downes & Ito, 2001).

For *L. monocytogenes*, Listeria Enrichment Broth was used as the enrichment medium (30 °C/24 h) and followed by inoculation through streaking in Oxford and Lithium chloride-phenylethanol-moxalactam mediums (37 °C/48 h). Typical *L. monocytogenes* colonies were checked after purification in Tryptic soy agar plus 0.6% yeast extract (TSAYE) through the use of Henry transillumination and motility (motility agar) (Downes & Ito, 2001). Further, presumptive colonies were confirmed by API *Listeria* (bioMérieux).

For aerobic mesophilic counts and coagulase positive staphylococci, results were expressed as log CFU/g, while for *Salmonella* spp. and *L. monocytogenes* results were expressed as presence or absence per 25 g. All the culture media used were from Oxoid (Basingstoke, UK), unless otherwise stated.

2.3.2. Alternative methods

The enumeration of coliforms and *E. coli* was performed after serial dilution in BPW and inoculation of 1 mL in Petrifilm EC plates, following incubation at 37 °C/48 h. Colonies of *E. coli* were blue to red–blue colonies with entrapped gas, while total coliforms were red colonies with entrapped gas plus. Total coliforms counts were the sum of total coliforms plus *E. coli* colonies.

For the detection of *Salmonella* spp. through Vidas (SLM) and 1,2 Test, 25 g of each MPV samples were diluted and incubated in BPW at 37 °C/24 h. One and 0.1 mL of pre-enrichment was inoculated in TT and RP broths which were incubated at 42 °C/8 h and 37 °C/8 h, respectively. For Vidas (SLM), 1 mL of TT and RP broths were transferred to M1 and M2 broths (bioMérieux) which were incubated at 42 °C/18 h. Then, 1 mL of each broth was transferred to sterile tubes and heated at 100 °C/15 min, cooled and read at Mini Vidas Immunoassay Analyzer (bioMérieux, France). For 1,2 Test, 1.5 mL of enriched TT broth was inoculated into the lateral 1,2 Test chamber. Antibody was added to the superior chamber and 1,2 Test kit was incubated at 37 °C/26 h. Presence of an immunoband yielded a positive result.

For Reveal, 25 g of each MPV samples were added to 200 mL of Revive medium (Neogen), which was used as pre-enrichment broth. Then, Revive medium was incubated at 37 °C/4 h. Rehydrated Rappaport broth (Neogen) (200 mL) was added to Revive medium previously inoculated with MPV samples, following incubation at 42 °C/18 h. Samples were taken and cooled to room temperature. One hundred and twenty micro liters of enriched broth was inoculated into the sample port of the test device. Reading was performed after 15 min, and positive samples were those presenting a red band in the control and test windows.

The detection of *L. monocytogenes* through Vidas (LMO) and VIP was performed using Fraser and modified Fraser broths at the first step. Incubation was carried out at 30 °C/24 h. Then, 1 mL of the first enrichment medium were transferred to tubes containing Fraser broth (bioMérieux, France) and BLEB, which were incubated at 30 °C/24 h. For Vidas, 500 μL were used for reading in Mini Vidas (bioMérieux, France). For VIP, 1 mL of BLEB was transferred to a sterile tube and heated at 100 °C/5 min, cooled to room temperature. One hundred micro liters were inoculated to the sample addition well and incubated for 10 min. Positive reactions were those in which the test and control windows presented a distinct black line.

For Reveal, Half Fraser broth Plus (Neogen) was used as pre-enrichment broth which was incubated at 30 °C/24 h. Further, 1 mL was added to buffered *Listeria* enrichment broth (BLEB) and incubated at 30 °C/24 h. Then, 2 mL of enriched BLEB was transferred to sterile tubes and heated at 80 °C/20 min. After cooling to room temperature, 135 μL of the broth was inoculated into the blue sample window of the test device. Reading was performed after 20 min, and positive samples were those presenting a blue band in the control and test windows. Results of the microbiological analysis using the alternative methods were expressed as presence or absence of *Salmonella* or *L. monocytogenes* per 25 g of MPV.

2.4. Statistical analysis

Statistical significant difference ($p \leq 0.05$) among the populations of total aerobic mesophilic microorganisms and total coliforms of different vegetables was assessed through analysis of variance and Tukey test. Statistical test was performed in Assistat version 7.5 free software (Campina Grande, Brazil) (Silva & Azevedo, 2002).

