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Application of Hexanal, (*E*)-2-Hexenal, and Hexyl Acetate To Improve the Safety of Fresh-Sliced Apples

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The aims of this work were to evaluate the effects of different concentrations of hexanal, (*E*)-2-hexenal, hexyl acetate, and their mixtures on the fate of pathogenic species such as *Escherichia coli*, *Salmonella enteritidis*, and *Listeria monocytogenes* inoculated in model systems as well as the antimicrobial activity against the target species of the chosen molecules when added to the packaging atmosphere of inoculated fresh-sliced apples. The result obtained in this work pointed out the potential use of compounds such as hexanal, (*E*)-2-hexenal, and hexyl acetate for both the extension of shelf life and an improvement of hygienic safety of "minimally processed foods". In fact, hexanal, (*E*)-2-hexenal, and hexyl acetate had a significant inhibitory effect against pathogen microorganisms frequently isolated from raw materials (*E. coli*, *S. enteritidis*, and *L. monocytogenes*) when inoculated in both model and real systems. In this last condition, these compounds, at the levels used (150, 150, and 20 ppm for hexanal, hexyl acetate, and (*E*)-2-hexenal, respectively), displayed a bactericide effect on *L. monocytogenes* and they exhibited significant extensions of lag phase of *E. coli* and *S. enteritidis* inoculated at levels of 10^4-10^5 CFU/g.

KEYWORDS: Pathogenic microorganisms; aromatic compounds; antimicrobial activity; fresh-sliced apples; safety

INTRODUCTION

Raw and minimally processed fruits and vegetables are sold to the consumer in a ready-to-use or ready-to-eat form. This product typology does not generally contain preservatives or antimicrobial substances and rarely undergoes any heat processing before consumption. However, a variety of pathogenic bacteria such as *Listeria monocytogenes*, *Salmonella* and *Shigella* spp., and some *Escherichia coli* strains, *Aeromonas hydrophila*, *Yersinia enterocolitica*, and *Staphylococcus aureus* may be present on fresh fruits and in related minimally processed refrigerated products (*1*−*3*). In fact, the number of documented outbreaks of human infections associated to the consumption of raw and minimally processed fruits and vegetables has considerably increased during the past decades (*4*). Changes in dietary habits, agronomic and processing practices, and sources of produces, as well as the emergence of pathogens previously not recognized for their association to raw materials, have enhanced the frequency of outbreaks (*5*). Moreover, the inefficacy of the sanitizers used, attributable to the inability of active components to reach microbial cell targets, makes the decontamination of raw fruits and vegetables difficult (*3*). Although a shelf life of several days under refrigerated conditions is requested for feasible transport and retail, minimally processed fruits are more perishable than raw materials. In fact, the presence of cut surfaces with a consequent release of nutrients, the absence of treatments able to ensure the microbial stability, the active metabolism of fruit tissue, and the confinement of final product enhance the growth extent of the naturally occurring microbial population (*6*). Moreover, the use of low acid fruits such as melon or tropical fruits can favor the proliferation of species such as *Salmonella* spp. and *E. coli* up to the infective threshold (*7*).

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, as well as the safety of foods, has notably increased. Previous literature reports proposed the inoculum of selected species of lactic acid bacteria to control the growth of spoilage and pathogenic microorganisms in vegetable-based salads (*8*). Also, plants and plant products can represent a source of natural alternatives to improve the shelf life and the safety of food. In fact, plants have a wide range of volatile compounds some of which are important flavor quality factors in fruits, vegetables, species, and herbs (*9*). A key role in the defense systems of fresh produce against decay microorganisms has been attributed to the presence of volatile compounds (*10*). The ability of plant volatiles to inhibit decay microorganisms is one of the reasons for interest in them as

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components of biological means for prolonging the shelf life of postharvest (*11*) or minimally processed fruits and vegetables. Moreover, the plant volatiles have been widely used as food flavoring agents and most of them are generally recognized as safe.

