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Impact of Plant Essential Oils on Microbiological, Organoleptic and Quality Markers of Minimally Processed Vegetables

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“Impact of plant essential oils on microbiological, organoleptic and quality markers of minimally processed vegetables”

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Running title**: Microbiological and sensory impact of EO’s on ready-to-eat vegetables**

Key words**: essential oils, spoilage, sensory, volatiles, lettuce, carrot, ready-to-eat**

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Abstract

The objectives of this study were to evaluate the efficacy of plant essential oils (EO's) for control of the natural spoilage microflora on ready-to-eat (RTE) lettuce and carrots whilst also considering their impact on organoleptic properties. Initial decontamination effects achieved using EO's were comparable to that observed with chlorine and solution containing oregano recorded a significantly lower initial TVC level than the water treatment on carrots ($p < 0.05$). No significant differences were found between the EO treatments and chlorine considering gas composition, color, texture and water activity of samples. The sensory panel found EO treatments acceptable for carrots throughout storage, while lettuce washed with the EO solutions were rejected for overall appreciation by Day 7. Correlating microbial and sensory changes with volatile emissions identified 12 volatile quality markers. Oregano might be a suitable decontamination alternative to chlorine for RTE carrots, while the identification of volatile quality markers is a useful complement to sensory and microbiological assessments in the monitoring of organoleptic property changes and shelf-life of fresh vegetables.

1. Introduction

Minimally processed fresh vegetables (MPFV) form an important component of a healthy diet and are a convenient way of increasing fresh produce consumption. Fresh vegetables are susceptible to microbial attack after harvest due to loss of natural resistance and their high water and nutrient content (Ippolito & Nigro, 2003), a problem which can be exacerbated by minimal processing. MPFV products are normally packaged in modified atmospheres and effective refrigerated temperature control during manufacture, distribution and retailing are required for maintaining the microbiological quality and safety of these products. Unfortunately, these steps do not either eliminate or delay microbial spoilage of these products entirely (Sapers, 2001). The dominating bacterial population on these products during low temperature storage mainly consists of species belonging to the *Pseudomonadaceae* and *Enterobacteriaceae* as well as some species belonging to the lactic acid bacteria (LAB) group (Ragaert, Devlieghere & Debevere, 2007).

Disinfection processes incorporating chlorine are often applied to fresh vegetables to enhance safety and shelf-life profiles, but its use has limitations and disadvantages, such as a reduced antimicrobial effectiveness or the possible formation of carcinogenic chlorinated compounds (Li, Brackett, Shewfelt & Beuchat, 2001; Martin-Diana, Rico, Barry-Ryan, Frias, Henehan & Barat, 2007). With increased concern about efficacy and toxicological safety of chemicals and synthetic preservatives, the demand for natural alternatives has increased. In this context, plant essential oils (EO’s) are attracting interest for their potential as natural food preservatives as they have Generally Recognised As Safe (GRAS) status and many of them display a wide spectrum of antimicrobial activity, with potential for control of foodborne pathogens and spoilage bacteria associated with ready-to-eat vegetables (Gutierrez, Rodriguez, Barry-Ryan & Bourke, 2008a). Oregano (*Origanum vulgare L.*) and thyme (*Thymus vulgaris L.*) oils, whose main components are carvacrol and thymol respectively, are characterized by strong antibacterial properties (Dorman & Deans, 2000; Elgayyar, Draughon, Golden & Mount, 2001; Burt, 2004; Oussalah, Caillet, Saucier & Lacroix, 2006; Gutierrez et al., 2008a). However, if EO's are expected to be widely applied as natural antimicrobials, the organoleptic impact should be considered as the use of naturally derived preservatives can alter the taste of food or exceed acceptable flavor thresholds (Hsieh, Mau & Huang, 2001; Nazer, Kobilinsky, Tholozana & Dubois-Brissonneta, 2005). Recently, it was observed that lettuce treated with oregano at 250 ppm was acceptable to a sensory panel as they did not find differences between this lettuce and that washed with chlorinated water (Gutierrez et al., 2008a). Furthermore, the use of oregano combined with thyme normally yields additive antimicrobial effects (Lambert, Skandamis, Coote & Nychas, 2001; Gutierrez, Barry-Ryan & Bourke, 2008b), thus, this combination could minimize the concentrations required, thereby reducing sensory impact.

MPFV manufacturers are often concerned with sensory improvement. Zhou et al., (2004) defined the shelf life of a green leafy vegetable as the length of time which it can maintain an appearance that appeals to the consumer: crisp green vegetable with little browning or wetness present. Sensory properties such as color, flavor and texture, play a key role in the consumer's choice of fresh prepared products. An issue associated with ready-to-eat vegetables is short shelf-life, which is usually no more than 8 days when stored in adequate conditions (Allende & Artes, 2003). Beyond day 7 of storage, these products present off-flavors, tissue softening and proliferation of microorganisms, making them more perishable than untreated material (Watada & Qui, 1999). Thus, optimizing the application of any novel natural preservation approach to shelf-life extension of MPFV requires that sensory analyses as well as other more objective methods, such as measurement of texture, color or water activity, and volatile emissions analysis are incorporated into the experimental design, in order to monitor possible changes on organoleptic properties. In this context, the identification of quality biomarkers among the volatile emissions from fresh vegetables can help to develop and optimize a rapid quality-monitoring method as well as an understanding of the origin and metabolic basis of volatile emission changes in MPFV during storage (Lonchamp, 2006). Little information is generally known about the relationship between the outgrowth of spoilage microorganisms, their production of metabolites, including volatiles, and the perception of the decay of minimally processed vegetables by consumers (Jacxsens, Devlieghere, Ragaert, Vanneste & Debevere, 2003).

Therefore, the objective of this study was to optimize the application of EO's for MPFV decontamination addressing control of spoilage microflora and improving shelf-life characteristics whilst also considering possible impact on organoleptic properties. Correlations between microbiological data, sensory analysis and volatiles emissions were investigated in order to determine volatile quality biomarkers.

2. Materials and methods

Essential oils

The EO's used in this study were oregano (*Origanum vulgare*) and thyme (*Thymus vulgaris*). They were selected based on previously reported efficacy (Gutierrez et al. 2008a), and were obtained from Guinness Chemical Ltd. (Portlaoise, Ireland) as pure CO_2 soluble supercritical fluid extracts.

Preparation of vegetable model products

Iceberg lettuce (*Lactuca sativa* sp.) and carrots (*Daucus carota* sp.) were purchased on the day of processing in a local retailer, and stored at 4ºC until use within 4 hours. To prepare the lettuce, the outer leaves were discarded and cores were removed. Heads were then cut using a stainless steel knife into pieces of approximately 1.5 square inch, to reflect retail packages of salad lettuce. Carrots were peeled and cut into 0.5 cm thick slices. Three separate treatment solutions were prepared using distilled water at room temperature. The concentrations were 250 ppm for oregano, 125 and 250 ppm for the combined mixture of oregano and thyme, respectively, and 120 ppm for chlorine. Prepared lettuce or carrot was placed in the appropriate treatment solution with gentle manual agitation for 2 min, prior to rinsing in distilled water for 1 min. The ratio of product to treatment solution was 1:10 w/v. Samples were then spin-dried for 6 minutes using an automatic salad spinner at room temperature (Dito Sama, Crypto Peerless, Halifax, UK) and packaged in 150g (lettuce) or 50g (carrot) quantities using 35 μm-thick oriented-polypropylene (OPP) bags of 20x25cm (Amcor Flexibles Europe, UK). Bags were then sealed using an impulse heat sealer (SMS 350, Packer Products, Basildon, UK) and stored at 4°C for 7 days. Unwashed samples and samples treated with distilled water alone were used as controls.

