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Shelf-life modelling for fresh-cut vegetables

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Abstract

The influence of various operations during the preparation of the 'ready-to-use' salads on the initial level and subsequent growth of microbial spoilage species was analyzed. The factors considered were the quality of the water used to wash the vegetables, the delay between the washing and cutting phases, and the level of active chlorine in the water used for washing. The dynamics of the processes and the effectiveness of the washing operations play the most important role in the reduction of the contamination level. Furthermore, the use of potential inhibiting ingredients, such as red chicory, and the antagonistic effect of *Lactobacillus plantarum* were all successful in reducing the growth of hazardous species, such as *Aeromonas hydrophila*. These effects were modelled on the basis of the results of the Central Composite Design (CCD) using clarified juices. From the results obtained, the inhibiting activity of red chicory was confirmed, but *A. hydrophila* seems to be counteracted by the presence of *L. plantarum*, which appears to negate the inhibiting effect of red chicory.

Keywords: Vegetable; Fresh cut; Shelf-life; Processing; Biological antagonism; Treatment

1. Introduction

In contrast to most processed foods whose shelf-life, with respect to the raw material, depends on the technological treatment, commercial 'ready-to-eat' salads are characterized by a shelf-life shorter than that of the original, unprocessed raw material. In fact, the higher nutrient availability of cut vegetables and the presence of different microbial populations typical of the individual ingredients, as well as the subsequent damage to the vegetable tissues caused by the rapid accumulation

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of CO_2 in the head space of the packed products, promote the biochemical and microbial instability of the product. The population colonizing the 'ready-to-eat' salads, includes *Pseudomonas* spp., *Xanthomonas* spp., *Enterobacter* spp., *Chromobacterium*

