Effects of Hexanal, *trans*-2-Hexenal, and Storage Temperature on Shelf Life of Fresh Sliced Apples

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

Istituto di produzioni e Preparazioni Alimentari, Università degli Studi 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 paper, the effects of hexanal and *trans*-2-hexenal, which are both natural molecules characterizing apple aroma, on the microbial population and on color retention of fresh sliced apples were evaluated. In particular, a central composite design (CCD) was developed to assess the individual and interactive effects of the chosen volatile molecules and storage temperatures on (i) the growth of the naturally occurring microflora, (ii) the evolution over time of an inoculated spoilage yeast (*Pichia subpelliculosa*), and (iii) the enzymatic browning reaction in minimally processed apples. The inclusion of hexanal and *trans*-2-hexenal in the storage atmosphere of apple slices determined a significant extension of shelf life also when *P. subpelliculosa* was inoculated at levels of 10^3 colony-forming units/g and abusive storage temperatures were used. In fact, the presence of these molecules in the packaging atmospheres considerably prolonged the lag phases of the inoculated yeast and reduced the growth potential of naturally occurring bacteria. Moreover, the addition to the modified atmosphere of low levels of the hexanal increased the color stability of the products up to 16 days.

Keywords: *Hexanal; trans-2-hexenal; minimally processed apples; shelf life*

INTRODUCTION

Ready to eat and ready to use refrigerated fruits have become an important area of potential growth in the rapidly expanding fresh-cut produce industry (Buta et al., 1999). However, deterioration of the fruit after minimal processing resulting from the presence of cut surfaces, the absence of treatments able to ensure microbial stability, and the active metabolism of plant tissue, as well as the confinement of the final products, make the minimally processed fruits more perishable than the original raw materials (Nguyen and Carlin, 1994). In particular, processing operations such as peeling, slicing, and shredding speed up deterioration of the raw materials through the destruction of plant cells, transferring the skin microflora to inner tissues and increasing the tissue respiration rate as well as the activation of enzymatic systems (Nicoli et al., 1994). Moreover, the destruction of tissue and the subsequent release of nutrients enhance the growth of naturally occurring microorganisms. The low pH of most fruits restricts the microflora to acid tolerant microorganisms (Brackett, 1987, 1994). Consequently, the recommended tests for assessment of sanitation and manufacturing practices for fruit are the enumeration of yeasts, molds, lactic acid bacteria, and Enterobacteriaceae (O'Connor-Shaw, 1994). Coliforms are part of the natural microflora of fruits and processing lines (O'Connor-Shaw, 1995). A variety of pathogenic microorganisms such as *Listeria monocytogenes*, *Salmonella* and *Shigella* spp., and enteropathogenic strains of *Escherichia coli*, *Aeromonas hydrophila*, *Yersinia enterocolitica*, and *Staphylococcus aureus* may be present on fresh fruits and in related minimally processed refrigerated products (Gould, 1992; Breidt and Fleming, 1997). Whether these pathogens grow and cause disease depends on the type of products, conditions of storage, and competing microflora.

Enzymatic browning of minimally processed apples has been regarded as the major problem affecting the utilization of the fruit (Buta et al., 1999). Browning is undesirable not only because of discoloration of the products but also because the associated reactions produce off-flavor. A great number of studies have been conducted to prevent browning, including treatment with reducing agents, acidulants, chelating agents, other chemicals, and heat (Martinez et al., 1995; Nicolas et al., 1994; Monsalve-Gonzalez et al., 1993; Nicoli et al., 1994; Svensson et al., 1977). The exploitation of natural compounds as inhibitors of enzymatic browning can meet the consumer preference for natural additives or ingredients. In particular, promising results were obtained with the combination of certain vegetable ingredients with each other, the dipping in pineapple juice, and the treatment with honey (Laurila et al., 1998; Oszmianski and Lee, 1990). Moreover, natural preservatives, for example, inhibitors produced by lactic acid bacteria, were proposed also as enzymatic browning inhibitors (Laurila et al., 1998). Besides, among the various natural compounds having antimicrobial activity, hexanal can be regarded as particularly promising in fresh apple slice based products. An enhancement of aromatic properties by its interconversion to other volatile compounds as well as significant extension of shelf life favoring the retention of the original color also under temperature abuse conditions was observed (Song

* Author to whom correspondence should be addressed (e-mail lanciott@kaiser.alma.unibo.it).
[†] Università degli Studi di Bari.
[‡] Università degli Studi di Bologna.

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et al., 1996, 1997; Lanciotti et al., 1999). According to Lanciotti et al. (1999), this volatile molecule, when included in the storage atmosphere of fresh sliced apples, totally inhibited, at 4 °C, the mesophilic bacteria and considerably prolonged lag phases of psychrotrophic bacteria. Also under temperature abuse conditions the hexanal strongly inhibited molds, yeasts, and mesophilic and psychrotrophic bacteria. Moreover, the hexanal induced a yeast selection favoring species having a reduced spoilage potential and inhibiting *Pichia subpelliculosa* and *Candida versatilis*, species responsible for off-flavor of fresh sliced apples packed in ordinary or modified atmospheres (Lanciotti et al., 1999).

