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

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

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24 **Abstract**

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

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47 **1. Introduction**

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

61 Disinfection processes incorporating chlorine are often applied to fresh vegetables
62 to enhance safety and shelf-life profiles, but its use has limitations and disadvantages,
63 such as a reduced antimicrobial effectiveness or the possible formation of carcinogenic
64 chlorinated compounds (Li, Brackett, Shewfelt & Beuchat, 2001; Martin-Diana, Rico,
65 Barry-Ryan, Frias, Henehan & Barat, 2007). With increased concern about efficacy and
66 toxicological safety of chemicals and synthetic preservatives, the demand for natural
67 alternatives has increased. In this context, plant essential oils (EO's) are attracting interest
68 for their potential as natural food preservatives as they have Generally Recognised As
69 Safe (GRAS) status and many of them display a wide spectrum of antimicrobial activity,

70 with potential for control of foodborne pathogens and spoilage bacteria associated with
71 ready-to-eat vegetables (Gutierrez, Rodriguez, Barry-Ryan & Bourke, 2008a). Oregano
72 (*Origanum vulgare L.*) and thyme (*Thymus vulgaris L.*) oils, whose main components are
73 carvacrol and thymol respectively, are characterized by strong antibacterial properties
74 (Dorman & Deans, 2000; Elgayyar, Draughon, Golden & Mount, 2001; Burt, 2004;
75 Oussalah, Caillet, Saucier & Lacroix, 2006; Gutierrez et al., 2008a). However, if EO's
76 are expected to be widely applied as natural antimicrobials, the organoleptic impact
77 should be considered as the use of naturally derived preservatives can alter the taste of
78 food or exceed acceptable flavor thresholds (Hsieh, Mau & Huang, 2001; Nazer,
79 Kobilinsky, Tholozana & Dubois-Brissonneta, 2005). Recently, it was observed that
80 lettuce treated with oregano at 250 ppm was acceptable to a sensory panel as they did not
81 find differences between this lettuce and that washed with chlorinated water (Gutierrez et
82 al., 2008a). Furthermore, the use of oregano combined with thyme normally yields
83 additive antimicrobial effects (Lambert, Skandamis, Coote & Nychas, 2001; Gutierrez,
84 Barry-Ryan & Bourke, 2008b), thus, this combination could minimize the concentrations
85 required, thereby reducing sensory impact.

86 MPFV manufacturers are often concerned with sensory improvement. Zhou et al.,
87 (2004) defined the shelf life of a green leafy vegetable as the length of time which it can
88 maintain an appearance that appeals to the consumer: crisp green vegetable with little
89 browning or wetness present. Sensory properties such as color, flavor and texture, play a
90 key role in the consumer's choice of fresh prepared products. An issue associated with
91 ready-to-eat vegetables is short shelf-life, which is usually no more than 8 days when
92 stored in adequate conditions (Allende & Artes, 2003). Beyond day 7 of storage, these

93 products present off-flavors, tissue softening and proliferation of microorganisms,
94 making them more perishable than untreated material (Watada & Qui, 1999). Thus,
95 optimizing the application of any novel natural preservation approach to shelf-life
96 extension of MPFV requires that sensory analyses as well as other more objective
97 methods, such as measurement of texture, color or water activity, and volatile emissions
98 analysis are incorporated into the experimental design, in order to monitor possible
99 changes on organoleptic properties. In this context, the identification of quality
100 biomarkers among the volatile emissions from fresh vegetables can help to develop and
101 optimize a rapid quality-monitoring method as well as an understanding of the origin and
102 metabolic basis of volatile emission changes in MPFV during storage (Lonchamp, 2006).
103 Little information is generally known about the relationship between the outgrowth of
104 spoilage microorganisms, their production of metabolites, including volatiles, and the
105 perception of the decay of minimally processed vegetables by consumers (Jacxsens,
106 Devlieghere, Ragaert, Vanneste & Debevere, 2003).

