Effect of Hexanal on the Shelf Life of Fresh Apple Slices

Rosalba Lanciotti,*[,†] Maria Rosaria Corbo,[†] Fausto Gardini,[‡] Milena Sinigaglia,[†] and Maria Elisabetta Guerzoni[‡]

Istituto di Produzioni e Preparazioni Alimentari, Università degli Studi di Bari (sede di Foggia), via Napoli 25, 71100 Foggia, Italy, and Dipartimento di Protezione e Valorizzazione Agroalimentare, Università degli Studi di Bologna, via S. Giacomo 7, 40126 Bologna, Italy

In this work the effects of hexanal, as a component of packaging atmosphere, on the shelf life of and evolution of naturally occurring microbial populations in fresh apple slices during storage at 4 and 15 °C were evaluated. Although hexanal had no bactericidal effects, in all conditions considered, this volatile molecule significantly extended the shelf life. In fact, the presence of hexanal in the storage atmosphere (at 4 °C) totally inhibited mesophilic bacteria and considerably prolonged the lag phase of psychrotrophic bacteria. Also, at 15 °C, hexanal strongly inhibited molds, yeasts, and mesophilic and psychrotrophic bacteria. Moreover, hexanal led to a yeast selection favoring species having a reduced spoilage potential due to their prevalent respiratory activity. When added to a modified atmosphere (70% N_2 and 30% CO_2), this molecule was also very effective in preventing browning reactions for at least 16 days at 15 °C. No changes in hue angle values were observed in samples packaged in modified atmosphere with hexanal, even after 16 days of storage at 4 °C.

Keywords: *Hexanal; minimally processed fruits; modified atmosphere; shelf life*

INTRODUCTION

Over the past few years, the demand for minimally processed fruit and vegetables has increased considerably, mainly attributable to features such as freshness and commodity. A shelf life of several days under refrigerated conditions is required for feasible transport and retail. However, the presence of cut surfaces and damaged plant tissues, the minimal processing able to ensure microbial stability, the active metabolism of the plant tissues, and the confinement of the final product make minimally processed fruits and vegetables more perishable than the raw materials (Nguyen and Carlin, 1994). In particular, activation of enzymatic systems can lead to changes of color, softening, flavor modification, and loss in nutritional value (Svensson, 1977; Nicoli et al., 1994). Moreover, the destruction of tissue and subsequent release of nutrients enhance growth of naturally occurring microorganisms. In fact, mesophilic bacteria counts of 10^3-10^6 CFU/g are frequent in minimally processed vegetables analyzed immediately after processing (Nguyen and Carlin, 1994; Guerzoni et al., 1997a). In retail outlets and in catering establishments, the mesophilic bacteria counts are more variable, ranging between 10^3 and 10^9 CFU/g (Nguyen and Carlin, 1994). Moreover, the presence of potential foodborne pathogens such as *Listeria monocytogenes*, *Yersinia enterocolitica*, *Escherichia coli*, and *Aeromonas hydrophila* is well documented (Brocklehurst et al., 1981; Archer and Kvenberg, 1988; Beaufort et al., 1992; Nguyen and Carlin, 1994). Most of the studies concerning the evaluation of shelf life and safety are referred to ready to use vegetables, which are characterized by longer biochemical (Nicoli et al., 1997) and different spoilage patterns than minimally processed fruits (Guerzoni et al., 1996).

Actually, the safety of ready to eat fruit and vegetable salads is based on maintaining the chill chain and hygienic practices, which are difficult to implement and control. The marketing of minimally processed fruits and vegetables is expected to increase, and the degree of safety obtained with the currently applied preservation methods seems to be insufficient for an expanded market (Carlin and Nguyen, 1997). In recent years there has been considerable pressure by consumers to reduce or eliminate chemically synthesized additives in foods. On the other hand, the interest in the possible use of natural alternatives as food additives to prevent bacterial and fungal growth and to extend the shelf life of foods has notably increased. Many naturally occurring compounds such as phenols, aldehydes, and organic acids present in plant extracts and in spices showed antimicrobial effects (Wilson and Wisniewski, 1989; Song et al., 1996; Caccioni et al., 1997; Gardini et al., 1997). Moreover, Caccioni et al. (1997) and Gardini et al. (1997) reported that the antimicrobial effects of molecules characteristic of apple flavor, such as esters, aldehydes, alcohols, and terpenes, were dependent on their vapor pressure rather than on their whole concentration in the system. The dependence of toxicity on vapor pressure, as proposed by Guerzoni et al. (1994) and Guerzoni et al. (1997b), could be exploited for packaged foods and for the generation of special atmospheres in unrefrigerated or refrigerated rooms.

In particular, six-carbon aldehydes are dominant compounds released by plant tissue through the lipoxygenase pathway after damage (Hildebrand et al., 1988). Their toxicity protects the wounded area from microorganisms causing decay. In particular, the effectiveness of hexanal as a metabolizable fungicide as well as the enhancement of the aroma production by the interconversion of hexanal to other aroma volatiles in minimally

* Author to whom correspondence should be addressed (e-mail lanciott@kaiser.alma.unibo.it).

† Università degli Studi di Bari.

‡ Università degli Studi di Bologna.

10.1021/jf990611e CCC: $18.00
Published on Web 10/09/1999

Table 1. Comparison between Gompertz Parameters for Psychrotrophic Bacteria at 4 and 15 °C

	Gompertz parameters[a]									
	4 °C					15 °C				
packaging	k	A	μ_{max}	λ	R^{2b}	k	A	μ_{max}	λ	R^2
under vacuum	0.80	5.56	0.42	1.34	0.982	0.80	5.90	0.75	1.62	0.992
modified atmosphere	0.80	3.06	0.44	7.36	0.990	0.80	3.25	0.53	7.04	0.994
modified atmosphere with hexanal	0.80	2.35	0.40	7.98	0.985	0.80	2.86	0.69	8.07	0.979
ordinary atmosphere	0.80	1.94	0.11	4.69	0.989	0.80	6.31	0.66	6.22	0.961
ordinary atmosphere with hexanal	0.80	0.31	0.31	7.69	0.992	0.80	3.78	0.52	7.07	0.983

[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 (Δ log[CFU/g]/day); λ, lag phase (days). [b] Correlation coefficient.

processed apples has been studied by Song et al. (1996, 1997) and Guerzoni et al. (1997).