The antimicrobial activity against spoilage species of hexanal and (*E*)-2-hexenal, which are components of the aroma of many fruits and vegetables, was already tested in model (*12*−*14*) as well as in real systems (*15*, *16*). In particular, the addition of hexanal and (*E*)-2-hexenal in storage atmosphere of fresh-sliced apples resulted in a positive effect on shelf life, due to their antimicrobial activity against naturally occurring spoilage species also when deliberately inoculated at levels of 10^3 CFU/g. Moreover, these molecules determined the enhancement of the aromatic properties, as well as the improvement of the original color retention of the packaged products (*15*−*18*). (*E*)-2-Hexenal was recognized as the principal antimicrobial agent from cashew apples and olive oil and proposed as an antimicrobial agent against *Salmonella* spp., *E. coli* and *Pseudeomonas aeruginosa* (*14*, *19*). Several studies showed a clinically useful role of this class of compounds, when used at nontoxic concentration, in the treatment of tumors due to the inhibition of glutathione-*S*-tranferase (*20*, *21*). Moreover, Kubo et al. (*22*) suggested the use of (*E*)-2-hexenal, in combination with other antimicrobial agents, in the eradication of *Helicobacter pilori* in patients affected by acute gastritis.

The aims of this work were to evaluate (i) the effects of different concentrations of hexanal, (*E*)-2-hexenal, hexyl acetate, and their mixtures on the fate of pathogenic bacteria such as *E. coli*, *S. enteritidis*, and *L. monocytogenes*, inoculated in model systems; and (ii) the antimicrobial activity against the target bacteria of the chosen molecules when added to the packaging atmosphere of inoculated fresh-sliced apples.

MATERIALS AND METHODS

Strain. The antimicrobial activity of hexanal, (*E*)-2-hexanal, and hexyl acetate was tested on the pathogenic strains *S. enteritidis* E5, *L. monocytogenes* Scott A, and *E. coli* DPV1, a nonenteropathogenic strain isolated from vegetables. The strains employed belonged to the collection of the Dipartimento di Protezione e Valorizzazione Agroalimentare of Bologna University.

Evaluation of Hexanal, (*E*)-2-Hexenal, and Hexyl Acetate Antimicrobial Activity in Model Systems. To evaluate the antimicrobial effects of three chosen volatile compounds against *S. enteritidis*, *E. coli*, and *L. monocytogenes*, the rapid method proposed by Caccioni et al. (*23*) was used. In fact, vials (10 mL capacity) containing 5 mL of plate count agar were superficially inoculated (10^4 CFU/vial) with the test microorganisms. Appropriate concentrations (calculated with respect to the total volume of the vials) of hexanal (purity 98%, Sigma Aldrich, Steinheim, Germany), (*E*)-2-hexenal (98%, Sigma Aldrich), and hexyl acetate (98%, Sigma Aldrich) (50, 100, 150, 200, and 250 ppm for hexanal and hexyl acetate and 20, 50, 100, and 200 ppm for (*E*)-2-hexenal) were put in the vials on 6 mm diameter filter paper disks (Antibiotica-Testblattchen, Shleicher and Schull, Dassel, Germany). The filter was soaked with the desired dose of the chosen molecules diluted in 9 μL of methanol. In the control samples, the paper disk was soaked with 10 μL of methanol. The compounds and methanol were previously sterilized by filtration (Millex-GS, Millipore, 0.22 μm, Molsheim, France). Vials were closed with butyl septa, paying attention to avoid contact between the disk and the medium, and incubated at 37 °C.

During incubation, microbial growth of pathogenic strains was monitored by gas chromatographic analysis of CO_2 evolved in the headspace of the sealed vials, which is an indirect index of the metabolic activity of the microorganisms (*12*). A gas chromatograph GC 6000 Vega Serie 2 (Carlo Erba Instrument, Milan, Italy), equipped with a hot wire detector and 2 m × 2 m i.d. glass-packed column filled with Poropak QS 80/100 mesh, was used for CO_2 detection in the headspace of the vials. The conditions for the analysis were the following: column temperature, 100 °C; injector temperature, 100 °C; detector temperature, 180 °C; filament temperature, 230 °C; carrier gas (He) flow rate, 40 mL/min. For each analysis, 3.5 min was required. The gas chromatograph was connected with a Headspace Autosampler model HS250 (Carlo Erba Instruments) equipped with a gas syringe Gastight 1750 (Hamilton, Bonaduz, Switzerland). During sampling, vials were maintained in the autosampler at 37 °C, and for each analysis, 0.2 mL of headspace was sampled. For each sample, three distinct and independent repetitions and two replicates have been considered. The variability coefficient never exceeded 5%.

For the evaluation of inoculum levels and for the confirmation of bactericidal effect of the tested molecules, Violet Red Bile Glucose Agar (VRBGA, Oxoid, Basingstoke, U.K.) and Listeria Selective Agar Base (LSA, Oxoid) were used for plate counting of *S. enteritidis*, *E. coli*, and *L. monocytogenes*, respectively.