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

112

113 **2. Materials and methods**

114 *Essential oils*

115 The EO's used in this study were oregano (*Origanum vulgare*) and thyme (*Thymus*
116 *vulgaris*). They were selected based on previously reported efficacy (Gutierrez et al.
117 2008a), and were obtained from Guinness Chemical Ltd. (Portlaoise, Ireland) as pure
118 CO₂ soluble supercritical fluid extracts.

119

120 *Preparation of vegetable model products*

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

138 *Microbiological Analysis*

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

150

151 *Quality markers studies*

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

157 A gas analyzer (MAPTEST 4000, Hitech Instruments Ltd., UK) was used to monitor %
158 levels of CO₂ and O₂ in the package during storage. Gas composition was measured using
159 a hypodermic needle inserted through an impermeable patch of polyvinylchloride (PVC)
160 adhesive septum fixed to the bags.

161 Color measurement was performed using a Color Quest XE colorimeter (Hunter Lab,
162 Northants, UK). The colorimeter was calibrated using a white reference tile ($L^* = 93.97$,
163 $a^* = -0.88$ and $b^* = 1.21$) and a light trap (black tile) under illumination conditions. Nine
164 random areas were measured thorough the packaging film, and the three CIELAB color
165 values (L^* , a^* and b^*) were recorded. The illuminant chosen was D65 and the observer
166 used was 10°. The variable L^* (lightness index scale) ranges from 0 for black to 100 for
167 white. The a^* scale measures the degree of red ($+a^*$) or green ($-a^*$) colors and the b^*
168 scale measures the degree of yellow ($+b^*$) or blue ($-b^*$) colors.

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

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

177

178 *2.5 Sensory analysis*

179 Sensory analysis was performed using a 10 member trained panel with an age range
180 of 25-40 years. The panel consisted of four females and six males who were trained to be
181 familiar with sensory properties of minimally processed lettuce and carrots. The sensory
182 testing method was an acceptance test in which the sensory parameters were scored on a
183 descriptive scale of 1-9. The sensory parameters investigated included the following: (i)

184 vegetable aroma; (ii) off-odor; (iii) color; (iv) browning; (v) texture; (vi) vegetable taste;
185 (vii) off-after taste; (viii) overall acceptability; and (ix) overall appreciation. Descriptions
186 for each score were as follows: 9 = like extremely or extremely high, 8 = like very much
187 or very high, 7 = like moderately or high, 6 = like slightly or lightly high, 5 = neither like
188 or dislike or neither high or low, 4 = dislike slightly or slightly low, 3 = dislike
189 moderately or low, 2 = dislike very much or very low, and 1 = dislike extremely or
190 extremely low. Testing was carried out in sensory analysis booths located adjacent to the
191 processing hall with appropriate lighting conditions and temperature of around 18-20°C.
192 Results were monitored using the Compusense® Five software (Release 4.4, Ontario,
193 Canada). Sensory trials were replicated twice.

194

195 *2.6 Volatile emission analysis: Solid-Phase Micro-Extraction (SPME) and Gas* 196 *chromatography-mass spectrometry (GC/MS) analysis*

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

205 A Varian 3800 GC (JVA Analytical Ltd., Ireland) with a 2200 Varian ion trap MS
206 was used to analyze the samples. SPME fiber injections were made splitless for 3 min

207 with the GC injection port temperature held at 250°C. Grade 5.0 helium, filtered through
208 a Gas Clean GC/MS filter (Varian), was used as the carrier gas at a constant flow rate of
209 2.0 ml/min. Volatile compounds were adsorbed by a fused-silica capillary column (CP-
210 Sil 8, JVA Analytical Ltd., Ireland) with a length of 30 mm, an inner diameter of 0.25
211 mm and a 0.25 µm film thickness. The initial column oven temperature was set at 30°C
212 and held at this temperature for 5 min. The temperature was then increased to 250°C at a
213 rate of 5°C/min and the final temperature of 250°C was maintained for 15 min. MS
214 analysis of the eluted compounds was then carried out using the technique of electron
215 impact ionization. The electron ion source energy used was 70 eV and the mass range
216 chosen was from 40 m/z to 350 m/z. Data were collected using the Varian software and
217 mass spectra of detected compounds were analyzed by library searching in the National
218 Institute of Standards and Technology (NIST) databases. Estimation of the volatile
219 compounds quantity was based on the areas of the peaks detected by MS. The headspace
220 concentration of a volatile compound was then expressed in percentage of total volatile
221 compounds detected or percentage of the total peak area. The compounds were identified
222 with high probabilities when compared with standards from the NIST database (similarity
223 coefficient or reverse similarity coefficient > 85%). Additional information was obtained
224 for the compounds detected using Flavornet, an online compilation of aroma compounds
225 found in human odor space.

