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# Determination of ready-to-eat vegetable salad shelf-life

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**Abstract**

The shelf-life of ready-to-eat vegetable salads established by manufacturers is usually 7–14 days depending on the type of vegetable, and is determined by loss in organoleptic qualities. A more objective method to predict shelf-life and spoilage would be desirable. The present study monitored the evolution of spoilage organisms in a mixed salad of red cabbage, lettuce and carrot stored at 4°C, 10°C and 15°C. Changes in carbon dioxide and oxygen concentrations and pH were also monitored. Predictive modelling was used to establish a theoretical shelf-life time as a function of temperature. Lactic acid bacteria at levels of $10^6$ cfu/g appeared to be related to both spoilage and theoretically-predicted shelf-life values. © 1997 Elsevier Science B.V.

*Keywords:* Ready-to use vegetables; Lactic acid bacteria; Psychrotrophic; Shelf-life; Modelling

## 1. Introduction

The predominant microbiological populations in ready-to-eat salads are comprised of the psychrotrophs *Pseudomonas* spp. and *Erwinia* spp., in addition to lactic acid bacteria including *Leuconostoc mesenteroides* (Clark and Burki, 1972; Willocx et al., 1993). Pectolytic strains of *Pseudomonas* have been reported to cause vegetable deterioration during storage (Denis and Picoche, 1986; Brocklehurst et al., 1987; Carlin et al., 1990). *Pseudomonas fluorescens* has been identified as a marker of spoilage of ready-to-eat vegetable salads (Willocx et al., 1993).

Numerous studies have investigated the effect of $CO_2$ and $O_2$ concentrations on *Pseudomonas* growth and have observed that $CO_2$ was an effective growth inhibitor at certain concentrations and temperatures (Clark and Burki, 1972; Wells, 1974; Gill and Tan, 1979; Enfors and Molin, 1980; Moir et al., 1993; Willocx et al., 1993). All of these reports have shown that as temperature fell, the inhibitory power of $CO_2$ increased.

Predictive microbiology could be an alternative approach for faster and less costly microbiological

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analyses. It relies on the development of mathematical equations that predict the rate of growth or decline of micro-organisms under a given set of environmental conditions. Mathematical models may be used to predict the shelf-life of food if they can predict the growth of spoilage micro-organisms.

The aim of this study was to investigate the potential value of predictive modelling in the estimation of the shelf-life of ready-to-eat vegetables salads.

## 2. Material and methods

### *2.1. Vegetable salad*

Mixed vegetable salad was composed of lettuce (75%), carrot (15%) and red cabbage (10%). Vegetables were sorted, cleaned and rinsed in potable water with 100 mg/l active chlorine at 2°C, then centrifuged (1000 rpm for 2 min) and mixed in the above ratio. After weighing, they were wrapped in 290 × 155 mm polypropylene film ($CO_2$ and $O_2$ transmission rates of 142 $cm^3/m^2/24$ h/atm and 44 $cm^3/m^2/24$ h/atm, respectively) without gas injection, and stored at 4°C.

### *2.2. Sampling*

A total of 144 samples of mixed vegetable salads prepared as described above were obtained from different batches at the production plant. They were stored at 4°C $\pm$ 0.5, 10°C $\pm$ 0.5 and 15°C $\pm$ 0.5. Two samples were examined for lactic acid and psychrotrophic bacteria every 12 h on the first 3 days, and then every 24 h, for a total period of 204 h of storage (8.5 days). On each day of analysis, gas composition and pH were determined.

### *2.3. Microbiological analyses*

Microbiological analysis consisted of 25 g of salad being diluted with 225 ml of 0.1% peptone solution and blended for 2 min in a Stomacher 400 (Seward, England). Serial decimal dilutions were made in dilution flasks with 90 ml 0.1% peptone solution (ICMSF, 1982).