Evaluation of Hexanal, (*E*)-2-Hexenal, and Hexyl Acetate Antimicrobial Activity in Fresh-Sliced Apples. Granny Smith apples (with a mean pH of 4.1) were hand-washed with drinkable water having added, according to Italian law, 0.2 mg/L of free chlorine. Then, they were peeled and sliced with sharp knifes. The slices were pretreated for 15 min with a solution containing 0.2% (w/w) citric acid and 1.0% (w/w) ascorbic acid and packaged in high barrier plastic bags—nylon/30 mm nylon and 120 mm of 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 ordinary and modified atmospheres (80% N_2, 20% CO_2). This atmosphere was chosen because it gave a longer shelf life to sliced fresh apples from previous literature reports (*15*, *24*). In fact, this condition allowed a good retention of the original color as well as a good microbial stability. The samples differed from each other for the inclusion in the ordinary or modified atmosphere of different levels of hexanal, (*E*)-2-hexenal, and hexyl acetate. The concentrations used, calculated taking into account the total bag volume, were as follows: 150, 150, and 20 ppm for hexanal, hexyl acetate, and (*E*)-2-hexenal, respectively. When used in mixture, the concentrations were 100, 100, and 50 ppm for hexanal, hexyl acetate, and (*E*)-2-hexenal, respectively. The appropriate concentrations of hexanal, hexyl acetate, and (*E*)-2-hexenal, or their mixtures were introduced in the atmosphere, before packaging, by means of 6 mm soaked diameter filter paper disk (Shleicher and Schull). The inoculum level of *E. coli*, *L. monocytogenes*, and *S. enteritidis* ranged between 10^4 and 10^5 CFU/g and was done just before sample packaging. For each condition, 20 repetitions were prepared and analyzed over time during storage at 20 °C. As controls, samples not inoculated that were stored under the same conditions were prepared as well as inoculated samples without volatile molecules stored at the same temperature. Aliquots of 10 g of the different samples were diluted with peptoned water (90 mL) and homogenized with Lab-blender 80 Stomacher (Medical, Seward, London, U.K.). The appropriate decimal serial dilutions were plated onto appropriate selective media, e.g., LSA and VRBGA. After the inoculum, incubation was performed at 37 °C for 48 h.

Microbiological data are the average of at least four different samples with identical inoculum atmosphere and stored for the same time. The variability coefficients, expressed as the percentage ratio between the standard deviation and the mean value, were less than 7%.

Data Analyses. Data in the headspace, expressed as percentage (v/v) or cell load data (expressed as Log CFU/g), collected during the incubation of model systems or during the storage of the products, were modeled according to the Gompertz equation modified by Zwietering et al. (*25*):

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

where, for the model system, y is the CO_2 percentage at time t, K is the initial level of the dependent variable (as %), A is the maximum percentage of CO_2 produced, μ_{max} is the maximum CO_2 production rate (as % CO_2/h), and λ is the lag time (h) for CO_2 production. For the real system, y is the Log [CFU/g], k is the initial level of the dependent variable to be modeled, A is the maximum bacteria cell load

Table 1. Growth Parameters of *S. enteritidis* Inoculated in Model Systems, with Different Concentration of Volatile Tested Molecules Added

molecule	concn (ppm)	Gompertz parameters[a] K	A	μ_{max}	λ	R^b
control[c]		1.00	30.38	3.41	15.71	0.99
hexanal	50	0.96	28.25	2.45	20.62	0.98
	100	*d*				
	200					
(*E*)-2-hexenal	50	0.80	22.93	1.59	16.17	0.99
	100					
	200					
	300					
hexyl acetate	50	0.62	27.15	2.38	19.25	0.99
	100					
	200					
	300					

[a] Gompertz parameters: *K* is the initial level of CO_2; *A* is the maximum percentage of CO_2 produced; μ_{max} is the maximum CO_2 production rate (as % CO_2/h); and λ is the lag time (h) for CO_2 production. [b] Correlation coefficient. [c] Sample without volatile molecules added. [d] Growth not detectable after 120 h of incubation.