The results of physical and microbiological analysis were assessed through principal component analysis (PCA) in a correlation matrix with the data centered into the average. The correlation matrix composed of 173 rows and 9 columns was elaborated. The rows were represented by the samples of MPV, while the columns corresponded to the rapid methods for detection of *Salmonella* (SALM1, SALM2, SALM3, SALM4) and *L. monocytogenes* (LIST1, LIST2, LIST3, LIST4), storage temperature (ST), days of storage at supermarket displays (days), and shelf-life of the MPVs. PCA was performed in XLSTAT for Windows 2010 software.

3. Results and discussion

The assessment of the microbiological safety of foods is safeguarded not only by the implementation of preventive strategies such as hygiene practices and Hazard Analysis Critical Control Points (HACCP), but also by evaluating whether a food attends the microbiological criteria set (Cocolin, Rajkovic, Rantsiou, & Uyttendaele, 2011). The compliance of the criteria is checked by industries and public health authorities through microbiological tests that must have an adequate cost-effectiveness, which is driven by good sensitivity, specificity, rapidness, and low cost. Although traditional methods present a well-documented sensitivity, the time needed to obtain results is most of times too long (Reiter et al., 2010). Rapidness is particularly more important when the target foods present a short shelf-life such as MPV. Thus, in this study, the microbiological quality and safety of MPV were assessed through the use of traditional and alternative methods. In addition to the microbiological data, information on the temperature and time of exposure at supermarkets' displays has been collected to gather data on the conditions MPV are exposed during commercialization in Campinas city, an important MPV consumption site in Brazil.

Table 1 depicts the average temperature of MPV vegetables at the time of collection and the average time of exposure in the supermarkets after processing. Data obtained indicate that temperature of the MPV packaged with perforated films at displays varied from 5 to 15 °C, with 22 and 24% of them being stored at 8 and 12 °C, respectively. On the other hand, temperature of storage of MPV packaged under modified atmosphere varied from 7 to 12 °C, with 49% of the samples being stored at 8 °C. To the microbiological point of view, the importance of temperature during MPV commercialization relies on the fact that variation of temperature in 5 °C may lead to doubling or triplication of growth rate of any foodborne pathogens contaminating these products. For instance, Koseki and Isobe (2005) reported an increase of the growth rate of *Salmonella* and *E. coli* 0157:H7 in ready-to-eat lettuce between 4 and 7 times with the increase of temperature from 10 to 15 °C. Data from literature have recommended different storage temperature values to ensure the microbiological quality and safety of MPV during storage such as 2–5 °C (Francis, Thomas, & O'Beirne, 1999), lower than 5 °C (Chitarra, 2000, p. 119) and 4 °C (Rosa, 2002, p. 120), among others. Although the best conditions for maintenance of MPV quality are established depending on quality of raw materials, type, and physiological response to the injuries caused by minimal processing (Pilon, 2003, p. 111), industries will establish a conservative temperature condition during storage that allow their commercialization, but at the same time do not impair the safety and quality of MPV. In Brazil, the maximum storage temperature recommended in labels of MPV is 7 °C, which is enough to keep the quality and safety of these products processed under strict hygienic conditions for at least 6 days, *i.e.*, the common shelf-life of these products in Brazil. The fact that temperature values recorded in this study are higher than the recommended by industries might lead to reduction of shelf-life of MPV and if pathogens are present, allow their growth posing risks to consumers. In fact, 63% of MPV in non-modified atmosphere packages and 80% of the MPV packaged under modified atmosphere were stored in the range of 7–10 °C, respectively. Several studies have shown that pathogens such as *Salmonella* and *E. coli* 1057:H7 may grow in ready-to-eat vegetables stored at temperature close to 10 °C (Koseki & Isobe, 2005). The concerns over the control of temperature during storage are increased because there is a lack of data and large uncertainty on the impact of consumer's practices and handling on the behavior of foodborne pathogens before MPV are consumed. Therefore, food handlers at the

Table 1
Temperature, gaseous composition, and average time of different types of MPV packages at supermarkets' displays.[a,b]

Vegetables	Packaging system	Gaseous composition (%)		Temperature at time of collection (°C)	Average time at supermarkets' displays (days)
		O_2	CO_2		
Chard	NM	–		9.6 ± 1.7	0.7 ± 0.5
Watercress	NM	–		8.1 ± 1.9	0.4 ± 0.5
Lettuce	NM	–		8.8 ± 1.0	0.8 ± 0.6
Chicory	NM	–		9.1 ± 2.2	0.1 ± 0.4
Collard green	NM	–		11.1 ± 1.4	0[c]
Cabbage	NM	–		11.3 ± 2.4	0[c]
Arugula	NM	–		10.7 ± 1.3	0.5 ± 0.5
Watercress	MA	6.6 ± 3.8	14.0 ± 3.2	9.2 ± 1.1	2.6 ± 1.8
Lettuce	MA	5.2 ± 1.7	10.6 ± 3.5	8.7 ± 1.2	2.9 ± 1.3
Escarole	MA	6.3 ± 2.5	12.1 ± 3.3	10.2 ± 1.2	2.3 ± 1.4
Arugula	MA	7.8 ± 1.7	11.2 ± 2.0	8.3 ± 1.4	2.6 ± 1.1