226

227 *2.7 Statistical analysis*

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

231 Linear regression analysis was used to determine correlations between changes in
232 volatile emissions, sensory properties and bacterial populations. A R^2 value higher than
233 0.90 was considered as indicator of satisfactory correlation between the factors and the
234 volatile compound analyzed was then considered as a marker of the sensory attribute or
235 the changes in bacterial population.

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

240

241 **3 Results and discussion**

242 *3.1 Effect of EO's and chlorine on the natural microflora of lettuce and carrots*

243 Survival of TVC, Enterobacteria, *Pseudomonas* spp. and LAB on treated lettuce
244 and carrots are indicated in Tables 1 and 2, respectively. The initial effect of EO's on
245 TVC, Enterobacteria and LAB was not significantly different ($p < 0.05$) to that obtained
246 using chlorine or water. The solution containing oregano recorded a significantly lower
247 initial TVC level than the water treatment on carrots. When oregano was combined with
248 thyme, the effect on bacteria was the same as that observed with the oregano alone ($p <$
249 0.05). Thus, from a microbiological point of view, oregano is a viable alternative to
250 chlorine as decontamination treatments. However, all treatments had a minimal

251 decontamination effect against *Pseudomonas* and did not maintain the initial decrease in
252 the remainder of the bacterial populations over the storage period. Uyttendaele, Neyts,
253 Vanderswalmen, Notebaert and Debevere (2004) reported that decontamination of carrots
254 with thyme accomplished a significant reduction of the indigenous flora but the
255 psychrotrophic aerobic flora recovered and multiplied during storage time. Bagamboula,
256 Uyttendaele and Debevere (2004) also observed limited reductions in the indigenous
257 flora of lettuce after decontamination treatment with thyme and attributed this to the
258 attachment of the indigenous flora and formation of biofilms on the surface of the lettuce
259 leaves. TVC found on fresh vegetables include a diverse microflora dominated by Gram-
260 negative bacteria, which are generally more resistant to the EO's than the Gram-positive
261 bacteria (Burt, 2004). In this respect, the combination of EO's with other natural
262 preservation methods as well as the improvement in packaging conditions might prolong
263 shelf-life of minimally processed vegetables.

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

273 washed with chlorine over 6 days, which was related to the sensitivity of these bacteria to
274 oxygen.

275

276 *3.2 Quality markers of lettuce and carrots treated with EO's and chlorine*

277 The gaseous composition in the bags containing samples washed with the EO
278 solutions were not significantly different ($p < 0.05$) to those recorded for samples treated
279 with chlorine. The initial conditions inside the OPP bags containing lettuce or carrots
280 were 20.9% O₂ and 0.1% CO₂. After 7 days of storage, the O₂ concentration was
281 approximately 12%, while the CO₂ concentration increased to 8-9% in both vegetables
282 type bags. The low concentration of LAB in lettuce could be attributed to these anaerobic
283 conditions, as previously observed by Klaiber et al. (2005), to the decontamination
284 methods used or to a synergistic effect of these two factors.

285 When color measurement was performed, no significant differences in lettuce color
286 values ($L^*a^*b^*$) were found between EO treatments and chlorine during the 7 days of
287 storage. With respect to carrots, samples treated with oregano in combination with thyme
288 were significantly ($p < 0.05$) darker ($L^* = 63.9 \pm 0.6$) than those washed with oregano
289 (61.6 ± 0.6) or chlorine (62.6 ± 0.4), but only on Day 1. During storage at 4°C for 7 days,
290 instrumental texture parameters and water activity values did not significantly ($p < 0.05$)
291 differ between the treatments (Data not shown).

