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Influence of process operations on shelf-life and microbial population of fresh-cut vegetables

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Cut lettuce salads and shredded carrots were prepared according to four different procedures in order to determine the influence of various operations on the shelf-life of these 'minimally processed' foods. In particular, the level of active chlorine used or its residue after washing as well as the processing time were considered. The results emphasize the role of free chlorine in reduction of the contamination level and its effectiveness toward *Pseudomonadaceae* and *Enterobacteriaceae*. Moreover a 12-h delay without refrigeration, after pre-washing or removal of ends, caused a lengthening of the processing time, enough to allow microbial proliferation and subsequent shortening of shelf-life. Shelf-life extension and the improvement of safety and quality of these products can be obtained by means of adequate processing operations.

Keywords: fresh-cut vegetables; process operations; shelf-life extension; growth modelling

Introduction

The foods known as 'IV gamma' products, according to a French denomination [12], are ready-to-use vegetables that consist of washed, sliced, chopped or grated raw vegetables. These products are packaged, stored at temperatures between 0°C and 5°C and must be distributed within a week [9].

The principal factors that affect the shelf-life and microbiology of raw prepared vegetables are: good agricultural practices in growing crops, good hygienic practices during harvesting, quality of washing water, packaging methods and materials, transporting and storage temperature [1,3,4,14,15,25].

The microbial population colonizing fresh-cut vegetables typically consists of *Pseudomonas* spp, *Xanthomonas* spp, *Enterobacter* spp, *Janthinobacterium* spp, yeasts, lactic acid bacteria, less frequently *Aeromonas hydrophila* and occasionally *Listeria monocytogenes* [2,6,16,19,22,27].

During the processes of peeling and cutting, intracellular oxidizing enzymes are released. In addition the surface of the vegetables is exposed to the air and to contamination by bacteria, yeasts and moulds. Therefore elevated respiration/transpiration rates and metabolic activities of spoilage microorganisms are the main reasons for shortened shelf-life [1,4,14,25].

Washing after peeling and cutting removes microbes and tissue fluids, and thus reduces microbial growth and enzymatic oxidation during storage. The microbiological and sensory quality of the washing water used must be good and its temperature low, ie below 5°C. By careful washing, the shelf-life of minimally processed vegetables can be prolonged by several days [1,3,4,15]. Furthermore, the effectiveness of washing can be improved using solutions containing chlorine or other antibacterial compounds [17,21,26].

The objective of the present work was to determine the effects of operations undertaken during processing on the shelf-life of cut lettuce and shredded carrots prepared according to four different procedures. Moreover the influence of the different operations on the composition of the microbial population was evaluated.

Materials and methods

Processing and storage of cut lettuce and shredded carrots

Packaged cut lettuce salads were processed according to four different procedures at a local company producing 'ready-to-eat-salads' (Figure 1). Figure 2 describes the four procedures used for preparation of packaged shredded carrots.

For both products, the main differences were: (I) treatment with a solution containing 150 ppm of free chlorine; (II) treatment with a solution containing 100 ppm of free chlorine; (III) treatment with a solution containing 100 ppm of free chlorine and washing after cutting for lettuce or shredding for carrots in order to reduce the residual chlorine concentration; (IV) the flow diagram is the same as III, but there was a 12-h delay at room temperature (15–18°C) after pre-washing or removal of ends for lettuce and carrots, respectively.

Cut lettuce and shredded carrots were packaged under ordinary atmosphere in a small polypropylene tray ($11 \times 8 \times 5$ cm, nominal thickness 30 μm) wrapped with polyethylene film (high gas permeability, nominal thickness 38 μm), heat sealed and stored at 5°C for 2 weeks.

Isolation and identification of salad and carrot flora

A 10-g sample from each batch of vegetables was diluted with 90 ml of sterile NaCl solution (0.9%) and homogenized with a Stomacher Lab-Blender 400 for 2 min.

Decimal dilutions, as required, were prepared with the same diluent and plated, in duplicate, on appropriate media.