Microbiological Analysis

Microbiological analyses were performed on Days 0, 2, 4 and 7. 10g of lettuce or carrots were transferred to Seward stomacher bags with 90 ml of Maximum Recovery Diluent (MRD) and stomached for 2 min on high. Serial dilutions were then prepared in MRD and spread on the following media: (i) Tryptic Soy Agar (TSA, Scharlau Chemie, Spain), for the enumeration of Total Viable Count (TVC); (ii) Man, Rogosa and Sharpe Agar (MRSA, Scharlau Chemie), for Lactic Acid Bacteria (LAB); (iii) Violet Red Bile Dextrose Agar (VRBDA, Scharlau Chemie), for Enterobacteria; (iv) and CN Selective Agar Base (CNA, Scharlau Chemie), for *Pseudomonas*. Inoculated plates were incubated for 48 h at 30°C (TSA, MRSA and CNA plates) or 37°C (VRBDA). Results were expressed as Log CFU/ml. Experiments were performed in duplicate and replicated twice.

Quality markers studies

Quality parameters were measured from samples treated with EO’s and compared to those obtained using chlorine on Days 1, 4 and 7. Unless otherwise stated, experiments were performed in duplicate and replicated twice. The parameters used were: pack headspace composition, color, texture, water activity, sensory analysis and volatile emission analysis.

A gas analyzer (MAPTEST 4000, Hitech Instruments Lts., UK) was used to monitor % levels of CO_2 and O_2 in the package during storage. Gas composition was measured using a hypodermic needle inserted through an impermeable patch of polyvinylchloride (PVC) adhesive septum fixed to the bags.

Color measurement was performed using a Color Quest XE colorimeter (Hunter Lab, Northants, UK). The colorimeter was calibrated using a white reference tile (L^* = 93.97, a^* = -0.88 and b^* = 1.21) and a light trap (black tile) under illumination conditions. Nine random areas were measured thorough the packaging film, and the three CIELAB color values (L^*, a^* and b^*) were recorded. The illuminant chosen was D65 and the observer used was 10°. The variable L^* (lightness index scale) ranges from 0 for black to 100 for white. The a^* scale measures the degree of red ($+a^*$) or green ($-a^*$) colors and the b^* scale measures the degree of yellow ($+b^*$) or blue ($-b^*$) colors.

Texture properties of lettuce and carrot discs were assessed using an Instron Universal Testing machine model 4464 (Instron Limited, High Wycombe, UK) fitted with a puncture cell. The speed setting for the experiment was 500 mm/min and maximum load for the puncture test was expressed in kN. For each treatment, data were obtained from 10 (carrot) or 40 (lettuce) pieces from a package and analyzed with the Instron series IX software for Windows.

Water activity was measured using the Aqualab Series 3 (Decagon Devices, Pullman, Washington, USA) at 23–24 °C.

2.5 Sensory analysis

Sensory analysis was performed using a 10 member trained panel with an age range of 25-40 years. The panel consisted of four females and six males who were trained to be familiar with sensory properties of minimally processed lettuce and carrots. The sensory testing method was an acceptance test in which the sensory parameters were scored on a descriptive scale of 1-9. The sensory parameters investigated included the following: (i) vegetable aroma; (ii) off-odor; (iii) color; (iv) browning; (v) texture; (vi) vegetable taste; (vii) off-after taste; (viii) overall acceptability; and (ix) overall appreciation. Descriptions for each score were as follows: 9 = like extremely or extremely high, 8 = like very much or very high, 7 = like moderately or high, 6 = like slightly or lightly high, 5 = neither like or dislike or neither high or low, 4 = dislike slightly or slightly low, 3 = dislike moderately or low, 2 = dislike very much or very low, and 1 = dislike extremely or extremely low. Testing was carried out in sensory analysis booths located adjacent to the processing hall with appropriate lighting conditions and temperature of around 18-20°C. Results were monitored using the Compusense® Five software (Release 4.4, Ontario, Canada). Sensory trials were replicated twice.

2.6 Volatile emission analysis: Solid-Phase Micro-Extraction (SPME) and Gas chromatography-mass spectrometry (GC/MS) analysis

The package headspace was analyzed using a solid phase micro extraction (SPME) device containing a fiber coated with polydimethylsiloxane (PDMS) film (Supelco, JVA Analytical Ltd., Ireland), following a procedure previously developed and validated using standard compounds in our laboratory (Lonchamp, 2006). Before extraction, an impermeable path of adhesive PVC was attached to each package and a hypodermic needle was used to perforate it. The SPME device was then inserted through the plastic adhesive, and the SPME fiber was exposed for five min and then retracted. The packaging film was resealed using another impermeable patch of PVC.

A Varian 3800 GC (JVA Analytical Ltd., Ireland) with a 2200 Varian ion trap MS was used to analyze the samples. SPME fiber injections were made splitless for 3 min with the GC injection port temperature held at 250°C. Grade 5.0 helium, filtered through a Gas Clean GC/MS filter (Varian), was used as the carrier gas at a constant flow rate of 2.0 ml/min. Volatile compounds were adsorbed by a fused-silica capillary column (CP-Sil 8, JVA Analytical Ltd., Ireland) with a length of 30 mm, an inner diameter of 0.25 mm and a 0.25 μm film thickness. The initial column oven temperature was set at 30°C and held at this temperature for 5 min. The temperature was then increased to 250°C at a rate of 5°C/min and the final temperature of 250°C was maintained for 15 min. MS analysis of the eluted compounds was then carried out using the technique of electron impact ionization. The electron ion source energy used was 70 eV and the mass range chosen was from 40 m/z to 350 m/z. Data were collected using the Varian software and mass spectra of detected compounds were analyzed by library searching in the National Institute of Standards and Technology (NIST) databases. Estimation of the volatile compounds quantity was based on the areas of the peaks detected by MS. The headspace concentration of a volatile compound was then expressed in percentage of total volatile compounds detected or percentage of the total peak area. The compounds were identified with high probabilities when compared with standards from the NIST database (similarity coefficient or reverse similarity coefficient > 85%). Additional information was obtained for the compounds detected using Flavornet, an online compilation of aroma compounds found in human odor space.

2.7 Statistical analysis

Statistical analysis was performed using SPSS 15.0 (SPSS Inc., Chicago, U.S.A). Means were compared using ANOVA followed by LSD testing at $p < 0.05$ level in order to follow changes over time as well as differences between treatments.

Linear regression analysis was used to determine correlations between changes in volatile emissions, sensory properties and bacterial populations. A R^2 value higher than 0.90 was considered as indicator of satisfactory correlation between the factors and the volatile compound analyzed was then considered as a marker of the sensory attribute or the changes in bacterial population.

Principal component analysis (PCA) was performed using the multivariate method on the Statgraphics software (version 2.1; Statistical Graphics Co., Rockville, USA) to obtain a visual overview of correlations between sensory attributes, microbiological analysis and the volatile markers.