292

293 *3.3 Sensory analysis of lettuce and carrots treated with EO's and chlorine*

294 The results of sensory analysis of EO and chlorine treatments are shown in Figure
295 1. Previous studies carried out in our laboratory (Gutierrez et al., 2008a; Gutierrez et al.,

296 2008b) showed that oregano oil was accepted by panelists at 250 ppm and that thyme oil
297 was only rejected at 500 ppm. These two EO's displayed additive anti-microbial effects
298 and the combination of 125 ppm of oregano oil and 250 ppm of thyme aimed at reducing
299 the sensory impact while maintaining the antimicrobial efficacy of the treatment. In this
300 study, carrots treated with oregano and oregano + thyme were accepted throughout the
301 storage period. Both EO treatments were suitable in terms of overall appreciation and no
302 significant differences were found between samples treated with the EO's and chlorine (p
303 < 0.05). However, on Day 1 the vegetable aroma perceived from samples treated with
304 oregano + thyme was significantly ($p < 0.05$) less intense than that of oregano or
305 chlorine. In this context, Valero and Giner (2006) observed a positive score for carvacrol
306 but a strong smell and flavor of thymol which minimized the degree of acceptance or
307 liking for carrot broth. The strong effect of thyme on sensory quality of chopped bell
308 peppers was also described by Uyttendaele et al. (2004).

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

319

320 *3.4 Volatile emission from lettuce and carrots treated with EO's and chlorine*

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

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

340 A wide variety of volatile organic compounds, including benzoic acids and phenols,
341 are emitted by the shikimic acid pathway and the phenylpropanoid acid pathway, which

342 are involved in enzymatic browning (Heath & Reineccius, 1986; Fischer & Scott, 1997;
343 Tomas-Barberan, Loaiza-Valverde, Bonfanti & Saltveit, 1997; Gil, Castaner, Fearers,
344 Artes & Tomas-Barberan, 1998). In this work, 5 phenolic compounds were identified
345 from carrots and lettuce (2,4-bis-1,1-dimethylethylphenol, 2,4-di-t-butyl-6-nitrophenol,
346 4,4,1-methyl-ethylenedene-bis-phenol, phenol and butylated hydroxytoluene), while 5-
347 methyl-phenyl-ester-benzoic acid was found from carrots, and 2-octyl-benzoic acid from
348 lettuce and carrots. The odor description of the benzoic acids is associated with flower,
349 honey, herb and sweetness (Table 3), so they may have participated in the development
350 of the aroma perceived from the fresh vegetables.

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

358 The main products of anaerobic metabolism, such as acetaldehyde or ethanol, are
359 also interesting volatiles since the values of these compounds seem to increase in stressful
360 conditions (Charron & Cantliffe, 1995; Lopez-Galvez, Peiser, Nie & Cantwell, 1997).
361 The alcohols 2-phenoxyethanol and cis-geraniol were detected from lettuce and carrots
362 and they are related to odors described as honey, lilac, rose or geranium (Table 3). 4-
363 methoxy-6,2-propenyl-1,3-benzodioxol was also detected but was specific for carrots
364 treated with oregano in combination with thyme. Increases in alcohol levels during

365 storage could be caused by fermentative reactions due to high CO₂ and/or low O₂
366 concentrations or due to microbiological activity (Ragaert et al., 2007). The
367 microbiological production of alcohol has been shown on a model medium of mixed-
368 lettuce agar (Jacxsens et al., 2003; Ragaert, Devlieghere, Devuyst, Dewulf, Van
369 Langenhove & Debevere, 2006).