In this paper, the effects of hexanal and *trans*-2-hexenal, which are both natural molecules characterizing apple aroma, on the microbial population and on color retention were evaluated. In particular, a three-variable five-level central composite design (CCD) was developed to assess the individual and interactive effects of the chosen volatile molecules and storage temperatures on (i) the selection and/or growth of the naturally occurring microflora, (ii) the evolution over time of an inoculated spoilage yeast (*Pichia subpelliculosa*), and (iii) the enzymatic browning reaction in fresh sliced apples. A more thorough knowledge of these aspects and their interaction can allow the exploitation of the results for the optimization of the atmosphere composition of minimally processed apples and extension of the shelf life also under unrefrigerated storage conditions.

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 free chlorine. They were then peeled and sliced with sharp knifes. 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 polyethylene (Tecnovac, Bergamo, Italy)—by means of S100-Tecnovac equipment. The film permeabilities to oxygen, carbon dioxide, and vapor were 1.25×10^{-3}, 2.79×10^{-3}, and 6.86 pmol s^{-1} m^{-2} Pa^{-1}. All of the samples were packaged in modified atmosphere (70% N_2, 30% CO_2). This atmosphere was chosen because, from previous literature reports (Lanciotti et al., 1999; Nicoli et al., 1994), it was determined to be appropriate for sliced fresh apples. In fact, these conditions allowed a good retention of the original color as well as a good microbial stability. The samples differed from one another in the inclusion in the modified atmosphere of different levels of hexanal and *trans*-2-hexenal and in the storage temperatures. Hexanal [H] and *trans*-2 hexanal [t-H] concentrations and storage temperature [T] were modulated according to a three-factor five-level CCD. The appropriate concentrations of hexanal and *trans*-2-hexenal were introduced in the atmosphere, before packaging, by means of 6 mm soaked diameter filter paper disks (Antibiotika, Schleiker & Shull, Dussell, Germany). Table 1 shows the levels of the three factors in the 17 runs of CCD. The inoculum with 10^3 colony-forming units (CFU)/g was performed in all of the runs of the CCD just before packaging. For each run 10 repetitions were prepared and analyzed over time. As controls were prepared uninoculated samples stored under the same conditions of the CCD as well as inoculated samples without volatile molecules stored at the same temperatures.

A simple, nonstructured, organoleptic evaluation was performed by five people to assess the compatibility of *trans*-2-hexenal with fresh sliced apples, as well as, during the storage, the organoleptic characteristics of the different runs of the CCD.

Isolation and Enumeration of Microorganisms. Aliquots of 10 g of the different samples were diluted with peptoned water (90 mL) and homogenized with a Lab-blender 80 Stomacher (Seward, London, U.K.). Appropriate decimal serial dilutions were plated onto specific media.The media and the conditions used were as follows: plate count agar (PCA; Biolife, Milan, 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.

Table 1. Levels of Hexanal and *trans*-2-Hexenal and Storage Temperatures in the 17 Runs of the Three-Factor Five-Level CCD

run	[H] (mmol/60 g of product)	[t-H] concn (mmol/60 g of product)	storage temp (°C)
1	0.030	0.006	10
2	0.090	0.006	10
3	0.030	0.018	10
4	0.090	0.018	10
5	0.030	0.006	20
6	0.090	0.006	20
7	0.030	0.018	20
8	0.090	0.018	20
9	0.060	0.012	15
10	0.060	0.012	15
11	0.000	0.012	15
12	0.120	0.012	15
13	0.060	0.000	15
14	0.060	0.024	15
15	0.060	0.012	5
16	0.060	0.012	25
17	0.060	0.012	15

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

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

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 experimenatl data were modeled through the Non Linear 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).

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

Table 2. Best Fit Equations Describing the Main, Interactive, and Quadratic Effects of Hexanal, *trans*-2-Hexenal, and Storage Temperatures on Viability Loss (Expressed as Log CFU/g), Lag Phase Length (Expressed in Days), and Dlag (Expressed in Days) of *P. subpelliculosa*

dependent variable	model	F^a	R^b	SE[c]
viability loss	$= 47.19[t\text{-}H] + 0.519[H][t\text{-}H] - 0.005[T]^2$ (eq 1)	87.5	0.97	0.15
lag phase length	$= 111.48[H] + 115.88[t\text{-}H] - 4.96[H][T]$ (eq 2)	34.1	0.94	1.68
Dlag	$= -217.14[t\text{-}H] - 2547.6[H][t\text{-}H] + 9.9[t\text{-}H][T] + 498.8[H]^{2+13849.9[t-H]^2}$ (eq 3)	28.6	0.95	0.93

[a] Fisher test value. [b] Regression coefficient. [c] Standard error.

