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Plastic Mulch Improves Microbial Quality and Shelf Life of Cold Stored Butter Lettuce (*Lactuca sativa* var. Lores)

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ABSTRACT

Differences in soil management during crop cultivation (plastic mulch or bare soil) are likely to influence microflora behavior during the post-harvest of the product. The evolution of native microflora and sensory quality analysis were assayed during cold storage of butter lettuce (*Lactuca sativa* var. Lores) from greenhouse cultivation using mulch and bare soil. All parameters analyzed were measured in three different zones of the complete lettuce head named as the external (outer leaves), middle (mid leaves) and internal (inner leaves) zones. The lettuce cultivation method had impact on the initial counts as well as on microbial evolution during refrigerated storage, playing a singular role on the sensorial quality and shelf life of each lettuce. The higher initial counts in mulch lettuce could be explained by the microclimate generated by the plastic mulch around the plant. While bacterial increment was observed from the beginning of the storage in the three zones of bare soil lettuce, cold storage had a bacteriostatic or bactericidal effect on mulch lettuce. Through OVQ indexes a significant higher shelf life was observed in mulch lettuce.

Keywords: cultivation method, lettuce quality, microbial evolution, native microflora, storage

INTRODUCTION

During crop growth, a number of factors may affect vegetable post-harvest quality. Vegetable quality is a combination of characteristics, attributes and properties which render value to this commodity for human consumption. Many pre-harvest and post-harvest factors such as genetic material, agro-ecological conditions, production technology, physiological stage at harvest, post-harvest technology and the interaction between them affect the composition of vegetable and the general quality of the product. Key to improving crop management is counting on information about the role that pre- and post-harvest factors play in vegetable quality. Chiesa *et al*. (2005) noted substantial differences in crop growth and yield of lettuce (*Lactuca sativa* var. Lores) grown under different production systems. Among the techniques utilized in lettuce cultivation, mulch application stands out, accounting for crop quality and productivity improvement. Verdial *et al*. (2001) reported several benefits to iceberg lettuce, cv. 'Lucy Brown' cultivation using mulch technology. Plastic mulches directly affect the plant microclimate by modifying the surface radiation budget, and by decreasing soil water loss (Liakatas *et al*. 1986; Muller 1991). Soil moisture and temperature increase, owed to plastic film mulching, change oil biological characteristics, enhance soil microbial biomass, cycle nutrients, and provide a more stable soil micro-environment (Li *et al*. 2004). To date, studies in vegetables, such as Iceberg lettuce, corn (*Zea mays* L.) and summer squash (*Cucurbita moschata* Duch.) have dealt with the analysis of plastic mulch effect on soil properties, such as weed control, soil moisture and temperature, soil solarization, soil microbial biomass (Li *et al*. 2004), soil profile aeration and nutrient availability (Muller 1991; Lamont 1999).

It is now well recognized that it is essential to consider vegetable microbiology during cultivation and harvesting as well as the potential changes during storage to ensure microbiological stability and safety in fresh vegetable. Horticultural crops are ecological niches for adverse and changing microflora. Fresh produce typically contains a complex mix of bacteria, fungi and yeast, which characterize the fruits or vegetables, and whose population and types are considerably variable (Zagory 1999). Regarding vegetables, microflora is dominated by soil organisms. Any change in the environmental conditions surrounding a product can lead to substantial changes in the microflora (Kader 1992). With respect to fresh produce, when the crop is evenly planted, any event can affect the safety and bacteriological quality of the final product (Brackett 1999). Production practices, such as fertilization, utilization of agrochemical, irrigation type and soil management, could have a great impact on the microbiological population (Ponce *et al*. 2002). Differences in soil management during crop cultivation, such as plastic mulch use or bare-soil cultivation methods, are likely to influence the microbiological population and/or microflora behavior during the post-harvest handling of the product. Further research is needed to compare the impact of different soil management techniques on vegetable microbial quality at harvest, and its evolution during post-harvest storage. General knowledge of native product microorganisms is crucial not only to predict and control any pathogens growth but also to advance specific technologies for the post-harvest handling of fresh vegetables.

The objectives of this research were (i) to compare native microflora evolution in butter lettuce var. Lores from the greenhouse cultivated using mulch and bare soil during storage under optimal conditions; (ii) and to evaluate the shelf life of each lettuce by means of a sensory quality analysis. Since the removal of external leaves during the trade of whole lettuce heads is a common practice, it is interesting to evaluate native microflora in different lettuce zones. For this purpose all parameters analyzed were measured in three different zones of the complete lettuce head. Correlation analyses between bacterial population and overall visual quality were also conducted.

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MATERIALS AND METHODS

Plant material and sample preparation

Heads of butter lettuce (*Lactuca sativa* var. Lores) were grown and harvested in Sierra de los Padres, Mar del Plata, Argentina. Lettuce was cultivated under cover in two conditions: on bare soil and with mulch technology in which a black plastic film separated the plants from the soil. Both types of cultures were exposed to the same light (800 μE m^{-2} s^{-1} of maximum photon photosynthetic flow), temperature (between 12-18°C) and irrigation water volume (60-70 L ha^{-1} with 1.5-2 Kg cm^{-2} of pressure). Once harvested, lettuce heads were immediately pre-cooled in refrigerated bags and transported to the laboratory within 1 h of harvesting. They were identified and weighted, and without any other conditioning operation, they were packed in polyethylene bags (O_2 permeability of 600 cm^3/m^2/d, CO_2 permeability of 4000 cm^3/m^2/d, and water vapor permeability of 4 g/m^2/d), placing two plants per bag (28 × 55 cm, useful volume: 4 L). Bags were sealed and stored in a refrigerated chamber at 0-2°C and 97-99% relative humidity (RH). On each sampling day, two bags, one from each condition (bare soil and mulch), were taken from the storage room. For each condition one lettuce was used to conduct the microbial population analysis while the other was used to perform the sensory acceptability analysis. Three independent experimental runs were performed and were continued up to 19 days of storage, a time at which samples from bare soil cultivation presented evidence of decay from sensory evaluations.

All parameters analyzed were measured in three different zones of the complete lettuce head, which were named the external (outer leaves), middle (mid leaves) and internal (inner leaves) zones. Since the internal zone is a compact area with a yellow core, the middle and external zones correspond to non-compacted leaves with light and dark green colour, respectively. In lettuce heads, each group consisted of 6 to 10 leaves.