3 Results and discussion

3.1 Effect of EO's and chlorine on the natural microflora of lettuce and carrots

Survival of TVC, Enterobacteria, *Pseudomonas* spp. and LAB on treated lettuce and carrots are indicated in Tables 1 and 2, respectively. The initial effect of EO's on TVC, Enterobacteria and LAB was not significantly different ($p < 0.05$) to that obtained using chlorine or water. The solution containing oregano recorded a significantly lower initial TVC level than the water treatment on carrots. When oregano was combined with thyme, the effect on bacteria was the same as that observed with the oregano alone ($p < 0.05$). Thus, from a microbiological point of view, oregano is a viable alternative to chlorine as decontamination treatments. However, all treatments had a minimal decontamination effect against *Pseudomonas* and did not maintain the initial decrease in the remainder of the bacterial populations over the storage period. Uyttendaele, Neyts, Vanderswalmen, Notebaert and Debevere (2004) reported that decontamination of carrots with thyme accomplished a significant reduction of the indigenous flora but the psychrotrophic aerobic flora recovered and multiplied during storage time. Bagamboula, Uyttendaele and Debevere (2004) also observed limited reductions in the indigenous flora of lettuce after decontamination treatment with thyme and attributed this to the attachment of the indigenous flora and formation of biofilms on the surface of the lettuce leaves. TVC found on fresh vegetables include a diverse microflora dominated by Gram-negative bacteria, which are generally more resistant to the EO's than the Gram-positive bacteria (Burt, 2004). In this respect, the combination of EO's with other natural preservation methods as well as the improvement in packaging conditions might prolong shelf-life of minimally processed vegetables.

LAB and Enterobacteria were not found above 10^2 CFU/ml throughout the storage period on lettuce and carrots, respectively (results not shown). Jacxsens et al. (2003) reported that vegetables containing naturally low concentrations of sugars, such as lettuce or endives, showed a spoilage dominated by Gram-negative microorganisms, while other vegetables with a higher content of carbohydrates, such as bell peppers or celery, suffered from a fast and intense growth of spoilage microorganisms dominated by LAB and yeasts. Furthermore, the growth of LAB did not reach the levels shown by Enterobacteria or *Pseudomonas* on carrots after 7 days of storage. Klaiber, Baur, Wolf, Hammes and Carle (2005) also observed a limited growth of LAB on minimally processed carrots washed with chlorine over 6 days, which was related to the sensitivity of these bacteria to oxygen.

3.2 Quality markers of lettuce and carrots treated with EO's and chlorine

The gaseous composition in the bags containing samples washed with the EO solutions were not significantly different ($p < 0.05$) to those recorded for samples treated with chlorine. The initial conditions inside the OPP bags containing lettuce or carrots were 20.9% O_2 and 0.1% CO_2. After 7 days of storage, the O_2 concentration was approximately 12%, while the CO_2 concentration increased to 8-9% in both vegetables type bags. The low concentration of LAB in lettuce could be attributed to these anaerobic conditions, as previously observed by Klaiber et al. (2005), to the decontamination methods used or to a synergistic effect of these two factors.

When color measurement was performed, no significant differences in lettuce color values ($L^*a^*b^*$) were found between EO treatments and chlorine during the 7 days of storage. With respect to carrots, samples treated with oregano in combination with thyme were significantly ($p <0.05$) darker ($L^* = 63.9 \pm 0.6$) than those washed with oregano (61.6 ± 0.6) or chlorine (62.6 ± 0.4), but only on Day 1. During storage at 4°C for 7 days, instrumental texture parameters and water activity values did not significantly ($p < 0.05$) differ between the treatments (Data not shown).

3.3 Sensory analysis of lettuce and carrots treated with EO's and chlorine

The results of sensory analysis of EO and chlorine treatments are shown in Figure 1. Previous studies carried out in our laboratory (Gutierrez et al., 2008a; Gutierrez et al., 2008b) showed that oregano oil was accepted by panelists at 250 ppm and that thyme oil was only rejected at 500 ppm. These two EO´s displayed additive anti-microbial effects and the combination of 125 ppm of oregano oil and 250 ppm of thyme aimed at reducing the sensory impact while maintaining the antimicrobial efficacy of the treatment. In this study, carrots treated with oregano and oregano + thyme were accepted throughout the storage period. Both EO treatments were suitable in terms of overall appreciation and no significant differences were found between samples treated with the EO's and chlorine ($p < 0.05$). However, on Day 1 the vegetable aroma perceived from samples treated with oregano + thyme was significantly ($p < 0.05$) less intense than that of oregano or chlorine. In this context, Valero and Giner (2006) observed a positive score for carvacrol but a strong smell and flavor of thymol which minimized the degree of acceptance or liking for carrot broth. The strong effect of thyme on sensory quality of chopped bell peppers was also described by Uyttendaele et al. (2004).

For lettuce, samples treated with EO's and chlorine were accepted throughout the 7 days of storage when considering sensory quality. However, lettuce washed with EO's were unsuitable in terms of overall appreciation by Day 7. The aroma and off-odors perceived from samples treated with EO's were significantly ($p < 0.05$) more intense than those of chlorine on Day 1, and the off-after taste of lettuce washed with oregano in combination with thyme was found to be significantly ($p < 0.05$) stronger that those of oregano or chlorine. By Day 7 samples treated with the EO combination had more intense off-odors than those perceived from lettuce treated with oregano or chlorine. Since the flavor of lettuce is weaker than that displayed by carrots, the sensory impact of EO's could be higher on lettuce.

3.4 Volatile emission from lettuce and carrots treated with EO's and chlorine

The number of volatiles that were detected and identified in passive MAP lettuce and carrots were 26 and 36, respectively (Table 3). Volatile compounds are secondary metabolites resulting from the degradation of primary metabolites, such as fats and fatty acids, peptides and amino acids, and carbohydrates. Some metabolic pathways produce volatile compounds in unprocessed horticultural produce, but most of them are either enhanced or activated as a consequence of the wound-induced stress following processing (Charron & Cantliffe, 1995; Choi, Tomas-Barberan & Salveit, 2005).

Terpenes were the main group of detected volatiles and different terpene profiles were found between lettuce and carrots. Eleven terpenes were specific to carrots (α-bergamotene, α-caryophyllene, α-curcumene, α-longipinene, β-ocimene, β-pinene, δ-elemene, γ-muurolene, γ-terpinene, p-cymene and pyronene), only one was specific to lettuce (dehydro-p-cymene) and ledene was detected from both vegetables**.** Terpenes are known to contribute to the fresh flavor of many vegetables (Fischer & Scott, 1997), therefore they are possible markers of the odor profile of ready-to-eat vegetables. Most of the identified terpenes are associated with odor descriptions that are generally accepted by consumers, such as wood, tea, warm, sweet, herb, pine or citrus (Table 3). However, some terpenes were related to off-odor profiles, such as the compounds α-longipinene, β-pinene, γ-terpinene or *p*-cymene, which are generally perceived as turpentine, gasoline or solvent.