($\tan^{-1} b^*/a^*$) 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 the Different Types of Atmospheres on *P. subpelliculosa* Growth in Apple Slices during Storage at the Different Temperature Levels of CCD. The addition of hexanal to ordinary atmosphere, but above all to modified atmosphere, significantly extended the shelf life of minimally processed apples (Lanciotti et al., 1999). Moreover, preliminary trials evidenced an antimicrobial activity in vitro not only of hexanal but also of its derivatives such as *trans*-2-hexenal, hexanol, and hexyl acetate (data not shown). A nonstructured sensorial analysis revealed the compatibility of *trans*-2-hexenal with the organoleptic characteristic of appld-based salad. To study the antimicrobial effects of the addition to the storage atmosphere (constituted by 70% N_2 and 30% CO_2) of different levels of hexanal and *trans*-2-hexenal, and their interaction with storage temperatures, a three-factor five-level CCD was developed on fresh sliced apples. Before packaging and storage at the different temperatures, the samples were inoculated with *P. subpelliculosa*, the species determined to be the one most frequently involved in the spoilage of minimally processed apples packaged in ordinary or modified atmospheres (Lanciotti et al., 1999). During storage at the different temperatures the growth of *P. subpelliculosa* was monitored. The growth data of this species showed that in some experimental conditions a significant reduction of *P. subpelliculosa* viable cell numbers over the first days of storage occurred. With the aim of evaluating the effects of the considered variables on the viability of *P. subpelliculosa*, the differences between the cell counts immediately after packaging (inoculum level) and the cell loads after 2 days of storage were calculated. Such differences were analyzed as a function of the variables of CCD. The best fit equation obtained is reported in Table 2 (eq 1). Positive values of viability loss indicate a viability decrease of the inoculated microorganism, whereas negative values indicate growth. As evidenced by Figure 1 the highest viability losses were obtained at the higher hexanal and *trans*-2-hexenal concentrations. However, also in the runs in which an initial viability reduction was observed, a subsequent growth of *P. subpelliculosa* occurred during the further storage. The cell loads of *P. subpelliculosa* in the different runs of CCD were modeled by using the Gompertz equation as modified by Zwietering et al. (1990). The lag phase lengths were analyzed in relation to the independent variables of the CCD to obtain a polynomial equation that describes the main, interactive, and quadratic effects of hexanal and *trans*-2-hexenal concentrations and temperature levels on these dependent variables (Table 2). According to eq 2 (Table 2) the lag phases, which ranged between 0.30 and 6.24 days, were positively affected by hexanal and *trans*-2-hexenal concentrations and negatively affected by the interaction [H][T]. The effects of the independent variables on lag phase extension were better evaluated using three-dimensional plots obtained by imposing a constant value (the central point of CCD) to one variable at time. As shown by parts a, b, and c of Figure 2, relative to the effects of the interactions [H][t-H], [H]-[T], and [T][t-H], respectively, on *P. subpelliculosa* lag phase, both volatile molecules used have a positive action resulting in the extension of the dependent variable. In fact, the highest values of lag phases were obtained for the highest levels of these two natural compounds. Whereas the antimicrobial activity of hexanal is increased by a rise in temperature, the effectiveness of *trans*-2-hexenal was decreased with the increase of temperature (Figure 2c). The latter results seem to be in disagreement with previously literature reports (Guerzoni et al., 1994, 1997; Gardini et al., 1997; Caccioni et al., 1997; Lanciotti et al., 1999). In fact, it has been reported that the inhibiting effectiveness of volatile molecules, including hexanal, was dependent and positively affected by their actual vapor pressure rather than their whole concentration in the system. The vapor pressure of a molecule, at a given concentration, depends on temperature and on its interaction with other solutes and provides a measure of the tendency to pass in the vapor phase. However, in the considered range, the temperature increase can have two opposite effects: it increases the vapor pressure of the considered molecules and, on the other hand, it reduces the lag phase of the inoculated yeast. Thus, to distinguish the direct effects of temperature on the *P. subpelliculosa* lag phase from its indirect effects on hexanal and *trans*-2-

