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Title: Aroma profile of Fuji apples treated with gelatin edible coating during their storage

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Abstract: This study aimed to detect possible changes in the volatile organic compounds (VOCs) of Fuji apples induced by gelatin-based edible coating (EC), during 21 days of storage at room temperature. VOCs were analyzed by solid-phase micro extraction-gas chromatography-mass spectrometry. Data were analyzed by one-way ANOVA and principal component analysis. Control apples showed a greater presence of total aldehydes and acids at 7 and 14 days, respectively, while coated apples were characterized by higher proportions of alcohols (from 1.3- to 2-fold) at 7 day till the end of the storage. The higher ethanol proportions detected in coated apples (154-fold higher after 7 days) indicate a likely partial anaerobiosis, confirmed by the lower CO2 emission (reaching -68 % after 21 days). Esters responsible of the varietal aroma of Fuji were identified also in coated fruits, suggesting that gelatin did not modify the typical aroma extensively. Acetate esters, normally increasing with maturity, were less concentrated in coated apples (-78 % 2-methylbutyl acetate and -73 % hexyl acetate, after 1 and 7 days respectively), suggesting a likely slowdown of the ripening due to the EC.

Further investigation is needed to improve this storage technology considering that aroma is an important determinant of food quality.

Pisa, 22 June 2017

Dear Professor Renard,

please find here enclosed the revised version of the manuscript “Aroma profile of Fuji apples treated with gelatin edible coating during their storage”, authors: Alessia Mannucci, Andrea Serra, Damiano Remorini, Antonella Castagna, Marcello Mele, Andrea Scartazza and Annamaria Ranieri.

In view of the increasing attention toward food quality and the research of tools to improve the shelf life and safety of produce, we investigated whether the use of gelatin-based edible coating could modify the aromatic profile of Fuji apple fruits during three weeks of storage. The results of this research are reported and commented in the present manuscript.

I state that all the material is original and that no part has been submitted as a printed article elsewhere.

Concerning the options for reproducing colour illustrations in the article, I choose the colour reproduction in the online version, and the black and white reproduction in the printed version.

Hoping that the manuscript will be suitable for publication in LWT – Food Science and Technology, I send my best regards.

Yours sincerely  
Annamaria Ranieri

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## \*Detailed Response to Reviewers

**Reviewer #1:** The manuscript now is much more clear about the influence of coating using this film on the aroma profile.

***We thank the reviewer for his/her positive evaluation***

**Reviewer #2:**

Corrections

Line 124 weighed

Line 342 that

Line 258 Mattinson,

Line 431 Elsabee, M.Z etc is not mentioned in the text?

***All the requested changes were done and the reference was deleted***

Statistics in Fig 1, 3 and 4 Using ANOVA in these data is disputable (wrong). You should select another subprogram in SAS for time-courses.

***Statistic was elaborated as requested and Fig. 3 and 4 were removed since changes in statistical analysis made them no more necessary.***

## Editor's corrections

I agree with reviewer 2:

ANOVA is NOT the correct statistics for time courses (you may use different fruits but it does not mean they are independent samples as in a time course it is assumed that the fruit measured at day 6 would have given at day 4 the same results as the fruit actually measured at day 4). As it is you may compare the fruits with /without coating at any given day using ANOVA, but NOT the fruits along a same time course.

***Statistic was elaborated as requested. As a consequence, the paragraphs "abstract", "statistical analysis" and "results and discussion" were modified and figs 3 and 4 were removed since changes in statistical analysis made them no more necessary. Tables were redrawn according to the new statistical analysis and one additional table was added as supplementary material.***

Borkh. should be in normal, not italics. Please check and correct throughout.

**Done**

L115: you obtained the gelating as a solution or as a solid? You state L117 that you melt delating sheets but L115 that the material contained 4.82 g/L gelatin, it is not logical

***Our starting material (gelatin sheets) was in solid state but in the text we reported the gelatin concentration of the solution prepared by the Chemical-Pharmaceutical Laboratory Tiaraju (4.82 g/L). Then we melted these sheets to obtain a final concentration of 1.5 g/L gelatin and we dipped apples in this solution that, at room temperature, dries becoming a solid film. To avoid misleading, we removed the initial gelatin concentration in the revised text.***

L168 VOCs

**Done**

L17ç etc: n-propylacetate n in italics

**Done**

L192 : NI ?