Microbiological studies

The enumeration and differentiation of microorganisms were conducted in the culture media and under the culture conditions described next. Mesophilic aerobic bacteria on Plate Count Agar (PCA) incubated at 30°C for 48-72 h (ICMSF 1983); psychrotrophic bacteria in the same medium at 5°C for 3–4 d (ICMSF 1983; Mossel and Moreno García 1985); Enterobacteriaceae and total coliforms in Eosin Metilen Blue (EMB) at 30–32°C for 24 h. Yeast and mold were counted in Yeast-Glucose-Chloramphenicol (YGC) medium at 25°C for 5 d (ICMSF 1983). Lactic acid bacteria were counted in MRS medium (de Man *et al*. 1960) at 30°C, 24 h. All culture media were from Britania (Buenos Aires, Argentina). In each experimental run and for all microbial populations, the three zones of each lettuce head were analyzed by duplicate on every sampling day.

Sensorial analysis

In each experimental run and for bare soil and mulch conditions, heads of lettuce were examined in the laboratory at the beginning of the experiment and after cold storage. Quality parameters evaluated were colour (shade and uniformity), brightness, texture, wilting, bacterial decay and physiological disorders, mainly midrib and edge browning. A nine-point scoring scale was employed to assess these parameters, in which 9 stood for excellent quality, 7 for good, 5 for fair (limit of acceptance), 3 for poor, and 1 for extremely poor quality (Artés and Martínez 1996). The average of these indexes was used to estimate the overall visual quality (OVQ). Immediately after lettuce was removed from storage conditions, a panel composed of 9 trained judges proceeded to the assessment. The judges were 30 to 55 years of age and belonged to the Food Engineering Group (UNMDP). They all had sensorial assessment experience in leafy vegetables. The 3-digit coded samples were randomly produced, one at a time, before the judges who offered their individual assessment sat around a table.

Statistical analysis

Data were analyzed using SAS software version 8.0 (SAS Inc., 1999). PROC GLM (General Linear Model Procedure) was used for the analysis of variance (ANOVA) for microbial populations counts (mesophilic, psychrotrophic, LAB, coliforms and yeast and molds) and OVQ. The experimental design was a split plot with one cultivation method for each plot. In each independent run all lettuces were harvested, transported to the laboratory and stored at optimal conditions within unnumbered bags. Each sampling day, bags from each cultivation method were retired from chamber in randomized way. The sources of variation were DAY (storage time, day of sampling), PLANT WITHIN DAY, CULTIVATION METHOD (mulch and bare soil) and CULTIVATION METHOD - DAY interaction. Differences between cultivation method and days of storage were determined by the Tukey multiple comparison test (P<0.05). PROC UNIVARIATE was used to validate ANOVA assumptions. Pearson's correlation coefficients were estimated among mesophilic aerobic bacteria, psychrotrophic bacteria, lactic acid bacteria, total coliforms, yeast and mold, and overall visual quality, with PROC CORR (Correlation Procedure). Correlations of P<0.05 were considered statistically significant and those of P<0.01, strongly significant.

Linear regression fitting for bacterial counts evolution and OVQ evolution was performed using SYSTAT 5.0 (SYSTAT INC. 1992).

RESULTS

Microbial evolution in mulch and bare soil lettuce heads

Mesophilic microorganisms

Mesophilic microorganisms provide an estimate of total viable population, and are indicative of endogenous microflora and contamination undergone by the material (Ponce *et al*. 2002). The bacteria found in fresh products are the same as those normally found in field produce (Zagory 1999).

Fig. 1A-C depicts the evolution of mesophilic population in external, middle and internal zones of mulch and bare soil lettuce heads. Initial counts for external, middle and internal zones were significantly different (P=0.0004, P=0.0380, and P=0.0410, respectively) under both cultivation methods, being 1.85 to 2.82 log higher in mulch lettuce than in bare soil lettuce.

The ANOVA applied to mesophilic aerobic bacteria data and carried out for each lettuce zone, yielded, in the three cases, a significant interaction (P<0.0001 for the external, and P=0.0032 for both the middle and internal zones) between the factors under analysis (CULTIVATION METHOD and DAY), thereby denoting that the cultivation methods differ in the evolution of mesophilic bacteria during storage. In addition, this interaction was indicative of different tendencies in each lettuce zone. Interactions behaved in an opposite manner as regards the external and middle zones. The evolution of mesophilic bacteria followed a significant linear increase for bare soil, represented by Log N = 0.23 t + 5.71, R^2 = 0.81, n = 30, P=0.0043 for the external zone up to day 12, and by Log N = 0.25 t + 5.65, R^2 = 0.93, n = 24, P=0.0493 for the middle zone up to day 8 of storage (**Fig. 1A, 1B**). Conversely, a significant decrease up to days 12 and 8 was observed for mulch lettuce, following a first order kinetics (Log N = -0.17 t + 8.23, R^2 = 0.86, n = 30, P=0.0126 for the external zone, and Log N = -0.22 t + 7.47, R^2 = 0.94, n = 24, P=0.029 for the middle zone) (**Fig. 1A, 1B**). After that, mesophilic counts remained steady for both cultivation methods until the end of storage. For the internal zone, the mesophilic evolution also increased linearly (P=0.0073) in bare soil lettuce up to day 8, and was represented by the following regression equation: Log N = 0.33 t + 5.69, R^2 = 0.91, n = 24. On the other hand, for mulch lettuce a decrease took place between days 5 and 12, following no specific kinetic order. For both cultivation methods, microbial counts remained steady in the internal zone