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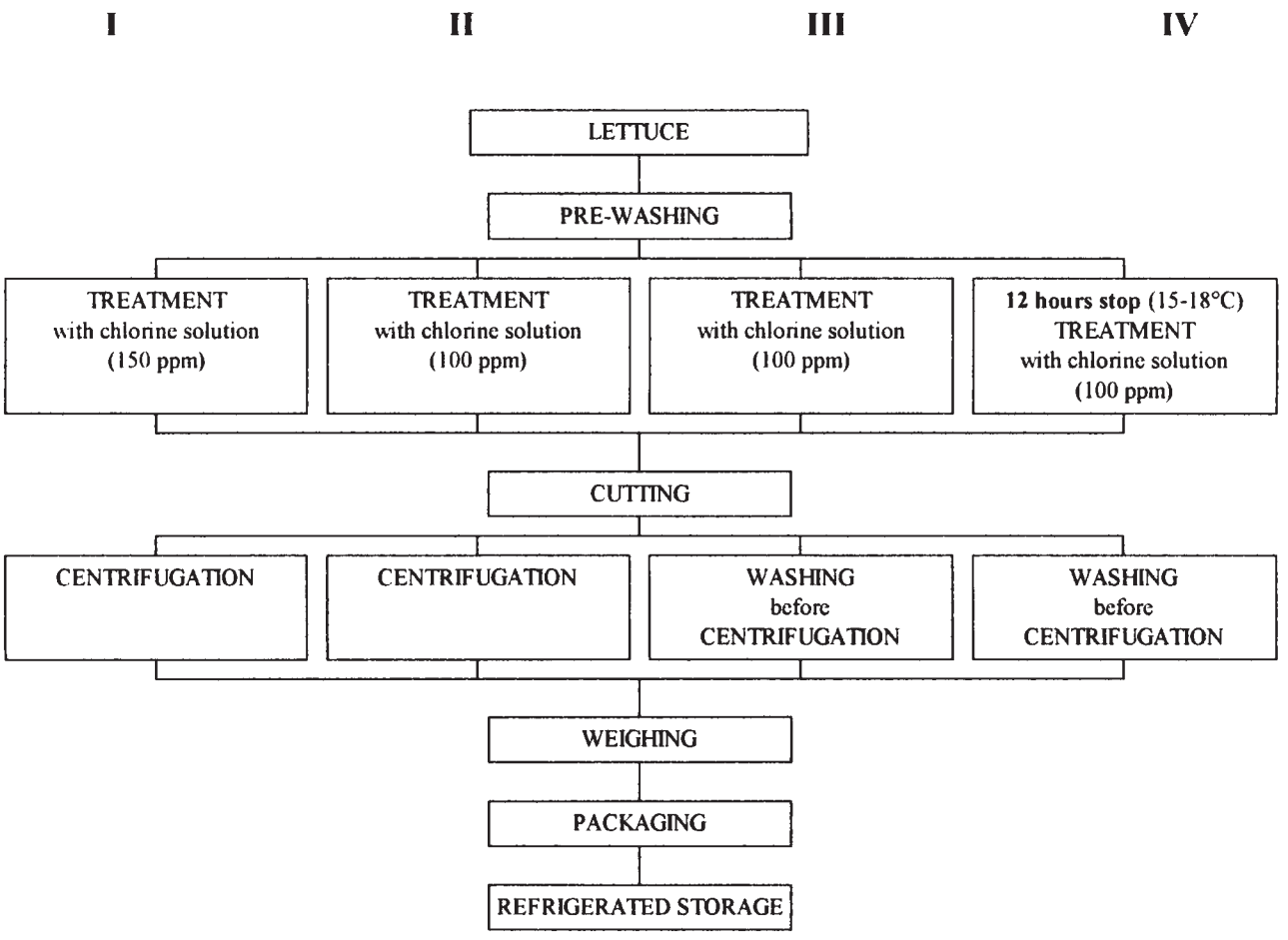

Figure 1 Diagram for the preparation of the four lines of packaged lettuce salads.

The media and the conditions of incubation were: Plate Count Agar (PCA, Oxoid) incubated at 5°C for 1 week for total psychrotrophic bacteria; Violet Red Bile Agar (VRBA, Oxoid) incubated at 37°C and 44°C for 24 h for total and fecal coliforms, respectively; MRS Agar (Oxoid) incubated anaerobically at 30°C for 4 days for lactic acid bacteria; Sabouraud Dextrose Agar (Oxoid) incubated at 28°C for 4 days for yeasts; Starch-Ampicillin Agar (SAA) [24], incubated at 28°C for 24–36 h for *Aeromonas hydrophila*. Only the colonies showing amylase activity on SAA were counted.

Colonies with different morphologies were selected from PCA plates incubated at 5°C and purified by repeated streaking on PCA. All strains were grouped on the basis of colony characteristics, cell shape, Gram-staining reaction, oxidase and catalase reactions as well as glucose utilization, according to the Hugh–Leifson test [13]. The classification schemes and the methods used were those of Collins *et al* [10].