#### *2.3.1. Lactic acid bacteria*

Lactic acid bacteria were counted using a pour plate method; Bacto Lactobacilli MRS agar (Difco Laboratories, Detroit, MI) was covered with an agar layer and incubated at 30°C for 48 h (Garg et al., 1990). Isolated lactic acid bacteria were identified using the API 50 CHL gallery (bioMérieux, Marcy l'Etoile, France).

#### *2.3.2. Aerobic psychrotrophic micro-organisms*

Aerobic psychrotrophic micro-organisms were counted using a surface plate count method on Plate Count Agar (PCA, Difco Laboratories, Detroit, MI) incubated at 7°C for 10 days (ICMSF, 1982). Isolates were identified by using API 20 E and API 20 EN (bioMérieux, Marcy l'Etoile, France), and PASCO (Difco Laboratories, Detroit, MI).

#### *2.3.3. Yeasts and moulds*

Yeast and moulds were counted using a surface plate count method on Potato Dextrose Agar medium (PDA, Difco Laboratories, Detroit, MI). Plates were incubated at 24°C for 3 days (ICMSF, 1982). Isolates were identified with API 20 C AUX (bioMérieux, Marcy l'Etoile, France).

All isolates to be identified using commercial identification kits were first subjected to Gram stain, oxidase and catalase analyses.

### *2.4. Determination of physicochemical and organoleptic parameters during refrigerated storage*

Concentrations of $CO_2$ and $O_2$ were measured by an Abiss model Pak 12 (Control y Suministros S.L., Barcelona, Spain). Gas samples (20 ml) were taken from the salad bags through a silicone septum, using hermetic gas syringes and following the technique described by the manufacturer (Instruction Manual, G20-B).

The pH was measured with a Crison pH/mv-meter digit 501 potentiometer (Crison Instruments, Barcelona, Spain) using a blended sample diluted 1/10 in a physiological saline solution (Stomacher 400, Seward, England).

Organoleptic characteristics were assessed by a panel using a hedonic scale of 0–4 (0, unacceptable; 1, poor; 2, fair; 3, good; and 4, very good) for 5 colour, texture, browning of the pieces and swelling of package (Strugnell, 1988).

### 2.5. Application of the predictive model to bacterial growth

In order to adjust the data to the curve, the 'DModel' (Baranyi et al., 1993a,b,c; Baranyi and Roberts, 1994) and 'Gomplamu' (FLAIR, 1993) programmes were used. Results were compared and that of the lowest residual mean square value was selected. Once the data were adjusted to the function chosen, the specific growth rate, lag time, maximum density level and time to reach $10^6$ cfu/g were estimated in order to calculate the possible mean shelf-life of the product.

### 2.6. Shelf-life determination

A hedonic grading of 2 was deemed to indicate the end of useful shelf-life as based on sensory attributes. This was defined as the time when the mean of the attributes fell to a value of 2 (King et al. (1986)) established a similar hedonic scale of 1–9 for asparagus with a value of 6 determining the shelf-life of the product, i.e. one above the midpoint on the hedonic scale). Comparison of model to actual counts and sensory results was performed.

The time ($t_s$) which each spoilage micro-organism took to reach $10^6$ cfu/g was calculated from the curve-adjustment equation.

A graph was made of the relationship between the natural logarithm of $t_s$ and temperature (K) (Singh, 1994), represented by the linear equation:

$$t_s = t_o * e^{-a*(T - T_O)}$$

where: $t_s$ is mean shelf-life time; $t_o$ is mean shelf-life time at reference; temperature, $T$ is temperature; $T_o$ is reference temperature and $a$ is the slope.

### 2.7. Statistical analyses

The 'Statgraphics' (v. 5.0, Statistical Graphic Corporation, US) program was used for statistical data analysis. Spearman and Kruskal-Wallis's non-parametric test was employed for the sensorial analysis (Steel and Torrie, 1960). Theoretical assumptions were verified by analyses of variance.