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

376

377 *3.5 Volatiles identified as quality markers*

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

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

402 Linear regression analysis for passive MAP carrots over the 7 days of storage
403 showed that β -ocimene was a marker of quality difference between chlorine and EO
404 treatments. 1,3-dehydro-5-methyl-2H-benzimidazol-2-one was also identified as a marker
405 of aroma difference between oregano in combination with thyme and the two other
406 treatments (oregano and chlorine) when PCA complemented linear regression analysis
407 (Fig. 2B). Browning was related to the increase over storage in TVC, LAB and
408 *Pseudomonas*. The volatiles ledene, α -bergamotene, α -caryophyllene, α -longipinene,
409 1,3-dehydro-5-methyl-2H-benzimidazol-2-one and thio-amino-butanamide were also
410 correlated to the increase in the spoilage bacteria population and consequently identified

411 as quality markers for all the treatments. Terpenes are generally synthesized by the
412 mevalonic acid pathway (Logan, Monson & Potosnak, 2000; Lee, Everts & Beynen,
413 2004) but also by some microorganisms (Charron & Cantliffe, 1995). Such a pathway
414 would then be in competition with the plant metabolism for the substrates and
415 intermediates of the mevalonic acid pathway and consequently alter the specific
416 organoleptic properties of fresh vegetables. When the quality marker volatiles for carrots
417 were grouped (Table 4B) and correlated to both sensory data and microbiological results,
418 the three following clusters were obtained: (1) α -caryophyllene, browning, TVC, LAB
419 and *Pseudomonas*; (2) 1,3-dehydro-5-methyl-2H-benzimidazol-2-one, acceptability,
420 appreciation, aroma and color; and (3) ledene and texture (Fig. 3B).

421 In general, the increase in TVC, Enterobacteria, *Pseudomonas* or LAB was
422 associated with losses of aroma, color and texture as well as with browning. Previous
423 studies reported that some flavor and visual defects can be induced by microbial growth
424 (Carlin, Nguyen-The, Cudennec & Reich, 1989; King, Magnuson, Torok & Goodman,
425 1991; Barry-Ryan & O'Beirne, 1998; Hao, Brackett, Beuchat & Doyle, 1999). Nguyen-
426 The and Prunier (1989) also found a relationship between the deterioration of leafy salads
427 and the growth of *Pseudomonas* spp. More recently, unacceptable changes of appearance
428 during storage of minimally processed artichoke and lettuce has been found due to a
429 psychrotrophic count exceeding 8 log cfu/g (Li et al., 2001; Gimenez, Olarte, Sanz,
430 Lomas, Echavarri & Ayala, 2003).

431

432

433

434 **4 Conclusion**

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

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

454 A detection method for quality markers of minimally processed vegetables has been
455 developed, based on the volatile emission changes and their correlation with sensory and
456 microbiological analyses. Further studies could include the development of an on-line

457 quality-monitoring method at industrial level to target specific volatiles, in order to
458 optimize the minimal processing and modified atmosphere packaging, with a view to
459 extending their shelf-life. This could include the development of intelligent or active
460 labels responding to specific changes in concentrations of selected volatile quality
461 markers. Investigation of enzymatic activities may also be of interest to further define the
462 metabolic pathways generating quality-related volatile compounds.

