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Effect of chitosan-lemon essential oil coatings on volatile profile of strawberries during storage

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Abstract

Chitosan coatings containing lemon essential oils were described as effective at controlling fruit fungal decay at 20ºC during 7 days. In this work, the GC-MS technique was used to characterise the volatile compounds of strawberries during cold storage in order to analyse the influence of fruit coatings with chitosan, containing or not lemon essential oil, on the volatile profile of the fruits. The coatings affected the metabolic pathways and volatile profile of the fruits. Pure chitosan promoted the formation of esters and dimethyl furfural in very short time after coating, while coatings containing lemon essential oil incorporated terpenes (limonene, γ-terpinene, p-cymene and α-citral) to the fruit volatiles and enhanced the fermentative process, modifying the typical fruit aroma composition. No effect of chitosan coatings was sensorially perceived, the changes induced by lemon essential oil were notably appreciated.

Keywords: biopolymer, film, volatile, storage, postharvest, essential oil, *Fragaria x ananassa*.

1. Introduction

Nowadays, essential oils (EOs) are increasingly applied in food preservation due to the interest of consumers in natural food additives. Essential oils are natural oily liquids obtained from plant material. These natural substances and their constitutive compounds

have antimicrobial (Burt, 2004; Bakkali, Averbeck, Averbeck, & Idaomar, 2008; Rivera Calo, Crandall, O'Bryan, & Ricke, 2015) and antioxidant properties (Xing et al., 2005; Perdones, Vargas, Atarés, & Chiralt, 2014). Lemon essential oil is citrus oil from *Citrus limon*, rich in volatile compounds, such as limonene and γ-terpinene (Caccioni, Guizzardi, Biondi, Renda, & Ruberto, 1998; Moufida & Marzouk, 2003). Limonene, which is the main compound of lemon essential oil, has the GRAS status of the US FDA (US EPA, 1994) and is used as a food additive or flavouring agent. Moreover, limonene exhibits fungicidal properties, including activity against common postharvest fungal pathogens of fruit (Combrick, Regnier, & Kamatou, 2011; Sharma, & Tripathi, 2008; Wilson, Solar, El Ghaouth, & Wisniewski, 1997). The use of EOs in food preservation is often limited because of their application costs and other disadvantages, e.i. their intense aroma and potential toxicity. An interesting approach to reduce the doses of essential oils while maintaining their effectiveness could be to incorporate these compounds into the formulation of edible coatings. Chitosan is one of the film-forming biopolymers with great compatibility with citrus essential oils (Sánchez-González, Chiralt, González-Martínez, & Cháfer, 2011). Chitosan is a cationic polysaccharide obtained from chitin by deacetylation in the presence of alkali that itself shows antimicrobial activity (Vargas & González-Martínez, 2010; Zheng & Zhu, 2003). Chitosan-based edible coatings were used to improve the postharvest quality and shelf-life of strawberries (Vargas, Albors, Chiralt, & González-Martínez, 2006; Hernández-Muñoz, Almenar, Del Valle, Vélez, & Gavara, 2008; Gol, Patel, & Ramana Rao, 2013; Wang & Gao, 2013). The ability of chitosan-based coatings to act as protective gas-barriers and modify the fruit's internal atmosphere may affect the fruit flavour and aroma. In this sense, previous studies showed that pure chitosan coatings can be used to maintain strawberry flavour during storage and to delay the production of off-flavours

(Almenar, Hernández-Muñoz and Gavara (2009). Lemon essential oil was also incorporated into chitosan-based coatings in order to improve their antimicrobial properties (Perdones, Sánchez-González, Chiralt, & Vargas, 2012) and both the development of the physicochemical quality of the fruit and the fungal decay of cold-stored strawberries as affected by coating application were reported. Chitosan coatings containing lemon essential oil induced a better preservation of the fruit in terms of fungal decay, although the oil impacted on the olfactory perception of the fruit.

The aim of this work was to study the influence of chitosan and chitosan-lemon essential oil coatings on the volatile profile of strawberry throughout cold storage, in order to discover the persistency of oil volatiles in the fruit and the influence of both the coating and the essential oil components on the development of the profile of volatile compounds in strawberry throughout cold storage.

2. Materials and Methods

2.1. Materials

Organically grown strawberries (*Fragaria×ananassa* cv. Camarosa), harvested at the same day, were selected according to shape, uniform size and colour as well as the absence of physical damage or fungal infection. Before coating, strawberries were washed with a solution of sodium hypochlorite (10 mg/L). A total of 200 fruits were used to conduct all the experiments.

To obtain coating-forming dispersions (CFD), high molecular weight chitosan (acetylation degree: 24.4%, viscosity in 1% (w/w) glacial acetic acid solution: 1.406 Pa·s), 98% acetic acid (Panreac Química, S.A., Castellar del Vallés, Barcelona, Spain) and lemon essential oil (Herbes del Molí, Alicante, Spain) were used.

75 Gas chromatography reference standards (corresponding to volatiles of Table 4) were
76 purchased from Sigma-Aldrich Corp. (St. Louis, MO). Absolute ethanol, used for lemon
77 essential oil dilution, was from VWR (Barcelona, Spain).

78 2.2. Preparation of the coating-forming dispersions

79 Three different coating-forming dispersions were prepared. 1% (w/w) chitosan was
80 dispersed in an aqueous solution of acetic acid 0.5% (v/w). Following overnight
81 agitation at 25 ºC, lemon essential oil (L) was added to the chitosan solution (CH) in a
82 CH:L ratio of 1:3. Both CH and CH.L dispersions were homogenised using a rotor-
83 stator homogenizer (Ultraturrax DI25 Yellow Line, IKA®, Germany) at 13,500 rpm for
84 4 min. After vacuum degasification at room temperature, CH.L CFD was submitted to a
85 second homogenization by means of a Microfuidizer® (M110-P, Microfluidics,
86 Newton, MA, USA) in a single pass at 165 MPa to obtain CH.LM coating.