Table 2. Growth Parameters of *E. coli* Inoculated in Model Systems, with Different Concentrations of Volatile Tested Molecules Added

molecule	concn (ppm)	Gompertz parameters[a] K	A	μ_{max}	λ	R^b
control[c]		2.11	27.67	1.24	3.63	0.99
hexanal	50	3.01	26.97	0.54	22.61	0.99
	100	1.78	18.29	0.19	34.54	0.98
	150	2.44	10.50	0.11	78.53	0.97
	200	*d*				
	250					
(*E*)-2- hexenal	20					
	50					
	100					
	200					
hexyl acetate	50	1.85	26.94	1.05	12.23	0.99
	100	1.66	29.30	0.81	12.44	0.99
	150	2.05	31.97	0.78	8.44	0.99
	200	2.15	28.15	0.35	11.03	0.99
	250	2.00	9.90	0.19	15.24	0.99

[a] Gompertz parameters: *K* is the initial level of CO_2; *A* is the maximum percentage of CO_2 produced; μ_{max} is the maximum CO_2 production rate (as % CO_2/h); and λ is the lag time (h) for CO_2 production [b] Correlation coefficient. [c] Sample without volatile molecules added. [d] Growth not detectable after 120 h of incubation.

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 Non Linear Regression Procedure of the statistic package Statistica for Windows (Statsoft, Tulsa, OK).

RESULTS

Evaluation of Hexanal, (*E*)-2-Hexenal, and Hexyl Acetate Antimicrobial Activity in Model Systems. To evaluate the antimicrobial activity of hexanal, (*E*)-2-hexenal, and hexyl acetate against *S. enteritidis*, *E. coli* and *L. monocytogenes*, the rapid method proposed by Caccioni et al. (*23*) was used. The Gompertz parameters obtained for the three species considered, i.e. *K*, λ, μ_{max}, and *A*, are reported in **Tables 1**−**3**. Fifty ppm of the chosen volatile molecules gave rise to a relevant increase of λ as well as to a reduction of μ_{max} and *A* values of *S. enteritidis*. Over this concentration, a bactericide effect was observed.

Table 3. Growth Parameters of *L. monocytogenes* Inoculated in Model Systems, with Different Concentrations of Volatile Tested Molecules Added

molecule	concn (ppm)	Gompertz parameters[a] K	A	μ_{max}	λ	R^b
control[c]		1.49	9.91	0.59	21.57	0.99
hexanal	50	3.48	6.86	0.25	29.12	0.98
	100	2.16	12.71	0.51	29.49	0.99
	150	3.39	5.19	0.13	32.02	0.97
	200	2.12	4.51	0.09	36.83	0.98
	250	*d*				
(*E*)-2-hexenal	20	2.30	8.92	0.28	50.42	0.99
	50					
	100					
	200					
hexyl acetate	50	1.08	7.77	0.53	21.16	0.99
	100	2.49	8.28	0.31	21.21	0.99
	150	1.80	5.59	0.19	23.07	0.99
	200	2.19	7.13	0.23	23.87	0.99
	250	3.00	4.56	0.18	31.43	0.99

[a] Gompertz parameters: *K* is the initial level of CO_2; *A* is the maximum percentage of CO_2 produced; μ_{max} is the maximum CO_2 production rate (as % CO_2/h); and λ is the lag time (h) for CO_2 production. [b] Correlation coefficient. [c] Sample without volatile molecules added. [d] Growth not detectable after 120 h of incubation.

E. coli and *L. monocytogenes* were more sensitive to (*E*)-2-hexenal than *S. enteritidis*. In fact, 20 and 50 ppm were bactericidal for *E. coli* and *L. monocytogenes*, respectively. In fact, no colony was detected by plating 0.1 g of sample in the appropriate media. However, these two species exhibited a lower sensitivity to hexanal and hexyl acetate. In fact, 200 and 250 ppm of hexanal were necessary, respectively, to determine the death of *E. coli* and *L. monocytogenes* cells. The increase of the hexyl acetate concentration did not result in a bactericidal effect on the last two species. The increase of its concentration provoked a reduction of *L. monocytogenes* growth potential but not of that of *E. coli* (as indicated by the Gompertz parameters).