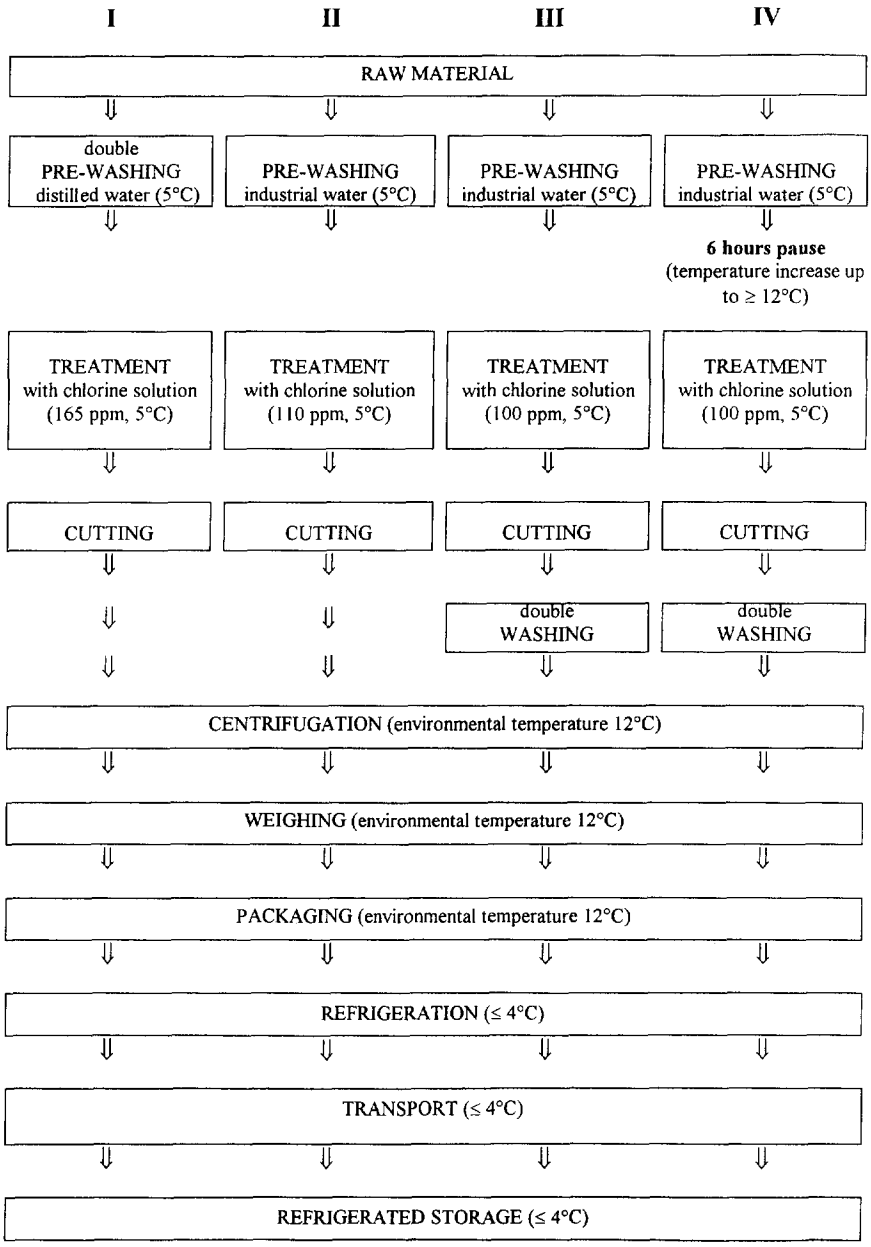

Fig. 1. Diagram for the preparation of the 4 lines of packaged salads of cut lettuce.

spp., yeasts, lactic acid bacteria, less frequently *Aeromonas hydrophila* and occasionally *Listeria monocytogenes*. (Lainé and Michard, 1988; Velani and Roberts, 1991; Beaufort et al., 1992; Marchetti et al., 1992; Carlin et al., 1995; Membré et al., 1995). The increase of the viable cell number of spoilage species, such as *Pseudomonas* spp., lactic acid bacteria or yeasts, which can rapidly attain a level $>10^7$ CFU/g, is accompanied by the accumulation of metabolites, such as ethanol, lactic acid, ethyl acetate, etc. The organoleptic changes due to such metabolic activity are associated with the enzymatic oxidation of the polyphenols of the injured tissues. Spoilage is detectable by sensory methods when the cell microbial number attains a threshold of about 10^7–10^8 CFU/g, depending on the species concerned and the ingredient characteristics. The shelf-life of the commercial products, terminating when the microbial population reaches 10^7 CFU/g, does not exceed 5–7 days under conditions of constant and controlled temperature (Marchetti et al., 1992). Taking into account the time needed for transport and the frequent thermal abuses during distribution, a minimum shelf-life of at least 6 days at 4°C can be regarded as a most important criterion for evaluating the feasibility and the economic convenience of these products for any processing enterprise. The most realistic methods for extending the shelf-life include minimizing the contamination level of the packed product after the processing phase and the retardation of microbial cell proliferation. The initial contamination of the various raw vegetables generally exceeds a level of 10^6 CFU/g of viable microbial cells depending on source of the product, season, etc. (Magnuson et al., 1990; Marchetti et al., 1992). The sequence of operations of the flow diagram from the raw materials to the packaged products reduces the initial contamination level by only one hundredfold, as shown by a survey on small enterprises producing these salads in Italy (Guerzoni, 1995: unpublished data). Good manufacturing practices, such as appropriate washing with cold (5°C), chlorinated water and use of an automatic and well-designed cutting machine can contribute to the reduction of the initial and subsequent microbial cell number. However, it is difficult to identify the critical control points over microbiological proliferation and hence to guarantee a reliable extension of the shelf-life of the products. Thus, only a strategic combination of procedures and their implementation can allow the effective improvement of the safety and quality of these minimally processed foods.

In this work, the individual and combined effects of the operations undertaken during the processing of raw material on the subsequent contamination level and the proliferation of micro-organisms during storage and marketing were analyzed. Moreover, the influence of a potential inhibiting ingredient and the antagonistic effect of *L. plantarum* on the growth of the hazardous species, *A. hydrophila*, were modelled.

2. Materials and methods

2.1. Characteristics of the samples

2.1.1. Experiment 1

Packaged, cut lettuce salads were processed at a local company producing 'ready-to-eat salads' according to 4 different flow diagrams. The essential differences between the 4 options are shown in Fig. 1 and were:

(I) double prewashing with water having a log CFU/ml <1 (in order to evaluate the importance of the water contamination), and treatment with a chlorine solution having 165 ppm of free chlorine;

(II) prewashing with industrial water (log CFU/ml ranging between 1 and 2) and treatment with a chlorine solution having 110 ppm of free chlorine;

(III) prewashing with industrial water and treatment with chlorine solution having 100 ppm of free chlorine;

(IV) prewashing with industrial water, a 6-h pause at 17–18°C, treatment with a chlorine solution having 100 ppm of free chlorine, and a second washing in order to eliminate the residue of the chlorine.