463

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467

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596

597 Figure Captions

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

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

606

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

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

615

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

626

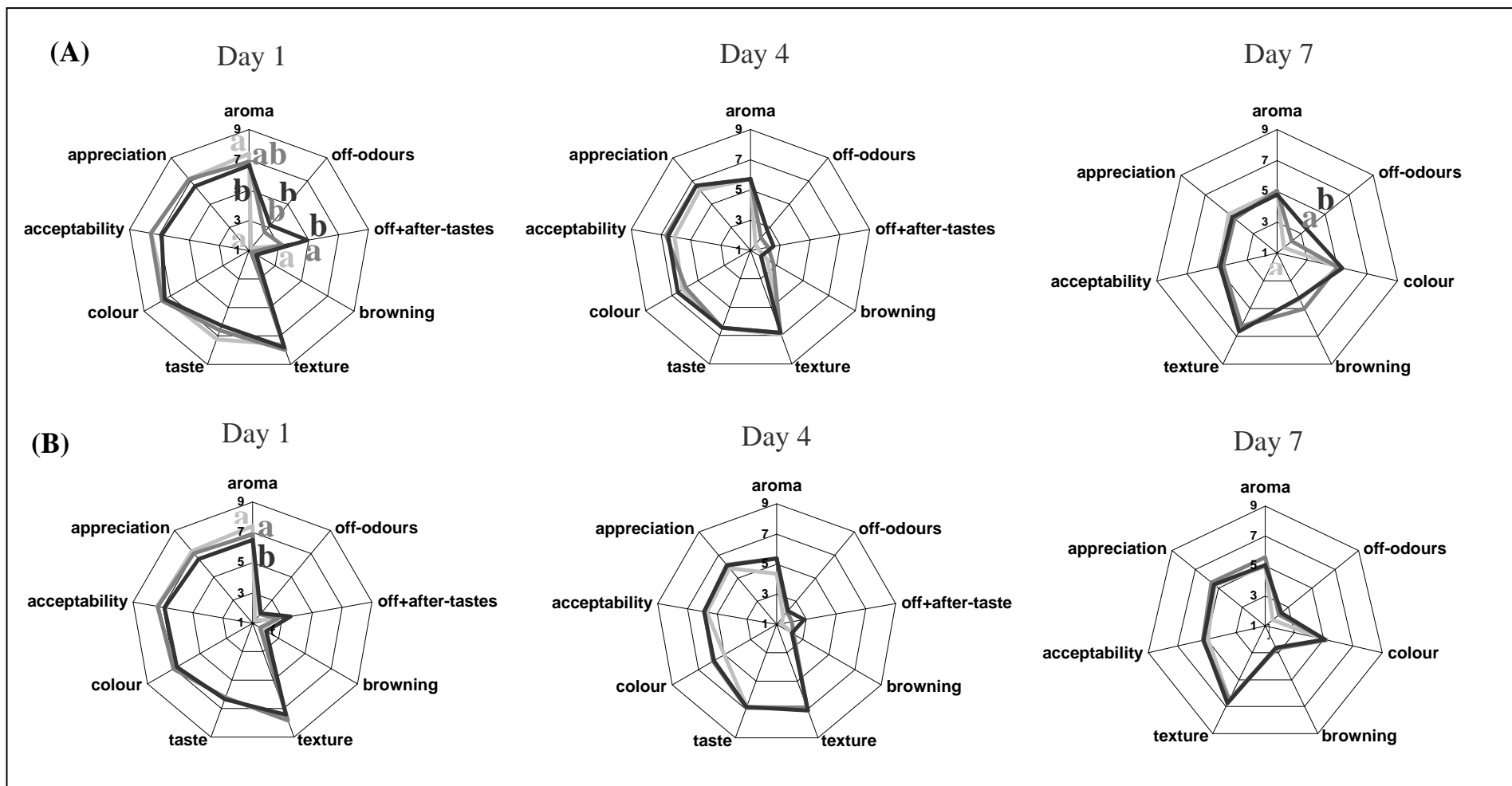


Fig. 1. Evolution of the sensory profile of lettuce (A) and carrots (B) treated with chlorine (—), oregano (—), or oregano and thyme (—) over 7 days. Different letters signify statistical differences between values ($p < 0.05$) for each attribute. Descriptions for each score were as follows: 9 = like 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.