## 3. Results and discussion

### 3.1. Behaviour of lactic acid and psychrotrophic bacteria in modified-atmosphere packaged salads during storage at different temperatures

In salads stored at 4°C, the initial counts of psychrotrophic and lactic acid bacteria were $1.07 \times 10^5$ cfu/g and $8 \times 10^2$ cfu/g, respectively (Figs. 1 and 2). Initial counts obtained in this study were relatively low compared to the levels found by Marchetti et al. (1992), who reported $10^8$ cfu/g of psychrotrophic bacteria, $10^5$–$10^7$ cfu/g of lactic acid bacteria and $10^4$–$10^5$ cfu/g of yeast. Carlin et al. (1989) found initial levels of $10^4$ cfu/g of lactic acid bacteria in carrot salads. Garg et al. (1990) recorded initial levels of $1.8 \times 10^5$ cfu/g of psychrotrophic bacteria in cabbage, $2.7 \times 10^5$ cfu/g in lettuce and $9.5 \times 10^4$ cfu/g in carrot salads. Results from the present study are similar to those of Garg et al. (1990), who reported $1.4 \times 10^3$ cfu/g of lactic acid bacteria in carrot salad.

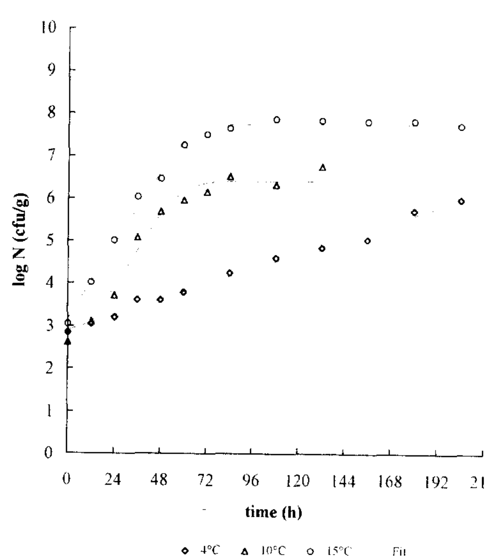


Fig. 1. Growth curves for lactic acid bacteria at 4, 10 and 15°C generated by the DModel program.

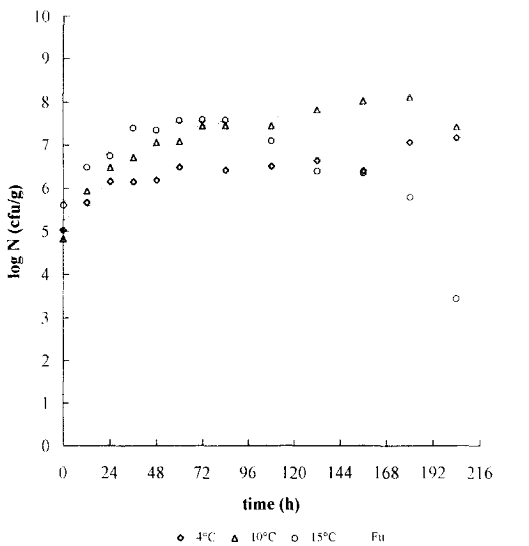


Fig. 2. Growth curves for psychrotrophic bacteria at 4, 10 and 15°C generated by the DModel program.

Differences in the initial counts may be due to various factors including salad composition, initial contents of raw matter, types of plastic film, modified or controlled atmospheres, and initial gas concentrations. Processing techniques also bear a great influence since, among other factors, washing contributes to the removal of a considerable amount of micro-organisms (Hobbs and Gilbert, 1978; Adams et al., 1989; Garg et al., 1990).