The water for all the washing treatments was prerefrigerated at 5°C. The processing time did not exceed 2.5 h in lines I and II, 3 h in line III and 8.5 h in line IV. For each line, 30 packs were examined immediately after processing and during the refrigerated storage.

2.1.2. Experiment 2

Two batches of commercial 'ready-to-use' vegetable salads, consisting of: grated carrots (C); mixed salad (A) containing carrots, red chicory, endive and lettuce (with a relative weight ratio of 1 : 1 : 1 : 1) (MSa); mixed salad containing carrots, endive, lettuce, rocket (*Eruca sativa*), red and green chicory (with a relative weight ratio of 1 : 1 : 1 : 1 : 1 : 1) (MSb) and cut endive (E), were purchased 1 day after packaging from a local company. Thirty 200-g samples of each product were placed on small polypropylene tray (nominal thickness 30 μm), wrapped in polyethylene film (nominal thickness 38 μm) and heat sealed. The internal atmosphere was not modified, and the packs were stored at 4°C (±1°C) in the dark. The samples were examined at various time intervals. The experimental data are based on the means of results from two packs.

2.2. Central composite design

In order to evaluate the effects of the storage temperature, initial level of lactic acid bacteria and the ratio between lettuce and red chicory on growth of *A. hydrophila*, these variables were modulated according to a Central Composite Design (CCD) (Box et al., 1978) and the combinations chosen are shown in Table 1. The model systems were based on clarified juices of the two different products. In this table, only the relative percentage of lettuce juice is reported, complemented to 100 with red chicory juice. The vegetables were homogenized, filtered, centrifuged at 18 000 rpm and pasteurized at 100°C for 15 min.

The model systems were inoculated with about 3.5 log CFU/ml of *A. hydrophila* and a different inoculum size of *L. plantarum*, as indicated in Table 1. The samples were incubated at different temperatures.

The growth over time of *A. hydrophila* and *L. plantarum* in the 17 combinations of the CCD (2 repetitions × combination) was analyzed by plate counting using specific media. The log CFU/ml data were analyzed according to the Gompertz equation modified by Zwietering et al. (1990):

$$y = k + A * \exp - \exp[\mu_{max} * e/A) * (\lambda - t) + 1]$$

Table 1
Combination of temperature, *Lactobacillus plantarum* inoculum size and lettuce juice percentage, complemented by red chicory juice, of the various runs of the Central Composite Design (CCD) approach

Experiment no.	*L. plantarum* inoculum size (as log CFU/g)	Temperature (°C)	Lettuce juice (%)
1	3.5	8	70
2	3.5	4	70
3	3.5	8	30
4	3.5	4	30
5	2.5	8	70
6	2.5	4	70
7	2.5	8	30
8	2.5	4	30
9	3	3	50
10	3	9	50
11	3	6	20
12	3	6	80
13	2	6	50
14	4	6	50
15	3	6	50
16	3	6	50
17	3	6	50

where 'y' is the log CFU/ml of *A. hydrophila* or *L. plantarum* at the time 't'; 'k' is the initial cell concentration as log CFU/g; 'A' represents the maximum cell number increase at the stationary phase, as log CFU/g; μ_{max} is the maximum growth rate as Δlog CFU/g per day, and λ is the length of the lag phase expressed in days.

Modelling was carried out in two stages. The first stage involved fitting the bacterial growth curve to estimate the above-indicated parameters with the Gompertz equation modified by Zwietering. At each combination of the CCD, the bacterial cell concentration was modelled as a function of time. The second stage of modelling was aimed at describing the variation of the growth parameters λ, A and μ_{max} as a function of the variables of the CCD.

A polynomial equation describing the effect of the independent variables (T, inoculum size and lettuce juice percentage) as individual or quadratic terms, and of their interactive effect on the growth parameters. A, λ and μ_{max} of both species was obtained.