Evaluation of Hexanal, (*E*)-2-Hexenal, and Hexyl Acetate Antimicrobial Activity in Packaged Fresh-Sliced Apples. During the incubation at 20 °C, the growth of *S. enteritidis*, *E. coli*, and *L. monocytogenes* in the different samples, packaged in ordinary or modified atmospheres supplemented with or without the tested aroma compounds, was determined by plate counting. *L. monocytogenes* was not able to grow in the real system also in the absence of the volatile molecule supplementation. However, while in the controls, its cell load remained quite constant over the storage at 20 °C; in the samples with volatile molecules added, no *L. monocytogenes* viable cells were detected after 4 days. The cell load data of *S. enteritidis* and *E. coli* were modeled according to the Gompertz equation as modified by Zwietering et al. (*25*). Hexanal, (*E*)-2-hexenal, and hexyl acetate or their mixtures, when added to the packaging atmosphere of fresh-sliced apples, had antimicrobial effects on these pathogenic species. In fact, as shown by **Figures 1**−**4**, all of the test molecules determined a relevant increase of the lag phases of the three pathogenic species. *E. coli* was more sensitive than *S. enteritidis* to the chosen levels of hexanal, (*E*)-2-hexenal, and hexyl acetate or their mixtures, as evidenced by the reduction of cell load detected in the packaged samples after 2 h of contact between the target cells and the volatile molecules. The most active molecule was (*E*)-2-hexenal, which induced viability decreases of 3.7 and 1.3 Log CFU/g in ordinary and modified atmospheres, respectively (**Figures 3** and **4**). This molecule exhibited the highest antimicrobial activity, among the tested molecules, and also against *S. enteritidis* inoculated

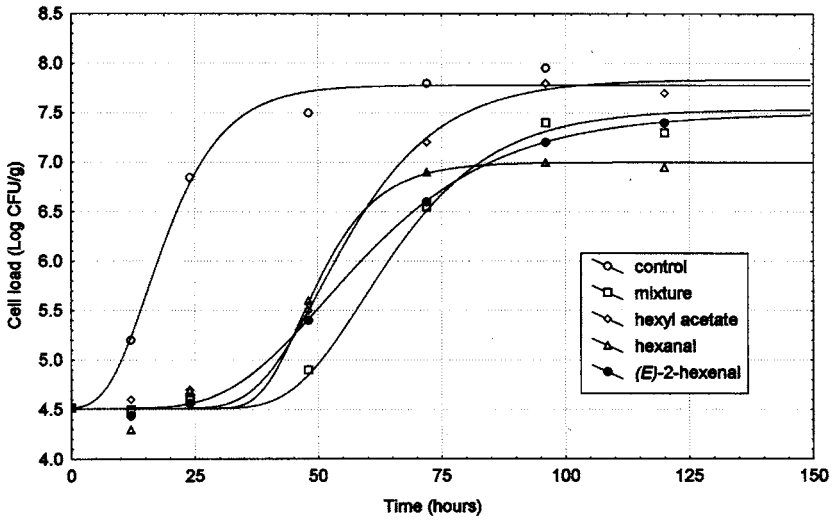

Figure 1. Effect of hexanal, (*E*)-2-hexenal, hexyl acetate, and their mixture on *S. enteritidis* inoculated in sliced apples packaged in an ordinary atmosphere.

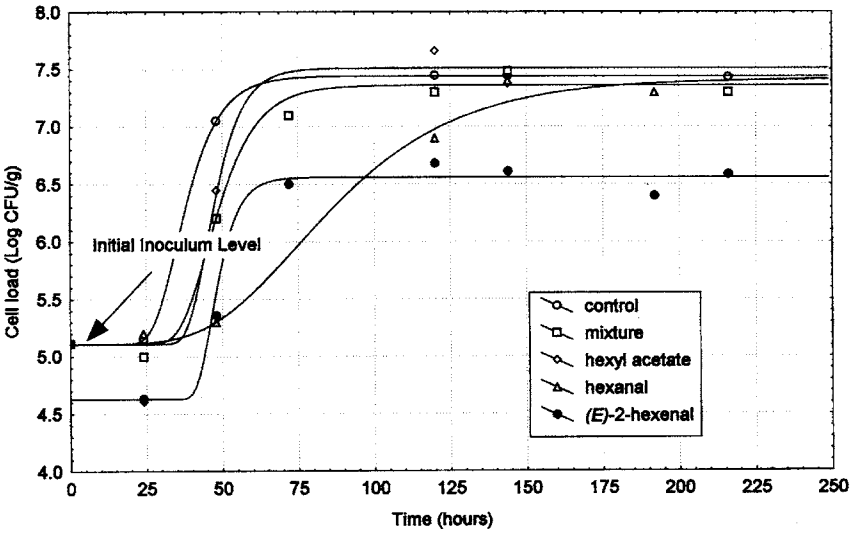

Figure 2. Effect of hexanal, (*E*)-2-hexenal, hexyl acetate, and their mixture on *S. enteritidis* inoculated in sliced apples packaged in a modified atmosphere.