[a] Where: NM is non-modified atmosphere package and MA is modified atmosphere.
[b] Where: values are average followed by standard deviation.
[c] An average time of "0" means that the package was collected in the moment the employees were filling the displays with new products.

supermarkets should be better trained and consumers informed of the importance of keeping the cold chain during the commercialization and consumption of MPV.

No relationship between the type of packaging system and temperature of storage has been found (Table 1). It is known that, when using modified atmosphere systems, the control of temperature should be even more restrict as the antimicrobial efficiency of carbon dioxide is closely related to low temperature (Devlieghere & Debevere, 2000). This may be related to the lack of knowledge of food handlers and consumers over the modified atmosphere technology, which may invalidate the efforts done to keep the quality and safety of MPV during commercialization.

All the bags of MPV packaged under modified atmosphere presented around 10–14% of CO_2 and less O_2 (Table 1). However, a variation of 1.6–16.4% and 5.4–22% for CO_2 and O_2 has been observed among the samples (data not shown). According to several authors, the concentration of O_2 and CO_2 in MPV may vary from 2–8% to 3–15%, respectively (Cantwell, 2002; Francis et al., 1999; Jacxsens, Devlieghere, Ragaert, Vanneste, & Debevere, 2003). As the vegetables keep their metabolism active during shelf-life, the increase of CO_2 concentration at the end of shelf-life can be considered normal. In this study, most of bags collected had an average of 2.5–3 days of storage, which may explain these results.

The populations of aerobic mesophilic microorganisms in the different types of MPV are shown in Table 2. The *F* test indicated the inexistence of a linear significant effect of temperature in the populations of aerobic mesophilic microorganisms in MPV packaged studied here. However, this may be due to the fact that the samples were collected few hours or days of their processing. Therefore, a major effect of temperature on the microbial population in these products could be more evident at the end of shelf-life. Although this possibility has not been studied here, the association between microbiological quality and safety of MPV and different times of exposure at displays of supermarkets is worthy of investigation as it could bring interesting data regarding the microbial ecology of these products and generate information to ensure the safety of these products.

All the MPV analyzed presented populations of aerobic mesophilic microorganisms higher than 4 $\log_{10}$ CFU/g, with collard green and cabbage showing the lowest counts ($p < 0.05$). The highest populations were found in watercress packaged regardless of the packaging system, which evidences an inherent contamination of this produce. From Table 2, it can be noticed that the type of packaging system did not influence the populations of aerobic mesophilic microorganisms. Although the populations of aerobic mesophilic in vegetables is high from the field, it is known that the longer the storage and the higher the temperature, the higher populations of these microorganisms in MPV at the end of shelf-life (Francis & O'Beirne, 2002).

A similar behavior observed for aerobic mesophilic microorganisms has been observed for population of total coliforms as no significant differences among the different types of MPV were observed (Table 2). It is known that the determination of total coliforms in MPV is not useful as the enumeration of fecal coliforms because these microorganisms are part of the normal microbiota of these products (Francis et al., 1999). On the other hand, only two samples out of 17 of minimally processed arugula were contaminated with *E. coli*. These samples showed levels of 3.5 and 3.1 $\log_{10}$ CFU/g of this microorganism. All the other samples of minimally processed watercress, lettuce, and escarole packaged under modified atmosphere presented counts of *E. coli* lower than 10^1 CFU/g, i.e., below the limit of quantification. Among the MPV packaged in perforated films, chard, watercress, lettuce, chicory, cabbage, and arugula did not present counts of *E. coli* above the limit of quantification of the method used (10^1 CFU/g). However, a total of 7 among 15 samples of collard greens presented contaminated with *E. coli* in the range of 1.4–3.3 log CFU/g (mean of 2.6 log CFU/g). Fecal coliforms and particularly *E. coli*, may efficiently indicate failures in sanitization process, although, their presence does not automatically imply the presence of a pathogen (Nguz, Shindano, Samapundo, & Huyghebaert, 2005).