In this work the effects of hexanal, as a component of packaging atmosphere, on the shelf life and on the evolution of naturally occurring microbial populations in fresh apple slices during storage at 4 and 15 °C were evaluated.

MATERIALS AND METHODS

Apple-Based Salad Preparation. Granny Smith apples were hand washed with drinkable water having, according to Italian law, 0.2 mg/L of free chlorine. They were then peeled and sliced with sharp knives. The slices were pretreated, for 15 min, with a solution containing 0.2% (w/w) of citric acid and 1.0% (w/w) of ascorbic acid and then packaged in high-barrier plastic bags [nylon/30 mm nylon and 120 mm of polyethylene (Tecnovac, San Paolo D'Argon, Bergamo, Italy)] by means of S100-Tecnovac equipment. The film permeabilities to oxygen, carbon dioxide, and vapor were 2.53, 7.11, and 0.014 g/100 cm^2/day. The samples were packaged as follows: under vacuum, ordinary atmosphere, modified atmosphere (70% N_2, 30% CO_2), ordinary atmosphere with hexanal (0.15 mmol/100 g), and modified atmosphere (70% N_2, 30% CO_2) with hexanal (0.15 mmol/100 g). After packaging, the samples were stored at 4 and 15 °C. The evolution of hexanal in the headspace as well as its conversion in other aroma compounds, during the storage, was gas chromatographically determined according to the method of Gardini et al. (1997).

A simple, nonstructured, organoleptic evaluation was performed by five people when the samples were opened for analyses.

Isolation and Enumeration of Microorganisms. The media and the conditions used were as follows: plate count agar (PCA; Biolife, Milano, Italy) incubated at 5 °C for a week or at 37 °C for 48 h for psycrotrophic bacteria and mesophilic bacteria, respectively; MRS agar (Biolife) incubated at 30 °C for 4 days under anaerobiosis for lactic acid bacteria; Sabouraud dextrose agar (Biolife) incubated at 28 °C for 4 days for yeasts; and malt extract agar (MEA, Biolife) incubated at 25 °C for 48 h for molds.

Microbiological data are the average of at least four repetitions. The variability coefficients, expressed as the percentage ratio between the standard deviation and the mean value, were <7%.

The cell load data, collected during the storage of the products, were modeled according to the Gompertz equation as modified by Zwietering et al. (1990)

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

where y is the log[CFU/g], k is the initial level of the dependent variable to be modeled, A is the maximum bacterial growth attained at the stationary phase, μ_{max} is the maximal growth rate (Δ log[CFU/g]/day), λ is the lag time (days), and t is the time.

The experimental data were modeled through the nonlinear regression procedure of the statistic package Statistica per Windows (Statsoft, Tulsa, OK).

Identification of Microorganisms. *Bacteria.* For the identification three colonies of each different bacterial morphological type were selected from the primary cultures and kept on PCA (Biolife) at 4 °C until they were identified. All bacteria strains were grouped on the basis of staining reaction, catalase test, oxidative−fermentative metabolism of glucose, motility reaction, cell shape, and spore formation by heating cultures at 80 °C for 10 min and successive plating on PCA according to the method of Collins et al. (1989). The isolates were identified at the species level, using the appropriate API identification system (BioMerieux, Marcy l'Etoile, France).

Yeasts. For the identification three colonies of each different yeast morphological type were selected from the primary cultures and kept on Sabouraud dextrose agar (Biolife) at 4 °C until they were identified. The isolates were characterized according to the method of van der Walt and Yarrow (1984) and by using the API ATB ID32C system (BioMerieux). Identification was carried out by comparing the test results with the tables of Kurtzman and Fell (1998).

Polyphenol Oxidase Activity. The enzymatic activity was measured on an enzymatic extract of sliced and homogenized apples, according to the method proposed by Nicoli et al. (1991). The homogenization was carried out in the presence of 0.5 M phosphate buffer (50:50 w/w) at pH 7 containing 5% (w/w) polyvinylpyrrolidone. Polyvinylpyrrolidone was used to eliminate the polyphenolic fraction of apples. The polyphenol oxidase activities were assayed at 475 nm by means of a Beckman DU 640 spectrophotometer (Beckman, Fullerton, CA) in 3 mL of incubation medium containing 100 μL of enzymatic extract, 5×10^4 M L-DOPA, and 0.1 M phosphate buffer at pH 4. The rate of enzymatic reaction was expressed as Δabsorbance/min.

Color Measurements. The color analysis was carried out on sliced apples. The changes in color during the storage at 4 and 15 °C were monitored by colorimetric measurements using a tristimulus colorimeter Chromameter-2 reflectance (Minolta, Japan) equipped with a CR-300 measuring head. The instrument was standardized against a white tile before each determination. Color was expressed as L and hue angle values ($\tan^{-1}$ b^*/a^*) as previously described by Nicoli et al. (1994). Data are the average of at least five repetitions. The variability coefficients, expressed as the percentage ratio between the standard deviation and the mean value, were <5%.

RESULTS AND DISCUSSION

Effects of Atmosphere Composition and Storage Temperature on the Growth of the Naturally Occurring Microorganisms. During refrigerated storage (4 °C) and under abuse conditions (15 °C) the growth of five microbial groups, such as total psychrotrophic bacteria, total mesophilic bacteria, lactic acid bacteria, yeasts, and molds, was evaluated. The cell load data were modeled according to the Gompertz equation as modified by Zwietering et al. (1990).