A wide variety of volatile organic compounds, including benzoic acids and phenols, are emitted by the shikimic acid pathway and the phenylpropanoid acid pathway, which are involved in enzymatic browning (Heath & Reineccius, 1986; Fischer & Scott, 1997; Tomas-Barberan, Loaiza-Valverde, Bonfanti & Saltveit, 1997; Gil, Castaner, Fearers, Artes & Tomas-Barberan, 1998). In this work, 5 phenolic compounds were identified from carrots and lettuce (2,4-bis-1,1-dimethylethylphenol, 2,4-di-t-butyl-6-nitrophenol, 4,4,1-methyl-ethyledene-bis-phenol, phenol and butylated hydroxytoluene), while 5-methyl-phenyl-ester-benzoic acid was found from carrots, and 2-octyl-benzoic acid from lettuce and carrots. The odor description of the benzoic acids is associated with flower, honey, herb and sweetness (Table 3), so they may have participated in the development of the aroma perceived from the fresh vegetables.

Oxidized phenolics are substrates of polyphenoloxidase, which generates polyphenols, responsible for browning when combined with amino acids to form melanins (Bassil, Makris & Kefalas, 2005). The ketones detected in this study from both vegetables were 1,3-dehydro-5-methyl-2H-benzimidazol-2-one and 2,3-dehydro-6-amino-indol-2-one, while pyrovalerone was specific to carrots, and 2,3-dehydro-3,5-dehydroxy-6-methyl-4H-pyran-4-one and 5-hydroxy-methyl-dehydro-furan-2-one were specific to lettuce.

The main products of anaerobic metabolism, such as acetaldehyde or ethanol, are also interesting volatiles since the values of these compounds seem to increase in stressful conditions (Charron & Cantliffe, 1995; Lopez-Galvez, Peiser, Nie & Cantwell, 1997). The alcohols *2*-phenoxyethanol and cis-geraniol were detected from lettuce and carrots and they are related to odors described as honey, lilac, rose or geranium (Table 3). 4-methoxy-6,2-propenyl-1,3-benzodioxol was also detected but was specific for carrots treated with oregano in combination with thyme. Increases in alcohol levels during storage could be caused by fermentative reactions due to high CO_2 and/or low O_2 concentrations or due to microbiological activity (Ragaert et al., 2007). The microbiological production of alcohol has been shown on a model medium of mixed-lettuce agar (Jacxsens et al., 2003; Ragaert, Devlieghere, Devuyst, Dewulf, Van Langenhove & Debevere, 2006).

Two isocyanates (2-methyl-m-phenylene ester isocyanic acid and 4-methyl-m-phenylene ester isocyanic acid) and one sulphide (diphenyl sulphide) were identified in the passive MA-packaged lettuce and carrots. These volatiles are usually related to undesirable odors, such as paint, cabbage or sulphur, in agreement with Smith, Song and Cameron (1998), who reported that the presence of dimethyl sulfide in 10 day-old ready-to-eat lettuce was responsible for the development of a putrid aroma.

3.5 Volatiles identified as quality markers

Carvacrol and thymol methyl ether were specific to the EO treatments for both vegetables (Table 4). Thymol was detected from lettuce and carrots treated with oregano combined with thyme. For lettuce, the volatiles α-caryophyllene, β-cadinene, γ-cadinene, caryophyllene oxide and p-cymene were specific to the treatment of oregano in combination with thyme. Caryophyllene oxide and p-cymene were also found from lettuce washed with the solution containing oregano. For carrots, 4-methoxy-6,2-propenyl-1,3-benzodioxol was specific for the treatment of oregano in combination with thyme. Carvacrol, thymol, caryophyllene and p-cymene are some of the main components of oregano and thyme EO's, and may have contributed to the off-odor and after-taste perceived by the panelists.

The linear regression and principle components analysis for passive MAP lettuce over the 7 days of storage showed that carvacrol and p-cymene were markers of appreciation difference between chlorine and the EO treatments (Fig. 2A). The volatile ledene and the sensory attribute browning were correlated for all the treatments (Fig. 2A). The losses of aroma, color and texture reported by sensory analysis were related to the increase in TVC, Enterobacteria and *Pseudomonas*, while the volatiles ledene, 1,3-dehydro-5-methyl-2H-benzimidazol-2-one, 2-methyl-m-phenylene ester isocyanic acid, thio-amino-butanamide, 2,4-di-t-butyl-6-nitrophenol, and 2,4-bis-1,1-dimethylethylphenol were found to be quality markers for all the treatments. The volatile quality markers identified for lettuce (Table 4A) were then correlated to both sensory data and microbiological results and the two following clusters were observed: (1) 2,4-di-t-butyl-6-nitrophenol, 1,3-dehydro-5-methyl-2H-benzimidazol-2-one and texture; (2) 2-methyl-m-phenylene ester isocyanic acid, off-odor, TVC, Enterobacteria and *Pseudomonas* (Fig. 3A).

Linear regression analysis for passive MAP carrots over the 7 days of storage showed that β-ocimene was a marker of quality difference between chlorine and EO treatments. 1,3-dehydro-5-methyl-2H-benzimidazol-2-one was also identified as a marker of aroma difference between oregano in combination with thyme and the two other treatments (oregano and chlorine) when PCA complemented linear regression analysis (Fig. 2B). Browning was related to the increase over storage in TVC, LAB and *Pseudomonas*. The volatiles ledene, α-bergamotene, α-caryophyllene, α-longipinene, 1,3-dehydro-5-methtyl-2H-benzimidazol-2-one and thio-amino-butanamide were also correlated to the increase in the spoilage bacteria population and consequently identified as quality markers for all the treatments. Terpenes are generally synthesized by the mevalonic acid pathway (Logan, Monson & Potosnak, 2000; Lee, Everts & Beynen, 2004) but also by some microorganisms (Charron & Cantliffe, 1995). Such a pathway would then be in competition with the plant metabolism for the substrates and intermediates of the mevalonic acid pathway and consequently alter the specific organoleptic properties of fresh vegetables. When the quality marker volatiles for carrots were grouped (Table 4B) and correlated to both sensory data and microbiological results, the three following clusters were obtained: (1) α-caryophyllene, browning, TVC, LAB and *Pseudomonas*; (2) 1,3-dehydro-5-methyl-2H-benzimidazol-2-one, acceptability, appreciation, aroma and color; and (3) ledene and texture (Fig. 3B).

In general, the increase in TVC, Enterobacteria, Pseudomonas or LAB was associated with losses of aroma, color and texture as well as with browning. Previous studies reported that some flavor and visual defects can be induced by microbial growth (Carlin, Nguyen-The, Cudennec & Reich, 1989; King, Magnuson, Torok & Goodman, 1991; Barry-Ryan & O'Beirne, 1998; Hao, Brackett, Beuchat & Doyle, 1999). Nguyen-The and Prunier (1989) also found a relationship between the deterioration of leafy salads and the growth of *Pseudomonas* spp. More recently, unacceptable changes of appearance during storage of minimally processed artichoke and lettuce has been found due to a psychrotrophic count exceeding 8 log cfu/g (Li et al., 2001; Gimenez, Olarte, Sanz, Lomas, Echavarri & Ayala, 2003).

4 Conclusion

The effectiveness of oregano as a decontamination treatment was comparable with that of chlorine. Moreover, when carrots were treated with oregano, the initial TVC concentration was significantly lower than in the water-treated samples. Since passive MAP carrot discs treated with the EO regimes were acceptable in terms of sensory quality and appreciation, oregano could offer a natural alternative for the washing and preservation of MPFV. Furthermore, as plant EO's are not only considered among the most important natural antimicrobial agents but also have antioxidant and anti-inflammatory activities (Longaray-Delamare, Moschen-Pistorello, Artico, Atti-Serafini & Echeverrigaray, 2005), they could be employed to extend shelf-life of minimally processed foods as well as confer other benefits to consumers health.