All the 172 samples of MPV analyzed in this study did not present counts of coagulase positive *Staphylococcus* above the limit of detection of the method (10 CFU/g) (data not shown). The counts of these microorganisms were always below the limit of quantification of the method used (10^1 CFU/g). The fact that most of studies on microbiological quality of MPV have not studied the populations and presence of coagulase positive *Staphylococcus* may be explained by their relative weak competition ability over the common microbiota of these products and by the fact that the production of enterotoxins does not take place below 10 °C (Oliveira, Miya, Sant'Ana, & Pereira, 2010).

Salmonella and *L. monocytogenes* are two important pathogens for public health and the association of the former with foodborne disease outbreaks has been constantly reported (Elviss et al., 2009; Harris et al., 2003). On the other hand, *L. monocytogenes* has been barely linked to foodborne disease outbreaks in which vegetables are the foods implicated (Harris et al., 2003), but it has been constantly isolated from vegetables (Cordano & Jacquet, 2009; Little et al., 2007; Oliveira, Ribeiro et al., 2010; Ponniah et al., 2010). Therefore, the evaluation of the presence of these microorganisms in MPV is of great relevance. The alternative methods used for detection of *Salmonella* and *L. monocytogenes* in the samples of MPV led to an overall higher prevalence of these pathogens in comparison to the traditional method (Table 3). No co-occurrence of

Table 2
Populations of aerobic mesophilic microorganisms and total coliforms in different types of MPV vegetables.[a,b]

Vegetables (*n*)	Type of packaging	Aerobic mesophilic microorganisms (log CFU/g)	Total coliforms (log CFU/g)
Chard (13)	NM	5.37 ± 0.99[a,b]	3.28 ± 0.61[a]
Watercress (15)	NM	6.51 ± 1.48[a]	2.98 ± 1.75[a]
Lettuce (13)	NM	6.57 ± 1.58[a]	2.81 ± 1.63[a]
Chicory (14)	NM	6.89 ± 0.87[a]	3.36 ± 1.20[a]
Collard green (15)	NM	4.02 ± 1.01[b]	3.08 ± 1.32[a]
Cabbage (15)	NM	4.00 ± 1.09[b]	2.53 ± 1.12[a]
Arugula (15)	NM	6.19 ± 0.65[a,b]	3.74 ± 1.10[a]
Watercress (18)	MA	6.39 ± 0.85[a]	3.15 ± 1.31[a]
Lettuce (18)	MA	5.93 ± 1.14[a,b]	1.02 ± 1.41[a]
Escarole (18)	MA	4.75 ± 0.85[a,b]	2.55 ± 1.69[a]
Arugula (18)	MA	6.18 ± 0.87[a,b]	2.86 ± 1.61[a]

[a] Different lowercase letters in a column indicate significant difference ($p < 0.05$) according to Tukey's test.
[b] Where: NM is non-modified atmosphere package and MA is modified atmosphere.

Table 3
Number of positive MPV samples for *Salmonella* and *L. monocytogenes* according to traditional and alternative methods.[a]

Microorganisms	Packaging system	Methods			
		Traditional	Vidas	1,2 test	Reveal
Salmonella	Perforated packages	0	4	0	29
	Modified atmosphere	1	1	0	13
Microorganisms	Packaging system	Methods			
		Traditional	Vidas	Vip	Reveal
L. monocytogenes	Perforated packages	0	0	1	0
	Modified atmosphere	2	1	2	1

[a] A total of 100 samples of MPV packaged in perforated films and 72 samples of MPV packaged under modified atmosphere were analyzed.

L. monocytogenes and Salmonella in the samples was observed by any of the four methods tested. Among the four different alternative methods evaluated for detection of *Salmonella* in MPV, high rates of positivity were observed when Reveal was used (Table 3). All the four tests used herein for the detection of *Salmonella* and *L. monocytogenes* are immunoassays, with Reveal and Vidas being based on enzyme-Linked immunoabsorbent assay (ELISA), while VIP and 1,2 Test are immunoprecipitation and antigen–antibody-based reactions, respectively (Jasson et al., 2010). The higher rates of positivity (29 and 18% for MPV packaged in perforated films and under modified atmosphere, respectively) when using Reveal in comparison to other alternative methods such as Vidas, VIP, traditional methods, and Reveal for detection of *Salmonella* in poultry slaughter houses have been reported by Reiter et al. (2010). According to these authors Reveal system detected twice more positive samples than the other methods which might be due to false-positive results. Chicory and collard green gave the high rates of positivity with 8 out of 14 and 15 samples, respectively, being positive for *Salmonella* according to Reveal. Among 18 samples of minimally processed watercress packaged under modified atmosphere, 9 were positive for the presence of *Salmonella* according to Reveal, while only one sample was confirmed by the traditional method and Vidas. Although Vidas is an automated method, a major attention must be paid when using Vidas, because it detects antigens rather than viable cells (Reiter et al., 2010). Therefore, there is the risk of rejecting lots of products that do not carry live cells of foodborne pathogens and that will not pose risks to food safety. However, it is a good option for quick determination of pathogens and release of lots when samples are negative for the pathogens under concern.