The Gompertz parameters relative to psychrotrophic bacteria in samples stored at 4 and 15 °C are reported in Table 1. The addition of hexanal to the storage

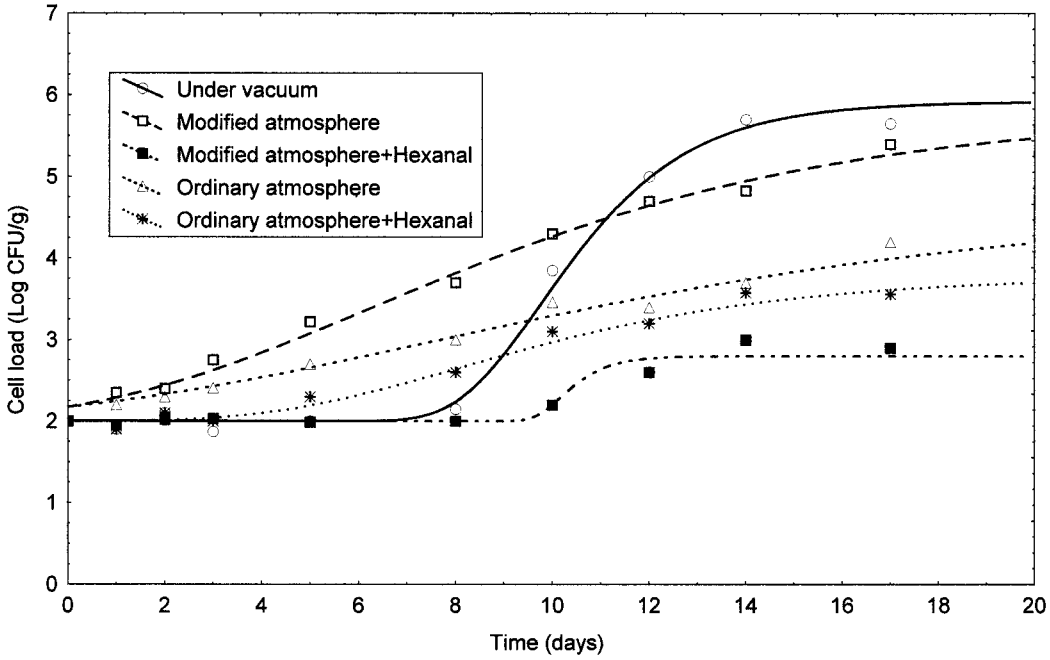

Figure 1. Evolution of yeast cell load during storage at 15 °C in relation to atmosphere composition.

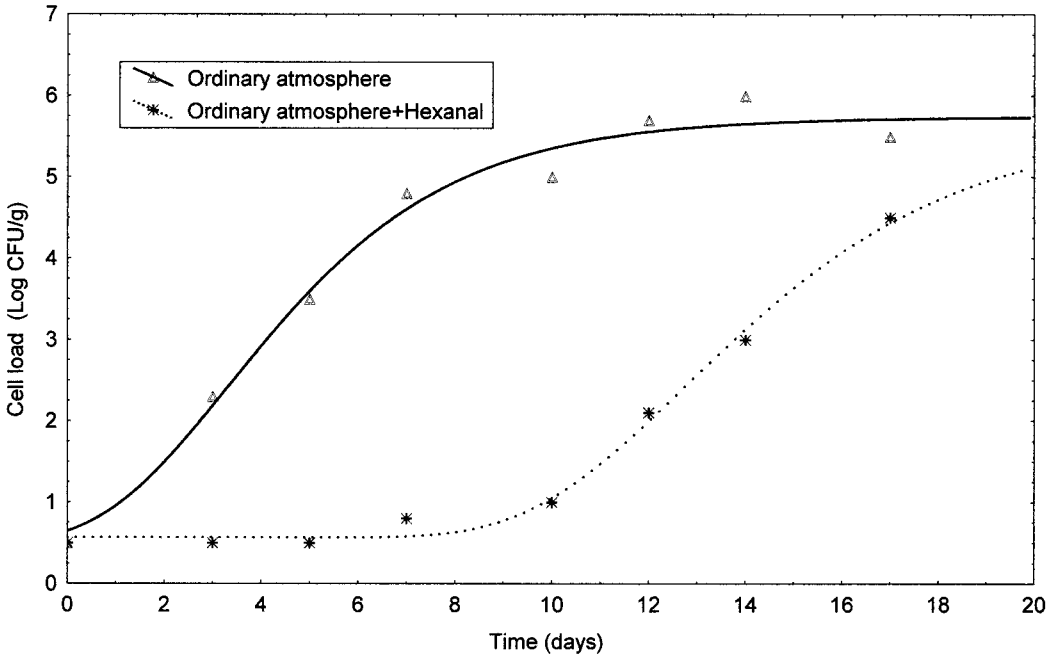

Figure 2. Evolution of mold cell load during storage at 15 °C in ordinary atmosphere and in ordinary atmosphere with hexanal.

atmosphere determined a significant reduction of the maximum cell load attained at the stationary phase, at both 4 and 15 °C, as well as the extension of the incubation phases.

The yeasts were not able to grow at 4 °C, whereas at 15 °C, probably favored by the selective action of the low pH value of apples, they had, as shown by Figure 1, very short incubation phases under both ordinary and modified atmospheres. Hexanal displayed a strong inhibition on yeasts as indicated by the great extension of the lag phase and the reduction of the maximum growth level. Hexanal proved to be active also against molds (Figure 2). However, this microbial group was an effective spoilage agent only in the samples stored at 15 °C.