The goodness-of-fit of the models obtained was evaluated using R^2 (multiple determination coefficients), i.e., the square of the multiple correlation coefficient (Pike, 1986), the Fisher F-test (and the derived P values), and the standard error to estimate (SE). Contour plots of the response surface as a function of two independent variables at a time (e.g., temperature and lettuce juice percentage), holding the other independent variable constant (e.g., inoculum size) at fixed levels (the intermediate value for instance), are helpful for understanding both the main and the interaction effects of the variables.

These plots can be easily derived from the polynomial model obtained by calculating the values assumed by one independent variable (e.g., temperature) when the second (lettuce juice percentage) varies (from the minimum to the maximum values considered),

and when the dependent variable (e.g., A) has given values (e.g., 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10 log CFU/g for the maximum levels (A) for *L. plantarum*) (Cheynier et al., 1983).

The statistical treatment of data was performed using the Statistica for Windows, Statsoft Inc.

2.3. Isolation and enumeration of micro-organisms

A 10-g sample from each pack was diluted with peptone (DIFCO) water (90 ml), homogenized with a Stomacher Lab-Blender 80 and an aliquot was plated in duplicate onto appropriate media. The media and the conditions were as follows: Plate Count Agar (PCA, Biolife) incubated at 5°C for a week for psychrotrophic bacteria; MRS Agar (Biolife) incubated under anaerobiosis at 30°C for 4 days for lactic acid bacteria; Violet Red Bile Agar (VRBA, Biolife) incubated at 37°C for total coliforms and at 42°C for fecal coliforms for 24 h; Sabouraud Dextrose Agar (Biolife) incubated at 28°C for 4 days for yeasts; and Starch-Ampicillin Agar (SAA), proposed by Palumbo et al. (1985), incubated at 28°C for 24–36 h for *A. hydrophila*. Only the colonies showing amylasic activity on SAA were counted.

3. Results

3.1. Experiment 1: Influence of the conditions of preparation

Four different flow diagrams (Fig. 1) for the preparation of packaged salads of cut lettuce were compared. The differences particularly concerned the quality of the water used to wash the vegetables, the delay between the washing and cutting phases and the level of active chlorine used or its residue after washing.

The dynamics of the process and the effectiveness of the washing operations played an important role on the reduction of the contamination level, as indicated in Table 2.

On the basis of the comparison of the microbial data, the two most important factors affecting the initial cell contamination seem to be the length of the processing time and the temperature. In fact, in line IV, whose products exhibited the highest contamination levels for the microbial groups analyzed, the lettuce was kept, after prewashing, for a 6-h period without refrigeration. Thus, the processing time was lengthy and the rate of the product flow was slow. During this delay, the temperature increased up to at least 12°C allowing microbial proliferation because of the high humidity level.

The absence of active chlorine seems to have a more important influence on the subsequent growth of the microbial population than on the initial contamination immediately after packing. In fact, a comparison of the changes over time of the lactic acid bacteria and fecal coliforms of the products II and III suggests that the total elimination of free chlorine in production line III, due to the double washing, allowed the survival and proliferation of these groups. In contrast, they tend to disappear in production line II.

On the basis of the Gompertz models, the time at 4°C necessary to attain a concentration of total psychrotrophic bacteria of 5×10^6 cell/g can range between about 1 and 11 days. This is indicated in Table 3, which also shows the time necessary to attain

Table 2
Initial contamination level and subsequent evolution of the microbial population of packaged cut lettuce in relation to the production processes I–IV [a]

Time (days)	Total coliforms [b]	Fecal coliforms [b]	Lactic acid bacteria [b]	Psychrotrophic bacteria [b]	Yeasts [b]
I					
0	3.85	3.41	2.23	3.72	1.00
3	4.23	2.00	1.00	4.88	2.48
5	4.67	1.95	1.00	5.49	2.78
10	4.82	<1.00	<1.00	7.00	3.85
II [a]					
0	3.53	2.74	1.30	4.11	1.00
3	3.58	2.48	<1.00	5.02	3.30
5	3.91	1.74	<1.00	5.18	3.70
10	4.54	<1.00	<1.00	7.01	4.48
III [a]					
0	2.95	2.22	1.70	4.37	2.78
3	4.26	2.56	2.40	6.20	3.68
5	5.11	2.90	2.40	6.70	4.20
10	5.20	2.90	2.38	7.80	4.60
IV [a]					
0	5.23	4.20	2.30	6.49	2.48
3	5.60	4.00	2.11	7.30	4.00
5	5.63	3.48	<1.00	7.88	5.00
10	7.70	3.37	<1.00	8.01	4.89