87 2.3. Application of coatings and sample preparation

88 Selected strawberries were randomly distributed into four groups of 50 strawberries
89 each. One group was used as a control, whose samples were immersed in an aqueous
90 solution of glacial acetic acid 0.5% (v/v) for 1 min, and the other three were treated with
91 each one of the coatings (CH, CH.L and CH.LM). Strawberry samples were dipped in
92 the corresponding CFD for 1 min, allowed to dry at room temperature for 1h and,
93 afterwards, cold-stored on PET trays in a climate chamber (EC1400, Radiber,
94 Barcelona, Spain) at 4 ± 1 ºC and 90% relative humidity (RH). The weight of the wet
95 coating in the samples was determined through their mass difference before and after
96 coating in order to evaluate the losses in lemon essential oil during the drying of the
97 coating and fruit storage. After 0, 7 and 15 days of storage, 5 strawberries per
98 formulation were randomly removed from the chamber and minced using an Ultraturrax

homogenizer at 8,500 rpm for 1 min. 60 g of the obtained puree were placed in propylene tubes and frozen at -20 ºC until the volatile analyses were carried out.

2.4. Characterization of the maturity index (MI) and respiration rate of strawberries

In the sample puree, maturity index was also determined through the measurement of the total soluble solids and acidity. Soluble solids were measured by means of a refractometer (3 T ABBE, ATAGO Co Ltd., Japan) at 22 ºC. Acidity (expressed as g of citric acid per 100 g of fruit) was measured following the method AOAC 942.15 (AOAC, 1995). MI was calculated as the quotient of total soluble solids and acidity. The respiration rate of the strawberries was evaluated at 5 ºC during storage following the methodology described by Vargas, Albors, Chiralt and González-Martínez (2006). Strawberry samples (about 150 g) were placed in 0.847 L hermetic glass jars with a septum in the lid for sampling gas in the headspace at different times. Gas sampling was carried out every 30 minutes by mea ns of a needle connected to a gas analyser (CheckMate 9900 PBI Dansensor, Ringsted, Denmark). Three replicates were performed for each formulation. Experimental points were considered in the time range where a linear relationship was observed between gas concentration and time. This means that no changes in the respiration pathway of the samples occurred in this period. Respiration rate of the samples in terms was determined from the slope of the fitted linear equation.

2.5. Volatile analysis

Volatile compounds were extracted by purge and trap thermal desorption (Peinado, Rosa, Heredia, Escriche, & Andrés, 2013). 500 µl of the internal standard 2-pentanol (10 mg/L) and 10 g of strawberry purée were placed into a purging flask and kept in a water bath at 45 ºC for 20 min. Throughout this time, purified nitrogen (100 ml/min)

was forced through a glass frit placed at the bottom of the flask. The volatile compounds were collected by the stream of bubbles, which passed through the sample and were trapped in a 100 mg porous polymer (Tenax® TA, 20-35 mesh) packed into a glass tube placed at the end of the system. The same procedure was used to characterize the volatile profile of lemon essential oil. To this end, 10 g of lemon essential oil dilution in water (1:1000) of a 3 % (w/v) ethanol absolute solution were used.

The aromatic extract was thermally desorbed by a direct thermal desorber (TurboMatrix TD, Perkin-Elmer TM, CT-USA). Desorption was performed under a 10 ml/min helium flow at 220 ºC for 10 min, and the volatiles were cryofocused in a cold trap at -30 ºC. After 1 min, the cold trap was heated up to 250 ºC (at a rate of 99 ºC) and volatiles were directly transferred onto the head of the capillary column.

GC-MS analysis was performed using a Finnigan TRACE^{TM} MS (ThermoQuest, Austin, USA). Volatile compounds were separated using a DB-WAX capillary column (1.0 μm x 0.32 mm x 60 m, SGE, Australia). Helium was used as the carrier gas at a constant flow rate of 1 ml/min. The oven was kept at an initial temperature of 40 ºC for 2 min. Then, the temperature was increased to 190ºC at a rate of 4 ºC/min, maintained for 5 min and finally increased to 230 ºC at 10 ºC/min. The MS interface and source temperatures were 250 and 200 °C, respectively. Electron impact mass spectra were recorded in impact ionisation mode at 70 eV and with a mass range of *m/z* 33–433. A total of 3 extracts were obtained for each sample.

The identification of isolated volatile compounds was tentatively carried out by comparing their mass spectra (*m/z* values of the most important ions) with spectral data from the National Institute of Standards and Technology 2002 library as well as published retention indices and spectral data. A solution of the homogenous series of

148 normal alkanes (C8–C20 by Fluka Buchs, Schwiez, Switzerland) was used to determine
149 the retention index.
150 The quantification of the 23 selected volatile compounds, selected on the basis of their
151 ratio and contribution to the aroma perception (Forney, Kalt, & Jordan, 2000; Jetti,
152 Yang, Kurnianta, Finn, & Quian, 2007; Larsen & Poll, 1992), was performed after
153 calibration by the standard addition method, in order to avoid the food matrix
154 composition effect. 10 g of thawed strawberry purée, 500 µl of internal standard 2-
155 pentanol (10 ml/L) and 10 different concentrations of the standards or of the essential
156 oil (limonene, γ-terpinene and p-cymene) were analysed in triplicate following the
157 procedure already described.
158 2.6. Sensory evaluation
159 Sensory evaluation was performed by a difference-from-control test with a seven-point
160 (-3 to 3) numerical category scale (Meilgaard, Civile and Carr, 1999). This test is
161 classified as an overall difference test and is used to determine if there is a difference
162 between one or more test samples and a control sample, while the size of the differences
163 can be quantified (score 0 means that there is no difference with respect to the control).
164 The sensory parameters (strawberry aroma and flavour) were evaluated by 30 untrained
165 panellists. Judges compared a coded sample with a control sample (non-coated
166 strawberry) and they evaluated the size of the differences against a seven-point scale.
167 All the coated and non-coated (blind) samples were compared with the control (non-
168 coated) sample.
169 2.7. Statistical analysis
170 The results were analysed by a multifactor analysis of variance (ANOVA) with a 95%
171 significance level using Statgraphics® Plus Centurion VII. Multiple comparisons were
172 performed through 95% Fisher's LSD intervals. Furthermore, a Principal Component