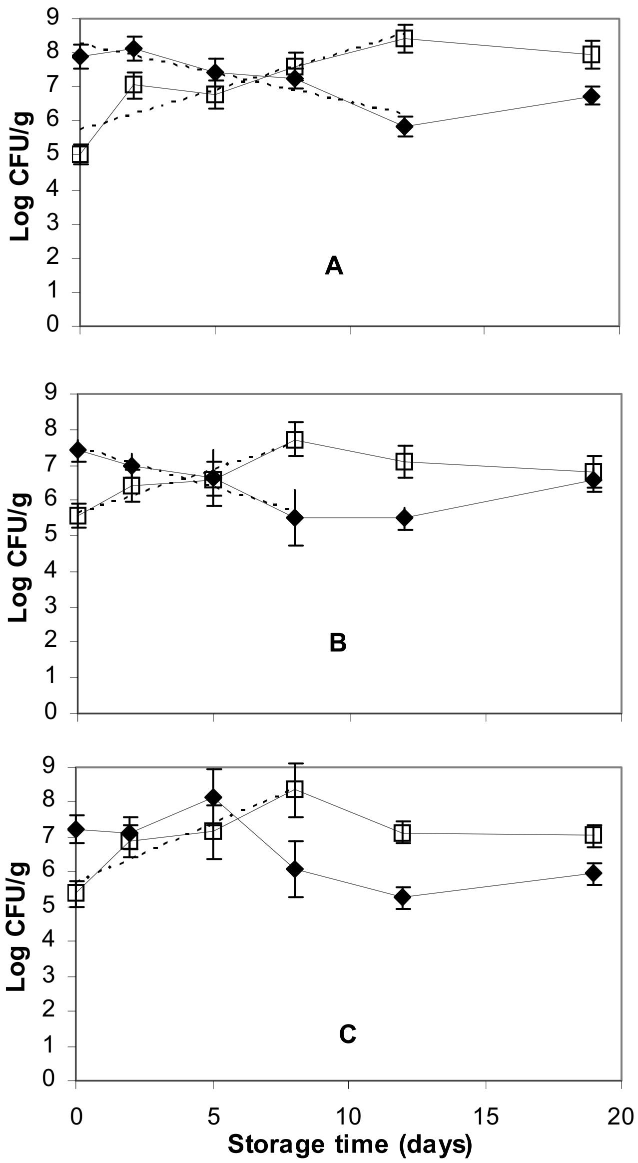

Fig. 1 Evolution of mesophilic bacteria counts in external (**A**), middle (**B**), and internal (**C**) zones of lettuce heads cultivated under different methods: bare soil (□) and mulch (♦). The data correspond to the LSMEAN (least square means, means estimators by least squares). The dotted lines correspond to the tendency lines. The error bars represent the deviation associated with each LSMEAN.

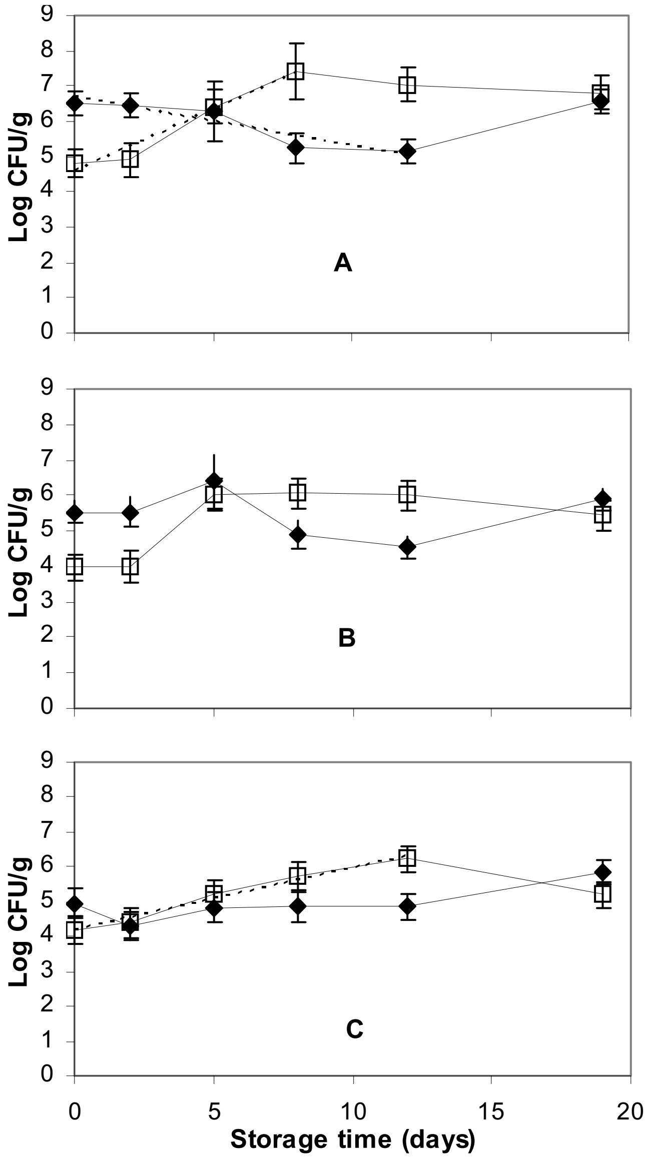

Fig. 2 Evolution of psychrotrophic bacteria counts in external (**A**), middle (**B**), and internal (**C**) zones of lettuce heads cultivated under different methods: bare soil (□) and mulch (♦). The data correspond to the LSMEAN (least square means, means estimators by least squares). The dotted lines correspond to the tendency lines. The error bars represent the deviation associated with each LSMEAN.

until the end of storage (**Fig. 1C**).

The different mesophilic evolution between both cultivation methods during the storage could be explained by the fact that, in mulch lettuce, bacteria proliferate in a more benign pre-harvest environment and this fact would enhance their susceptibility to cold storage.

Psychrotrophic bacteria

Psychrotrophic microorganisms constitute a major microorganism group in fresh vegetables, since, despite being responsible for a small percentage of the initial microflora, they tend to predominate in the chill temperatures recommended for the storage of these commodities (ASHRAE 1994).

The evolution of psychrotrophic bacterial population under both cultivation methods in each lettuce zone is presented in **Fig. 2A-C**. For the external and middle zones, initial counts in both cultivation methods differed significantly (P=0.0050 and 0.0010, respectively), the counts being 1.70 and 1.56 log higher for mulch lettuce than for bare soil lettuce, respectively. As noticed for mesophilic counts, higher initial microbial counts could be explained by the higher temperatures and moisture regime to which mulch lettuce was subjected. For the internal zone, initial counts in mulch lettuce were 1.2 times higher than those for bare soil, the difference being non-significant.