[a] See Fig. 1.
[b] Bacteria and yeast populations expressed as log CFU/g.

a 5×10^6 cell/g level for total coliforms. A level of 5×10^6 cell/g for psychrotrophic bacteria, chosen as a criterion for establishing the expiry date, is severe and applies to high quality products. Although processing of line IV is not realistic under normal conditions, the results emphasize the importance of processing time particularly when, for relatively short periods, the temperature exceeds 12°C. In fact, the coliforms can attain in the stationary phase a maximum level $>10^7$ CFU/g only in lettuce packs processed according to line IV (Table 3).

The ability of a process to significantly reduce the initial contamination level is a factor that determines the shelf-life of the resulting products. A satisfactory shelf-life (8–11 days) can be obtained with appropriate combinations of temperature and washing procedures.

3.2. Experiment 2: Influence of salad constituents on the initial load and growth of selected microbial groups

Two batches of 4 commercial products based on different combination of ingredients, were analyzed for the initial count of the total psychrotrophic bacteria, total coliforms, lactic acid bacteria and *A. hydrophila*. Their subsequent fate at 4°C was followed by

Table 3
Influence of the processing operations I–IV on the Gompertz parameters and predicted shelf-life of packaged lettuce

	K[a]	A	μ_{max}	λ	R^2	T[b]
I[c]						
Total coliforms	3.84	5.00	0.25	1.4	0.991	–[d]
Psychrotrophic bacteria	3.70	9.10	0.37	0	0.999	8.22
II						
Total coliforms	3.53	4.57	0.15	2.32	0.999	–[d]
Psychrotrophic bacteria	3.74	7.90	0.28	0	0.999	11.21
III						
Total coliforms	2.95	5.20	1.15	1.84	0.999	–[d]
Psychrotrophic bacteria	4.37	7.89	2.09	2.12	0.999	3.29
IV						
Total coliforms	5.40	7.63	1.80	4.92	0.995	5.65
Psychrotrophic bacteria	6.21	8.41	0.29	0	0.999	1.65

[a] Gompertz equation parameters: K, initial load (log CFU/g); A, maximum bacteria growth attained at the stationary phase (log CFU/g); μ_{max}, maximal growth rate; λ, lag phase (days); R^2, regression coefficient of the Gompertz equation obtained.
[b] Predicted shelf-life as the time (days) necessary to attain a 5×10^6 CFU/g level.
[c] See Fig. 1.
[d] The cellular concentration did not attain 5×10^6 CFU/g level.

analyzing duplicate samples for up to 10 days from processing. The microbial cross-contamination, due to a convergence of different products in the same packs, seems to play an important role in the initial contamination (K parameter in the Gompertz equation). In fact, the K values for total coliforms, psychrotrophic bacteria and *A. hydrophila*, were the highest in the mixed salad based on 6 ingredients (MSb). The data from the two batches of the same product were averaged (Table 4).

The growth data, as log CFU/g, were analyzed according to the Gompertz equation. In Table 4, the initial cell levels and the Gompertz parameters, i.e., maximum level of log CFU/g attained (A), maximum growth rate (μ_{max} as log CFU g^{-1} day^{-1}), lag phase (λ) (days) and the predicted values of the time (days) necessary to attain a cell population of 5×10^6 CFU/g, are reported. This elapsed time, calculated for the psychrotrophic bacteria, can be taken as a measure of the potential shelf-life. The A parameter, as the maximum cell level attained in the stationary phase, can be regarded as a measure of the potential of the system for sustaining microbial spoilage. As a consequence of the higher initial microbial contamination of the product, MSb had a shorter shelf-life than the product MSa.