***The abbreviation was explicated in the revised text***

L223 : SI units g/L or g/kg ? not %

***Percentage was corrected as SI (g/L)***

L385: limits

**Done**

Reference list: put issue numbers for ALL or NONE of the references.

***All references are now written without issue numbers***

L486: check reference, it is a total book or a chapter (the "In" implies a chapter) total number of pages or identify the chapter (and its pages)

L473: total number of pages?

***This reference was omitted in the revised manuscript***

Fig 1, fig 3, fig 4: eliminate the black border; use the conventional "Entity (unit)" for the legend of the axes; do not use ANOVA along a time course (eliminate the letters)

***Figs 3 and 4 were deleted. Fig 1 was redrawn as requested.***

Fig 5: Figures should still be legible printed in B&W: the blue and red print the same pale grey; use different symbols (full / empty for example), not different colours (specially as they are a bit pale)

***Following your suggestion, the blue and red colours were removed and substituted with full and empty symbols.***

## Highlights

- Decreased levels of acids and aldehydes were detected in coated apples
- Acetate esters behavior suggests a slowdown of the ripening in coated apples
- Gelatin application increased ethanol percentage and lowered CO<sub>2</sub> emission
- Esters responsible of the Fuji varietal aroma were identified also in coated apples

1 **Aroma profile of Fuji apples treated with gelatin edible**  
2 **coating during their storage**

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18



19 **Abstract**

20 This study aimed to detect possible changes in the volatile organic compounds (VOCs)  
21 of Fuji apples induced by gelatin-based edible coating (EC), during 21 days of storage  
22 at room temperature. VOCs were analyzed by solid-phase micro extraction-gas  
23 chromatography-mass spectrometry. Data were analyzed by one-way ANOVA and  
24 principal component analysis. Control apples showed a greater presence of total  
25 aldehydes and acids at 7 and 14 days, respectively, while coated apples were  
26 characterized by higher proportions of alcohols (from 1.3- to 2-fold) at 7 day till the end  
27 of the storage. The higher ethanol proportions detected in coated apples (154-fold  
28 higher after 7 days) indicate a likely partial anaerobiosis, confirmed by the lower CO<sub>2</sub>  
29 emission (reaching -68 % after 21 days). Esters responsible of the varietal aroma of Fuji  
30 were identified also in coated fruits, suggesting that gelatin did not modify the typical  
31 aroma extensively. Acetate esters, normally increasing with maturity, were less  
32 concentrated in coated apples (-78 % 2-methylbutyl acetate and -73 % hexyl acetate,  
33 after 1 and 7 days respectively), suggesting a likely slowdown of the ripening due to the  
34 EC.

35 Further investigation is needed to improve this storage technology considering that  
36 aroma is an important determinant of food quality.

37

38 *Keywords*

39 *Malus × domestica* Borkh., GC/MS, CO<sub>2</sub> emission, storage, volatile organic compounds