Growth modelling

Growth parameters were estimated by modelling growth curves according to the Gompertz equation modified by Zwietering *et al* [29]:

$$y = k + A \times \exp - \exp\left[(\mu_{max} \times e/A) \times (\lambda - t) + 1\right.$$

where y is the growth extent as log CFU g^{-1} at the time t; k is the initial cell concentration as log CFU g^{-1}; A represents the difference in cell concentration between inoculum and stationary phase, as log CFU g^{-1}, μ_{max} is the maximum growth rate as Δ log CFU g^{-1} per day; and λ is the length of the lag phase expressed in days. The statistical treatment of data was performed using Statistica for Windows (Statsoft Inc).

Results and discussion

Cut lettuce salads

The samples of cut lettuce prepared according to four different flow diagrams (Figure 1), were analyzed for initial total counts of total psychrotrophic bacteria, yeasts, lactic acid bacteria (LAB), total and fecal coliforms and *Aeromonas hydrophila*.

The cell loads of all microbial groups analyzed were lowest in lettuce handled according to I (treatment with 150 ppm of free chlorine) and highest in products of IV treated with 100 ppm chlorine and kept, after prewashing, for a 12-h period without refrigeration (data not shown), and the predicted shelf-lives ranged from about 11 to 3 days. As shown by Guerzoni *et al* [11], the dynamics of the process and the extent of washing operations play an important role in reduction of the contamination level. The