The evolution over time of mesophilic bacteria in relation to atmosphere composition and storage temperature is reported in Figures 3 and 4. At 4 °C mesophilic bacteria were able to proliferate only in the samples stored under vacuum. In the other conditions this microbial group showed a viability reduction. The addition of hexanal accelerated the viability decline. In the samples stored at 15 °C the mesophilic bacteria grew in ordinary atmosphere and modified atmosphere and under vacuum. On the other hand, in the presence of hexanal, viability decreases to 2.5 and 1.8 log CFU/g, in the samples stored in ordinary and modified atmospheres, respectively, were observed. However, in these samples after ~11 days of storage an increase of cell numbers to 3.5 log CFU/g was observed. The viability

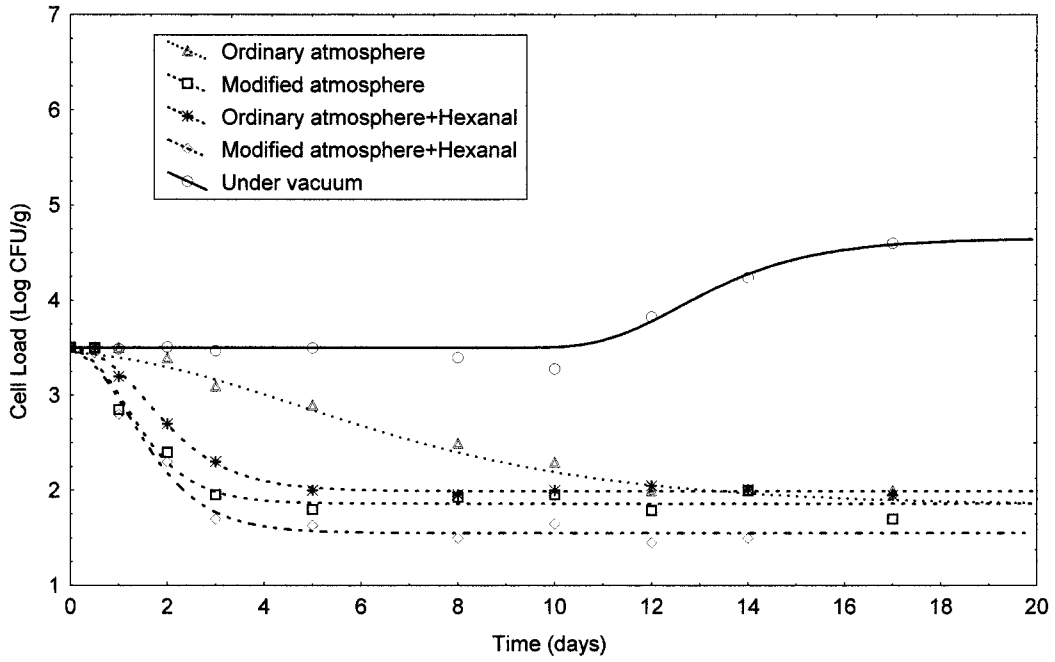

Figure 3. Evolution of mesophilic bacteria cell load during storage at 4 °C in relation to atmosphere composition.

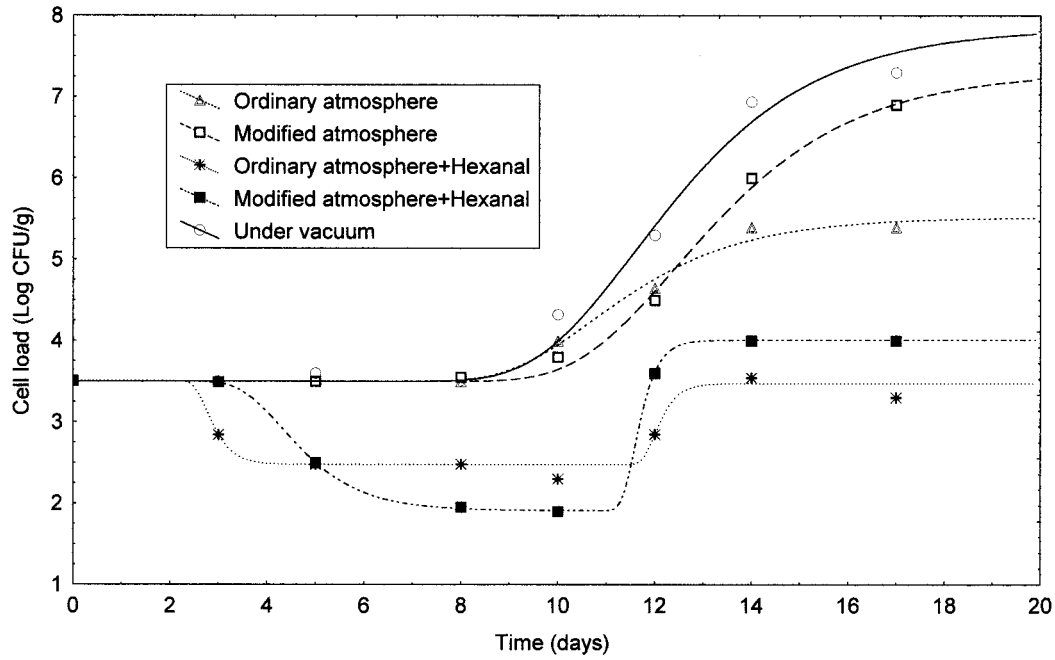

Figure 4. Evolution of mesophilic bacteria cell load during storage at 15 °C in relation to atmosphere composition.

loss of mesophilic bacteria at 4 °C can be attributed to the combined action of low temperature, low pH value, and CO_2 tissue permeation as well as to CO_2 increased solubility, at 4 °C with respect to 15 °C, in the thin water layer surrounding the fruit tissues. Such behavior has not been reported in the literature concerning minimally processed vegetables, especially for higher pH values.