ANOVA applied to psychrotrophic bacteria population data, in each lettuce zone, showed a significant interaction (P=0.0020 for the external, P=0.0051 for the middle, and P=0.0500 for the internal zones, respectively) between the factors considered in the analysis. Just like in the mesophilic case, the interaction type differed in each lettuce zone. For the external zone, psychrotropic evolution was crossed. For bare soil lettuce, an initial increment was observed (P=0.002) up to day 8 (Log N = 0.35 t + 4.57, R^2 = 0.96, n = 24), remaining constant thereafter (**Fig. 2A**). For mulch lettuce, a decrease (P=0.0015) following a first order kinetics up to day 12 (Log N = -0.16 t + 6.68, R^2 = 0.90, n = 30) was observed and then a notorious increment (P=0.0157) was detected, reaching similar values to those at day 0. The increments were similar to those reported by Roura *et al.* (2003) during the storage of fresh Romaine lettuce. Regarding the middle zone, psychrotrophic bacteria followed a typical growth curve for bare soil lettuce, while no significant changes were observed in bacterial counts during mulch lettuce storage (**Fig. 2B**). For the internal zone, while a linear increment was observed up to day 12 for bare soil, represented by Log N = 0.18 t + 4.17, R^2 = 0.98, n = 30, no changes were noted in mulch lettuce (**Fig. 2C**). For bare soil lettuce, the tendency line drawn for psychotropic bacteria evolution in the external zone was significantly different (P<0.05) from the evolution monitored in the internal zone, the rate of growth doubling in the outer leaves with respect to the inner leaves. This would indicate that outer leaves constitute a more appropriate growth substrate than inner leaves (compact and clear leaves), probably owing to the greater nutrient and oxygen availability.

While psychrotrophic bacteria increments were observed from the beginning of storage in the three bare soil lettuce zones, cold storage had a bacteriostatic (middle and internal) or bactericidal (external) effect on mulch lettuce. Once again and in agreement with the behavior exhibited by mesophilic bacteria, low-temperature storage provided a sufficient barrier to limit psychrotrophic bacteria in mulch lettuce.

Lactic acid bacteria (LAB)

The role of LAB in maintaining vegetables quality remains unclear. Breidt and Fleming (1997) advocated that LAB acts as a bio-control agent in minimally processed refrigerated foods. LAB may exert antimicrobial effects through one or more of the following mechanisms: lowering pH (Raccach *et al*. 1979), generating hydrogen peroxide (Gomez *et al*. 2002), competing for nutrients, and possibly producing antimicrobial compounds, such as bacteriocins (Klaenhammer 1988; Harris *et al*. 1989).

Fig. 3A-C shows the evolution of LAB in external, middle and internal zones of mulch and bare soil lettuces. Initial counts in both cultivation methods did not differ significantly for external and middle zones; yet, in the internal zones, the initial counts in both cultivation methods were significantly different (P=0.049), being higher for bare soil lettuce (4.15 log) than for mulch (2.57 log).

ANOVA applied to LAB population data showed a significant CULTIVATION METHOD × DAY interaction (P<0.0001 for the external, P=0.0032 for the middle, and P=0.0003 for the internal zones). The interaction in the external zone was evident after 8 days of storage (**Fig. 3A**). For bare soil lettuce, a significant increase (P<0.0001) was noticed in LAB counts between days 8 and 12, remaining constant thereafter. Regarding mulch lettuce, no significant differences in LAB counts were observed during storage time.

Interaction in the middle zone was noticed since storage started. LAB counts in bare soil lettuce remained unaltered up to day 8, afterwards, a significant increase (P=0.0023) between days 8 and 12 was detected. However, LAB counts for mulch lettuce increased linearly (P=0.0008) up to day 8 (Log N = 0.24 t + 3.23, R^2 = 0.96, n = 24), decreasing significantly (P=0.0183) between days 8 and 12, and remaining stable afterwards (**Fig. 3B**).

The interaction observed in the internal zone was expressed from time 0 of storage. A linear increase (P=0.0024) in LAB counts up to day 12, represented by Log N = 0.21 t + 3.76 (R^2 = 0.86, n = 30), was observed in bare soil lettuce, while, for mulch lettuce, an early significant rise (P=0.0392) was noticed (**Fig. 3C**) up to day 2, remaining constant to the end of storage.

At day 19 of storage, LAB counts in the three zones of bare soil lettuce were significantly higher (2.2-3.9 log) if compared to those for mulch lettuce, the largest difference being perceived in the external zone.

Total coliforms

Coliforms imply hygiene at the production stage as well as in cold chain maintenance. Soil, irrigation water or improper handling may explain the contamination of fresh vegetables by coliforms (Barriga *et al*. 1991; Francis and O'Beirne 1998).

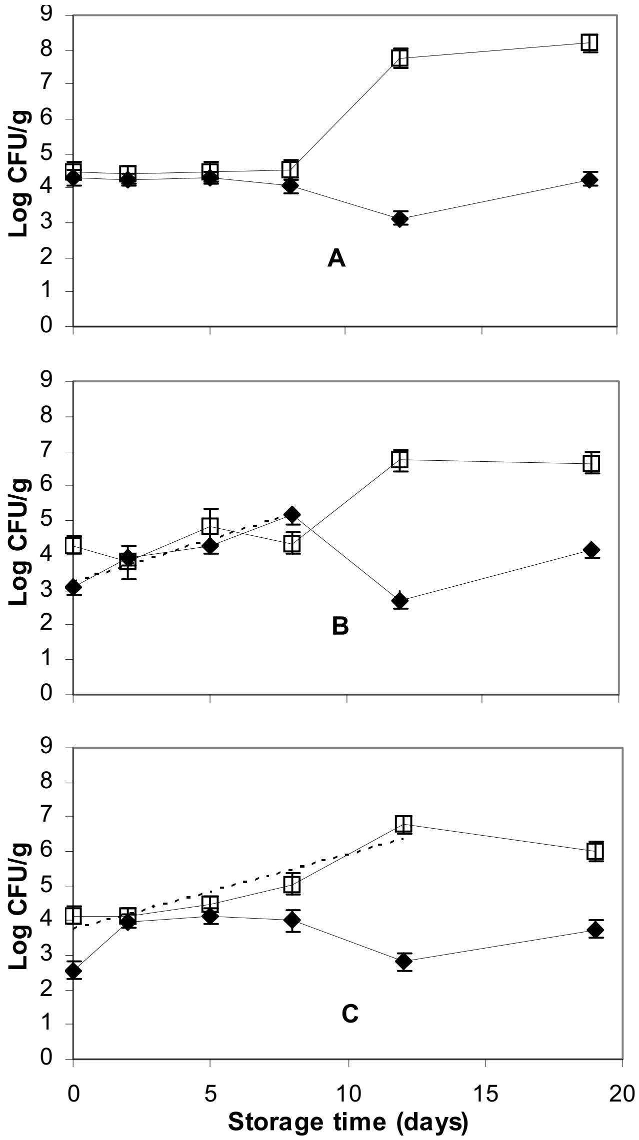

Fig. 3 Evolution of LAB bacteria counts in external (**A**), middle (**B**), and internal (**C**) zones of lettuce heads cultivated under different methods: bare soil (□) and mulch (♦). The data correspond to the LSMEAN (least square means, means estimators by least squares). The dotted lines correspond to the tendency lines. The error bars represent the deviation associated with each LSMEAN.