The lactic acid bacteria (LAB) showed the highest growth rate (μ_{max}) and the highest A value for carrots, probably due to the sugar content of this ingredient. On the other hand, the viability of *A. hydrophila* decreased over time in carrots as indicated by the negative sign of the μ_{max} value. Probably psychrotrophic species able to grow at about 5°C were included in the naturally occurring microbial population. The times necessary to attain a cell level of 5×10^6 cell/g were calculated by solving the various Gompertz

Table 4
Influence of the composition of commercial salads on the product stability

Microbial group	Product	K	A	μ_{max}	T^a	T^b
Total coliforms	Carrots (C)	1.00	8.59	1.65	3.81	3.81
	Mixed salad (MSa)	4.43	6.43	1.04	–[c]	–
	Mixed salad (MSb)	5.08	7.62	0.94	2.11	4.85
	Endive (E)	3.26	5.72	0.46	–	–
Psychrotrophic bacteria	Carrots (C)	3.76	10.29	1.14	2.58	3.26
	Mixed salad (MSa)	5.82	7.25	0.62	3.67	18.65
	Mixed salad (MSb)	6.13	8.86	0.63	0.88	6.08
	Endive (E)	5.27	7.83	0.34	4.27	12.14
Lactic acid bacteria	Carrots (C)	4.34	8.30	1.6	3.77	4.72
	Mixed salad (MSa)	3.76	6.01	0.97	–	–
	Mixed salad (MSb)	3.75	7.74	1.59	3.91	4.50
	Endive (E)	3.86	5.88	0.61	–	–
Aeromonas hydrophila	Carrots (C)	2.60	2.00[d]	−1.90	–	–
	Mixed salad (MSa)	1.99	3.98	0.84	–	–
	Mixed salad (MSb)	3.24	4.15	0.26	–	–
	Endive (E)	1.00	1.89	0.90	–	–

[a] Shelf-life in days calculated according to the Gompertz equation obtained on the basis of the experimental data and whose parameters are reported (K, A, μ_{max}) as the time necessary to attain a level of 5×10^6 CFU/g.
[b] Shelf-life in days calculated according to the Gompertz equation obtained for the same product, the K value of which is reported as the time necessary to attain a level of 5×10^6 CFU/g solving the equation for a hypothetical initial load of 10^3 CFU/g.
[c] The cell concentration did not attain a level of 5×10^6 CFU/g.
[d] The viable cell number decreased.

equations, and the parameters for the 4 products and for the 4 microbial species or groups are reported in Table 4. The fourth column in Table 4 deals with the predictions obtained on the basis of the actual values of the initial contamination level (K) of the packaged products. The last column deals with predictions obtained by substituting, in the same equations, hypothetical initial level (K) of 10^3 CFU/g for the various micro-organisms and for each product. With respect to the actual commercial products, a reduction of the initial level to 10^3 CFU/g could extent the shelf-life, at 5°C for up to 18 days, depending on the constituents. The shorter shelf-life of shredded carrots (3 days) can be attributed to its high sugar content. However, the high level of lactic acid bacteria, always associated to this ingredient, can lead to an improvement of safety as lactic acid production limits the proliferation of the pathogen (Marchetti et al., 1992). Thus, the threshold could be extended to more than 5×10^6 CFU/g for this product.

3.3. Central composite design: Evaluation of the antimicrobial effect of red chicory

In a previous study, Marchetti et al. (1992) observed that vegetables, such as red chicory, had an antimicrobial effect on spoilage bacteria and potentially dangerous species, such as *A. hydrophila*. A Central Composite Design (CCD) was developed in

Table 5
'Best-fit' equations for the prediction of maximum cell level attained at the stationary phase (A), maximum growth rate (μ_{max}) as CFU/g in days and lag phase (λ) for *Aeromonas hydrophila* and *Lactobacillus plantarum* inoculated in clarified juice in relation to the composition of the combinations of the CCD. T, temperature (°C); L, *Lactobacillus plantarum* inoculum size (log CFU/ml); Lg, lettuce juice percentage concentration of red chicory (complement to 100)

Eq. 1: *Aeromonas hydrophila*			
$A = 2.85\ L - 0.059\ L \times Lg + 0.185\ Lg$			
$R^2 = 0.993$	SE = 0.825	$F = 662.96$	$P = 0.00001$
$\mu_{max} = 0.364\ T - 0.122\ T \times L + 0.016\ L \times Lg - 0.0003\ Lg^2$			
$R^2 = 0.973$	SE = 0.257	$F = 116.40$	$P = 0.00001$
$\lambda = -3.18\ T + 0.243\ T^2 + 7.63\ L - 1.26\ L^2$			
$R^2 = 0.737$	SE = 1.272	$F = 9.10$	$P = 0.00100$
Eq. 2: *Lactobacillus plantarum*			
$A = 2.17\ T - 0.11\ T^2 + 0.065\ L - 0.011\ T \times L$			
$R^2 = 0.995$	SE = 0.679	$F = 706.62$	$P = 0.00001$
$\mu_{max} = 7.95\ L - 0.149\ L^2$			
$R^2 = 0.933$	SE = 0.287	$F = 104.32$	$P = 0.00001$
$\lambda = 0.667\ L$			
$R^2 = 0.558$	SE = 0.186	$F = 20.19$	$P = 0.0004$