173 Analysis (PCA) was applied to describe the relationships between the quantified volatile
174 compound and the different treatments during storage, using Unscrambler 10.X
175 software.

176 **3. Results and discussion**

177 The volatile compounds identified in non-coated (control) strawberry samples before
178 storage are shown in Table 1, together with the Retention Index. A total of 57 esters, 16
179 alcohols, 7 aldehydes, 3 ketones, 2 terpenes, 1 aromatic hydrocarbon and 2.5-dimethyl-
180 4-methoxy-3(2H) furanone (DMF) were identified in the strawberry volatile profile.
181 Taking into account the relative area of the different peaks in the chromatograms with
182 respect to that of the internal standard, the weight percentage of each compound family
183 was estimated; as followed 76.85% for esters, 11.4% for alcohols and 8.85% for
184 aldehydes (Table 2). The rest of the compounds, including DMF, were in minority.
185 Similar volatile profiles were reported for strawberries of the same variety by different
186 authors (Jetti, Yang, Kurnianta, Finn, & Quian, 2007; Peinado, Rosa, Heredia, Escriche,
187 & Andrés, 2013). In every case, the esters were found to be the major compounds.
188 The main compounds of the used lemon essential oil (Table 2) were D-limonene (51 ± 2
189 %), γ-terpinene (15.0 ± 0.7 %), β-pinene (4.7 ± 1.0 %), myrcene (3.8 ± 0.3), p-cymene
190 (3.1 ± 0.5 %), sabinene (2.6 ± 0.5 %) and α-citral (2.4 ± 0.2 % %), coinciding with that
191 reported by other authors (Caccioni, Guizzardi, Biondi, Renda, & Ruberto, 1998;
192 Espina et al., 2011). For limonene and α-citral, antimicrobial properties were reported
193 (Burt, 2004). Some of the identified volatile compounds of lemon essential oil were also
194 present as minor compounds in non-coated strawberry samples were also; in particular,
195 the aldehyde nonanal and the monoterpene β-linalool.
196 Strawberries, prior to coating, showed an average maturity index (MI) of 8.7 ± 0.8. Both
197 storage and the type of coating influenced how much this value increased throughout

198 cold storage. The highest increase was observed in non-coated samples (control) and in
199 those coated with CH; the average MI values for these treatments was 12.5 ± 1.2 at 15
200 storage days. The addition of lemon essential oil to CH coatings promoted a delay in
201 ripening, especially in samples coated with CH.LM, which reached a MI value of 9.6 ±
202 0.3 after 15 days of storage. The detected ripening patterns correspond well with the
203 respiratory behaviour reported in Table 3, where a significantly lower respiration rate
204 both in terms of oxygen and carbon dioxide production was detected in samples with
205 coatings containing lemon essential oil. Moreover, the addition of lemon essential oil to
206 the coatings led to a significant increase in the respiratory quotient of the samples,
207 which reached the highest values at 7 storage days, whereas no significant effect of
208 coating with CH was observed on the respiration pattern of the samples. Coherently, the
209 levels of acetaldehyde and ethanol, which are volatile compounds related with a
210 fermentative metabolism, were also higher at 7 storage days in samples treated with
211 lemon essential oil, although they decreased after 15 storage days (Table 3). At the end
212 of the storage period, the concentration level of ethyl acetate was significantly lower in
213 these samples as compared to uncoated or CH coated samples. This behaviour suggests
214 that the physiological pathways of the plant cells could be affected by the contact with
215 the essential oil compounds, which may also influence the volatile biosynthesis and
216 aroma profile, regardless of the incorporation of new volatiles passing from the lemon
217 essential oil to the samples. The effect of the cellular stress provoked by different
218 treatments such as, osmotic treatments, on the volatile profile of strawberries has been
219 demonstrated in previous studies (Talens, Escriche, Martínez-Navarrete & Chiralt,
220 2002). A relevant role of enzyme activity in syntheses of volatiles in strawberries has
221 been also reported (Zabetakis & Holden, 1997). In fact, no modifications in the volatile
222 profile were observed in previously blanched strawberry samples submitted to different