The evolution of total coliforms in the external, middle and internal zones of mulch and bare soil lettuce heads is presented in **Fig. 4A-C**. No significant differences were recorded in the initial microbial counts (4.42-5.52 log) between the different cultivation methods employed in each lettuce zone.

ANOVA applied to total coliforms data showed a significant interaction (P<0.0001 for the external, P=0.0013 for the middle, and P=0.0037 for the internal zones) between the factors considered in the analysis. The interaction between CULTIVATION METHOD and DAY was expressed in the external zone from the beginning of storage. Total coliform counts in bare soil lettuce increased linearly up to day 8 (Log N = 0.35 t + 4.88, R^2 = 0.99, n = 24, P=0.0015); afterwards, a significant decline (P=0.0021) was identified between days 8 and 12 to finally remain constant up to the end of storage. No significant changes were detected for mulch lettuce during storage (**Fig. 4A**). The middle zone in bare soil and mulch lettuce did not differ largely in the overall coliform counts up to day 12. The interaction was evident after 12 days of storage, when a significant increase (P=0.0106) was noted in mulch lettuce, while in bare soil lettuce bacterial counts kept their initial

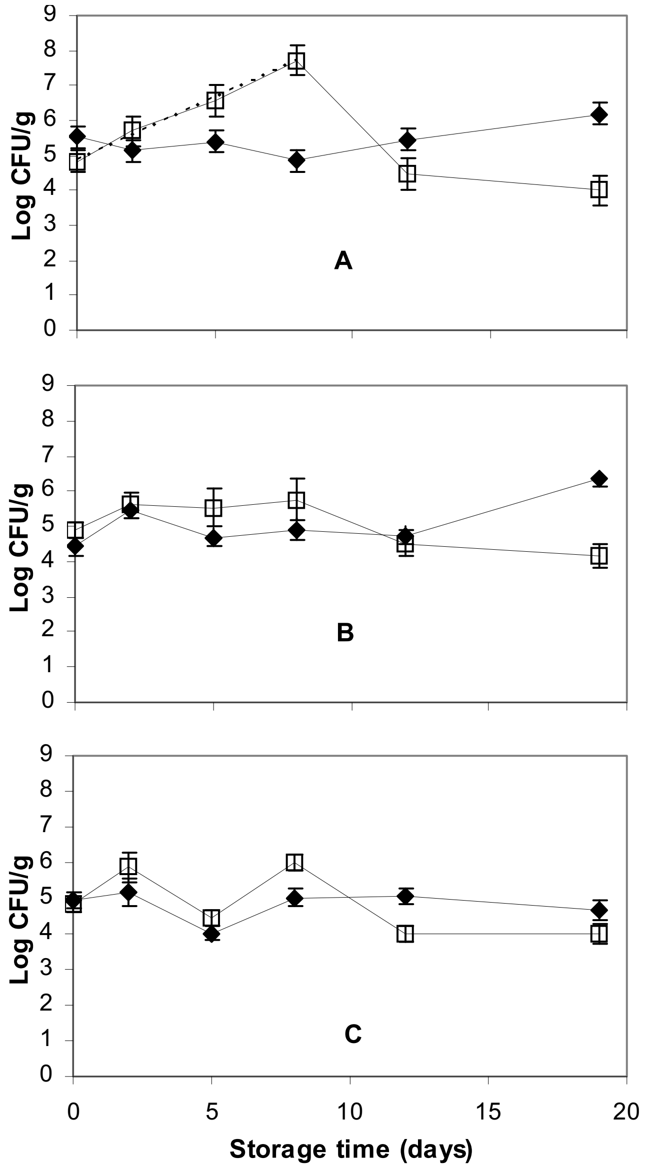

Fig. 4 Evolution of coliforms bacteria counts in external (**A**), middle (**B**), and internal (**C**) zones of lettuce heads cultivated under different methods: bare soil (□) and mulch (♦).The data correspond to the LSMEAN (least square means, means estimators by least squares). The dotted lines correspond to the tendency lines. The error bars represent the deviation associated with each LSMEAN.

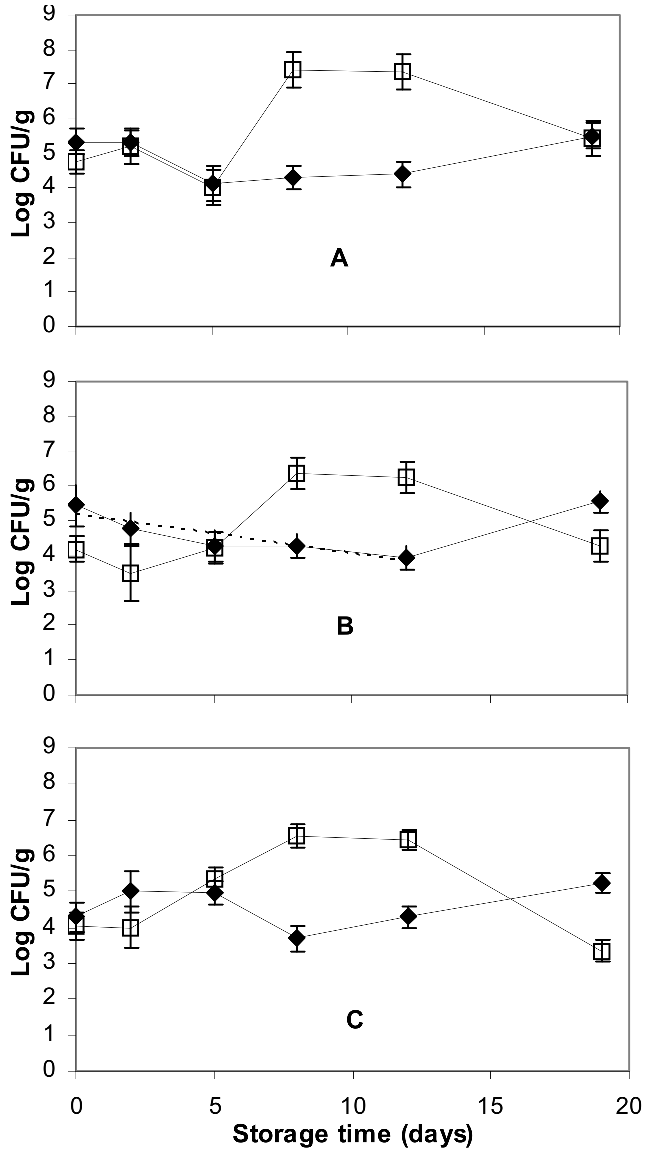

Fig. 5 Evolution of yeast and mold counts external (**A**), middle (**B**), and internal (**C**) zones of lettuce heads cultivated under different methods: bare soil (□) and mulch (♦).The data correspond to the LSMEAN (least square means, means estimators by least squares). The dotted lines correspond to the tendency lines. The error bars represent the deviation associated with each LSMEAN.