From 14 to 18 minimally processed chicory and arugula samples, only one sample of each was positive for *L. monocytogenes*. However, this pathogen was detected by Vip, Reveal and traditional methods in watercress packaged under modified atmosphere, while in escarole *L. monocytogenes* was detected by both Vidas and traditional methods (Table 3). Although the alternative methods gave several positive results (Table 3), in only three cases a confirmation by the traditional methods was obtained. As all the alternative tests used for the detection of *Salmonella* and *L. monocytogenes* herein methods require supplementary confirmation (Jasson et al., 2010); the presence of *Salmonella* was confirmed in only one sample (watercress), while *L. monocytogenes* was present in two samples (watercress and escarole). It should be stressed that the three MPV samples were packaged under modified atmosphere. It should be stressed that according to the traditional method, none of samples packaged in perforated films were positive for both pathogens studied. Several studies have reported low prevalence or no detection of *Salmonella* and *L. monocytogenes* in MPV worldwide. Giusti et al. (2010) reported no isolation of *L. monocytogenes* from MPV collected in Italy; while a low prevalence of *Salmonella* (1 positive sample out of 265 collected) has been found. A higher prevalence of *Salmonella* was reported by Abadias et al. (2008) in MPV from Spain. According to these authors, four samples harbored this pathogen out of 300 samples taken, while *L. monocytogenes* was isolated from two samples. In Brazil, four and one of the 181 samples were positive for *Salmonella* and *L. monocytogenes*, respectively (Fröder et al., 2007). In the current study, it should be highlighted that only a limited number of samples were positive for the presence of pathogens. In addition, because an enrichment step was included for detection, no information is available on the bacterial concentration that influences the safety of the products. Therefore, any indications of the hygienic practices adopted during the processing of the MPV analyzed and on the quality of the fresh produce acquired for minimal processing should be made with care.

The PCA model explained 40.53% of the variability in the data, with 20.96% and 19.57% of the variation demonstrated in the first (CP1) and second (CP2) principal components, respectively (Fig. 1).

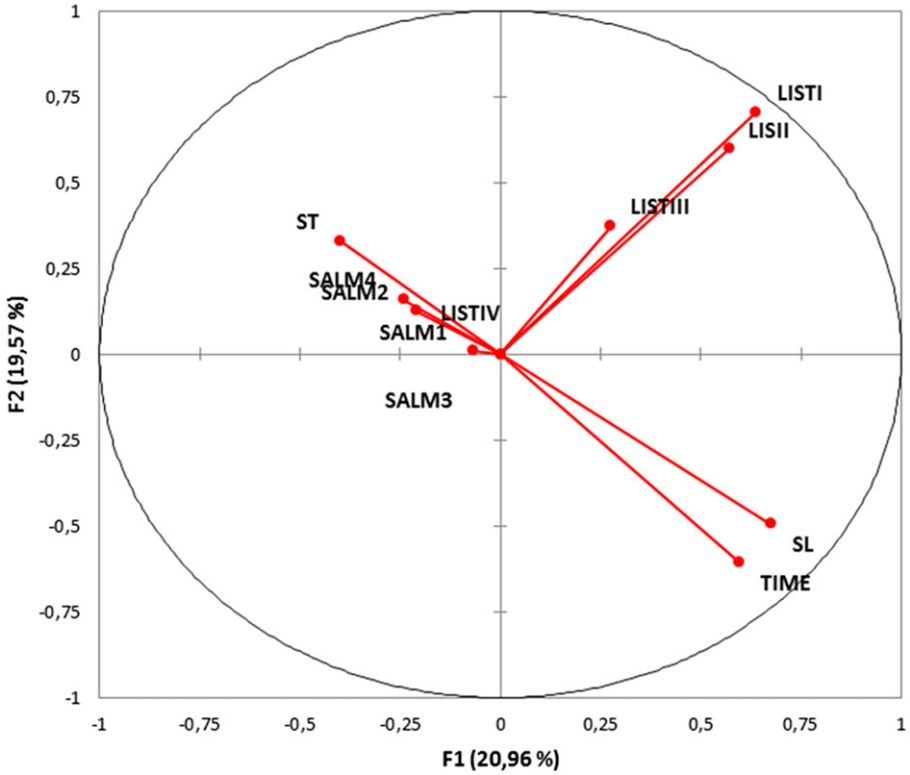

Fig. 1. Principal component analysis of detection of *Salmonella* and *Listeria monocytogenes* in 172 MPV samples collected in Brazil.