223 treatments (Moreno, Chiralt, Escriche & Serra, 2000). In this sense, active coatings can
224 provoke changes in the cellular synthesis of volatiles, associated with the chitosan or
225 essential oil interactions with the plant cells, which will induce cellular stress and
226 changes in enzyme activity.
227 Table 4 shows the amount (expressed as mg/kg of strawberry) of some selected volatile
228 compounds present in the strawberry profile for the different treatments and storage
229 time. In general, a decrease in the concentration of esters and aldehydes during storage
230 was observed for all treatments. Alcohols decreased during storage for all treatments,
231 except 1-hexanol and 3-hexen-1-ol whose concentration increased when coatings
232 containing lemon essential oil were applied. Nevertheless, no relevant impact of these
233 alcohols on the strawberry flavour has been reported. The concentration of DMF
234 notably increased in all the samples during storage, especially in the CH-coated
235 strawberries, but this hardly occurred in samples treated with CH.L. This occurs in line
236 with the different ripening behaviour observed for the different samples: CH coating did
237 not significantly affect the ripening index whereas coatings with lemon essential oil
238 slowed down the cell respiration while promoting the fermentative process, thus
239 reflecting the influence of lemon essential oil compounds on the cell physiological
240 pathways.
241 The concentration of monoterpenes, which mainly came from lemon essential oil,
242 decreased during storage, thus indicating that a progressive loss of the exogenous
243 volatile compounds of strawberry occurred throughout the storage time. Likewise, their
244 concentration was much lower than that expected from the initial concentration in the
245 CFD. Table 5 shows the estimated losses of the lemon essential oil components during
246 the coating formation, determined both from the concentration value in the newly
247 coated samples (0 storage days) and that deduced from the mass of the wet coating of

248 each sample and the lemon essential oil compound concentration in the CFD. The latter
249 was estimated from the wt% of lemon essential oil in the CFD and its concentration of
250 volatiles commented on above. In the same way, the losses of these compounds after 7
251 and 15 day storage times were evaluated and shown in Table 5. A high percentage loss
252 was obtained in every case due to the volatility of the compounds and their
253 simultaneous evaporation with water during the coating drying step. The smallest loss
254 was detected for limonene, although no significant differences were detected among
255 treatments for the four evaluated compounds. CH.LM coatings led to significantly
256 higher essential oil losses in terms of the 4 evaluated volatiles ($p<0.05$). The lower
257 viscosity of the CH.LM coating-forming dispersion (64 mPa·s, Perdones et al., 2012) as
258 compared to CH.L (247 mPa·s, Perdones et al., 2012) could facilitate the diffusion of
259 lemon essential oil to the coating surface and its subsequent evaporation during the
260 drying step of the coating (Sánchez-González, Cháfer, González-Martínez, Chiralt, &
261 Desobry, 2011).
262 Limonene, γ-terpinene and p-cymene losses progressed during storage, reaching final
263 average loss values of 93%, whereas all α-citral was completely lost after 7 days of
264 storage. Despite these high lemon EO losses, the remaining amount oil (38 ± 5 mg
265 lemon essential oil/kg strawberry) was enough to control fungal decay in strawberries
266 (Perdones et al., 2012), and to alter the physiological pathways in strawberries, as
267 previously stated.
268 In order to properly analyse the correlation between volatile composition and coating
269 application during storage, a Principal Component Analysis was carried out, taking into
270 account all the quantified volatiles (Tables 3 and 4). Figure 1 shows the typical plot
271 where the two functions, PC-1 and PC-2, explained 67 % of the total variability, PC-1
272 explaining a greater percentage. The location of the different treatments-times in the

plot shows a good grouping of the treatments and times, exhibiting marked differences among the different samples due to both coating treatment and storage time. Likewise, plotting the different compounds allows us to find the compound group, which has greater weight in the volatile profile of the different samples. In this sense, it is remarkable that, after 15 storage days, all the samples appeared as only one wide group, near ethyl acetate, which indicates that the most characteristic volatile compounds in each one notably disappeared throughout time, giving rise to a similar final profile in every case. However, marked differences were observed for newly coated samples, which appeared in well separated groups, in terms of both PC-1 and PC-2 functions. PC-1 mainly separates samples treated with lemon essential oil, both microfluidized and non-microfluidized, which are located nearer to the terpenes coming from the lemon essential oil. Likewise, samples coated with CH are nearer the location of ester, in agreement with the higher concentration of these compounds.

Differences in the volatile profile of the samples submitted to the different treatments persisted after 7 storage days, although they all migrate in the plot towards a position near to that of the final time. At 7^{th} day of storage, the samples treated with CFD containing lemon essential oil were closer to the acetaldehyde and ethanol coordinates. This is in agreement with the fermentative process that is promoted by the essential oil, as previously commented on.

The sensory test revealed a significantly different perception of the panellists regarding the aroma and flavour of samples treated with coatings containing essential oils as compared to non-coated and CH coated samples. However, the panellists did not differentiate between the non-coated and CH coated samples in terms of these attributes. As deduced from the analysis of volatiles, differences in the aroma and flavour perception must be attributed not only to the presence of lemon essential oil compounds

298 in the strawberry fruit, but also to the different development of the characteristic
299 volatiles of strawberry in line with the physiological alterations in the fruit.
300
301 **4. Conclusion**
302 Chitosan coatings, with and without lemon essential oil, affected the strawberry volatile
303 profile which, in turn, had an impact on the perception of the fruit aroma and flavour.
304 The coatings affected the metabolic pathways of the fruit. Particularly, pure chitosan
305 promoted ester and 2.5-dimethyl-4-methoxy-3(2H) furanone formation in very short
306 time after coating, which could enhance the aroma perception. However, the addition of
307 lemon essential oil to the coatings not only incorporated the lemon essential oil terpenes
308 into the fruit volatiles, but also promoted changes in the cell physiology, enhancing the
309 fermentative process, thus modifying the typical fruit volatile composition. Whereas the
310 effect of chitosan coatings was not sensorially perceived, probably because the
311 differences were within the range of consumer tolerance, the changes induced by lemon
312 essential oil were notably appreciated. Therefore, although lemon essential oil
313 contributed to the prevention of fungal decay, the impact of its application was negative
314 from the point of view of the quality of the fruit's aroma.