However, the application of EO's on ready-to-eat vegetables requires further studies incorporating additional hurdles such as active MAP as well as extensive sensory screening in order to ensure the overall quality of the product, whilst retaining food safety. The potential nutraceutical properties of EO's in product application studies also merit further investigation. Although EO's used in this study might replace or reduce the concentration of chlorine or other chemical preservatives, panelists rejected lettuce washed with the EO treatments at the end of the storage period for overall appreciation. The combination of EO's with other natural preservatives might minimize doses and consequently reduce impact on organoleptic properties of fresh vegetables.

A detection method for quality markers of minimally processed vegetables has been developed, based on the volatile emission changes and their correlation with sensory and microbiological analyses. Further studies could include the development of an on-line quality-monitoring method at industrial level to target specific volatiles, in order to optimize the minimal processing and modified atmosphere packaging, with a view to extending their shelf-life. This could include the development of intelligent or active labels responding to specific changes in concentrations of selected volatile quality markers. Investigation of enzymatic activities may also be of interest to further define the metabolic pathways generating quality-related volatile compounds.

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Figure Captions

Figure 1. Evolution of the sensory profile of lettuce (A) and carrots (B) treated with chlorine (—), oregano (—), or oregano and thyme (━) over 7 days.

Different letters signify statistical differences between values ($p < 0.05$) for each attribute. Descriptions for each score were as follows: 9 = like extremely or extremely high, 8 = like very much or very high, 7 = like moderately or high, 6 = like slightly or lightly high, 5 = neither like or dislike or neither high or low, 4 = dislike slightly or slightly low, 3 = dislike moderately or low, 2 = dislike very much or very low, and 1 = dislike extremely or extremely low. No tasting was carried out at day 7.

Figure 2. 3-D PCA plots of the volatile quality markers (Y axis) and sensory attributes (X axis) of passive MA-packaged lettuce (A) and carrots (B). Clusters are indicated by circles. Volatiles quality markers included in the graphics are α-bergamotene (bergamote), α-caryophyllene (humelene), α-longipinene (longipine), β-ocimene (ocimene), 1,3-dehydro-5-methyl-2H-benzimidazol-2-one (azolone), 2-methyl-m-

phenylene ester isocyanic acid (cyanic2), 2,4-bis-1,1-dimethylethylphenol (ethylphenolphenol), 2,4-di-t-butyl-6-nitrophenol (nitrophenol), carvacrol, ledene, and *p*-cymene (cymene). Judgment and quality are appreciation and acceptability, respectively.

Figure 3. 3-D PCA plots of the volatile quality markers (Z axis), sensory attributes (X axis) and changes in bacterial populations (Y axis) of passive MA-packaged lettuce (A) and carrots (B). Clusters are indicated by circles. Volatiles quality markers included in the graphics are α-bergamotene (bergamote), α-caryophyllene (humelene), α-longipinene (longipine), β-ocimene (ocimene), 1,3-dehydro-5-methyl-2H-benzimidazol-2-one (azolone), 2-methyl-m-phenylene ester isocyanic acid (cyanic2), 2,4-bis-1,1-dimethylethylphenol (ethylphenolphenol), 2,4-di-t-butyl-6-nitrophenol (nitrophenol) and ledene. Bacterial populations comprise TVC (tvc), Enterobacteria (entero), Pseudomonas (pseudo) and LAB (lab). Judgment and quality are appreciation and acceptability, respectively.

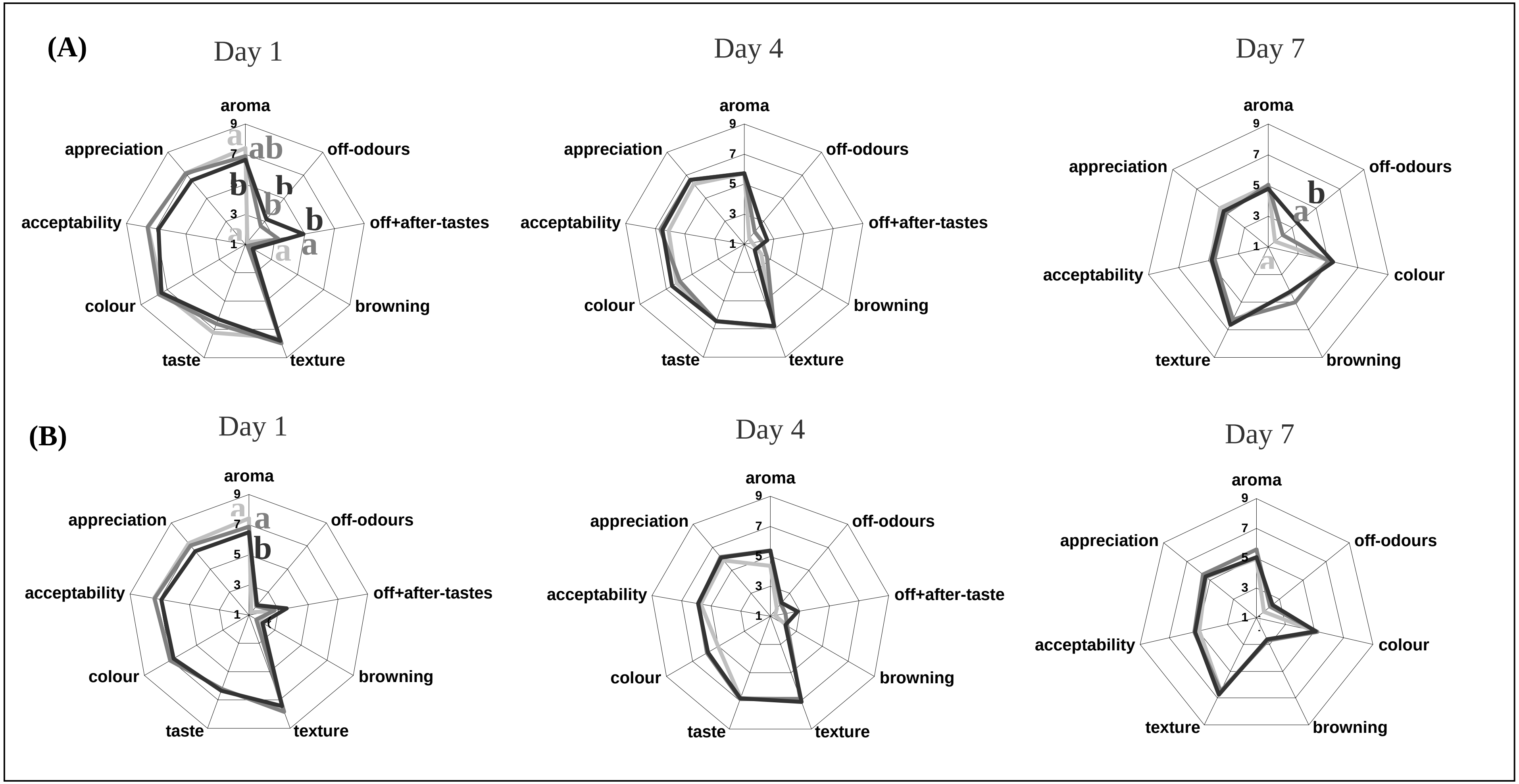
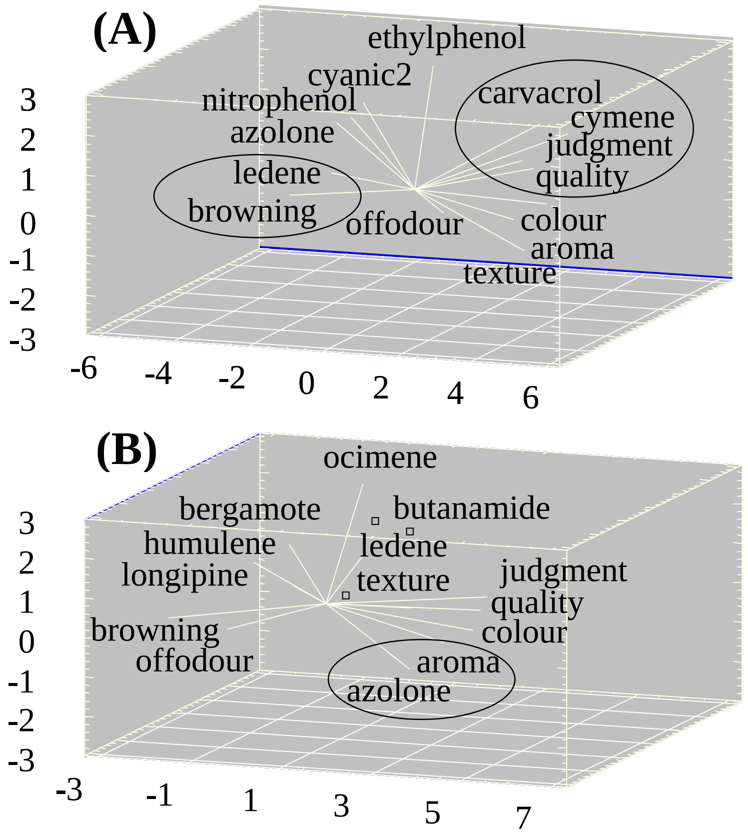