order to evaluate the relative importance of the ratio of the red chicory with respect to lettuce, the storage temperature and the presence of an antagonistic species, such as *L. plantarum*. The various model systems were based on clarified juices inoculated with different relative ratios of *A. hydrophila* and *L. plantarum*. The growth data over time of both strains, relative to all the combinations of the CCD, were modelled according to the Gompertz equation. The parameters (A, μ_{max} and λ) obtained for the various runs of the CCD, and for both species, were analyzed in order to obtain polynomial equations describing the effects of the individual and interactive effects of inoculum size, relative ratio of *L. plantarum*, and temperature on the growth of both species (Table 4).

The results confirmed the inhibiting effect of red chicory. In fact, the growth of *A. hydrophila* increased with the increase of lettuce juice percentage with respect to that of red chicory (Eq. 1, Table 5). On the other hand, *L. plantarum* did not have any inhibiting action on the potential pathogen, as indicated by the Eq. 1 in which the L factor (*L. plantarum* inoculum size) shows a positive coefficient. In fact, *A. hydrophila* seems to be positively affected by the presence of *L. plantarum*, which favored its growth at the highest level of red chicory. Fig. 2 is the contour plot relative to the effect of the interaction of the inoculum size of *L. plantarum* and of the lettuce percentage on the maximum cell number increase (A) of *A. hydrophila*. This hazardous species was favored either by an elevated *L. plantarum* inoculum size, when the relative percentage of red chicory was high, or by high lettuce juice concentration and a low *L. plantarum* inoculum, as indicated by the two relative maxima. In contrast, the cell increase (A) of *A. hydrophila* showed two relative minima, one when *L. plantarum* inoculum size was high

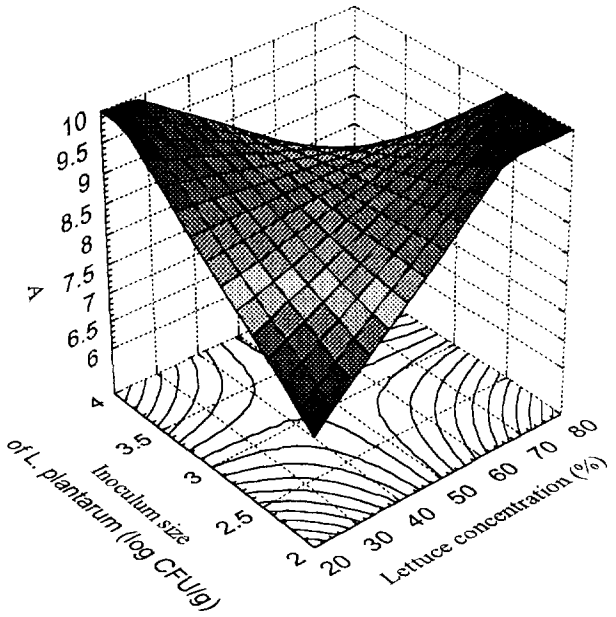

Fig. 2. Contour plots for the interaction size of *L. plantarum* with lettuce concentration on *A. hydrophila* maximum cell number increase (A), as log CFU/g, at the stationary phase.

in the presence of a low level of red chicory, or another when the red chicory level was high accompanied by a restricted *L. plantarum* inoculum. *L. plantarum* can display a competitive action only when red chicory was at low relative level. Moreover the modest competitive action of *L. plantarum* could be due to its dependence on temperature. In fact, according to Eq. 2, the final growth extent (A) of *L. plantarum* was affected more by temperature than by its initial inoculum size, as indicated by the coefficients of these terms in Eq. 2 (Table 5) and as shown by the Fig. 3 which is the contour plot relative to the interactive effect of the inoculum size of *L. plantarum* and the temperature on the maximum cell increase (A) of *L. plantarum*. *L. plantarum* seems to be unaffected by its inoculum size. In fact, the extent of proliferation at the stationary phase (A) increases as the temperature rises, and at $T \geq 8$°C can reach 10 log CFU/g.