PCA indicated that the shelf-life of the MPV was not correlated with the detection of pathogens by a specific method of analysis. In conclusion, it has been observed that the microbiological quality of MPV commercialized in Campinas is generally satisfactory. Besides, the choice of microbiological method should be based not only on resource and time issues, but also on parameters such as sensitivity and specificity for the specific foods under analysis.

Acknowledgments

The authors would like to thank the São Paulo Research Foundation (FAPESP), the National Council for Scientific and Technological Development (CNPq) and the Coordination for the Improvement of Higher Education Personnel (CAPES) for the financial support.

References

Abadias, M., Usall, J., Anguera, M., Solsona, C., & Viñas, I. (2008). Microbiological quality of fresh, minimally-processed fruit and vegetables, and sprouts from retail establishments. *International Journal of Food Microbiology, 123*, 121–129.

Aguado, V., Vitas, A. I., & García-Jalón, I. (2004). Characterization of *Listeria monocytogenes* and *Listeria innocua* from a vegetable processing plant by RAPD and REA. *International Journal of Food Microbiology, 90*, 341–347.

Balagué, C., Khan, A. A., Fernandez, L., Redolfi, A. L., Aquili, V., Voltattorni, P., et al. (2006). Occurrence of non-O157 Shiga toxin-producing *Escherichia coli* in ready-to-eat food from supermarkets in Argentina. *Food Microbiology, 23*, 307–313.

Bhagwat, A. A. (2004). Rapid detection of *Salmonella* from vegetable rinse-water using real-time PCR. *Food Microbiology, 21*, 73–78.

Bohaychuk, V. M., Bradbury, R. W., Dimock, R., Fehr, M., Gensler, G. E., King, R. K., et al. (2009). A microbiological survey of selected Alberta-grown fresh produce from farmers' markets in Alberta, Canada. *Journal of Food Protection, 72*, 415–420.

Cantwell, M. (2002). Postharvest handling systems: minimally processed fruits and vegetables. In A. A. Kader (Ed.), *Postharvest technology of horticultural crops* (pp. 277–281). Davis: University of California, Dir. Agric. Nat. Res.

Cengiz, M. F., Certel, M., Karakaş, B., & Göçmen, H. (2007). Residue contents of captan and procymidone applied on tomatoes grown in greenhouses and their reduction by duration of a pre-harvest interval and post-harvest culinary applications. *Food Chemistry, 100*, 1611–1619.

Chitarra, M. I. F. (2000). *Processamento mínimo de frutos e hortaliças*. Lavras: UFLA/FAEPE.

Cocolin, L., Rajkovic, A., Rantsiou, K., & Uyttendaele, M. (2011). The challenge of merging food safety diagnostic needs with quantitative PCR platforms. *Trends in Food Science and Technology, 22*, S30–S38.

Cordano, A. M., & Jacquet, C. (2009). *Listeria monocytogenes* isolated from vegetable salads sold at supermarkets in Santiago, Chile: prevalence and strain characterization. *International Journal of Food Microbiology, 132*, 176–179.

De Boer, E., & Beumer, R. R. (1999). Methodology for detection and typing of foodborne microorganisms. *International Journal of Food Microbiology, 50*, 119–130.

Devlieghere, F., & Debevere, J. (2000). Influence of dissolved carbon dioxide on the growth of spoilage bacteria. *LWT – Food Science and Technology, 33*, 531–537.

Downes, F. P., & Ito, K. (2001). *Compendium of methods for the microbiological examination of foods*. Washington, DC: APHA.

Elviss, N. C., Little, C. L., Hucklesby, L., Sagoo, S., Surman-Lee, S., de Pinna, E., et al. (2009). Microbiological study of fresh herbs from retail premises uncovers an international outbreak of salmonellosis. *International Journal of Food Microbiology, 134*, 83–88.

Feldsine, P. T., Kerr, D. E., Shen, G., & Lienau, A. H. (2009). Comparative validation study to demonstrate the equivalence of a minor modification to AOAC method 996.09 visual immunoprecipitate (VIP®) for *E. coli* 0157:H7 method to the reference culture method. *Journal of AOAC International, 92*, 1416–1420.