Fig. 1. Evolution of the sensory profile of lettuce (A) and carrots (B) treated with chlorine (—), oregano (—), or oregano and thyme (—) over 7 days. Different letters signify statistical differences between values ($p < 0.05$) for each attribute. Descriptions for each score were as follows: 9 = like extremely or extremely high, 8 = like very much or very high, 7 = like moderately or high, 6 = like slightly or lightly high, 5 = neither like or dislike or neither high or low, 4 = dislike slightly or slightly low, 3 = dislike moderately or low, 2 = dislike very much or very low, and 1 = dislike extremely or extremely low. No tasting was carried out at day 7.



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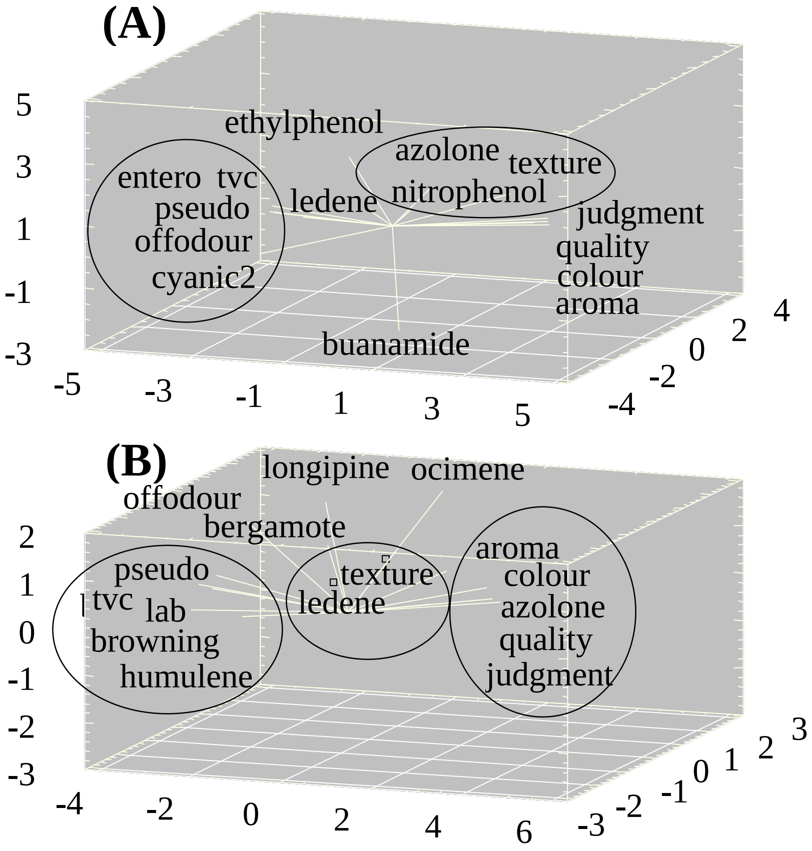

Fig. 3. 3-D PCA plots of the volatile quality markers (Z axis), sensory attributes (X axis) and changes in bacterial populations (Y axis) of passive MA-packaged lettuce (A) and carrots (B). Clusters are indicated by circles. Volatiles quality markers included in the graphics are α-bergamotene (bergamote), α-caryophyllene (humelene), α-longipinene (longipine), β-ocimene (ocimene), 1,3-dehydro-5-methyl-2H-benzimidazol-2-one (azolone), 2-methyl-m-phenylene ester isocyanic acid (cyanic2), 2,4-bis-1,1-dimethylethylphenol (ethylphenolphenol), 2,4-di-t-butyl-6-nitrophenol (nitrophenol) and ledene. Bacterial populations comprise TVC (tvc), Enterobacteria (entero), Pseudomonas (pseudo) and LAB (lab). Judgment and quality are appreciation and acceptability, respectively.

Table 1

Survival of TVC, Enterobacteria and *Pseudomonas* on prepared lettuce salad treated with EO's or chlorine