315
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320
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414

415 **Table 1.** Identified volatile compounds in strawberry samples and Retention Index
416 (RI). Compounds have been ordered within each category according to the ratio
417 $Area_{compund}/Area_{total}$

Compounds	RI	Compounds	RI
Esters 76.85%		butyl 3-methyl hexanoate	1,475
ethyl acetate	915	hexyl 3-methyl butanoate	1,661
methyl butanoate	1,010	methyl 2-methyl propanoate	945
2-hexenyl acetate	1,354	3-methyl-2-butenyl acetate	1,275
ethyl 2-methyl butanoate	1,073	methyl heptanoate	1,319
ethyl hexanoate	1,250	ethyl 3-octenoate (Z)	1,513
hexyl acetate	1,290	methyl 2-hexenoate	1,316
ethyl propanoate	980	methyl octanoate	1,408
isoamyl acetate	1,139	ethyl decanoate	1,656
butyl acetate	1,095	*Aldehydes 11.4%*	
ethyl butanoate	1,059	2-hexenal (E)	1,249
4-hexenyl acetate	1,338	hexanal	1,106
methyl acetate	858	acetaldehyde	646
ethyl 2-methyl propanoate	987	nonanal	1,417
ethyl 3-methyl butanoate	1,089	2-nonenal	1,568
ethyl 2-butenoate (Z)	1,187	decanal	1,524
methyl hexanoate	1,205	octanal	1,311
methyl propanoate	931	*Alcohols 8.85%*	
ethyl 1-methyl acetate	922	ethanol	959
propyl acetate	999	1-hexanol	1,372
propyl 2-methyl acetate	1,033	1-butanol, 3-methyl	1,229
methyl 3-methyl butanoate	1,040	2-hexen-1-ol (E)	1,427
octyl acetate	1,493	1-penten-3-ol	1,183
methyl 2-methyl butanoate	1,033	1-butanol	1,170
S-methyl thioacetate	1,077	1-octanol	1,575
3-hexenyl acetate	1,328	1-propanol, 2-methyl	1,119
2-pentenyl acetate (Z)	1,245	3-hexen-1-ol (Z)	1,407
ethyl 2-hexenoate (E)	1,368	2-penten-1-ol (Z)	1,342
pentyl acetate	1,191	1-pentanol	1,271
ethyl 2-methylthio acetate	1,479	1-hexanol, 2-ethyl	1,506
ethyl 2-methyl-2-butenoate	1,260	2-octen-1-ol (E)	1,556
ethyl octanoate	1,452	1,5-pentanediol, 3-methyl	1,385
methyl 3-hexenoate	1,323	2-undecanol	1,596
octyl 2-methyl butanoate	1,644	2-propanol	950
ethyl benzoate	1,708	*Ketones 2.11%*	

butyl butanoate	1,234	2-pentanone	1,005
hexyl butanoate	1,433	2-propanone	848
methyl methacrylate	1,133	6-methyl-5-heptene-2-one	1,363
ethyl pentanoate	1,151	*Monoterpenes 0.57%*	
octyl butanoate	1,635	β-linalool	1,564
pentyl butanoate	1,281	*Furans 0.11%*	
benzyl acetate	1,768	2,5-dimethyl-4-methoxy-3(2H) furanone	1,632
2-hexenyl isovalerate (E)	1,498	*Aromatic hidrocarbons 0.06%*	
hexyl 2-methyl butanoate	1,442	ethyl benzene	1,285
methyl 2-methylene butanoate	1,216		

Table 2. Identified volatile compounds in the lemon essential oil and Retention index (RI). Compounds have been ordered within each category according to the ratio $Area_{compund}/Area_{total}$

Compound	**RI**	**Compound**	**RI**
Monoterpenes 95.45%		*Sesquisterpenes 2.51%*	
limonene	1.218	β-bisabolene	1.751
γ-terpinene	1.264	isocaryophyllene	1.633
β-pinene	1.122	valencene	1.756
myrcene	1.174	β-farnesene	1.713
p-cymene	1.293	α-caryophyllene	1.708
sabinene	1.134	β-bisabolol	1.639
α-citral	1.768		
geranyl acetate	1.778	*Ketones 1.26%*	
neryl acetate	1.748	2-pentanone	1.005
β-citral	1.717	5-hepten-2-one, 6 methyl	1.363
α-bergamotene	1.608	3-buten, 2-one	976
β-phellandrene	1.227		
terpinolene	1.303	*Aldehydes 0.62%*	
trans-β-Ocimene	1.248	decanal	1.524
β-linalool	1.564	nonanal	1.417
α-thujene	1.043	octanal	1.311
camphene	1.115	hexanal	1.106
cis-limonene oxide	1.479		
α-pinene	1.038	*Esters 0.26%*	
β-citronellal	1.505	citronellyl acetate	1.680
eucalyptol	1.229		
trans-limonene oxide	1.491	*Aromatic hydrocarbons 0.16%*	
α-terpinene	1.195	4-mehylstyrene	1.389
α-terpineol	1.726		
cis-β-terpineol	1.575	*Alcohols 0.03%*	
4-terpineol	1.488	2-nonanol	1.429
3-carene	1.079		
α-phellandrene	1.179		
2,6-dimethyl-3,5,7-octatriene-2-ol (E)	1.472		

Table 3. Evolution of the respiration rate (RR) and respiratory quotient (RQ) of strawberry samples and of the content of volatiles related with fermentative metabolism during storage. Mean values and standard deviation, in brackets.