Initial lactic acid bacteria populations were approximately $8 \times 10^2$ cfu/g and by the end of the analysis period had increased linearly to $9.6 \times 10^5$ cfu/g in samples kept at 4°C (Fig. 1). These micro-organisms seemed to find the medium very favourable for their growth; indeed, their growth was even greater than that of the psychrotrophic bacteria (Fig. 2), increasing by 3 log as opposed to the 2 log achieved by the psychrotrophs. Lactic acid bacteria are probably favoured by an increase in the $CO_2$ content inside the package. Marchetti et al. (1992) described the growth of lactic acid bacteria at 5°C in carrot salads which started at $3.16 \times 10^5$ cfu/g and reached approximately $10^8$ cfu/g at 7 days (168 h). The same authors found a slower growth of these micro-organisms in mixed salad (carrot, red and white chicory and rocket) which started at approximately $7 \times 10^6$ cfu/g and reached $5 \times 10^7$ cfu/g. Manvell and Ackland (1986) observed that in mixed salads (lettuce, carrot, cabbage, onion and green peppers) stored at 7°C, the lactic acid bacteria rose from 3 decimal logarithms to about 8 log in about 8 days (192 h).

Among the lactic acid bacteria, *Lactobacillus brevis* (22%) and *Leuconostoc mesenteroides* (16%) were isolated in the highest proportion (Table 1). This coincided with results reported by Carlin et al. (1989), who also observed that *Leuconostoc mesenteroides* spoiled carrot salads to a greater degree due to lactic fermentation.

Initial psychrotrophic bacteria count started at $1.07 \times 10^5$ cfu/g and increased slowly to $1.48 \times 10^7$ cfu/g by the end of the observation period in the samples kept at 4°C (Fig. 2). Abdul-Raouf et al. (1993) reported psychrotrophic bacteria in modified-atmosphere salads of approximately $10^9$ cfu/g at 14 days, having started at $7.76 \times 10^4$ cfu/g. Manvell and Ackland (1986) observed that in salads stored at 7°C the psychrotrophic bacteria count rose from 5 decimal logarithms to approximately 9 log at 8 days (192 h).

The species most frequently isolated in this study were *Pseudomonas fluorescens* (19%), followed by *Pseudomonas putida* (8%) (Table 1). This predominance of the genus *Pseudomonas* among the psychrotrophic bacteria has been described elsewhere (Marchetti et al., 1992; Willocx et al., 1993).

No moulds were detected in the salads, possibly due to the modified atmosphere in which the $CO_2$ rose quickly and the $O_2$ was consumed at or before 48 h. Other authors such as Manvell and Ackland (1986) have, however, isolated moulds.

No yeasts were detected initially but after 156 h of storage, levels of approximately $10^3$ cfu/g were obtained. Marchetti et al. (1992) found initial levels of around $10^5$ and $7 \times 10^6$ cfu/g, which rose to approximately $5 \times 10^6$ and $7 \times 10^8$ cfu/g, depending on salad type. Manvell and Ackland (1986) reported that 17% of isolates in salads were yeasts as opposed to 78% bacteria and 5% moulds. However, Garg et al. (1990) found few yeasts or moulds. The species found are indicated in Table 1.

Table 1
Psychrotrophic and lactic acid bacteria and yeasts identified during storage of vegetable salads

| Psychrotrophic bacteria | % | Lactic acid bacteria | % | Yeasts | % |
|---|---|---|---|---|---|
| *Enterbacter intermedium* | 1 | *Lactobacillus curvatus* | 2 | *Candida valida* | 4 |
| *Pasterella haemolytic* bio T | 1 | *Lactococcus lactis* | 2 | *Geotrichum* spp. | 4 |
| *Pseudomonas* spp. | 1 | *Lactococcus fermentum* | 4 | *Hansenula anomala* | 4 |
| *Pseudomonas aeruginosa* | 1 | *Lactobacillus plantarum* | 7 | *Rhodotorula glutinis* | 4 |
| *Pseudomonas picketti* bio 1 | 1 | *Lactobacillus paracasei* | 11 | *Torulopsis* spp. | 4 |
| *Pseudomonas putrefasciens* | 1 | *Lactobacillus mesenteroides* | 16 | *Candida lambica* | 11 |
| *Pseudomonas stutz/mendo* gp | 1 | *Leuconostoc brevis* | 22 | *Trichosporon* spp. | 18 |
| *Salmonella chloraesuis* | 1 | N.I. | 36 | N.I. | 50 |
| *Acinetobacter* spp. | 3 | | | | |
| *Enterobacter agglomeran* | 3 | | | | |
| *Pasturella urae* | 3 | | | | |
| *Plesiomonas shigelloides* | 3 | | | | |
| *Pseudomonas maltophila* | 3 | | | | |
| *Staphylococcus cohnii* | 4 | | | | |
| *Acinetobacter anitratus* | 5 | | | | |
| *Chromobacterium violaceum* | 7 | | | | |
| *Pseudomonas putida* | 8 | | | | |
| *Pseudomonas fluorescens* | 19 | | | | |
| N.I. | 33 | | | | |