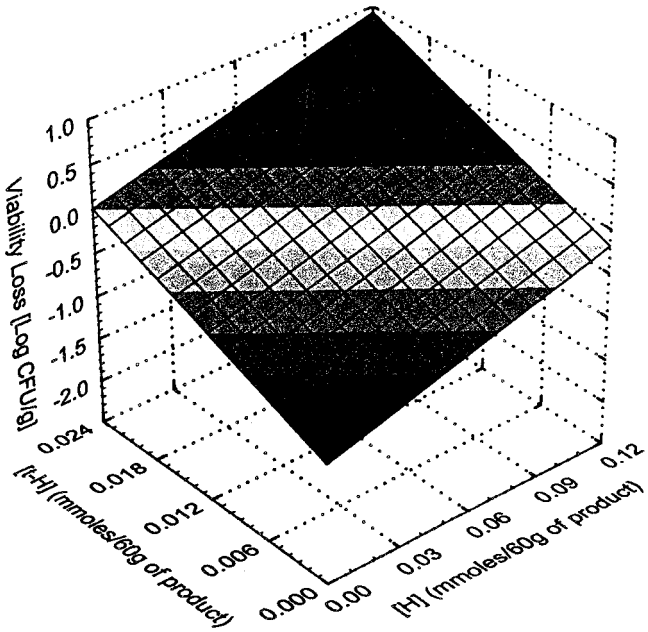

Figure 1. Effects of the interaction [H][t-H] on viability loss of *P. subpelliculosa*.

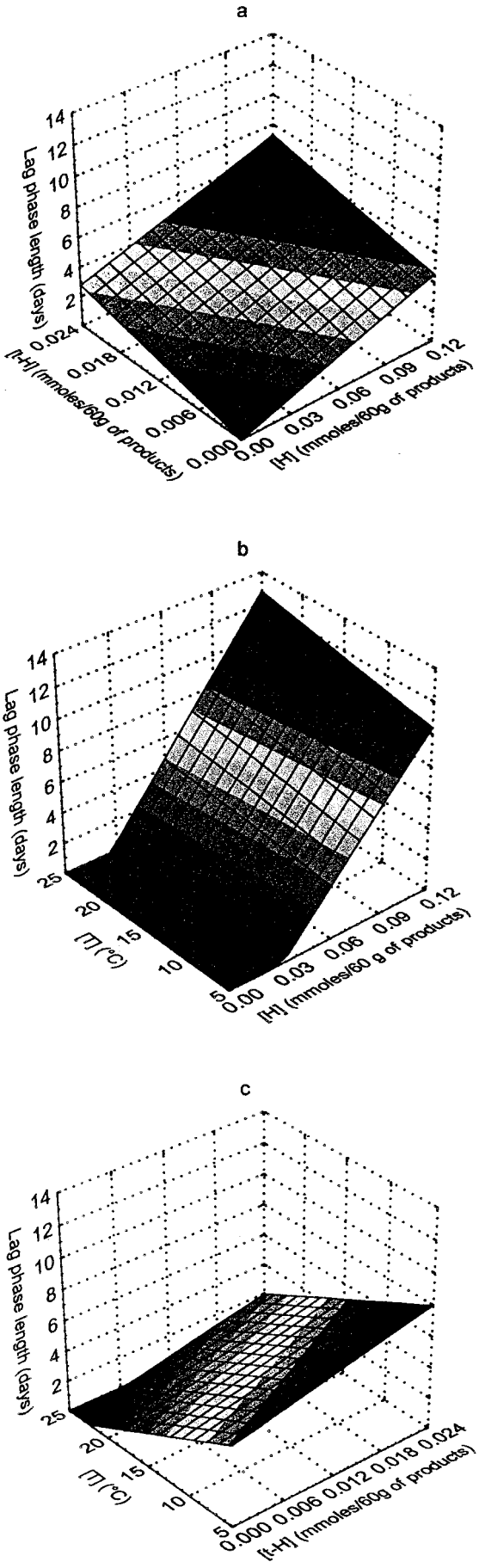

Figure 2. Effects of the interactions [H][t-H] (a), [H][T] (b), and [T][t-H] (c) on *P. subpelliculosa* lag phase length.