Effects of Atmosphere Composition and Storage Temperature on the Microbial Population. Considering an apple as an ecosystem, it can be expected that each unit operation including washing, peeling, slicing, etc., modifies the composition of the microbial population. It is well-known that the initial microflora present on the fruit and vegetable surface mainly included molds such as *Penicillium* spp., *Rhizopus* spp., *Alternaria* spp., and *Trichoderma* spp. and yeasts such as *Metschnikowia* spp., *Debaryomyces* spp., and *Candida* spp. (Ngyyen and Carlin, 1994). Under our experimental conditions, such naturally occurring genera and species were replaced, immediately after washing, peeling, and slicing, by Gram-positive bacteria. In particular, *Staphylococcus* spp. and *Bacillus amyloliquefaciens* became the most frequent bacterial species, whereas *Cryptococcus albidus* and *Debaryomyces hansenii* were the most commonly occurring yeasts. Packaging and refrigerated storage under the different atmospheres determined further modification of the composition of the microbial population. The frequencies of the different species changed during storage in relation to both atmosphere composition and storage temperature. In particular, in the products stored in ordinary atmosphere, at both 4 and 15 °C, the bacterial population was

Table 2. Composition of Aerobic Mesophilic Bacteria in Ordinary Atmosphere with Hexanal during Storage at 4 °C

processing and storage phase	frequency of identified species[b]
before peeling	*a*
immediately after packaging	93%[b] *Staphylococcus* spp. 7% *B. amyloliquefaciens*
1−10 days of storage	35% *Staphylococcus* spp. 30% *B. amyloliquefaciens* 35% *Pediococcus* spp.
>10 days of storage	30% *R. aquatilis* 70% *Acinetobacter* spp.

[a] Before peeling are not isolated total aerobic mesophilic bacteria. [b] Calculated as [(number of isolated species/total number of species isolated) × 100].

represented principally by *Bacillus* spp. In particular, at 4 °C the most frequent species (having a frequency of 44%) during the first 10 days of storage was *B. amyloliquefaciens*. The other bacteria identified belonged to *Bacillus macerans* and *Bacillus licheniformis* as well as to *Enterobacter agglomerans* (having frequencies of 22, 22, and 12%, respectively). An extended storage, over 10 days, of the products determined the appearance of *Acinetobacter* spp., which attained a frequency of 60%.

The inclusion of hexanal in the atmosphere of the samples stored at 4 °C resulted in a higher homogeneity of the bacterial population and favored the growth of lactic acid bacteria belonging to *Pediococcus* spp. (Table 2). Similar results were obtained in the samples stored at 15 °C. Also, the packaging under modified atmosphere made the bacterial population quite homogeneous during the storage at both 4 and 15 °C. The addition of hexanal to the modified atmosphere (30% of CO_2 and 70% of N_2) stimulated the proliferation of Gram-negative bacteria and particularly *Rahnella aquatilis*. In fact, *R. aquatilis*, associated with *E. agglomerans* and *Erwinia herbicola*, is the most frequent of the *Enterobacteriaceae* in minimally processed fruit and vegetables (Nguyen and Carlin 1994; Bennik et al., 1998). Moreover, O'Conner-Shaw et al. (1995) isolated *R. aquatilis*, *Enterobacter cloacae*, and *Klebsiella pneumoniae* in mango-based salads. The specificity of these microorganisms to this kind of product has been attributed to their physiology and to the availability of specific carbon sources such as inositol and pinitol.

Under our experimental conditions, the growth of opportunistic species such as *R. aquatilis* and *E. agglomerans* was favored by the reduction of the O_2 level in the storage atmosphere (under vacuum, modified atmosphere, and modified atmosphere with hexanal added).

Metschinikowia pulcherrima, Debaryomyces hansenii, Candida spp., and *Cryptococcus* spp. were the most representative species having frequencies of 20, 55, 15, and 10%, respectively. Many different yeast species, including *Candida* spp., *Cryptococcus* spp., *Rhodotorula* spp., *Thricosporon* spp., *Pichia* spp., and *Torulaspora* spp., have been identified on minimally processed fruits and vegatables (Marchetti et al., 1992). The source of contamination is generally attributed to fruit and vegetable raw materials.

Processing operations resulted in significant modifications of the yeast population. In fact, immediately after packaging *C. albidus* and *D. hansenii* were the most frequently isolated species (65 and 35%, respectively). At 4 °C, the occurrence of these species did not change during the storage time and were not affected by the atmosphere composition. On the other hand, after 10−12 days of storage at 15 °C, fermentative species such as *Pichia subpelliculosa* and *Candida versatilis* were selectively favored by the packaging in ordinary and modified atmospheres without hexanal added. The presence of hexanal in the atmosphere did not allow this selection; therefore, *D. hansenii* and *C. albidus* predominated during the storage time.

Effects of Atmosphere Composition on Color and Polyphenol Oxidase Activity. The effects of the different types of atmosphere on the browning reaction of apple slices during the storage at 4 and 15 °C are shown in Figure 5. It can be noted that the hexanal, when added to a modified atmosphere (70% N_2 and 30% CO_2), was very effective in preventing browning reaction for at least 17 days at 15 °C. When stored at 4 °C, no changes in hue angle values were observed in samples packaged in modified atmosphere with hexanal, even after 17 days of refrigeration. Otherwise, a marked decrease in hue angle values was observed, within 8 days, in samples stored in ordinary atmosphere, and the retention of the original color, in agreement with previous literature studies (Nicoli et al., 1994), was higher using vacuum and modified atmosphere packaging. On the other hand, the inhibition of enzymatic browning can be performed through direct action on the enzyme (heat, aromatic carboxyl acids, halide ions), on secondary reactions affecting the *o*-quinones (ascorbic acid, cysteine, etc.), or by removing the two substrates involved, that is, oxygen and phenols (Nicolas et al., 1994). Also, the *L* values had the same behavior as hue angle values. In fact, no significant decrease over time of *L* values was observed in the samples stored at 4 °C in modified atmosphere supplemented with hexanal, whereas the highest *L* value decreases were detected in samples stored in ordinary atmosphere (data not shown).