values throughout storage time (**Fig. 4B**). Similar values were recorded for total coliform counts in bare soil and mulch internal zones from day 0 to day 8 of storage. Subsequently, interaction was attained by a significant (P=0.0018) decrease in total coliform counts from bare soil lettuce (**Fig. 4C**).

It should be noted that at the end of the storage, coliform counts for mulch lettuce were approximately 2 log higher than those for bare soil lettuce. After 8 days of storage, coliform counts in bare soil lettuce decreased (**Fig. 4A-C**), along with an increment in LAB counts (**Fig. 3A-C**). However, such a rise in LAB was not observed for mulch lettuce. In bare soil lettuce LAB could exert a bio-control (Vescovo *et al*. 1996; Gómez *et al*. 2002; Settanni and Corsetti 2008) action over the total coliform population.

Yeast and mold

Depending on plant resistance, strain virulence, the competing microflora and ambient conditions, yeast and mold can act as either strict or latent parasites. They may present a profound change in the rate of growth after harvest when the plant resistance is diminished, and lead to rapid spoilage (Ponce *et al*. 2002).

Fig. 5A-C shows yeast and mold evolution for each zone of mulch and bare soil lettuce heads. For external and internal zones, no significant differences were founded in the initial counts regarding both cultivation methods. Conversely, significant differences (P=0.001) were detected between initial counts in the middle zone, where counts in mulch lettuce were 5.45 log and in bare soil lettuce were 4.19 log.

ANOVA applied to yeast and mold data showed a significant CULTIVATION METHOD × DAY interaction for external (P=0.0027), middle (P=0.0015), and internal zones (P=0.0006). For external zone and both cultivation methods, no significant changes were observed in yeast and mold counts up to day 5. The interaction became evident after this time period. Yeast and mold counts from bare soil lettuce increased significantly (P=0.0171) between days 5 and 8, remaining constant up to day 12, when a significant decrease (P=0.001) was recorded. No significant changes in yeast and mold counts were detected for mulch lettuce along storage (**Fig. 5A**).

Ever since the beginning of storage, the interaction observed in the middle zone was evident. In bare soil lettuce, yeast and mold counts followed a typical growth curve with a lag phase up to day 5, a significant (*P*=0.0005) increase up to day 8, and a significant decrease (*P*=0.005) between days 12 and 19. In mulch lettuce, a linear significant decrease (*P*=0.001) was identified up to day 12 (Log N = -0.11 t + 5.15, R^2 = 0.84, n = 30), rebounding after that to initial values (**Fig. 5B**).

The interaction in the internal zone for bare soil lettuce yielded a typical growth curve, with a significant rise (*P*=0.045) between days 2 and 8, and a significant decrease (*P*=0.0163) between days 12 and 19. For mulch lettuce, no significant changes were detected in yeast and mold counts during storage (**Fig. 5C**).

Yeast and mold evolution in bare soil lettuce indicated the presence of a lag phase in the three zones, followed by an increase, a stationary phase and, finally, a decline, each stage with different duration. Similar evolution profiles in yeast and mold were reported by Roura *et al.* (2003) regarding Romaine lettuce. Barriga *et al.* (1991) and King *et al.* (1991), who worked with Iceberg lettuce, reported similar results respect to yeast and molds, too.

Sensorial analysis

Different quality components comprise lettuce OVQ, such as fresh-looking appearance, bright green color, crispness, and essentially the absence of browning. The members of the sensory panel rated samples with OVQ scores below 5 as unacceptable.

Fig. 6A-C represents the evolution of OVQ in external, middle and internal zones of mulch and bare soil lettuce heads during refrigerated storage. Throughout the sensorial analyses, each lettuce zone from both cultivation methods was scored independently, so no comparison was made among the zones. Regarding fresh lettuce, each zone presented typical organoleptic characteristics differentiated mainly by leaf size, color and texture. Even though zones differed in their sensorial characteristics, their initial attributes were rated with the maximum score (9). Independently of the cultivation method applied, no significant differences were identified in each lettuce zone. This demonstrated that the initial sensorial characteristics remained unaltered although both lettuce heads were grown under different soil managements.

ANOVA applied to OVQ data showed a significant CULTIVATION METHOD × DAY interaction (*P*<0.0001 for external, *P*=0.005 for middle, and *P*=0.0015 for internal zones). This proved that lettuce behaved differently under different cultivation methods during storage. For the external zone, a significant decrease (OVQ = -0.25 t + 9.75, R^2= 0.93, n = 12, *P*<0.0001) was observed in mulch lettuce after a 5-day induction period, during which no significant changes were found with regard to the different quality components of the outer leaves. The outer leaves of bare soil lettuce also decreased significantly (OVQ = -0.33 t + 9.32, R^2 = 0.99, n = 15, *P*<0.0001) in OVQ parameters, yet after a shorter induction period of 2 days.

Different sensory quality scores reflected the differences in the induction period and the rate of quality loss between mulch and bare soil lettuce after 19 days of storage. Whilst mulch outer leaves reached an OVQ score near commercial acceptability, the poor quality of bare soil outer leaves fell within rejection limits (**Fig. 6A**).

The pattern obtained for the middle zone was similar to that for external zone in bare and mulch lettuce. After an induction period the OVQ for both types of lettuce heads decreased significantly (*P*=0.0144 and *P*=0.0032 for mulch and bare soil, respectively). Once again, mulch lettuce had the longer induction period (8 days) compared with bare soil lettuce (2 days). At the end of storage, OVQ scores for middle leaves of mulch lettuce were above the acceptability limits, but middle leaves for bare soil presented rejection scores (**Fig. 6B**).