The effect of temperature on the growth of micro-organisms was evident (Fig. 1). From the initial lactic acid bacteria count of approximately $10^3$ cfu/g, at all storage temperatures, it was seen that bacteria increased in number more rapidly in samples stored at 15°C and that bacterial growth diminished with lower storage temperature. In salads stored at 10°C, the count increased more rapidly than at 4°C, reaching $10^6$ cfu/g at 84 h, subsequently remaining between log 6 and 7 until the end of the observation period. In salads stored at 15°C, the counts increased until they reached $1.81 \times 10^7$ cfu/g at 60 h and from then on they remained roughly the same until 204 h. It was noted that while lactic acid bacteria count rose as temperature increased, increased $CO_2$ concentration did not appear to limit growth and in fact seemed to encourage it. This agrees with the observations made by other authors (Jones, 1989; Carlin et al., 1990), for whom a modified atmosphere might favour the growth of lactic acid bacteria. Hence, temperature would seem to be the constraining factor and not $CO_2$. Carlin et al. (1990) found final counts (at 14 days) ranging from $10^4$–$10^8$ cfu/g in salads kept at 10°C. This wide variability was not observed in the present study, where variation coefficients ranged between 0.5% and 21% throughout storage.

Psychrotrophic bacteria numbers rose from about $1.33 \times 10^5$ cfu/g to $4.79 \times 10^7$ cfu/g in salads kept at 10°C. In those stored at 15°C, counts reached $2.65 \times 10^7$ cfu/g at 36 h. They remained at that level until about 84 h, subsequently falling at 204 h to $2.75 \times 10^3$ cfu/g (Fig. 2). If we compare the growth of the psychrotrophic bacteria at 4°C and at 15°C, and starting from the same concentration (about $10^5$ cfu/g), it can be seen that salads stored at 4°C recorded levels of over $10^7$ cfu/g, while at 15°C these levels decreased to $2.75 \times 10^3$ cfu/g. Here, the influence of various factors, including $CO_2$ concentration, pH and temperature, was noted. Gas evolution varied considerably between samples stored at different temperatures. Fig. 4 shows the evolution of carbon dioxide and oxygen at 4°C, 10°C and 15°C. The $CO_2$ concentration at 15°C (52.67%) seemed to be the cause of the decreasing, alongside the effect of a drop in pH (near 4) which also occurred. This decrease in pH in foods stored in a modified atmosphere has also been described by Daniels et al. (1985), Farber (1991) and Abdul-Raouf et al. (1993).

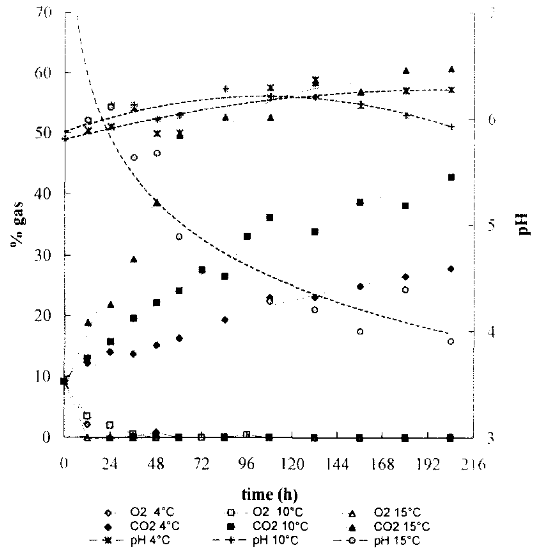


Fig. 3. Evolution of pH and gas concentrations in salads stored at different temperatures.