CFD	Time (days)	RR [O_2] ($mg \cdot kg^{-1} \cdot h^{-1}$)	RR [CO_2] ($mg \cdot kg^{-1} \cdot h^{-1}$)	RQ	acetaldehyde (mg/kg)	ethyl acetate (mg/kg)	ethanol (mg/kg)
Control	0	26 (4)^{ab2}	14.1 (0.5)^{ab1}	0.55 (0.06)^{a1}	6.8 (0.8)^{a1}	11 (2)^{a1}	18.8 (0.3)^{a1}
	7	12.5 (1.2)^{b2}	15.45 (1.09)^{b1}	1.24 (0.06)^{a2}	9.4 (1.0)^{a12}	26 (4)^{b2}	13.24 (0.13)^{a1}
	15	16 (3)^{b1}	17 (2)^{b1}	1.07 (0.09)^{b2}	15.4 (1.6)^{a2}	23.5 (1.4)^{b2}	40 (3)^{a2}
CH	0	22 (2)^{a3}	12 (1.3)^{a1}	0.54 (0.02)^{a1}	20 (4)^{b1}	13.7 (0.5)^{a1}	46 (12)^{b1}
	7	11.4 (1.2)^{b2}	12.7 (1.4)^{b1}	1.112 (0.012)^{a2}	14.4 (1.6)^{ab1}	19.1(1.3)^{a2}	32.5 (1.6)^{b1}
	15	16 (4)^{b1}	13 (3)^{b1}	0. 85 (0.03)^{a3}	14 (7)^{a1}	22.6 (0.9)^{ab2}	46 (4)^{a1}
CH.L	0	28 (4)^{b2}	19 (2)^{c2}	0.69 (0.05)^{a1}	18 (7)^{bc1}	11 (2)^{a1}	32 (13)^{ab1}
	7	3.7 (1.2)^{a2}	7 (2)^{a1}	2 (0.2)^{c2}	19.6 (1.4)^{bc1}	23 (2)^{ab2}	43 (8)^{b1}
	15	7 (3)^{a1}	7.3 (0.2)^{a1}	1.6 (0.2)^{c3}	15.28 (0.02)^{a1}	18 (3)^{a2}	47 (15)^{a1}
CH.LM	0	24 (2)^{ab2}	16.6 (0.3)^{bc2}	0.71 (0.06)^{a1}	10.93 (0.15)^{a1}	12 (3)^{a1}	20 (3)^{a1}
	7	5.5 (0.3)^{a2}	8.7 (1.3)^{a1}	1.57 (0.14)^{b2}	25 (3)^{c2}	22.1 (1.3)^{ab2}	41 (4)^{b2}
	15	6 (2)^{a1}	9 (3)^{a1}	1.5 (2)^{c2}	16 (7)^{c1}	14.9 (0.8)^{a1}	36.8 (0.7)^{a2}

$^{a, b, c, d}$ different superscripts within a column indicate significant differences among different treatments for the same storage time according to ANOVA test ($p < 0.05$).

$^{1, 2, 3}$ different superscripts within a column indicate significant differences due to storage time for a determined treatment according to ANOVA test ($p < 0.05$).

CH: chitosan, L: lemon essential oil, M: microfluidized.

Table 4. Concentration (mg/kg strawberry) of the different volatile compounds quantified in strawberry samples as a function of storage time for samples uncoated (control) and coated with chitosan (CH), chitosan-lemon essential oil (CH.L) and the microfluidized chitosan-lemon essential oil (CH.LM). Mean values and standard deviation, in brackets.

Compound	Time (days)	Control	CH	CH.L	CH.LM
Esters					
ethyl propanoate	0	0.72 (0.07)^{a2}	2.1 (0.6)^{c3}	1.6 (0.2)^{b2}	0.81 (0.08)^{a2}
	7	0.50 (0.04)^{ab12}	1.18 (0.05)^{c2}	0.42 (0.08)^{a1}	0.930 (0.108)^{bc2}
	15	0.17 (0.03)^{a1}	0.210 (0.012)^{a1}	0.036 (0.003)^{a1}	0.030 (0.004)^{a1}
methyl butanoate	0	2.58 (0.03)^{a2}	3.7 (0.9)^{a3}	2.797 (0.012)^{a2}	2.56 (0.07)^{b2}
	7	1.88 (0.08)^{b2}	1.52 (0.06)^{b2}	0.213 (0.015)^{a1}	0.685 (0.016)^{a1}
	15	0.103 (0.012)^{a1}	0.06 (0.03)^{a1}	0.020 (0.010)^{a1}	0.0214 (0.0015)^{a1}
ethyl butanoate	0	1.63 (0.05)^{a2}	1.92 (0.15)^{ab2}	2.17 (0.17)^{b2}	2.3 (0.3)^{b2}
	7	1.69 (0.12)^{a2}	3.22 (0.12)^{c3}	2.2 (0.4)^{b2}	3.1 (0.2)^{c3}
	15	0.8 (0.3)^{a1}	0.95 (0.04)^{a1}	0.24 (0.06)^{b1}	0.27 (0.06)^{b1}
methyl hexanoate	0	0.18 (0.04)^{a2}	0.53 (0.09)^{c2}	0.27 (0.05)^{ab2}	0.34 (0.15)^{b2}
	7	0.103 (0.000)^{a12}	0.064 (0.003)^{a1}	0.016 (0.007)^{a1}	0.046 (0.009)^{a1}
	15	0.0343 (0.0017)^{a1}	0.0101 (0.0018)^{a1}	0.007 (0.003)^{a1}	0.007 (0.003)^{a1}
ethyl hexanoate	0	0.96 (0.02)^{a1}	6.0 (0.6)^{d2}	4.0 (0.7)^{c2}	2.6 (0.4)^{b3}
	7	0.514 (0.014)^{a1}	0.71 (0.12)^{ab1}	0.63 (0.03)^{ab1}	1.3 (0.2)^{b2}
	15	0.45 (0.16)^{a1}	0.26 (0.03)^{a1}	0.1262 (0.0103)^{a1}	0.143 (0.018)^{a1}
hexyl acetate	0	0.916 (0.015)^{a}	1.58 (0.05)^{b}	0.88 (0.18)^{a}	0.7 (0.3)^{a}
	7	0.55 (0.06)^{bc}	0.74 (0.09)^{c}	0.10 (0.10)^{a}	0.31 (0.09)^{ab}
	15	0.20 (0.08)^{a}	0.122 (0.014)^{a}	0.024 (0.003)^{a}	0.02 (0.03)^{a}
Aldehydes					
hexanal	0	0.69 (0.16)^{b2}	0.8 (0.4)^{b2}	0.059 (0.009)^{a1}	0.10 (0.06)^{a12}
	7	0.88 (0.04)^{b2}	0.83 (0.03)^{b2}	0.22 (0.11)^{a1}	0.34 (0.08)^{a2}
	15	0.25 (0.04)^{a1}	0.12 (0.04)^{a1}	0.056 (0.006)^{a1}	0.0336 (0.0009)^{a1}
2-hexenal	0	2.0 (0.2)^{a2}	4.9 (1.0)^{b3}	1.62 (0.01)^{a2}	1.5 (0.5)^{a3}
	7	2.0 (0.3)^{b2}	2.9 (0.4)^{c2}	0.61 (0.18)^{a1}	0.77 (0.10)^{a2}
	15	0.54 (0.03)^{a1}	0.25 (0.07)^{a1}	0.18 (0.04)^{a1}	0.14 (0.06)^{a1}
nonanal**	0	0.068 (0.008)^{a12}	0.0463 (0.0009)^{a1}	0.241 (0.015)^{b2}	0.053 (0.014)^{a12}
	7	0.0447 (0.0006)^{a2}	0.078 (0.004)^{b2}	0.0513 (0.0018)^{a1}	0.070 (0.011)^{ab2}
	15	0.08 (0.03)^{b1}	0.038 (0.003)^{a1}	0.034 (0.009)^{a1}	0.0347 (0.0015)^{a1}
Alcohols					
1-penten-3-ol	0	0.28 (0.05)^{b2}	0.442 (0.009)^{c2}	0.066 (0.015)^{a1}	0.107 (0.009)^{a1}
	7	0.141 (0.004)^{b1}	0.21 (0.02)^{c1}	0.068 (0.008)^{a1}	0.12 (0.04)^{b1}
	15	0 (0)^{a3}	0 (0)^{a3}	0 (0)^{a2}	0 (0)^{a2}
	0	0.049 (0.013)^{b3}	0.017 (0.009)^{a2}	0.0074 (0.0006)^{a1}	0.0067 (0.0002)^{a2}