For both cultivation methods, lettuce internal zone showed an extended induction period (12 days) during which the OVQ scores remained high, and then fell significantly (*P*=0.0385 and *P*=0.0015) by the end of storage. The latest OVQ scores were above acceptability limits, being higher for mulch inner leaves in relation to bare soil (**Fig. 6C**).

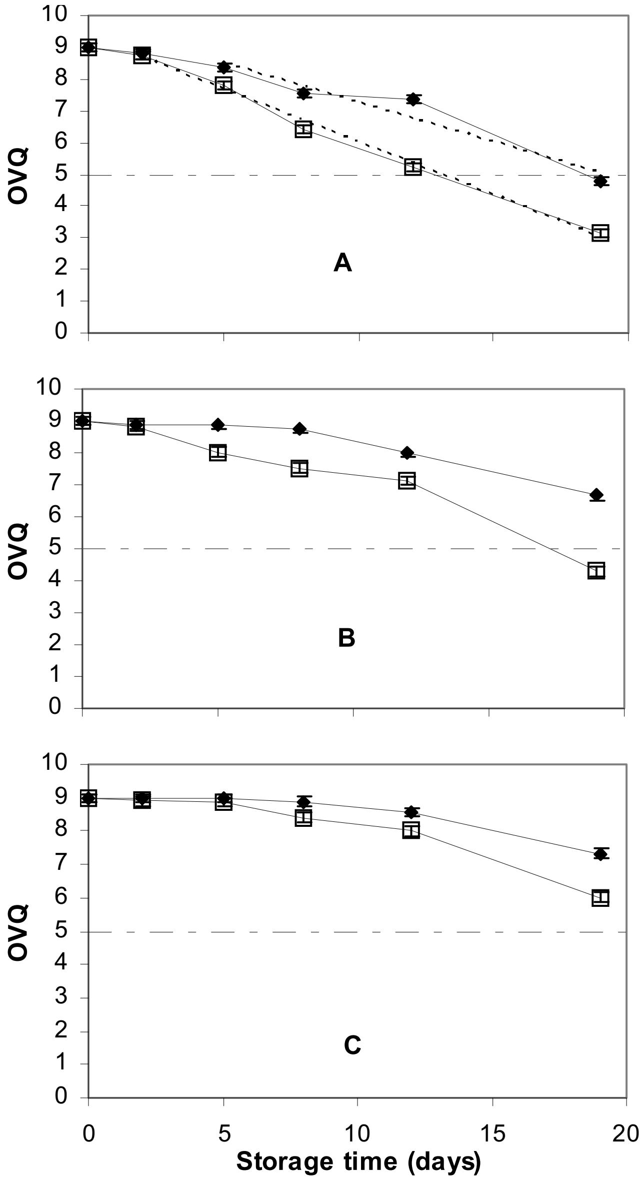

Fig. 6 Evolution of OVQ in external (**A**), middle (**B**), and internal (**C**) zones of lettuce heads cultivated under different methods: bare soil (□) and mulch (♦). The data correspond to the LSMEAN (least square means, means estimators by least squares). The dotted lines correspond to the tendency lines. The error bars represent the deviation associated with each LSMEAN.

In external zone, for both cultivation methods the first-change panelists perceived a decrease in leaf brightness. At day 8, outer leaves from bare soil lettuce presented moderate discoloration and slight browning in their external edges, presenting acceptable texture, reaching a mean OVQ score of 6.4. At this point in time, the panel rated the visual quality of middle and inner leaves as good for both cultivation methods, with slight defects not objectionable. OVQ score ranged from 7.5 (middle) and 8.7 (inner) for bare soil and 8.4 (middle) and 8.9 (inner) for mulch lettuce. At day 19, while the outer leaves from mulch lettuce presented moderate and objectionable ruptures in leaf tissues, suggesting an increase in their mechanical fragility, bare soil outer leaves showed an extended browning in their midribs, extreme mechanical fragility with complete loss of texture and a widespread surface discoloration. Such defects accounted for a final score of 4.8 for mulch lettuce, reaching

Table 1 Pearson's correlation coefficients (r) between OVQ and bacterial population (mesophilic, psychrotrophic, LAB, coliforms, and yeast and mold) corresponding to the different lettuce zones under both cultivation methods.

OVQ	Mesophilic	Psychrotrophic	LAB	Coliforms	Yeast and mold
External zone – BARE SOIL	-0.773	-0.743	-0.897*	0.396	-0.444
Middle zone – BARE SOIL	-0.427	-0.474	-0.797*	0.684	-0.182
Middle zone – MULCH	0.203	-0.013	0.058	-0.785*	-0.346
Internal zone – MULCH	0.523	-0.887*	-0.034	0.096	-0.445

* $P<0.05$, ** $P<0.01$.

the acceptability limit, and 3.15 for bare soil lettuce. At this time (day 19), mid leaves of mulch lettuce showed slight objectionable browning in their external edges, preserving their texture, which resulted in a mean score of 6.7. However, a low OVQ score (4.3) was registered for bare soil lettuce, in which the presence of moderate browning in the midribs affected its quality, rendering it unmarketable. For both cultivation methods, the internal zone maintained its good color, brightness and texture features, reaching OVQ scores above the acceptability limits by the end of the storage time.

According to OVQ scores, the shelf life was 19 and 12 days for the external zone for mulch and bare soil lettuce heads, respectively. Regarding the middle zone, shelf life exceeded 19 days for mulch and 17 days for bare soil. For both cultivation methods, the internal zone shelf life was above 19 days.

Correlation analysis

Table 1 represents Pearson's correlation coefficients between OVQ and the different populations analyzed, for each lettuce zone under different cultivation methods.

For mulch lettuce, OVQ did not correlate with any bacterial population in the external zone throughout the complete storage time. For the external zone of bare soil lettuce, OVQ correlated negatively with lactic acid bacteria (r = -0.897, *P*=0.015). Regarding the middle zone, mulch OVQ negatively correlated with total coliforms (r = -0.785, *P*=0.045), while bare soil correlated negatively with lactic acid bacteria (r = -0.797, *P*=0.044). For the internal zone of mulch lettuce, changes in the OVQ correlated only with psychrotrophic bacteria (r = -0.887, *P*=0.019). In bare soil lettuce, OVQ did not correlate with any bacterial population up to day 19 (**Table 1**).