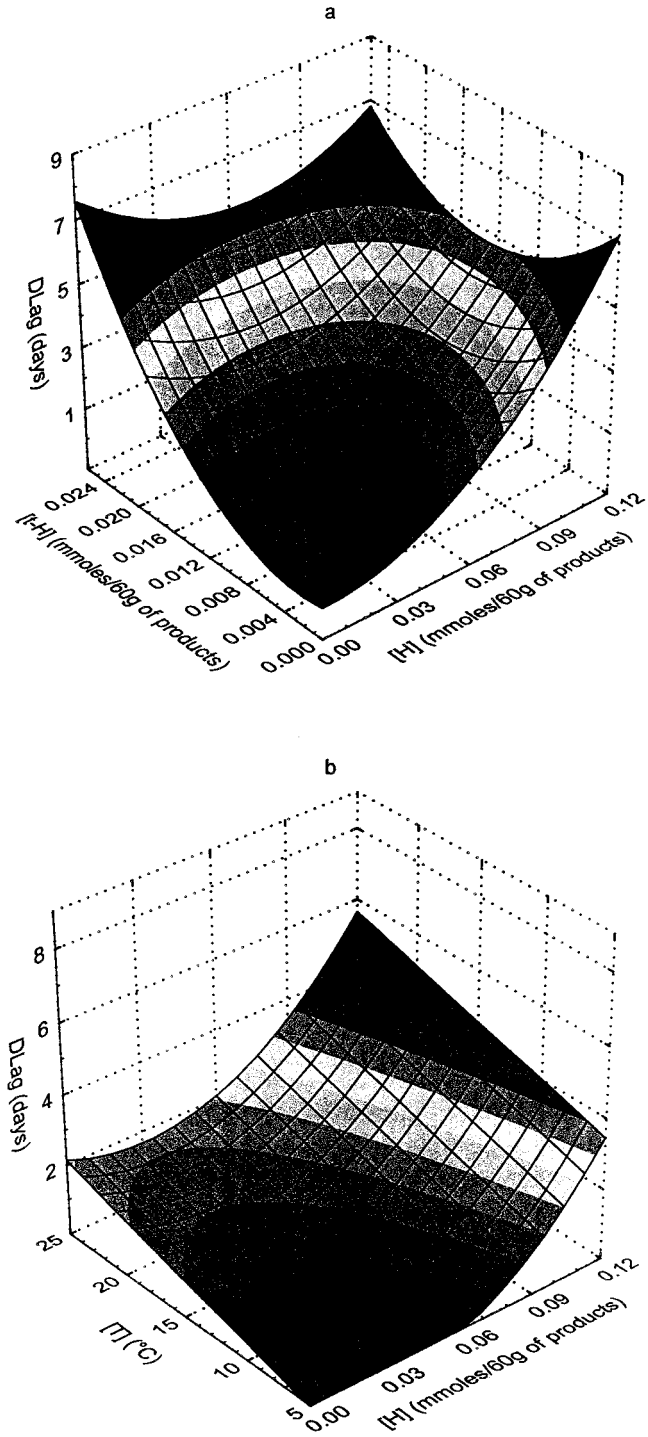

Figure 3. Effects of the interactions [H][t-H] (a) and [T][t-H] (b) on *P. subpelliculosa* Dlag. Dlag represents the differences between the lag phases of the inoculated yeast in the samples with the considered volatile molecules and the lag phases in the samples stored at the same temperatures but without volatiles.

hexenal vapor pressures, the differences between the lag phases of the inoculated yeast in the samples with the considered volatile molecules and the lag phases in the samples stored at the same temperatures but without both volatiles were calculated and here reported as DLag. A polynomial equation describing the individual and interactive effects of the three independent variables on the values of DLag was obtained (eq 3, Table 2). All of the independent variables significantly affected Dlag. Particularly, as shown by Figure 3a, the

Table 3. Best Fit Equations Describing the Main, Interactive, and Quadratic Effects Effects of Hexanal, *trans*-2-Hexenal, and Storage Temperatures on Cell Increases after 16 Days of Storage of Mesophilic, Psychrotrophic, and Lactic Acid Bacteria

dependent variable	model	F^a	R^b	SE^c
psychrothrophic bacteria	$= 126.69[\text{t-H}] + 0.75[\text{T}] - 6101[\text{t-H}]^{2-0.02[\text{T}]^2}$ (eq 1)	593.35	0.99	0.51
lactic acid bacteria	$= 38.9[\text{H}] + 24.1[\text{t-H}][\text{T}] - 376.1[\text{H}]^{2-12408.4[\text{t}-\text{H}]^2}$ (eq 2)	43.30	0.96	1.02
mesophilic bacteria	$= 0.32[\text{T}] - 2012.68[\text{t-H}]^{2-0.01[\text{T}]^2}$ (eq 3)	139.72	0.98	0.50

[a] Fisher test value. [b] Regression coefficient. [c] Standard error.

inhibiting action against *P. subpelliculosa* increased when both hexanal and *trans*-2-hexenal concentrations augmented. However, as shown by Figure 3b, the temperature played a positive role also in the increase of the effectiveness of *trans*-2-hexenal in the extension of the lag phase of the yeast with respect to the control incubated at the same temperatures but without volatile molecules in the storage atmospheres. Thus, Figures 2b and 3b account for an indirect effect of the temperature both on hexanal and on *trans*-2-hexenal antimicrobial activity and confirm that their effectiveness depends on the vapor pressure of molecules, which is, in turn, dependent on temperature.

Effects of the Different Types of Atmospheres on Naturally Occurring Microflora Growth in Apple Slices during Storage at the Different Temperature Levels of CCD. During the storage, in addition to the monitoring of the inoculated *P. subpelliculosa*, the growth of mesophilic and psychrotrophic bacteria and lactic acid bacteria was evaluated. For the three microbial groups the differences between the cell concentrations after 16 days of storage and the cell loads detected immediately after packaging were modeled as a function of the independent variables of CCD. The best fit equations obtained are reported in Table 3. As evidenced by eqs 1 and 2, hexanal did not significantly affect the growth of mesophilic and psychrotrophic bacteria, whereas *trans*-2-hexenal had, when added at the higher concentrations, an inhibiting action (negative coefficients of quadratic terms).