4. Conclusion

The safety and the shelf-life of the 'ready-to-eat' salads depend on many factors, including water quality, ingredients, their history, production technology and interaction among the microbial groups. Thus, the shelf-life extension and safety of the commercial products can be significantly improved only by means of a coordinated strategy of actions along the whole of the production line. The rapidity of the processing, the washing effectiveness and the continuity of the refrigeration principally affect the extent of salad contamination. On the other hand, the choice of the constituents, their combination and relative ratios, as well as the elimination of the washing step after

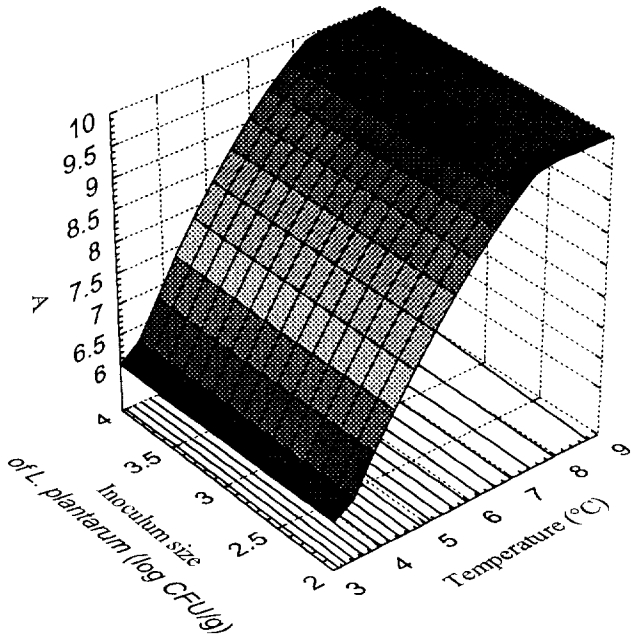

Fig. 3. Contour plots for the interaction of inoculum size of *L. plantarum* with temperature on *L. plantarum* maximum cell number increase (*A*), as log CFU/g, at the stationary phase.

cutting seem to be the most important factors able to control the proliferation during storage. The potentiality of some vegetable species, such as red chicory, probably endowed with natural antimicrobial components in addition to the anthocyans, can contribute to the reduction of the spoilage rate or growth of hazardous psychrotrophes such as *A. hydrophila* during refrigerated storage.

The inoculum of competitive micro-organisms requires further study concerning the autogonistic mechanisms between microbial species and their interaction with the salad constituents. The competitive advantage of LAB in carrots can be exploited with an appropriate inoculum in order to induce rapid product acidification. The organoleptic changes associated with LAB growth can be regarded as a commercial opportunity. However, a LAB inoculum can only result in modest to uncertain success with less sugary vegetables. Moreover, according to the results of the experimental design, the presence of a high initial level of *L. plantarum*, inoculated in order to inhibit *A. hydrophila* by means of lactic acid production, reduced the inhibiting effect of a high relative concentration of red chicory and did not result in the prevention of the growth of *A. hydrophila*. This agrees with the previous results of Marchetti et al. (1992) which suggested that the complexity of the microbial population can reduce or emphasize the bioactivity of some vegetable ingredients on specific target organisms. In fact, the selective success of an antagonistic microflora, and particularly of the LAB species, is conditioned by environmental factors, such as sugar availability, nutrient competition, temperature and tolerance to chemical constituents. The lactic acid bacteria, which

are frequently proposed as useful competitors of pathogenic or spoilage species, are sensitive to anthocyanins, constituents of red chicory, and are generally unable to grow at temperatures lower than 5–6°C. Thus non-psychrotrophic antagonistic strains or species of bacteria cannot be guaranteed to exert effective control of psychrotrophic pathogens in refrigerated products.

The compatibility of a wider selection of ingredients, the role of different gas mixtures in the pack atmosphere during storage, and the interaction between various products with their bacterial contaminants are all factors that could be investigated. Their evaluation should improve the safety and quality necessary for lightly processed products to be more widely accepted in the marketplace.

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