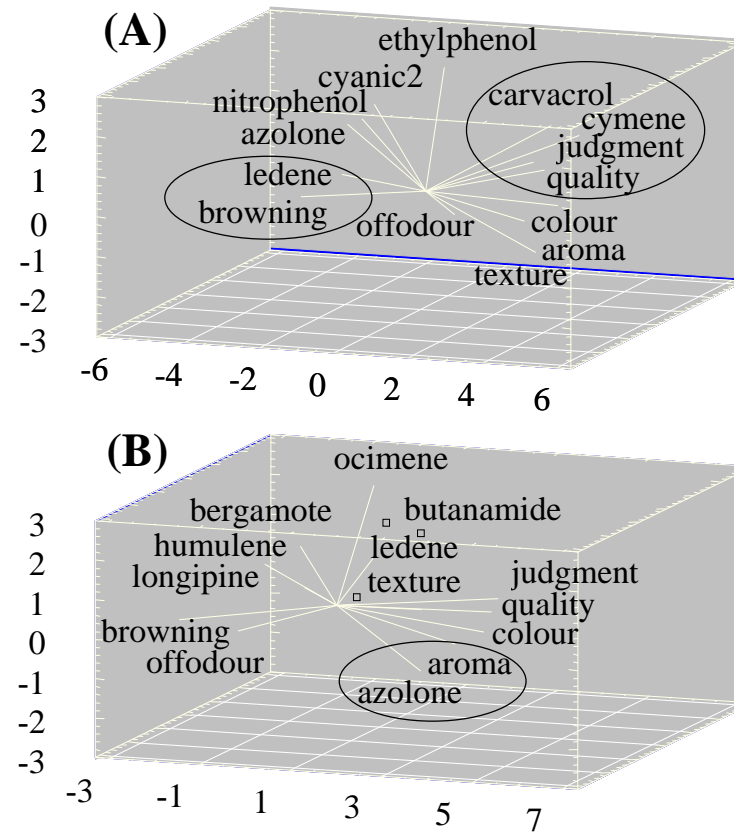


Fig. 2. 3-D PCA plots of the volatile quality markers (Y axis) and sensory attributes (X axis) of passive MA-packaged lettuce (A) and carrots (B). Clusters are indicated by circles. Volatiles quality markers included in the graphics are α -bergamotene (bergamote), α -caryophyllene (humulene), α -longipinene (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.

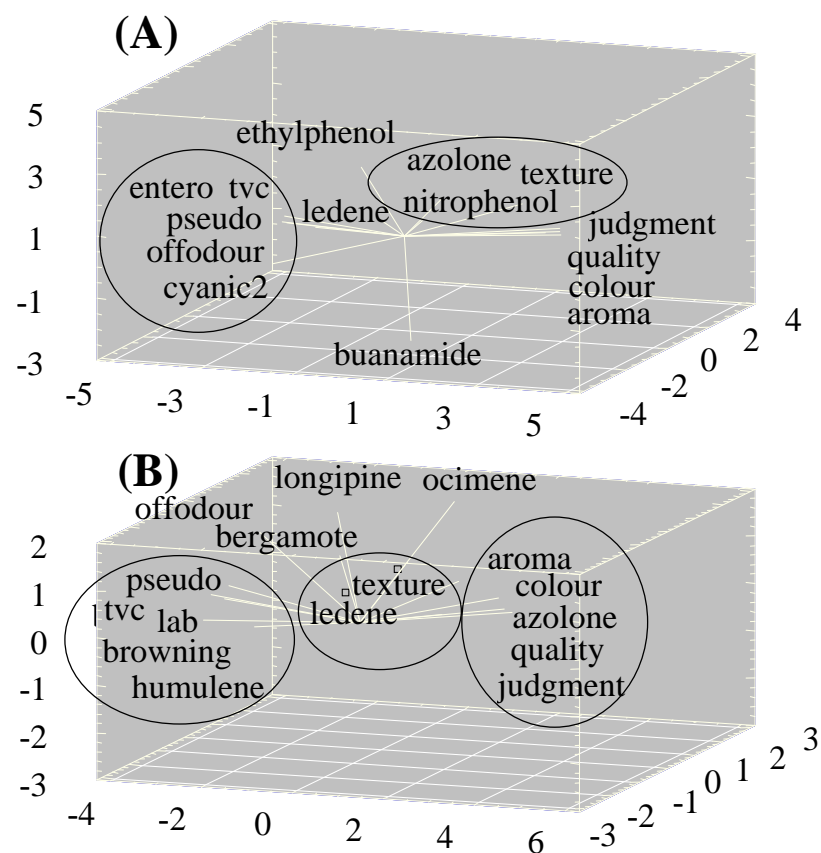


Fig. 3. 3-D PCA plots of the volatile quality markers (Z axis), sensory attributes (X axis) and changes in bacterial populations (Y axis) of passive MA-packaged lettuce (A) and carrots (B). Clusters are indicated by circles. Volatiles quality markers included in the graphics are α -bergamotene (bergamote), 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-ethylenedene-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	▲●	▲●	▲●	▲●	▲●	▲●	▲●	▲●	▲●
<i>p</i> -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	
<i>p</i> -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).