The lactic acid bacteria cell concentrations after 16 days of storage affected all of the variables of the CCD. As shown by Figure 4 both of the volatile molecules used exhibited a positive effect on the growth of this microbial group up to thresholds of 0.015 and 0.07 mmol/60 g of product for *trans*-2-hexenal and hexanal, respectively. Above these concentrations the considered volatile molecules had a inhibiting action. The growth of lactic acid bacteria could not be considered negatively for the shelf life of minimally processed vegetables. In fact, previous literature reports proposed the inoculum of selected species of lactic acid bacteria to control the growth of spoilage and pathogenic microorganisms on vegetable-based salads (Vescovo et al., 1996; Romick, 1994; Laurila et al., 1998; Breidt and Fleming, 1997). However, it can be claimed that all three of the microbial groups considered showed during the storage limited cell load increases. In fact, also after 16 days of storage, their proliferation (with respect to the cell loads immediately after packaging) did not exceed values of 3, 3.5, and 4.0 Log CFU/g for lactic acid bacteria, mesophilic, and psychrotrophic bacteria, respectively (also in the more favorable conditions). This can be attributed not only to the use in all of the considered runs of the CCD of nonconventional modified atmospheres (including hexanal and *trans*-2-hexenal) but also to the competition of the inoculated yeast. In fact, for samples stored under the same conditions (of temperature, hexanal, and *trans*-2-hexenal levels) of the CCD but uninoculated (without *P. subpelliculosa*), the three microbial groups after 16 days of storage attained cell levels significantly higher. In particular, the increases with respect to the initial levels were 4.0, 5.2, and 5.0 Log CFU/g for lactic acid bacteria, mesophilic, and psychrotrophic bacteria, respectively.

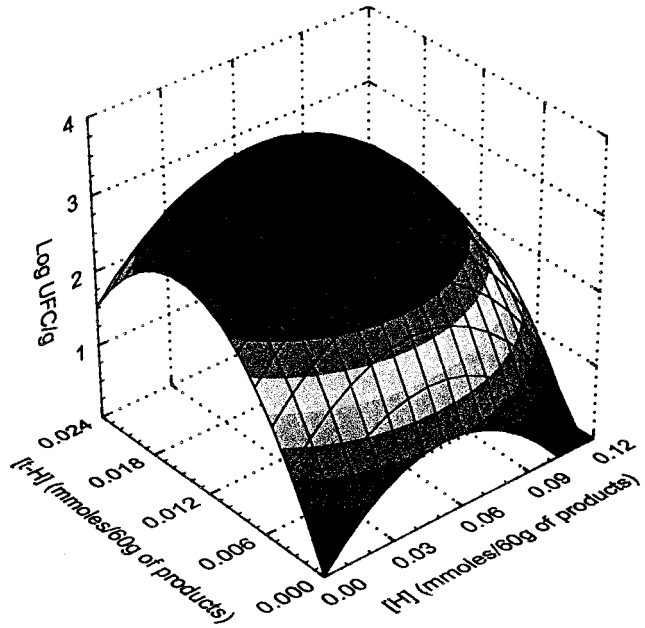

Figure 4. Effects of the interaction [H][t-H] on the growth of lactic acid bacteria.

Effects of Atmosphere Composition and Storage Temperature on Microbial Population. During the storage at the different temperatures, the identification of naturally occurring bacteria and yeasts was carried out. Although the frequency values of the different identified species changed during the storage in relation to both atmosphere composition and storage temperatures, the bacterial population was constituted principally by Enterobacteriaceae. In fact, the most represented species were, in all of the runs of CCD, *Enterobacter cloacae*, *E. agglomerans*, *Klebsiella oxytoca*, and *Rhanella aquatilis*. In the uninoculated samples stored without the addition of the volatile molecules, in addition to the previously reported species, *Bacillus amyloliquefaciens*, *B. macerans*, and *B. licheniformis* were found. The high frequency of Enterobacteriaceae in minimally processed fruits and vegetables is not surprising (Nguygen and Carlin, 1994; O'Conner-Show et al., 1994, 1995; Bennik et al., 1998; Gillian and O'Beirne, 1998; Gillian et al., 1999). The specificity of these microorganisms to this kind of products has been attributed to their physiology and to the occurrence of specific carbon sources and to a major sugar availability, with respect to the vegetable produces (O'Conner-Show et al., 1994, 1995). As already observed by Lanciotti et al. (1999), also the use of modified atmosphere (constituted by 70% N_2 and 30% CO_2) supplemented or not