40

## 41 **1. Introduction**

42 Fuji apple (*Malus × domestica* Borkh.) is one of the most consumed cultivar worldwide  
43 thanks to its typical sweetness, crunchiness and aroma. Apples are well known as a huge  
44 source of phytochemicals like flavonoids and phenolic acids (Francini & Sebastiani,  
45 2013), which play an important role in the antioxidant defense of the human organism.  
46 Food packages are essential in increasing the shelf life of fresh foods, like fruits,  
47 preserving their safety and quality. Nowadays the most common package materials, like  
48 polyethylene terephthalate (PET), polyvinylchloride (PVC) and others, are produced  
49 from petrochemical plastics, which are not totally biodegradable and recyclable  
50 (Siracusa, Rocculi, Romani, & Rosa, 2008). Because of their high environmental  
51 impact, many recent studies have investigated new materials, more eco-friendly and  
52 user-friendly, to preserve food, such as edible coatings (ECs). ECs are prepared from  
53 edible materials, such as lipids, proteins or polysaccharides; they are applied and formed  
54 directly on the food product by spraying, dipping or brushing, constituting a thin layer  
55 around (Guilbert, Gontard, & Gorris, 1996; Dhall, 2013; Falguera, Quintero, Jiménez,  
56 Muñoz, & Ibarz, 2011). They can be consumed along with foods, but they can be easily  
57 removed as well, if the consumer does not like to eat them. Edible coatings can be  
58 obtained from natural biopolymers, also derived from wastes of agro-food industry, so  
59 they have low impact on the environment and may reduce the plastic packaging waste.  
60 The use of coatings for fruits is not a new concept: waxes have been used from a long  
61 time in China to prevent water transpiration loss and it has been reported that in 1930's  
62 hot-melt paraffin wax was used as EC for apples and pears (Dhall, 2013). In many  
63 studies, researchers demonstrated the effectiveness of ECs in decreasing weight loss,  
64 prolonging conservation and preventing deterioration of perishable fruits (Dhall, 2013;  
65 Falguera et al., 2011; Del-Valle, Hernández-Muñoz, Guarda, & Galotto, 2005; Elasbee  
66 & Abdu, 2013; Zhang, Wang, Hu, & Liu, 2015).

67 Among the film-forming proteins, gelatin, obtained by hydrolysis of collagen, has effective barrier properties against oxygen and carbon dioxide (Jiang, Liu, Du, & Wang, 68 2010). Gelatin-starch coatings prolonged the postharvest shelf life of avocado (Aguilar- 69 Méndez, San Martín-Martínez, Tomás, Cruz-Orea, & Jaime-Fonseca, 2008) and 10% 70 gelatin coating, besides delaying ripening of mango fruit by suppressing the activity of 71 softening enzymes, allowed the retention of higher ascorbic acid and phenolic content 72 as compared with control (Gol & Ramana Rao, 2013). In the last few years, many scientific 73 publications focused on physical and organoleptic changes in fruits covered with 74 ECs, but at the best of our knowledge, only very few papers deal with aroma profile of 75 coated apple fruit (Maya-Meraz et al., 2014; Sepulveda & Olivas, 2016; Olivas, 76 Mattinson, & Barbosa-Cánovas, 2007)), and no paper assessed possible changes in 77 volatile organic compounds (VOCs) induced by gelatin coating. 78 VOCs contributing to apples aroma are over 300 and they belong to different chemical 79 classes including carboxylic esters, alcohols, aldehydes, ketones, acids, terpenes and 80 ethers (Ferreira, Perestrelo, Caldeira, & Câmara, 2009). However, only about 20 of 81 them are character impact compounds (Dixon & Hewett, 2000) and each individual 82 molecule has its own odor threshold and concentration. Fruit aroma is cultivar-specific 83 (Dixon & Hewett, 2000) but it depends also from pre- and post-harvest conditions like 84 seasonal variation (López, Lavilla, Riba, & Vendrell, 1998) or harvest date and storage 85 technology (Echeverría, Fuentes, Graell, Lara, & López, 2004b). The major volatiles in 86 apples are esters, which are synthesized by esterification of alcohols and acyl-coA from 87 fatty acids or amino acids-pathways; another important group is represented by 88 alcohols, derived from fatty acids and amino acids metabolism and lipoxygenase (LOX) 89 activity, this latter also producing aldehyde volatiles (Dixon & Hewett, 2000). 90 From a quality point of view, it is extremely important that the aroma profile of produce 91 treated with EC is preserved to ensure good palatability. Therefore, this work aimed to 92

93 verify, by means of HS-SPME and GC/MS technique, whether a gelatin-based ECs  
94 induced changes in the VOCs profile of Fuji apples during three weeks of storage at  
95 room temperature to simulate home conditions.