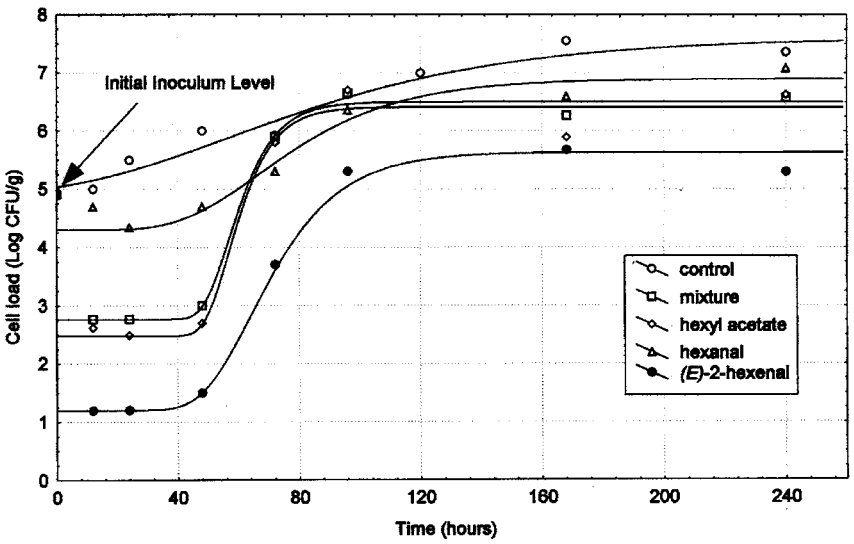

Figure 3. Effect of hexanal, (*E*)-2-hexenal, hexyl acetate, and their mixture on *E. coli* inoculated in sliced apples packaged in an ordinary atmosphere.

in fresh-sliced apples packaged under a modified atmosphere. In the samples packaged in an ordinary atmosphere, hexanal was the volatile molecule endowed with the highest inhibitory effects. In fact, the addition of 150 ppm to the packaging atmosphere of this molecule determined, in addition to a significant increase of the *S. enteritidis* lag phase, a remarkable reduction of the maximum cell load attained in the stationary phase.

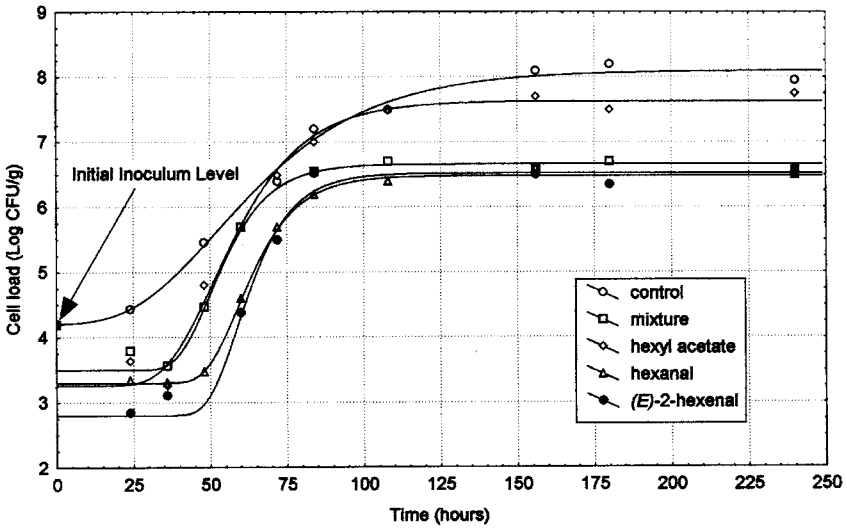

Figure 4. Effect of hexanal, (*E*)-2-hexenal, hexyl acetate and their mixture on *E. coli* inoculated in sliced apples packaged in a modified atmosphere.