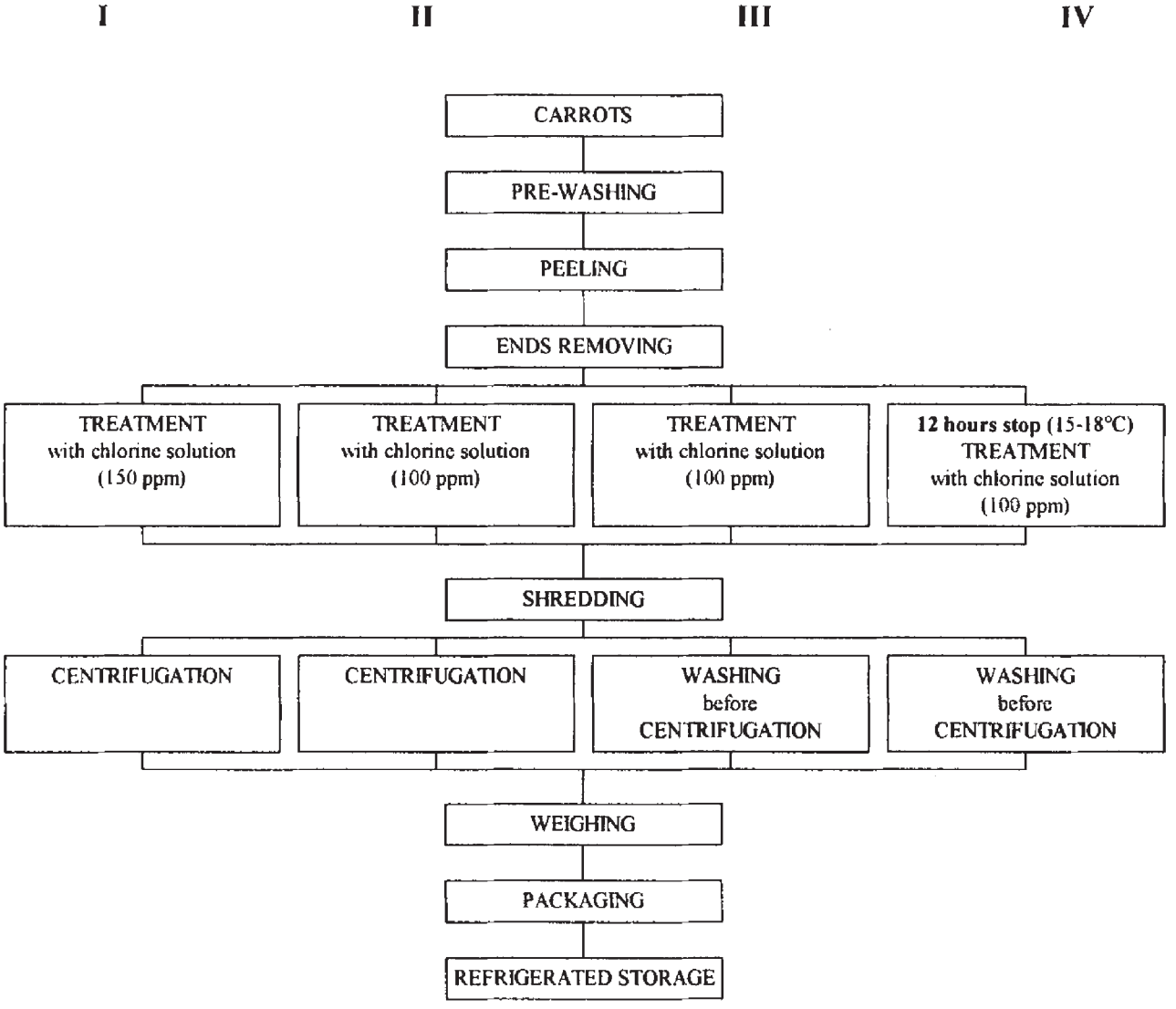

Figure 2 Diagram for the preparation of the four lines of packaged shredded carrots.

quality of the water used to wash the vegetables, the delay between the washing and cutting phases, the level of active chlorine used or residue of the washing, the process time and the temperature have an important influence on the initial load and subsequent evolution of the microbial population. *A. hydrophila*, commonly associated with water and fresh vegetables, was absent in all the samples analyzed.

In order to evaluate the effects of chlorine treatment on the composition of the microbial population, the frequency of psychrotrophic bacteria isolated and identified at family or genus level was determined in relation to different production lines. The frequency of *Pseudomonadaceae* and *Enterobacteriaceae* increased with the diminution of the active chlorine used for washing, while this antibacterial compound did not seem to have an influence on the frequency of *Moraxella* spp and *Vibrionaceae*. Moreover, the highest frequencies of *Janthinobacterium* spp and *Flavobacterium* spp, typical spoilage microorganisms, in lettuce of line IV could be attributed to temperature abuse after the pre-washing step.

Shredded carrots

The samples of shredded carrots, processed according to four different flow diagrams (Figure 2) were analyzed for the same microbial groups determined in lettuce.

As observed for packaged cut lettuce salads, the different process operations played a key role in the contamination levels (Table 1). Samples produced according to line IV showed the highest initial cell loads for all the microbial groups analyzed with the exception of lactic acid bacteria. Guerzoni *et al* [11] observed that elimination of free chlorine, by washing after cutting the lettuce, allowed survival and proliferation of fecal coliforms and lactic acid bacteria. As far as fecal coliforms are concerned, our results agree with those of Guerzoni *et al* [11]; however, lactic acid bacteria survived and multiplied in the carrots of I and II, whereas they disappeared in III and IV. This could be linked to the high sugar concentration of carrots [7,8,28] making lactic acid bacteria more competitive in I and II, while the washing step after shredding, by eliminating the free chlorine, increased the competitiveness of the

Table 1 Initial cell loads and subsequent evolution during storage of the microbial population of packaged shredded carrots in relation to production lines

Time (days)	Log CFU^{-1}				
	Psychrotrophic bacteria	Total coliforms	Fecal coliforms	Lactic acid bacteria	Yeasts
I[a]					
0	4.78	2.84	1.54	2.69	2.00
3	5.89	3.52	<1.00	2.50	2.32
5	7.00	5.53	<1.00	2.69	2.95
8	7.95	7.50	<1.00	3.39	3.48
15	8.04	7.50	<1.00	4.00	3.40
II					
0	4.82	3.30	2.00	2.60	2.01
3	6.40	3.85	<1.00	3.70	2.25
5	7.00	6.48	<1.00	3.48	3.00
8	8.10	7.20	<1.00	4.84	3.51
15	8.10	7.51	<1.00	4.80	3.80
III					
0	5.02	3.40	2.18	1.78	2.12
3	6.40	5.30	2.25	<1.00	2.50
5	7.10	7.05	2.30	<1.00	3.25
8	8.21	7.90	2.90	<1.00	3.64
15	8.41	7.90	2.90	<1.00	4.00
IV					
0	5.48	3.85	2.30	1.65	2.03
3	7.45	5.30	2.04	<1.00	2.65
5	8.42	7.00	2.10	<1.00	3.45
8	8.80	8.20	2.00	<1.00	3.89
15	9.04	8.20	2.05	<1.00	4.18

[a]See Figure 2.

microflora characterizing the 'IV gamma' products. These microorganisms, which were responsible for product spoilage, did not allow lactic acid bacteria to survive. *A. hydrophila*, was also absent in all carrot samples.

The growth data, as log CFU g^{-1}, relative to psychrotrophic bacteria, were analyzed according to the Gompertz equation as modified by Zwietering *et al* [29].