2-penten-1-ol (Z)	7	0.015 (0.006)^{bc2}	0.026 (0.005)^{c2}	0 (0)^{a1}	0.011 (0.002)^{b12}
	15	0 (0)^{a1}	0 (0)^{a1}	0 (0)^{a1}	0 (0)^{a1}
	0	0.6 (0.2)^{c2}	0.33 (0.11)^{b1}	0.06 (0.03)^{a1}	0.049 (0.002)^{a2}
1-hexanol	7	0.131 (0.012)^{a1}	0.491 (0.006)^{b2}	0.12 (0.04)^{a1}	0.27 (0.06)^{a1}
	15	0.164 (0.003)^{a1}	0.23 (0.03)^{a1}	0.174 (0.007)^{a1}	0.137 (0.011)^{a12}
	0	0.065 (0.013)^{c2}	0.0295 (0.0013)^{b3}	0.006 (0.003)^{a1}	0.010 (0.002)^{a1}
3-hexen-1-ol (Z)	7	0.0086 (0.0009)^{ab1}	0.0170 (0.0009)^{b2}	0.0053 (0.0019)^{a1}	0.0095 (0.0003)^{ab1}
	15	0.00230 (0.00006)^{a1}	0.005 (0.002)^{a1}	0.0026 (0.0004)^{a1}	0.0026 (0.0005)^{a1}
	0	0.93 (0.03)^{c2}	0.5 (0.3)^{b2}	0.07 (0.03)^{a1}	0.09 (0.05)^{a1}
2-hexen-1-ol (E)	7	0.11 (0.02)^{a1}	0.37 (0.02)^{b2}	0.06 (0.04)^{a1}	0.143 (0.004)^{a1}
	15	0 (0)^{a1}	0 (0)^{a1}	0 (0)^{a1}	0 (0)^{a1}
Monoterpenes					
	0	-	-	160 (40)^{a2}	71 (15)^{b1}
limonene*	7	-	-	91 (6)^{a1}	59 (4)^{a1}
	15	-	-	47 (4)^{a1}	35 (8)^{a1}
	0	-	-	39 (8)^{a3}	19 (5)^{b2}
γ-terpinene*	7	-	-	21.1 (0.8)^{a2}	14.8 (0.7)^{a1}
	15	-	-	8.5 (0.9)^{a1}	7 (2)^{a1}
	0	-	-	6.7 (1.8)^{b3}	3.3 (0.7)^{a2}
p-cymene*	7	-	-	4.3 (0.4)^{a2}	2.6 (0.4)^{a12}
	15	-	-	1.3 (0.2)^{a1}	1.2 (0.4)^{a1}
	0	0.29 (0.09)^{a1}	0.68 (0.17)^{a2}	1.3 (0.6)^{b1}	0.72 (0.03)^{a12}
β-linalool**	7	0.05 (0.02)^{a1}	0.169 (0.010)^{a1}	1.5 (0.4)^{b1}	1.2 (0.3)^{b2}
	15	0.066 (0.004)^{a1}	0.096 (0.006)^{a1}	0.220 (0.014)^{a2}	0.27 (0.03)^{a1}
	0	-	-	3.1 (1.1)^{a2}	1.31 (0.12)^{b2}
α-citral*	7	-	-	0 (0)^{a1}	0 (0)^{a1}
	15	-	-	0 (0)^{a1}	0 (0)^{a1}
Furans					
	0	7.5 (1.1)^{a1}	4.7 (0.9)^{a1}	6.6 (1.2)^{a1}	2.9 (0.9)^{a1}
DMF	7	5.13 (0.18)^{a1}	5.8 (0.3)^{a1}	6.7 (1.9)^{a1}	6.5 (1.8)^{a12}
	15	11 (3)^{a2}	25 (3)^{b2}	8 (5)^{a1}	10 (3)^{a2}