In general, a marked difference in appearance was observed between salads stored at 4°C and at 15°C (Fig. 3), and confirmed statistically (Strugnell, 1988). At 4°C, a 'very good' appearance was scarcely reduced to 'good' at 60 h, whereas salads stored at 15°C had a generally poor appearance at the same time lapse. With regard to vegetable colour, at 108 h salads stored at 4°C began to look pale, whereas those kept at 15°C had paled at 60 h. Little brownness was observed in the cuts in general. Flabbiness of the leaves at 84 h was noted in salads stored at 15°C but not in those kept at 4°C. After 84 h storage at 15°C the swelling of the bag was very noticeable whereas at 4°C swelling did not occur until 180 h.

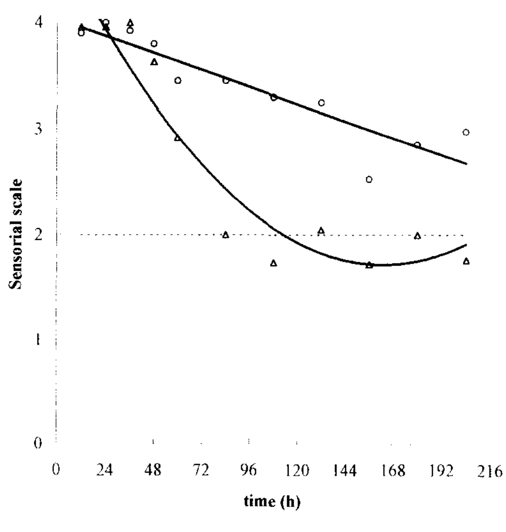


Fig. 4. Evolution of sensory attributes in salads stored at 4 and 15°C.

The adjustment of lactic acid and psychrotrophic bacteria evolution by the DModel Program equation gave a lower square residual error than that of Gompertz and was thus selected. Specific growth rate estimates were: at 4°C, 0.061 $h^{-1}$ and 0.035 $h^{-1}$; at 10°C: 0.090 $h^{-1}$ and 0.176 $h^{-1}$; at 15°C: 0.124 $h^{-1}$ and 0.167 $h^{-1}$ for psychrotrophic bacteria and lactic acid bacteria, respectively.

In order to prepare predictive models for different temperatures, specific growth rate vs. temperature was expressed by the equation described by Ratkowsky et al. (1982). Ratkowsky's equation is an especially suitable model for low temperatures. The square root of the specific growth rate ($\sqrt{\mu}$) was plotted against temperature in order to obtain the minimum temperature ($T_{min}$) as in the equation:

$$\sqrt{\mu} = b*(T - T_{min})$$

where $\mu$ is the specific growth rate; $b$ is the slope; $T$ is the temperature considered; $T_{min}$ is the minimum temperature of growth of this micro-organism.

On applying the Ratkowsky equation the result was:

For lactic acid bacteria with a $T_{min}$ of 266.11 K

$$\sqrt{\mu} = 0.0209*(T - 266.11) \quad r^2 = 0.76$$

For psychrotrophic bacteria with a $T_{min}$ of 250.85 K

$$\sqrt{\mu} = 0.0094*(T - 250.85) \quad r^2 = 0.998$$

### *3.2. Determination of commercial shelf-life. Relationship between organoleptic and physicochemical changes and the presence of spoilage bacteria*

The commercial shelf-life of a ready-to-eat vegetable salad varies from 6–14 days, depending on the ingredients and on the manufacturer's criteria (based on sensory appearance). The samples analysed in this study had a shelf-life of 7 days assigned to them.