Table 4. Best Fit Equations Describing the Main, Interactive, and Quadratic Effects of Hexanal, *trans*-2-Hexenal, and Storage Temperatures on the Changes of Hue Angle (Expressed as $\tan^{-1}$ *b/*a**) and *L** Values of Fresh Sliced Apples after 16 Days of Storage**

dependent variable	model	F^a	R^b	SE[c]
hue angle	$= 679.78[H]^{2-5.73[H][T]+0.47[T]}$ (eq 1)	74.55	0.97	1.39
*L**	$= -0.146[T] - 5.80[H][T] + 1251.4[H][t\text{-}H] + 413.5[H]^{2+0.025[T]^2}$ (eq 2)	30.38	0.96	0.79

[a] Fisher test value. [b] Regression coefficient. [c] Standard error.

with volatile molecules contributed to a further selection of species able to grow.

In the samples inoculated with the test yeast, *P. subpelliculosa* represented the sole yeast species, whereas in the uninoculated samples *Debariomyces hansenii* and *Cryptococcus albidus* were identified. In the uninoculated samples stored without volatile molecules, at the end of storage the yeast population was constituted by *Candida versatilis* and *P. subpelliculosa*. According to previous literature reports the inclusion of hexanal led to a yeast selection, in the uninoculated samples, favoring species having a reduced spoilage potential due to their prevalent respiratory activity (Lanciotti et al., 1999). In the inoculated samples the addition of the test volatile molecules determined a reduction of the growth potential of *P. subpelliculosa*.

Effects of the Different Types of Atmospheres on the Browning Reaction of Apple Slices during Storage at the Different Temperature Levels of CCD. The color modification for each run of CCD was evaluated as the differences between the hue angle and *L** values immediately after packaging and those after 16 days of storage. These two dependent variables were modeled as a function of hexanal and *trans*-2-hexenal concentrations and storage temperatures. The best fit equations obtained are reported in Table 4. The hue angle values were significantly affected only by the temperature, as an individual term, and hexanal, as a quadratic term, as well as by the interaction [H][T]. *L** values were influenced by the same terms, by *trans*-2-hexenal, and by the quadratic term of temperature. The effect of the interaction [T][H] on the modification of hue angle values is shown in Figure 5. The increase of hexanal concentration in the storage atmosphere determined a reduction of browning. The effects of hexanal on the retention of the original color was enhanced by the increase of storage temperature. As expected, without hexanal the enzymatic activity was increased by the rise of temperature. Also, the *L** values had the same behavior as hue angle values (Figure 6a). The inclusion of *trans*-2-hexenal in the storage atmosphere seems to have, at the highest concentration, a very weak but negative effect on the color stability (Figure 6b,c). Consequently, the good retention of the color in the samples stored also under abusive temperatures can be attributable to hexanal. The conversion of hexanal to hexanol, as already reported by Lanciotti et al. (1999), could be the key to understanding its effect on browning prevention. In fact, the aliphatic alcohols are regarded as inhibitiors of polyphenol oxidase (Valero et al., 1990). Another possible target of hexanal could be the phenylalanine ammonia-lyase, a key enzyme for the polyphenol biosynthesis, the production of which can be activated by tissue injuries and ethylene (Martinez and Withaker, 1995; Lanciotti et al., 1999).

According to a nonstructured sensorial analysis, the samples stored in atmospheres containing hexanal and *trans*-2-hexenal were positively appreciated for their flavor.

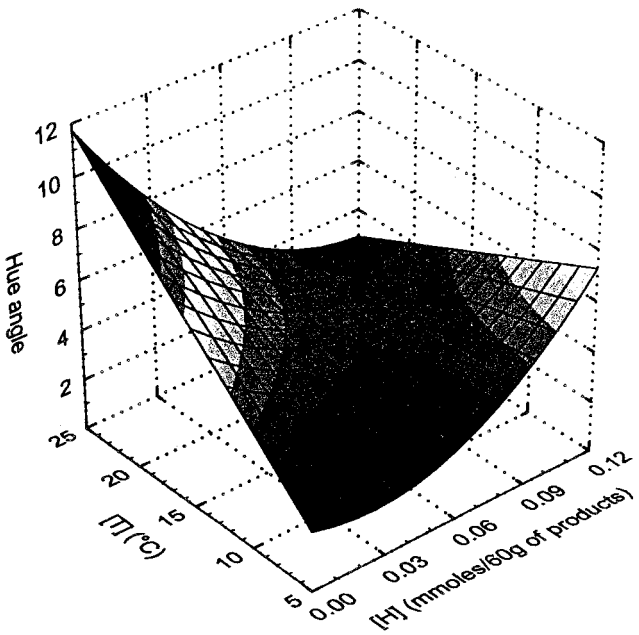

Figure 5. Effects of the interaction [H][T] on the changes of hue angle values (expressed as $\tan^{-1}$ *b**/*a**) after 16 days of storage.