DISCUSSION

In the present work two facts were analyzed. Native microbial initial counts and their evolution across storage of lettuce cultivated under two different methods were analyzed. In addition, overall visual quality evolution along storage was determined and correlation analysis between each bacterial population and OVQ were analyzed. Besides the cultivation method utilized, several factors could account for the differences in OVQ evolution in lettuce zones, including higher exposure of outer leaves to environmental factors (light and oxygen), likely to speed-up deterioration; variations in phenolic metabolism among zones, which could denote differential browning potentials; different levels of phenylalanine ammonia lyase activity in each zone; and differences in CO_2 sensitivity.

Rate of quality loss was different depending on the cultivation method, a fact detected in each lettuce zone. The different responses of microbial population to refrigerated storage, as a result of their pre-harvest history, have had technological implications on fresh produce handling. From a sensory quality viewpoint, the lack of or limited bacterial increment observed in mulch lettuce during refrigerated storage was beneficial. On the other hand, except for coliforms, bacterial populations from bare soil lettuce presented, at least, a growth phase during the refrigerated storage. Such bacterial growth, even under low temperature conditions, would indicate that microorganisms get energy and nutrients from the substrate vegetable, lowering lettuce quality and causing bacterial decay.

The differences revealed for each cultivation method (initial microbial counts, evolution and tolerance to refrigeration temperatures of some microbial population under study) played a singular role in sensorial quality and shelf life of lettuce. The higher initial counts yielded by mulch lettuce could be explained by the microclimate generated by the plastic mulch around the plant. The extent to which soil mulching with plastic films increases soil temperature depends on soil characteristics, mulch properties and environmental conditions (Gutkowsky and Terranova 1991). Soil temperatures under black plastic mulch are generally around 1.7-2.8°C higher during the daytime if compared to those of bare soil (Lamont 1999; Fontanetti Verdial *et al*. 2001). Surface radiation budget is also modified, thereby decreasing soil water loss (Liakatas *et al*. 1986). In this way, lettuce heads grown under mulch conditions were exposed at higher temperature and moisture regimens than bare soil lettuce heads.

In addition, the conditions under which vegetables are grown led to different responses to low temperatures storage in indigenous microflora. The fact that mulch lettuce microflora evolved under a more benign and controlled environment than that of bare soil lettuce would render it more susceptible to adverse conditions under storage. The microorganisms that proliferated under more changing conditions during pre-harvest, such as bare soil lettuce, may have developed greater resistance to stress factors like low temperatures. Bacteria can encounter a variety of physical adverse conditions during their lifetime, and their survival relies on the induction of specific or general protection mechanisms. Microorganism's adaptation to hostile environmental conditions has the potential to alter cellular physiology so much so that the organism becomes more resistant to further stress (Bunning *et al*. 1990).

It is worth mentioning that despite the higher initial microbial counts obtained for mulch cultivated lettuce with respect to bare soil lettuce, no initial differences in the sensorial characteristics of both lettuce types were registered. In that regard, Zagory (1999) indicated that, in many cases, total bacterial numbers bear little or no relationship with product quality or shelf life. Yet the storage behavior of native microflora in both lettuce heads types greatly differed in response to low temperatures. In this way the initial bacterial population numbers were not directly associated with lettuce quality or shelf life, however microbial evolution during storage had a direct impact on it. In bare soil lettuce, the bacterial population with highest impact on the sensorial quality of lettuce leaves was lactic acid bacteria. The decrease in overall visual quality correlated with the increase in LAB population in the three lettuce zones. The role that LAB plays in keeping the quality of vegetables remains unclear. Carlin *et al*. (1990) have associated LAB with the spoilage of specific commodities; Tirilly and Thouvenot (1994) reported that *Lactobacillus* spp. was one of the most frequent microorganisms responsible for plants alteration. On the other hand, Brocklehurst *et al*. (1987) claimed that LAB are not necessarily destructive to plant tissue; and Breidt and Fleming (1995) have advocated that LAB act as biocontrol agents in minimally processed refrigerated foods. Before drawing any conclusion in this regard, it should be noticed that the results from Pearson's correlation analysis indicate that these two events (the increase in LAB population and the decrease in overall visual quality throughout storage) are not causative but concurrent phenomena.

Nevertheless, the LAB species involved should be identified if the interest is in promoting or arresting their growth during bare soil lettuce storage.

OVQ in external and internal zones were significantly correlated with psychrotrophic bacteria population in mulch lettuce, though differently. While the correlation was positive for external zone, was negative for internal one. Once more, the role that psychrotrophic microorganisms play in keeping vegetable quality is uncertain. Some authors reported that psychrotrophic bacteria were associated with quality loss (Maxcy 1982), whereas others have connected them with high-quality ratings (Labadie and Dousset 1988; Barriga *et al*. 1991).

OVQ from the mulch middle zone was negatively correlated with total coliforms. Coliforms are a group of bacteria indicators related to "quality" or "safety" of common plant and root colonizers, including plant pathogens, human pathogens and a diverse group of soil and plant residents. While studying physicochemical, microbial and sensorial parameters as indexes to assess minimally-processed carrots quality, Lavelli *et al*. (2006) found that total coliforms reached the concentration threshold quicker when compared to other microbial population, thereby proposing this bacterial population as estimators of carrot sticks shelf-life.

CONCLUSION

This research demonstrates that the cultivation method (bare soil and mulch) introduces differences in the initial counts, evolution and tolerance to refrigeration temperatures for native microflora of Butter lettuce. Furthermore, the evolution of overall visual quality and shelf life depends on this pre-harvest factor. In this way, the proposed objectives were accomplished.

We found higher initial counts for all microbial populations in mulch lettuce and this fact could be explained by the microclimate generated by the plastic mulch around the plant when it has not yet been harvested.

Most bacterial populations from lettuce grown in mulch presented a decline or little growth under refrigerated storage. On the other hand, with the exception of coliforms, the populations from lettuce grown on bare soil presented growth during refrigerated storage.

The different responses of microbial population to refrigerated storage as a result of their pre-harvest history had a direct impact on the overall visual quality. Also critical in ensuring an extended lettuce shelf life is the ability of certain microbial population to adapt to hostile environmental conditions by virtue of resistance mechanisms previously developed during pre-harvest agricultural practices. Hence, the first handling step to assure a successful post-harvest vegetable management would imply the selection of pre-harvest conditions favoring the colonization of sensible microflora.

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