The predicted curves fitted well the experimental points as indicated in Figure 3 showing growth curves of psychrotrophic bacteria in relation to the production processes. In Table 2 the Gompertz parameters and the predicted values of the time (days) necessary to attain a cell population of 5×10^6 CFU g^{-1} are reported. This time can be considered as a measure of the potential shelf-life. The predicted shelf-lives ranged from about 5 to 9 days; these results emphasize the fundamental role of the free chlorine concentration as well as of the processing temperature. In fact, the products of IV, characterized by absence of free chlorine and thermal abuse after pre-washing, presented the shortest shelf-life.

A significant number of psychrotrophic bacteria were identified at family or genus level and the frequency was calculated in relation to different production processes. The isolates belonged to *Enterobacteriaceae*, *Pseudomonadaceae*, *Vibrionaceae*, *Moraxella* spp, *Acinetobacter* spp, *Jan-*

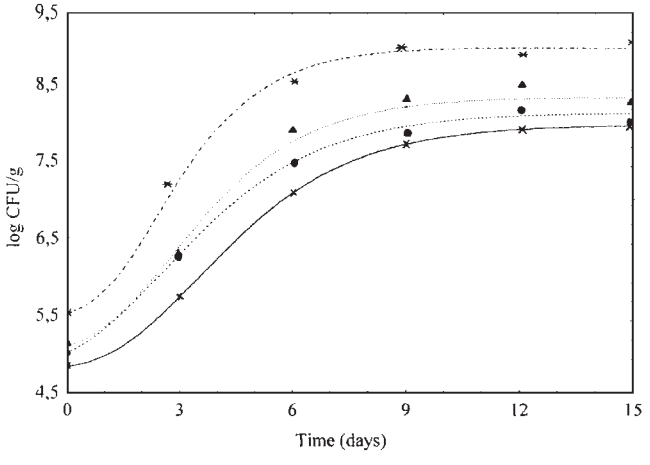

Figure 3 Growth curves of psychrotrophic bacteria fitted with the Gompertz equation in relation to the production line of shredded carrots: —×— I; --●-- II; ---▲--- III; ---∗--- IV.

Table 2 Influence of the processing operation on the growth of psychrotrophic bacteria and predicted shelf-life of packaged shredded carrots

Line[a]	K[b]	A	μ_{max}	λ	R^2	T[c]
I	4.78	8.04	0.50	1.09	0.999	9.29
II	4.82	8.21	0.52	0.13	0.992	8.05
III	5.02	8.41	0.60	0.67	0.992	7.48
IV	5.48	9.04	0.80	0.75	0.999	5.81

[a]See Figure 2.
[b]Gompertz equation parameters: K, initial load (log CFU g^{-1}); A, maximum cell extent attained at the stationary phase (log CFU g^{-1}); μ_{max}, maximal growth rate; λ, lag phase (days); R^2, regression coefficient of the Gompertz equation obtained.
[c]Predicted shelf-life as the time (days) necessary to attain 5×10^6 CFU g^{-1} level.

thinobacterium spp and *Flavobacterium* spp. The products of I and II were characterized by greater bacterial heterogeneity, while the microfloral composition of carrots produced according to IV was relatively homogeneous. *Pseudomonadaceae*, *Enterobacteriaceae* and *Acinetobacter* spp were the only microorganisms identified. Moreover, *Moraxella* spp and *Vibrionaceae* disappeared in both III and IV. This could be due to elevated frequency of *Pseudomonadaceae* and *Enterobacteriaceae* that inhibited growth of microbial spoilage microorganisms (*Moraxella* spp, *Vibrionaceae*, *Flavobacterium* spp and *Janthinobacterium* spp) and lactic acid bacteria (Table 1).

Conclusion

The agricultural practices and hygienic conditions during harvesting, processing, packaging, transport and storage, undoubtedly influence the initial microbial population [5,20]. The microbial flora of minimally processed foods such as ready-to-eat vegetables is of great concern, as it involves both spoilage and safety problems [9,18,23]. In these systems which fall into the low acid range food category, the high humidity and the large number of cut surfaces can provide ideal conditions for multiplication of spoilage microorganisms, and do not present sufficient 'hurdles' for the growth of more hazardous species.

In this work we have demonstrated the effectiveness of chlorine treatment towards *Pseudomonadaceae* and *Enterobacteriaceae*. Moreover a 12-h delay without refrigeration after pre-washing or end removal, although not likely under normal operating conditions, caused sufficient extension of the processing time to allow microbial proliferation and subsequent shortening of shelf-life.

Although the microorganisms analyzed in this study do not represent a public health concern, shelf-life extension and the improvement of the safety and quality of these 'minimally processed foods' are important criteria for the economic convenience of production and distribution. These objectives can be obtained by means of adequate processing operations.

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