The residual enzymatic activities of polyphenol oxidase, expressed as lag phase, determined on apple slices stored under the different atmospheres at 4 °C are reported in Table 3. These results indicated that the combination of modified atmosphere and hexanal was very effective, delaying the enzymatic reaction also after the opening of the packages. However, after the lag phases, the enzymatic reaction started with a rate that was similar in all of the considered conditions. Similar data were obtained in the case of samples stored at 15 °C. These results highlight that the different atmospheres, including hexanal, have a temporary inhibiting activity at least up to the opening of pouches. The gas chromatographic analyses indicated that hexanal tended to disappear from the headspace of the samples within 2−3 days of storage, due to both its partition in the apple tissues and its conversion in hexanol and hexyl acetate (data not shown). According to Song et al. (1996), hexanal can be actively converted by apple tissues to other aroma volatiles such as hexanol and hexyl acetate. The conversion of hexanal to hexanol could be the key to understanding its effect on browning prevention. In fact, the aliphatic alcohols are regarded as inhibitors of the polyphenol oxidase (Valero et al., 1990). Their activity seems to be more related to the aliphatic chain than to alcoholic function. Otherwise, inhibition activity on polyphenol oxidase has never been attributed to aldehydes.

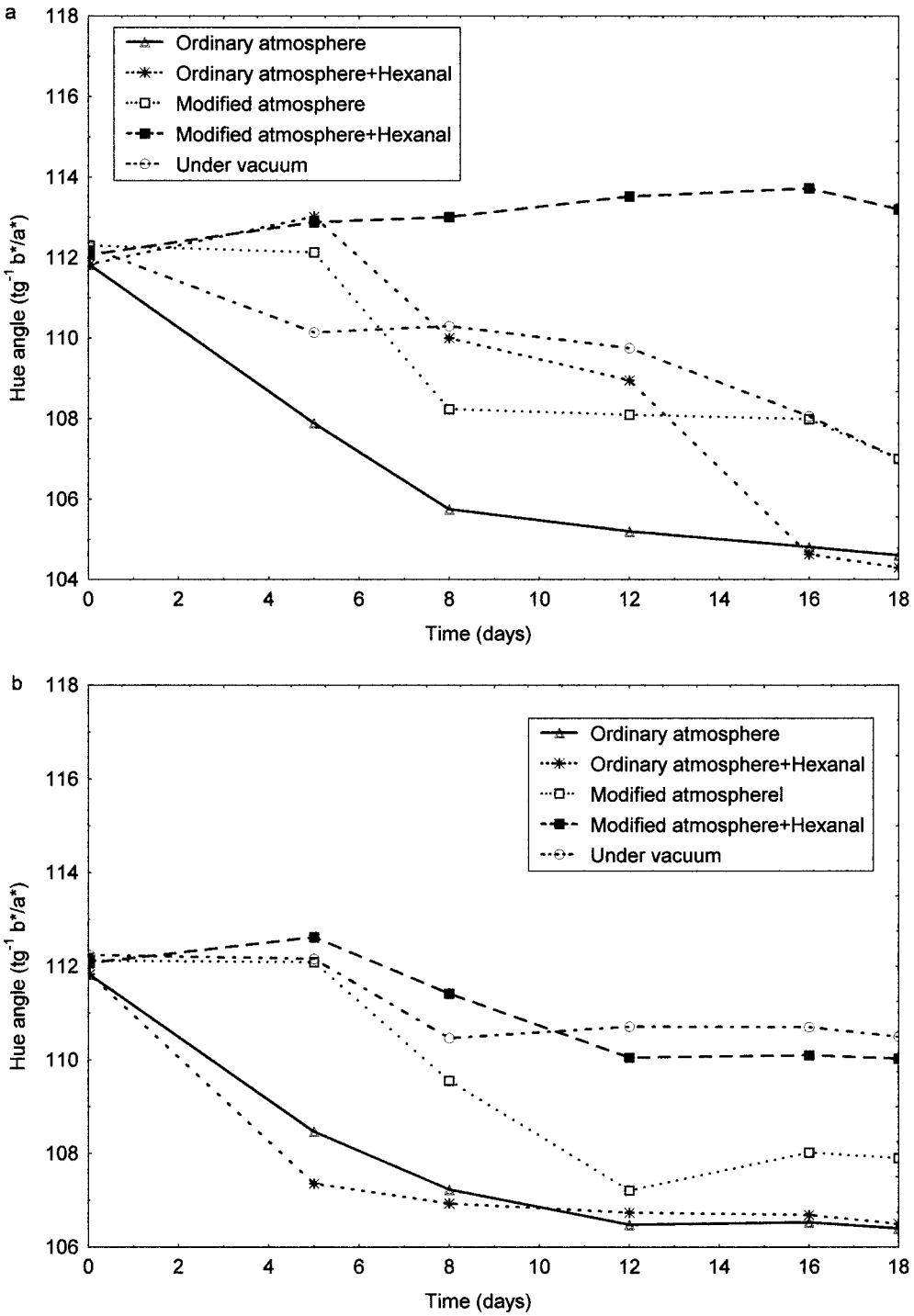

Figure 5. Changes in hue angle values during storage at 4 °C (a) and 15 °C (b) in relation to atmosphere composition.

In general, hexanal is regarded as an undesirable molecule because it is an oxidation indicator. On the other hand, although its concentration depends on variety and ripening phase, it is one of the molecules that characterize apple flavor. According to a nonstructured sensorial analysis, the samples under modified atmosphere containing hexanal could be recognized and positively appreciated for flavor and color retention.