DISCUSSION

The results of this work pointed out the potential use of compounds such as hexanal, (*E*)-2-hexenal, and hexyl acetate for the improvement of safety of minimally processed fruits. In fact, these substances had a significant inhibitory effect against pathogenic microorganisms frequently isolated from raw materials (*E. coli*, *S. enteritidis*, and *L. monocytogenes*) when inoculated in both model systems and fresh-sliced apples. In this last condition, these compounds, at the levels used, displayed a bactericidal effect on *L. monocytogenes*. Moreover, they exhibited significant lag phase extension of *E. coli* and *S. enteritidis* inoculated at levels of 10^4-10^5 CFU/g (exceeding the usual contamination level of raw materials).

The precise action of all of these antimicrobial compounds is not yet clear. (*E*)-2-Hexenal unlikely acts as a surfactant but likely permeates by passive diffusion across the plasma membrane. Once inside cells, its α,β-unsaturated aldehyde moiety reacts with the biologically important nucleophilic group. This aldehyde moiety is known to react with sulfydryl groups mainly by 1,4-additions under physiological conditions (*14*). Sulfydryl groups in proteins and lower molecular weight compounds such as glutathione are known to play a key role in living cells. Although the precise targets in microbial cells remain unclear, the toxicity of these molecules, analogously to other volatile molecules, seems to be dependent on its affinity with the membrane phospholipidic bilayer (*26*).

Previous literature data (*12*, *15*, *23*) proved that the antimicrobial activity of hexanal, (*E*)-2-hexenal, and hexyl acetate is dependent on their vapor pressure and, consequently, positively affected by a rise in temperature. 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 (*26*), and its actual hydrophobicity, which provides an inverse measure of the water molecules surrounding its polar groups (*26*, *27*). A temperature rise increases the tendency to pass in the vapor phase and, consequently, the antimicrobial effects of molecules such as esters, aldehydes, terpenes, alcohols, and hydrocarbons (*23*, *28*, *29*). Thus, the effect of temperature on the increase of vapor pressure and of the toxicity can compensate for the eventual interruption of the chilling chain.

Among the tested molecules, (*E*)-2-hexenal and hexanal were the most active molecules against the pathogenic species tested. However, their toxicity was affected by atmosphere composition. In fact, their antimicrobial activity was higher in ordinary atmosphere than under modified atmosphere, probably due to the more severe oxidative damage to microbial cells. As reported by Dodd et al. (*30*), in general, physicochemical stresses determined an oxidative stress as the result of an imbalance that occurs when the survival mechanisms are unable to deal adequately with the reactive oxygen species in the cells.

The sensitivity to the aroma compounds used varied with the target species considered and was affected by the system composition. In fact, *S. enteritidis* and *E. coli* showed an intrinsic major growth potential, attributable also to the low pH of the raw materials, in fresh-sliced apples, with respect to *L. monocytogenes*. O'Conner-Shaw et al. (*7*) evidenced a high specificity of Enterobacteriaceae for fruit and vegetable products attributable to their physiology and to the availability of specific carbon sources such as inositol and pinitol. The supplementation with aroma compounds reduced significantly the growth potential of the Gram-negative species considered also in real system.

The sensitivity of the tested Gram-negative bacteria to (*E*)-2-hexenal and hexanal makes these molecules particularly interesting as antimicrobial agents. In fact, the inherent or acquired resistance of this microbial group to many antimicrobial agents makes their control in the environment and in food materials difficult. The resistance of Gram-negative bacteria is mainly due to the outer membrane, which acts as an efficient permeability barrier against macromolecules and hydrophobic substances (*31*) as well as to the high content in cyclopropanic fatty acids of the inner membrane (*32*). The use of several chelating agents, such as ethylenediaminetetraacetic acid and other substances, has been proposed to destabilize the lipopolysaccharide layer of the outer membrane of Gram-negative bacteria (*31*, *33*, *34*). Unfortunately, those agents that cause outer membrane permeabilization are often too toxic to be used as food ingredients. The citric acid used in the sliced apple pretreatment probably enhanced the outer membrane destabilization, due to its chelating activity. Neverthless, small hydrophobic compounds such as six carbon aldehydes can enter, throughout porin proteins, into the deeper parts of Gram-negative bacteria without any alteration to the permeability of the outer membrane (*31*). This gives an extra value for the aroma compounds considered as antimicrobial agents in foods, because its action is naturally strong against Gram-negative bacteria. Moreover, the effectiveness at low levels (also under abuse temperature conditions), the natural occurrence in several fruits

and edible vegetables, and the possibility of using in unregulated doses as flavoring agents makes hexanal, (*E*)-2-hexenal, and hexyl acetate good candidates as antimicrobial agents to improve the safety of minimally processed fruits.

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