Fröder, H., Martins, C. G., De Souza, K. L. O., Landgraf, M., Franco, B. D. G. M., & Destro, M. T. (2007). Minimally processed vegetable salads: microbial quality evaluation. *Journal of Food Protection, 70*, 1277–1280.

Francis, G. A., & O'Beirne, D. (2002). Effects of vegetable type and antimicrobial dipping on survival and growth of *Listeria innocua* and *E. coli*. *International Journal of Food Science and Technology, 37*, 711–718.

Francis, G. A., Thomas, C., & O'Beirne, D. (1999). The microbiological safety of minimally processed vegetables. *International Journal of Food Science and Technology, 34*, 1–22.

Giusti, M., Aurigemma, C., Marinelli, L., Tufi, D., De Medici, D., Di Pasquale, S., et al. (2010). The evaluation of the microbial safety of fresh ready-to-eat vegetables produced by different technologies in Italy. *Journal of Applied Microbiology, 109*, 996–1006.

Harris, L. J., Farber, J. N., Beuchat, L. R., Parish, M. E., Suslow, T. V., Garrett, E. H., et al. (2003). Outbreaks associated with fresh produce: incidence, growth, and survival of pathogens in fresh and fresh-cut produce. *Comprehensive Reviews in Food Science and Food Safety, 2*, 78–141.

Ilic, S., Odomeru, J., & LeJeune, J. T. (2008). Coliforms and prevalence of *Escherichia coli* and foodborne pathogens on minimally processed spinach in two packing plants. *Journal of Food Protection, 71*, 2398–2403.

Islam, M., Morgan, J., Doyle, M. P., Phatak, S. C., Millner, P., & Jiang, X. (2004). Fate of *Salmonella enterica* serovar Typhimurium on carrots and radishes grown in fields treated with contaminated manure composts or irrigation water. *Applied and Environmental Microbiology, 70*, 2497–2502.

Jacxsens, L., Devlieghere, F., Ragaert, P., Vanneste, E., & Debevere, J. (2003). Relation between microbiological quality, metabolite production and sensory quality of equilibrium modified atmosphere packaged fresh-cut produce. *International Journal of Food Microbiology, 83*, 263–280.

Jasson, V., Jacxsens, L., Luning, P., Rajkovic, A., & Uyttendaele, M. (2010). Alternative microbial methods: an overview and selection criteria. *Food Microbiology, 27*, 710–730.

Khoo, C.-H., Cheah, Y.-K., Lee, L.-H., Sim, J.-H., Salleh, N. A., Sidik, S. M., et al. (2009). Virulotyping of *Salmonella enterica* subsp. *enterica* isolated from indigenous vegetables and poultry meat in Malaysia using multiplex-PCR. *Antonie Van Leeuwenhoek, International Journal of General and Molecular Microbiology, 96*, 441–457.

Koseki, S., & Isobe, S. (2005). Prediction of pathogen growth on iceberg lettuce under real temperature history during distribution from farm to table. *International Journal of Food Microbiology, 104*, 239–248.

Little, C. L., & Gillespie, I. A. (2008). Prepared salads and public health. *Journal of Applied Microbiology, 105*, 1729–1743.

Little, C. L., Taylor, F. C., Sagoo, S. K., Gillespie, I. A., Grant, K., & McLauchlin, J. (2007). Prevalence and level of *Listeria monocytogenes* and other *Listeria* species in retail pre-packaged mixed vegetable salads in the UK. *Food Microbiology, 24*, 711–717.

Meldrum, R. J., Little, C. L., Sagoo, S., Mithani, V., McLauchlin, J., & de Pinna, E. (2009). Assessment of the microbiological safety of salad vegetables and sauces from kebab take-away restaurants in the United Kingdom. *Food Microbiology, 26*, 573–577.

Nguz, K., Shindano, J., Samapundo, S., & Huyghebaert, A. (2005). Microbiological evaluation of fresh-cut organic vegetables produced in Zambia. *Food Control, 16*, 623–628.

Noriega, E., Newman, J., Saggers, E., Robertson, J., Laca, A., Díaz, M., et al. (2010). Antilisterial activity of carrots: effect of temperature and properties of different carrot fractions. *Food Research International, 43*, 2425–2431.

Oktay, H. I., & Heperkan, D. (2006). Evaluation of ISO method and VIDAS automated system for identifying *Listeria* and *Salmonella* in selected foods. *Journal of Rapid Methods and Automation in Microbiology, 14*, 133–145.