CONCLUSION

Although hexanal had non bactericide effects, in all of the considered conditions this volatile molecule significantly extended the shelf life. In fact, the presence of hexanal in the storage atmosphere totally inhibited, at 4 °C, the mesophilic bacteria and considerably prolonged the lag phase of psychrotrophic bacteria. Also, at abuse temperature, the hexanal strongly inhibited molds, yeasts, and mesophilic and psychrotrophic bacteria. Moreover, the hexanal led to a yeast selection favoring species having a reduced spoilage potential due to their prevalent respiratory activity. The toxicity of hexanal, analogously to other volatile molecules, seems to be linked to its affinity with the microbial membranes.

The ability of a potentially active molecule to interact with the hydrophobic cell membrane can be regarded as the result of its intrinsic hydrophobicity, which

Table 3. Effect of Atmosphere Composition, during Storage at 4 °C, on the Residual Polyphenol Oxidase Activity[a] in Apple Tissues

	enzymatic lag phase (min)				
phase	OA[b]	OA + hexanal	MA	MA + hexanal	UV
before pretreatment[c]	4.0	4.0	4.0	4.0	4.0
after pretreatment	4.0	4.0	4.0	4.0	4.0
1 h after packaging	4.0	4.0	9.2	19.2	5.8
24 h after packaging	3.8	7.5	10.0	20.0	42.0
120 h of storage	4.2	4.2	10.0	29.1	42.0
192 h of storage	2.0	1.0	11.3	29.0	22.5
288 h of storage	2.5	0.0	6.7	26.0	35.5
384 h of storage	0.0	0.0	7.5	27.2	42.0

[a] As an indirect measure of the enzymatic activity, the time necessary to have, after the atmosphere removal, an increase in absorbance at 475 nm was taken. [b] OA, ordinary atmosphere; OA + hexanal, ordinary atmosphere with hexanal added (0.15 mmol/100 g of product); MA, modified atmosphere (70% N_2, 30% CO_2); MA + hexanal, modified atmosphere (70% N_2, 30% CO_2) with added hexanal (0.15 mmol/100 g of product); UV, under vacuum. [c] Fifteen minute treatment with a 0.2% (w/w) citric acid and 1.0 (w/w) ascorbic acid solution.

increases with the hydrocarbon chain length (Guerzoni et al., 1994), and its actual hydrophobicity, which provides an inverse measure of the water molecules surrounding its polar groups (Guerzoni et al., 1994, 1997b). Temperature rise increases the volatility and consequently the antimicrobial effects of molecules such as esters, aldehydes, terpenes, alcohols, and hydrocarbons (Walker et al., 1975; Walter et al., 1991; Caccioni et al., 1997; Gardini et al., 1997). Thus, the effect of temperature on the increase of vapor pressure and, consequently, of the toxicity, can compensate for the eventual interruption of the chilling chain.

Enzymatic browning of minimally processed apples has been regarded as one of the major problems affecting their production and commercial success. The inclusion in the modified atmosphere of 0.15 mmol of hexanal in 100 g of product increased, particularly at 4 °C, color stability of the sliced apples up to 17 days. To develop an appropriate strategy able to control the polyphenol oxidase activity, it is necessary to understand the action mechanism and the type of inhibition exercised by hexanal or its derivative hexanol. Another possible target of hexanal could be the phenyalanine ammonia lyase, which is involved in the biosynthesis pathway of the phenolic compounds. In fact, both mechanical injuries and ethylene presence enhance its activity. In agreement with Martinez and Withaker (1995), the control of this enzyme and thereby the biosynthesis of phenolic compounds at the site of injury to the fruit are important in the control of enzymatic browning caused by processing. Although these preliminary results and previous literature studies (Song et al., 1996, 1997) provide evidence for the positive effect of hexanal on the flavor of fresh apple slices, deeper investigations are necessary to assess the organoleptic features also in relation to apple variety, storage and transport conditions, and film package permeability.

LITERATURE CITED

Archer, D. L.; Kvenberg, J. E. Regulatory significance of *Aeromonas* in foods. *J. Food Saf.* **1988**, *9*, 53−57.

Beaufort, A.; Poumeyrol, G.; Rudelle, S. Fréquence de contamination par *Listeria* et *Yersinia* d'une gamme de produits de 4ème gamme. *Rev. Gen. Froid*. **1992**, *82*, 28−32.

Bennik, M. H. J.; Vorstman, W.; Smid, E. J.; Gorris, L. G. M. The influence of oxygen and carbon dioxide on growth of prevalent *Enterobacteriaceae* and *Pseudomonas* species isolated from fresh and controlled-atmosphere-stored vegetables. *Food Microbiol.* **1998**, *15*, 459−469.

Brocklehurst, T. F.; Lund, B. M. Properties of pseudomonads causing spoilage of vegetables stored at low temperature. *J. Appl. Bacteriol*. **1981**, *50*, 259−263.

Caccioni, D. R. L.; Gardini, F.; Lanciotti, R.; Guerzoni, M. E. Antifungal activity of natural compounds in relation to their vapour pressure. *Sci. Aliments* **1997**, *17*, 21−84.

Carlin, F.; Nguyen, C. Control of spoilage and pathogenic microorganisms in minimally processed and mildly heat processed vegetables. *Proceedings of Joint Workshop COST 914-COST915 "Non Conventional Methods for the Control of Postharvest Disease and Microbiological Spoilage"*; 1997; pp 1−6.

Collins, C. H.; Lyne, P. M.; Grange, J. M. *Collins and Lyne's Microbiological Methods*; Butterworth: London, U.K., 1989.

Gardini, F.; Lanciotti, R.; Caccioni, D. R. L.; Guerzoni, M. E. Antifungal activity of hexanal as dependent on its vapor pressure. *J. Agric. Food Chem*. **1997**, *45*, 4297−4302.