Bacterial population	**Day 0**			**Day 2**			**Day 4**			**Day 7**		
TVC												
Oregano	**5.12**	± 0.10	ab	**6.60**	± 0.38	c	**7.64**	± 0.44	d	**8.26**	± 0.57	d
Oregano + Thyme	**4.96**	± 0.04	a	**6.29**	± 0.15	c	**7.74**	± 0.25	d	**8.16**	± 0.59	d
Chlorine	**4.68**	± 0.40	a	**6.30**	± 0.26	c	**7.17**	± 0.44	cd	**7.89**	± 0.26	d
Water	**4.91**	± 0.02	a	**6.10**	± 0.31	bc	**7.50**	± 0.23	d	**7.98**	± 0.43	d
Untreated	**5.59**	± 0.40	b	**6.69**	± 0.06	c	**6.91**	± 0.17	c	**7.41**	± 0.09	d
Enterobacteria												
Oregano	**3.27**	± 0.21	ab	**4.99**	± 0.54	c	**5.26**	± 0.03	c	**6.20**	± 0.27	d
Oregano + Thyme	**3.54**	± 0.15	ab	**4.81**	± 0.45	c	**5.83**	± 0.90	cd	**6.12**	± 0.49	d
Chlorine	**2.89**	± 0.06	a	**4.39**	± 0.60	c	**4.97**	± 0.54	cd	**5.52**	± 0.70	cd
Water	**3.82**	± 0.62	bc	**4.94**	± 0.40	c	**5.29**	± 0.01	c	**5.99**	± 0.06	d
Untreated	**4.51**	± 0.10	c	**5.30**	± 0.74	c	**5.50**	± 0.46	cd	**6.66**	± 0.60	d
Pseudomonas												
Oregano	**2.69**	± 0.76	a	**5.18**	± 0.05	b	**5.96**	± 0.06	bc	**6.89**	± 0.27	c
Oregano + Thyme	**3.31**	± 0.71	a	**5.72**	± 0.97	bc	**6.01**	± 0.96	bc	**6.79**	± 0.73	c
Chlorine	**2.28**	± 0.74	a	**5.40**	± 0.29	b	**5.96**	± 0.17	c	**6.51**	± 0.36	c
Water	**3.34**	± 0.48	a	**5.19**	± 0.56	b	**6.40**	± 0.07	c	**6.86**	± 0.39	c
Untreated	**3.86**	± 0.56	a	**5.65**	± 0.42	bc	**5.66**	± 0.89	bc	**5.99**	± 0.11	c

Counts are expressed in Log cfu ml^{-1} (+/- standard deviation). Means followed by different letters are significantly different ($p<0.05$) for each bacterial population. The concentrations used for each treatment were the following: oregano (250 ppm), oregano + thyme (125 ppm + 250 ppm), and chlorine (120 ppm). Lettuce washed with distilled water and unwashed lettuce were used as controls.

Table 2

Survival of TVC, LAB and *Pseudomonas* on prepared carrot discs treated with EO's or chlorine

Bacterial population	**Day 0**			**Day 2**			**Day 4**			**Day 7**		
TVC												
Oregano	**3.77**	± 0.26	a	**3.96**	± 0.23	a	**5.18**	± 0.37	c	**6.09**	± 0.27	d
Oregano + Thyme	**4.47**	± 0.56	abc	**4.50**	± 0.49	abc	**5.65**	± 0.25	cd	**6.10**	± 0.24	d
Chlorine	**4.22**	± 0.22	ab	**4.50**	± 0.41	ab	**5.22**	± 0.22	cd	**5.88**	± 0.07	d
Water	**4.83**	± 0.13	bc	**5.25**	± 0.56	bc	**6.19**	± 0.15	d	**6.63**	± 0.15	d
Untreated	**5.09**	± 0.16	c	**5.29**	± 0.34	c	**6.33**	± 0.46	d	**6.57**	± 0.27	d
LAB												
Oregano	**2.39**	± 0.01	a	**2.94**	± 0.69	ab	**3.96**	± 0.24	b	**3.77**	± 0.03	b
Oregano + Thyme	**3.14**	± 0.44	ab	**3.38**	± 0.44	ab	**3.56**	± 0.31	b	**3.55**	± 0.37	b
Chlorine	**3.30**	± 0.56	ab	**3.35**	± 0.07	ab	**3.41**	± 0.75	b	**3.68**	± 0.56	b
Water	**3.20**	± 0.65	ab	**3.25**	± 0.84	ab	**3.64**	± 0.20	b	**3.14**	± 0.37	b
Untreated	**3.44**	± 0.14	b	**3.18**	± 0.82	ab	**3.99**	± 0.52	b	**3.39**	± 0.57	b
Pseudomonas												
Oregano	**3.54**	± 0.41	a	**3.97**	± 1.24	a	**5.02**	± 1.31	ab	**5.91**	± 1.15	b
Oregano + Thyme	**3.43**	± 0.93	a	**4.55**	± 0.33	a	**5.27**	± 0.69	b	**5.81**	± 0.39	b
Chlorine	**3.87**	± 1.15	a	**4.67**	± 1.41	a	**5.17**	± 1.42	b	**5.95**	± 1.11	b
Water	**3.87**	± 1.35	a	**4.83**	± 1.22	a	**5.58**	± 1.10	ab	**5.82**	± 1.30	b
Untreated	**4.27**	± 1.03	a	**4.82**	± 0.40	a	**5.75**	± 0.76	ab	**6.01**	± 0.43	b

Counts are expressed in Log cfu ml^{-1} (+/- standard deviation). Means followed by different letters are significantly different ($p<0.05$) for each bacterial population. The concentrations used for each treatment were the following: oregano (250 ppm), oregano + thyme (125 ppm + 250 ppm), and chlorine (120 ppm). Lettuce washed with distilled water and unwashed lettuce were used as controls.

Table 3

Volatile compounds identified in passive MA-packaged lettuce (●) and carrots (▲) treated with oregano, oregano with thyme or chlorine

Volatile compound name	**Odor description**[a]	**Oregano**			**Oregano + Thyme**			**Chlorine**		
		Day 1	Day 4	Day 7	Day 1	Day 4	Day 7	Day 1	Day 4	Day 7
α-bergamotene	Wood, warm, tea	▲	▲	▲	▲	▲	▲	▲	▲	▲
α-caryophyllene	Wood	▲	▲	▲	▲●	▲	▲	▲	▲	▲
α-curcumene	Herb	▲	▲	▲	▲	▲	▲	▲	▲	▲
α-longipinene	Pine, turpentine	▲	▲	▲	▲	▲	▲	▲	▲	▲
β-cadinene	Thyme, wood				●	●	●			
β-ocimene	Sweet, herb	▲	▲	▲	▲	▲	▲	▲	▲	▲
β-pinene	Pine, resin, turpentine	▲			▲			▲		
δ-elemene	Wood	▲	▲	▲	▲	▲	▲	▲	▲	▲
γ-cadinene	Thyme, wood				●	●	●			
γ-muurolene	Herb, wood, spice	▲	▲		▲	▲		▲	▲	
γ-terpinene	Gasoline, turpentine	▲	▲	▲	▲	▲	▲	▲	▲	▲
1,3-dehydro-5-methyl-2H-benzimidazol-2-one	Paint		●	▲●		●	▲●		●	▲●
2-diethoxymethyl-1H-imidazole	Fruit	▲●						▲●		
2-methyl-m-phenylene ester isocyanic acid	Paint	▲●	▲●	▲●	▲●	▲●	▲●	▲	▲	▲●
2-octyl-benzoic acid	Lettuce, herb, sweet	▲●			▲●			▲●		
2-phenoxyethanol	Honey, spice, rose, lilac	▲●	▲●	▲●	▲●	▲●	▲●	▲●	▲●	▲●
2,3-dehydro-3,5-dehydroxy-6-methyl-4H-pyran-4-one	Caramel	●	●		●	●		●	●	
2,3-dehydro-6-amino-indol-2-one	Mothball, burnt	▲●	▲●	▲●	▲●	▲●	▲●	▲●	▲●	▲●
2,4-bis-1,1-dimethylethylphenol	Phenol	▲●	▲●	▲●	▲●	▲●	▲●	▲●	▲●	▲●
2,4-di-t-butyl-6-nitrophenol	Sweet	▲●	▲●	▲●	▲●	▲●	▲●	▲●	▲●	▲●
4-methoxy-6,2-propenyl-1,3-benzodioxol	Spice					▲	▲			

[a] Compound odour reported in the database http://www.flavornet.org. Volatiles compounds identified in MA-packaged lettuce and carrots are indicated with circles and triangles, respectively.