480 $^{a, b, c, d}$ different superscripts indicate significant differences among coating treatment at a given storage time according to ANOVA
481 test ($p < 0.05$).
482 $^{1, 2, 3}$ different superscripts indicate significant differences due to storage time for a given coating treatment according to ANOVA
483 test ($p < 0.05$).
484 * compound also identified in lemon essential oil.
485 ** compound identified in both lemon essential oil and in strawberry samples.
486 CH: chitosan, L: lemon essential oil, M: microfluidized.
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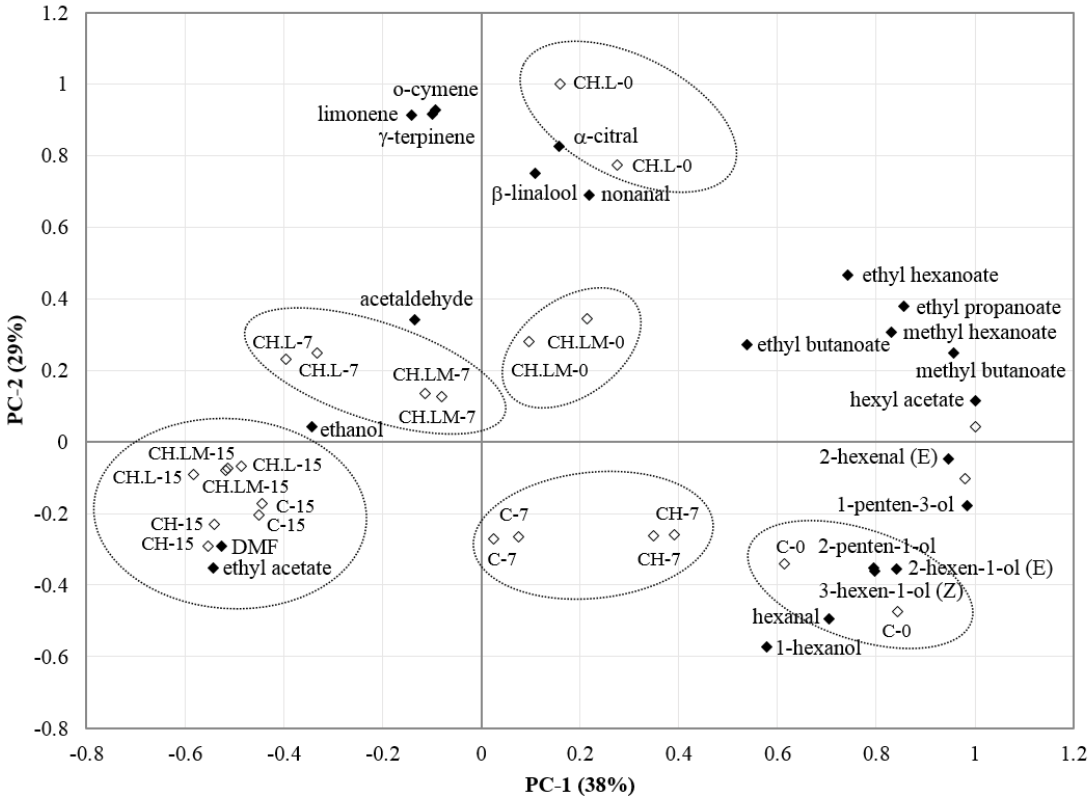
Table 5. Loss of essential oil compounds (wt %) during coating formation and storage.

CFD	Time (days)	Limonene (T_b = 176ºC)	γ-terpinene (T_b = 183ºC)	p-cymene (T_b = 178ºC)	α-citral (T_b = 229ºC)
CH.L	0	63 (10)^{a1}	65 (9)^{a1}	70 (8)^{a1}	83 (5)^{a1}
	7	78 (6)^{a2}	81 (5)^{a2}	81 (5)^{a2}	100 (0)^{a2}
	15	89 (3)^{a3}	92 (2)^{a3}	94.3 (1.6)^{a3}	100 (0)^{a2}
CH.LM	0	83 (3)^{b1}	84 (3)^{b1}	86 (3)^{b1}	92.9 (1.4)^{b1}
	7	86 (3)^{b12}	87 (3)^{b1}	89 (2)^{b1}	100 (0)^{a2}
	15	91.7 (1.6)^{a2}	93.6 (1.3)^{a2}	94.98 (0.99)^{a2}	100 (0)^{a2}

$^{a, b}$Different superscripts in a column indicate significant differences among coating treatments at a determined time according to ANOVA test ($p < 0.05$).

$^{1, 2, 3}$ Different superscripts in a column indicate significant differences due to storage time for a given treatment according to ANOVA test ($p < 0.05$).

L: lemon essential oil, M: microfluidized.



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507 **Figure 1.** Principal Component analysis of strawberry quantified volatile compounds
508 and treatments (coating-storage time). C: control, CH: chitosan coating, CH.L: chitosan-
509 lemon essential oil coating, CH.LM: microfluidized chitosan-lemon essential oil
510 coating.

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