Guerzoni, M. E.; Lanciotti, R.; Sinigaglia, M.; Anese, M.; Lerici, C. R. Influence of some selected ions on system water activity and on ethanol vapour pressure and its inhibitory action on *Saccharomyces cerevisiae*. *Can. J. Microbiol.* **1994**, *40*, 1051−1056.

Guerzoni, M. E.; Gianotti, A.; Corbo, M. R.; Sinigaglia, M. Shelf life modelling for fresh-cut vegetables. *Postharvest Biol. Technol*. **1996**, *9*, 195−207.

Guerzoni, M. E.; Gardini, F.; Corbo, M. E.; Lanciotti, R.; Sinigaglia, M. Modelling of growth of microorganisms on minimally processed fruits and vegetables in relation to technological and environmental conditions. *Proceedings of Joint Workshop COST 914-COST915 "Non Conventional Methods for the Control of Postharvest Disease and Microbiological Spoilage"*; 1997a; pp 15−20.

Guerzoni, M. E.; Nicoli, M. C.; Massini, R.; Lerici, C. R. Ethanol vapour pressure as a control factor during alcoholic fermentation. *World J. Microbiol. Biotechnol.* **1997b**, *13*, 11−16.

Hildebrand, D. F.; Hamilton-Kemp, C. S.; Legg, C. S.; Bookjans, G. Plant lipoxigenase: occurrence, properties and possiblke functions. *Curr. Top. Plant. Biochem. Physiol.* **1988**, *7*, 210−219.

Kuttzman, C. P.; Fell, J. W. *The Yeasts: A Taxonomic Study*; Elsevier: Amsterdam, The Netherldands, 1998.

Marchetti, R.; Casadei, M. A.; Guerzoni, M. E. Microbial population dynamics in ready to use vegetable salads. *Ital. J. Food Sci.* **1992**, *2*, 97−101.

Martinez, M. V.; Withaker, J. R. The biochemistry and control of enzymatic browning. *Trends Food Sci. Technol.* **1995**, *6*, 195−205.

Nguygen, C.; Carlin, F. The microbiology of minimally processed fresh fruits and vegetables. *Crit. Rev. Food Sci. Nutr.* **1994**, *34*, 371−401.

Nicolas, J. J.; Richard-Forget, F. C.; Goupy, P. M.; Amiot, M. J.; Aubert, S. Y. Enzymatic browning reactions in apple and apple products. *Crit. Rev. Food Sci. Nutr.* **1994**, *34*, 109−157.

Nicoli, M. C.; Elizalde, B. E.; Pitotti, A.; Lerici, C. R. Effect of sugars and Maillard reaction products on polyphenoloxidase and peroxidase in food. *J. Food Biochem*. **1991**, *15*, 169−184.

Nicoli, M. C.; Anese, M.; Severini, C. Combined effects in preventing enzymatic browining reactions in minimally processed fruits. *J. Food Qual.* **1994**, *17*, 221−229.

Nicoli, M. C.; Manzano, M.; Anese, M.; Lerici, C. R. Shelf life of ready to use fresh apple slices using different combined technologies. *Proceedings of Joint Workshop COST 914-COST915 "Non Conventional Methods for the Control of Postharvest Disease and Microbiological Spoilage"*; 1997; pp 33−39.

O'Conner-Shaw, R. E.; Gurthrie, J. A.; Dunlop, K. J.; Roberts, R. Coliforms in processed mango: significance and control. *Int. J. Food Microbiol*. **1997**, *25*, 51−61.

Song, J.; Leepipattanawit, R.; Beaudry, R. Hexanal vapour is a natural, metabolizable fungicide: inhibition of fungal activity and enhancement of aroma biosynthesis in apple slice. *J. Am. Soc. Horic. Sci.* **1996**, *121*, 937−942.

Song, J.; Deng, W.; Fan, L.; Verschoor, J.; Beaudry, R.; Gonry, J. R. Aroma volatiles and quality changes in modified atmosphere packaging. *Proceedings "Fresh-cut fruits and vegetables and MAP" Symposium*; University of California: Davis, CA, 1997; Vol. 5, p 19.

Svensson, S. Inactivation of enzymes during thermal processing. In *Physical, Chemical and Biological Changes in Food Caused by Thermal Processing*; Hoyem, T., Kvale, O., Eds.; Applied Science: London, U.K., 1977; pp 202−234.

Valero, E.; Varon, R.; Garcia-Carmona, F. Inhibiton of grape polyphenol oxidase by several aliphatic alcohols. *J. Agric. Food Chem.* **1990**, *38*, 1097−1103.

Van der Walt, J. P.; Yarrow, D. Y. Methods for isolation, maintenance, classification and identification of yeasts. In *The Yeasts: A Taxonomic Study*; Kreger-van Rij, N. J. W., Ed.; Elsevier: Amsterdam, The Netherlands, 1984; pp 45−104.

Walker, J. D.; Seesman, P. A.; Colwell, R. R. Effect of South Louisiana crude oil and no. 2 fuel oil on growth heterotrophic microorganisms, including proteolytic, chitinolytic and cellulolytic bacteria. *Environ. Pollut.* **1975**, *9*, 13−33.

Walter, U.; Beyer, M.; Klein, J.; Rehm, H. J. Degradation of pyrene by *Rhodococcus* sp. UW1. *Appl. Microbiol. Biotechnol.* **1991**, *34*, 671−676.

Wilson, C.; Wisniewski, M. E. Biological control of postharvest diseases of fruits and vegetables: an emerging technology. *Annu. Rev. Phytopathol.* **1989**, *27*, 425−441.

Zwietering, M. H.; Jongenberger, I.; Roumbouts, F. M.; van't Riet, K. Modelling of bacterial growth curve. *Appl. Environ. Microbiol.* **1990**, *56*, 1875−1881.

Received for review June 8, 1999. Revised manuscript received August 16, 1999. Accepted August 31, 1999.

JF990611E