Table 3 (Continued)

Volatile compounds identified in passive MA-packaged lettuce (●) and carrots (▲) treated with oregano, oregano with thyme or chlorine

Volatile compound name	**Odor description**[a]	**Oregano**			**Oregano + Thyme**			**Chlorine**		
		Day 1	Day 4	Day 7	Day 1	Day 4	Day 7	Day 1	Day 4	Day 7
4-methyl-1,3-benzene-diamine	Paint			▲●			▲●			▲●
4-methyl-m-phenylene ester isocyanic acid	Paint	▲●	▲●	▲●	▲●	▲●	▲●	▲●	▲●	▲●
4,4,1-methyl-ethyledene-bis-phenol	Not described	▲●	▲●	▲●	▲●	▲●	▲●	▲●	▲●	▲●
5-methyl-phenyl-ester-benzoic acid	Flower, honey		▲	▲		▲	▲		▲	▲
5-hydroxy-methyl-dehydro-furan-2-one	Spice		●			●			●	
Butylated hydroxytoluene	Phenol			▲			▲			▲
Caryophyllene oxide	Wood	▲●	▲●	▲●	▲●	▲●	▲●	▲	▲	▲
Carvacrol	Citrus, warm	▲●	▲●	▲●	▲●	▲●	▲●			
Cis-geraniol	Rose, geranium	▲●	▲		▲●	▲		▲●	▲	
Dehydro-p-cymene	Citrus, pine	●			●			●		
Diphenyl sulphide	Cabbage, sulphur	▲●	▲●	▲●	▲●	▲●	▲●	▲●	▲●	▲●
Isobornyl formate	Green, earth, camphor	▲			▲			▲		
Ledene	Not described	▲●	▲●	▲●	▲●	▲●	▲●	▲●	▲●	▲●
p-cymene	Solvent, gasoline, citrus	▲●	▲●	▲	▲●	▲●	▲	▲	▲	▲
Phenol	Phenol	▲	▲		▲	▲		▲	▲	
Pyrovalerone	Wet	▲	▲	▲	▲	▲	▲	▲	▲	▲
Pyronene	Wood, wet	▲	▲	▲	▲	▲	▲	▲	▲	▲
Thio-amino-butanamide	Not described	▲●	▲●	▲●	▲●	▲●	▲●	▲●	▲●	▲●
Thymol	Citrus, warm, mint				▲●	▲●	▲●			
Thymol methyl ether	Herbal	▲●			▲●	▲	▲			

[a] Compound odour reported in the database http://www.flavornet.org. Volatiles compounds identified in MA-packaged lettuce and carrots are indicated with circles and triangles, respectively.

Table 4

Evolution of quality markers of passive MA-packaged lettuce (A) or carrots (B) treated with oregano, oregano with thyme or chlorine over 7 days of storage

Quality marker volatile	**Oregano**			**Oregano + Thyme**			**Chlorine**		
	Day 1	Day 4	Day 7	Day 1	Day 4	Day 7	Day 1	Day 4	Day 7
(A)									
1,3-dehydro-5-methyl-2H-benzimidazol-2-one	0.00	1.21 ± 1.71	6.05 ± 0.55	0.00	3.33 ± 2.36	4.75 ± 3.87	0.00	1.75 ± 0.88	2.83 ± 1.24
2-methyl-m-phenylene ester isocyanic acid	1.39 ± 0.99	1.09 ± 0.77	4.02 ± 2.03	1.56 ± 1.11	2.40 ± 1.70	5.83 ± 4.13	0.00	0.00	2.45 ± 1.54
2,4-bis-1,1-dimethylethylphenol	1.18 ± 0.55	1.81 ± 0.04	1.68 ± 0.08	1.98 ±0.71	2.19 ± 0.46	1.59 ± 0.00	1.35 ± 0.14	1.75 ± 0.42	1.84 ± 0.00
2,4-di-t-butyl-6-nitrophenol	0.78 ± 0.70	1.26 ± 0.03	1.21 ± 0.21	1.05 ± 0.88	1.27 ± 0.04	1.16 ± 0.00	0.89 ± 0.68	1.09 ± 0.04	1.16 ± 0.00
Carvacrol	11.94 ± 9.57	8.88 ± 1.10	4.56 ± 2.16	11.13 ± 3.84	8.62 ± 7.52	5.48 ± 3.91	0.00	0.00	0.00
p-cymene	3.04 ± 1.59	2.96 ± 2.00	0.00	3.99 ± 0.57	2.89 ± 0.74	0.00	0.00	0.00	0.00
Ledene	0.69 ± 0.42	1.83 ± 1.13	2.02 ± 0.45	0.29 ± 0.19	1.83 ± 0.98	1.41 ± 1.03	2.25 ± 1.08	2.24 ± 0.26	1.23 ± 0.65
Thio-amino-butanamide	3.19 ± 0.00	3.24 ± 0.40	3.29 ± 0.67	3.17 ± 0.00	2.98 ± 1.17	3.30 ± 0.25	3.95 ± 0.00	2.26 ± 0.56	3.83 ± 0.27
(B)									
α-bergamotene	0.87 ± 0.31	0.98 ± 0.18	1.19 ± 0.00	0.58 ± 0.04	1.23 ± 0.09	0.91 ± 0.39	0.95 ± 0.00	1.02 ± 0.09	0.98 ± 0.00
α-caryophyllene	4.20 ± 0.95	5.62 ± 0.66	2.83 ± 1.87	7.01 ± 5.08	7.65 ± 1.23	10.28 ± 6.89	10.06 ± 2.01	6.63 ± 0.87	5.49 ± 0.89
α-longipinene	0.15 ± 0.00	0.74 ± 0.00	1.18 ± 0.00	0.25 ± 0.00	0.74 ± 0.00	0.90 ± 0.00	0.20 ± 0.00	0.75 ± 0.00	1.02 ± 0.00
β-ocimene	2.20 ± 0.74	2.54 ± 0.91	2.08 ± 1.10	1.74 ± 0.20	2.76 ± 0.85	1.81 ± 0.75	1.72 ± 0.57	1.78 ± 0.27	1.68 ±0.31
1,3-dehydro-5-methyl-2H-benzimidazol-2-one	0.00	0.00	0.82 ± 0.58	0.00	0.00	4.56 ± 3.23	0.00	0.00	1.34 ± 0.00
Ledene	6.89 ± 0.47	5.30 ± 0.34	2.08 ± 0.95	9.25 ± 4.07	7.26 ± 0.12	4.98 ± 1.58	8.88 ± 0.18	4.95 ± 0.83	4.08 ± 0.26
Thio-amino-butanamide	3.18 ± 0.00	3.60 ± 0.53	2.71 ± 0.64	2.32 ± 0.00	2.97 ± 0.69	3.07 ± 0.80	4.36 ± 0.00	2.82 ± 0.41	2.67 ± 1.10

Estimation of the volatile compounds quantity was based on the areas of the peaks detected by MS. The headspace concentration of a volatile compound was then expressed in percentage of total volatile compounds detected or percentage of the total peak area (+/- standard deviation).