In the present study, the acceptance by the consumer of the product when the mean values of the sensorial attributes analysed reached value 2 was taken as a reference. Thus, salads stored at 4°C were still acceptable at 204 h (the end of the experimental period) but at 84 h of storage at 15°C they were no longer acceptable. Bolin et al. (1977) reported that at 17 days chopped lettuce salads stored at 5°C, and at 11 days those kept at 10°C, lost the optimal sensorial characteristics required for sale.

At the moment when salad stored at 15°C was regarded as being unacceptable (84 h), psychrotrophic and lactic acid bacteria counts lay between $3.55 \times 10^7$ and $5.5 \times 10^7$ cfu/g, respectively. The maximum level of micro-organisms to be detected as an indicator of imminent food spoilage should thus be lower than this value. In salads stored at 4°C, still in acceptable conditions at 204 h, counts ranged between $9.33 \times 10^5$ and $5.25 \times 10^6$ cfu/g. The value of $10^6$ cfu/g suggested by various authors as a microbiological value for the determination of the end of a product's shelf-life is thus in line with the present results. In most countries a criterion has been established whereby ready-to-eat products should contain $< 10^6$ cfu/g or ml of mesophiles at the consumption stage and that there should also be an absence of pathogenic micro-organisms or their toxins which are a risk to health (Notermans et al., 1993).

Fig. 5 shows the natural logarithm of mean shelf-life times versus temperature. The values reached by lactic acid bacteria coincide with, or are nearest to, the moment of food spoilage. Taking this into account, it is possible to set a maximum lactic acid bacteria count of $10^6$ cfu/g as an indicator of the beginning of spoilage. The predictions of a product's shelf-life (Table 2) would indicate that at 4°C, storage could be as long as 209 h, which would be substantially longer than the 144 h (7 days from manufacture) established by the manufacturer.

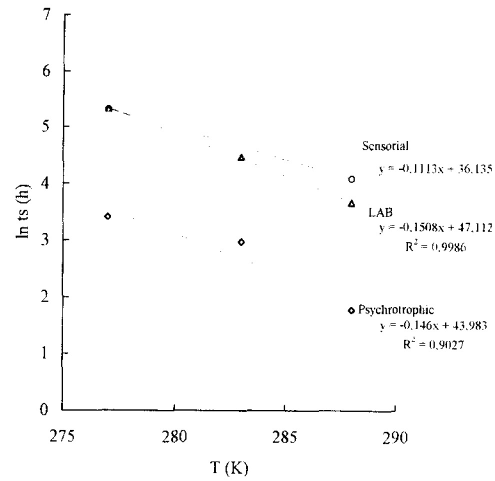


Fig. 5. Linear regression lines of the mean life-time (ln ($t_s$)) for lactic acid (LAB) and psychrotrophic bacteria and for sensorial characteristics by temperature.


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Table 2
Prediction value of ready-to-use vegetable salad shelf-life time (h) according to storage temperature[a]

| T (K) | Psychrotrophic[b] | LAB[b] | Sensorial[c] |
|---|---|---|---|
| 277 | 35 | 209 | 201 |
| 278 | 30 | 179 | 180 |
| 279 | 26 | 154 | 161 |
| 280 | 22 | 133 | 144 |
| 281 | 19 | 114 | 129 |
| 282 | 17 | 98 | 115 |
| 283 | 14 | 84 | 103 |
| 284 | 12 | 73 | 92 |
| 285 | 11 | 62 | 83 |
| 286 | 9 | 54 | 74 |
| 287 | 8 | 46 | 66 |
| 288 | 7 | 40 | 59 |

[a] Expressed as the time required for psychrotrophic and lactic acid bacteria to reach a level of $10^6$ cfu/g, resulting in a loss of optimum sensorial characteristics[c].
[b] Time (h) taken by microorganisms to reach $10^6$ cfu/g.
[c] Time (h) taken to reach spoilage value (2), as defined by the mean of the sensorial attributes.

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