CONCLUSION

The inclusion of hexanal and *trans*-2-hexenal in the storage atmosphere of fresh sliced apples determined a significant extension of shelf life also when a spoilage yeast such as *P. subpelliculosa* was inoculated at levels of 10^3 CFU/g and abusive storage temperatures were used. In fact, the presence of these volatile molecules in the packaging atmospheres considerably reduced the growth potential of the inoculated yeast, prolonging significantly its lag phase. Moreover, also the growth of naturally occurring bacteria was negatively affected by the inclusion of hexanal and *trans*-2-hexenal in the packaging atmosphere. In the presence of such molecules, also the increase of temperature played a positive role in the extension of the microbiological stability of the products due to the increase of the vapor pressure of the volatile molecules added and consequently their toxicity against microorganisms. In fact, the toxicity of volatile molecules is reported to be linked to its affinity with the microbial membranes (Guerzoni et al., 1994, 1997; Gardini et al., 1997). 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 increases with the hydrocarbon chain length, and its actual hydrophobicity, which provides an inverse measure of the water molecules surrounding its polar groups (Guerzoni et al., 1994, 1997). A rise in the temperature increases the volatility and, consequently, the antimicrobial effects of molecules such as esters, aldheydes, 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

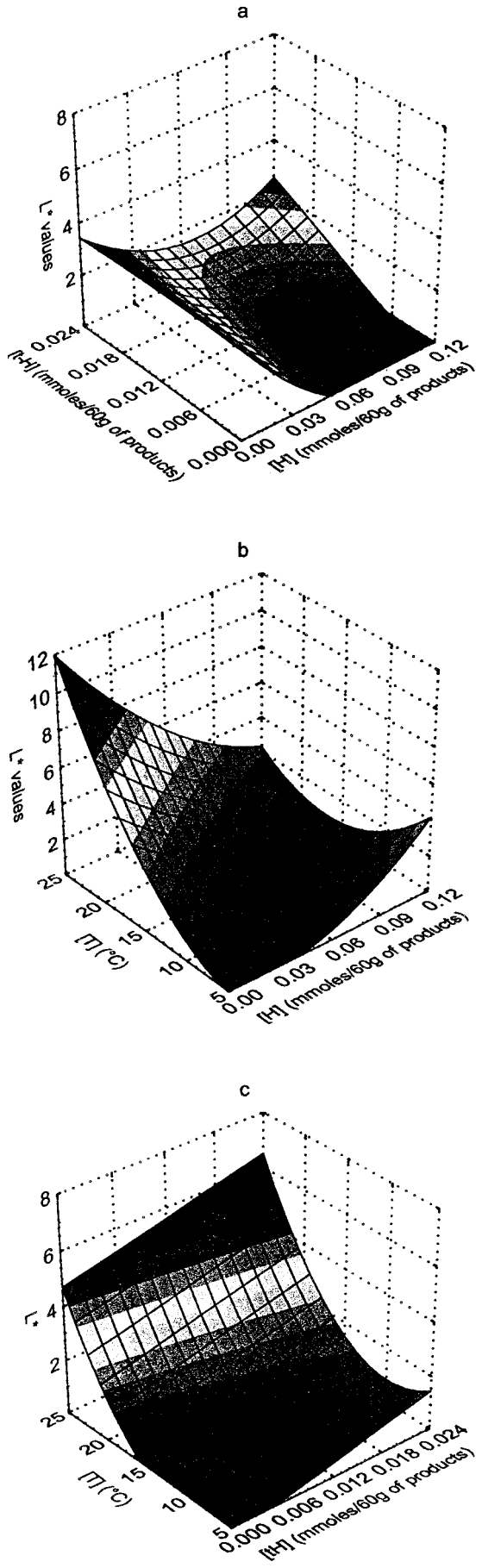

Figure 6. Effects of the interactions [H][t-H] (a), [H][T] (b), and [T][t-H] (c) on the changes of L^* after 16 days of storage.

and, consequently, the toxicity can compensate 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 low levels of hexanal increased the color stability of the sliced apples by up to 16 days. The bioconversion of hexanal to hexanol has been proposed as one of the possible keys to explain its effects on browning prevention. The bioconversion requires a diffusion of the hexanal through the intact fruit tissues with a rate depending on molecule vapor pressure. This mechanism can explain the positive interaction between temperature increase and hexanal concentration on color stability. Therefore, it can be presumed that hexanal action on polyphenol oxidase can be due to the prevention of enzyme production or the inhibition of the preformed enzyme.

The use of a CCD made possible the evaluation of the individual and interactive effects both of volatile molecules added and of storage temperatures on microbial stability as well as on color changes of the products. This approach was useful in the identification of the combination of temperatures, hexanal, and *trans*-2-hexanal concentrations, which corresponded to the maximal extension of shelf life in terms of microbial stability and color retention.

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