Oliveira, A. M., Miya, N. T. N., Sant'Ana, A. S., & Pereira, J. L. (2010). Behavior and enterotoxin production by coagulase negative *Staphylococcus* in cooked ham, reconstituted skimmed milk, and confectionery cream. *Journal of Food Science, 75*, M475–M481.

Oliveira, M. A., Ribeiro, E. G. A., Bergamini, A. M. M., & Martinis, E. C. P. (2010). Quantification of *Listeria monocytogenes* in minimally processed leafy vegetables using a combined method based on enrichment and 16S rRNA real-time PCR. *Food Microbiology, 27*, 19–23.

Pilon, L. (2003). *Estabelecimento da vida útil de hortaliças minimamente processadas sob atmosfera modificada e refrigeração*. Master degree dissertation. Piracicaba: University of Sao Paulo, ESALQ-USP.

Ponniah, J., Robin, T., Paie, M. S., Radu, S., Ghazali, F. M., Kqueen, C. Y., et al. (2010). *Listeria monocytogenes* in raw salad vegetables sold at retail level in Malaysia. *Food Control, 21*, 774–778.

Reiter, M. G., López, C., Jordano, R., & Medina, L. M. (2010). Comparative study of alternative methods for food safety control in poultry slaughterhouses. *Food Analytical Methods, 3*, 253–260.

Rosa, O. O. (2002). *Microbiota associada a produtos hortícolas minimamente processados comercializados em supermercados*. PhD thesis. Lavras: Federal University of Lavras, UFAL.

Salleh, N. A., Rasul, G., Hassan, Z., Reezal, A., Isa, S. H., Nishibuchi, M., et al. (2003). Incidence of *Salmonella* spp. in raw vegetables in Selangor, Malaysia. *Food Control, 14*, 475–479.

Sant'Ana, A. S., Landgraf, M., Destro, M. T., & Franco, B. D. G. M. (2011). Prevalence and counts of *Salmonella* spp. in minimally processed vegetables in Sao Paulo, Brazil. *Food Microbiology, 28*, 1235–1237.

Shi, X., Wu, Z., Namvar, A., Kostrzynska, M., Dunfield, K., & Warriner, K. (2009). Microbial population profiles of the microflora associated with pre- and post-harvest tomatoes contaminated with *Salmonella* Typhimurium or *Salmonella* Montevideo. *Journal of Applied Microbiology, 107*, 329–338.

Silbernagel, K., Jechorek, R., Carver, C., Barbour, W. M., & Mrozinski, P. (2003). Evaluation of the BAX® system for detection of *Salmonella* in selected foods: collaborative study. *Journal of AOAC International, 86*, 1149–1159.

Silbernagel, K. M., Jechorek, R. P., Carver, C. N., Horter, B. L., & Lindberg, K. G. (2003). 3M™ Petrifilm™ staph express count plate method for the enumeration of *Staphylococcus aureus* in selected types of processed and prepared foods: collaborative study. *Journal of AOAC International, 86*, 954–962.

Silva, F. A. S., & Azevedo, C. A. V. (2002). Versão do programa computacional Assistat para o sistema operacional Windows. *Revista Brasileira de Produtos Agroindustriais, 4*, 71–78.

Sitohy, M., & Osman, A. (2010). Antimicrobial activity of native and esterified legume proteins against gram-negative and gram-positive bacteria. *Food Chemistry, 120*, 66–73.

Sivapalasingam, S., Friedman, C. R., Cohen, L., & Tauxe, R. V. (2004). Fresh produce: a growing cause of outbreaks of foodborne illness in the United States, 1973 through 1997. *Journal of Food Protection, 67*, 2342–2353.

Vitas, A. I., & Garcia-Jalon, V. A. E. I. (2004). Occurrence of *Listeria monocytogenes* in fresh and processed foods in Navarra (Spain). *International Journal of Food Microbiology, 90*, 349–356.

Yeh, K.-S., Tsai, C.-E., Chen, S.-P., & Liao, C.-W. (2002). Comparison between VIDAS automatic enzyme-linked fluorescent immunoassay and culture method for salmonella recovery from pork carcass sponge samples. *Journal of Food Protection, 65*, 1656–1659.

Zhang, Z.-Y., Liu, X.-J., Yu, X.-Y., Zhang, C.-Z., & Hong, X.-Y. (2007). Pesticide residues in the spring cabbage (*Brassica oleracea L. var. capita*ta) grown in open field. *Food Control, 18*, 723–730.