96

## 97 **2. Material and methods**

### 98 *2.1 Plant material and experimental design*

99 Apple fruits (*Malus domestica* Borkh. L. cv. Fuji) were obtained from a commercial  
100 orchard (Marchetti Anna Paola, province of Pisa). After one month of cold storage,  
101 fruits of uniform size and free from any visual symptoms of diseases were washed,  
102 dried and randomly separated into two groups: the first group was coated with gelatin  
103 while the second one, uncoated, represented the control. Apples were stored at room  
104 temperature ( $20 \pm 1^\circ\text{C}$ ), to simulate home storage conditions, and samples were  
105 collected at four different times after coating treatment: 1, 7, 14 and 21 days. After this  
106 period, visual signs of ageing, as wrinkled skin, appeared on some fruits. For each  
107 storage time and coating treatment, 3 fruits were used for aroma analysis and 5 for  
108 measurement of fruit gas exchanges (64 fruits in total). Each fruit represented an  
109 independent biological replicate.

110

### 111 *2.2 Edible coating*

112 Edible coating was prepared using the gelatin sheets discarded during the production of  
113 soft gelatin capsules, obtained from the Chemical-Pharmaceutical Laboratory Tiaraju  
114 (Santo Ângelo, Rio Grande do Sul, Brasil). The EC solution was prepared at 1.5 g/L  
115 final gelatin concentration by melting gelatin sheets in water at  $60^\circ\text{C}$  until complete  
116 dissolution. After cooling at  $40^\circ\text{C}$ , Tween 20 (0.01 g/L) and potassium sorbate (0.01  
117 g/L) were added. The edible coating was applied by dipping the apples into the solution  
118 and let them dry at room temperature.

119

120 *2.3 Headspace solid-phase micro extraction (HS-SPME) procedure*

121 The HS-SPME procedure was done according to Ferreira et al. (2009), with some  
122 modifications. Briefly, each apple was weighed and, after removing core but keeping  
123 pulp and peel, it was cut into small pieces within a beaker filled with saturated calcium  
124 chloride (1/1 apple weight/CaCl<sub>2</sub> volume) to inhibit enzyme activity and homogenized  
125 with a mixer. Two grams of the mixture were put into a glass vial and closed with  
126 aluminum cap provided with a PTFE-septum. The vial was placed in a water bath at 50°  
127 C for 15 minutes. VOCs were collected by using a  
128 divinylbenzene/carboxen/polydimethylsiloxane (DVB/Carboxen/PDMS) Stable Flex  
129 SPME fiber (50/30 μm; 2-cm long) (Supelco, Bellefonte, PA, USA). The SPME fiber  
130 was first preconditioned for 15 min in the GC injection port at 250°C and then exposed  
131 to headspace for 30 min, after which the fiber was retracted prior removal from the  
132 sample and then inserted into the GC system. Before every sampling, the fiber was  
133 preconditioned and blank runs were done in-between to check the absence of volatile  
134 residues on it.

135

136 *2.4 GC/MS analysis*

137 The fiber was inserted into the injector of a single quadrupole GC/MS apparatus  
138 (TRACE GC/MS, Thermo-Finnigan, Waltham, MA, USA) set at 250° C, 3 minutes in  
139 splitless mode, keeping the fiber into the injector for 15 min in order to obtain the  
140 complete desorption. The GC program conditions were the same as those described by  
141 Ferreira et al. (2009). The GC apparatus was coupled with a Varian CP-WAX-52  
142 capillary column (60 m x 0.32 mm; coating thickness 0.5 μm). The transfer-line and the  
143 ion source were both set at 250° C. The filament emission current was 70 eV. A mass  
144 range from 32 to 300 *m/z* was scanned at a rate of 1.6 amu/sec. The acquisition was

145 carried out by electron impact, using the Full Scan (TIC) mode. Three replicates (n = 3)  
146 per sample were run. For the determination of LRI a C<sub>8</sub>-C<sub>20</sub> series was used (Sigma-  
147 Aldrich).

148 The VOCs were identified in three different ways: by comparison with the mass spectra  
149 of the Wiley library (version 2.0-11/2008); by injection of authentic standards  
150 previously analyzed and stored in the database; by calculation of LRI (Linear Retention  
151 Index) and comparing with those obtained in literature. For those compounds of which  
152 authentic standards were not used, the identification is to be considered tentative. Data  
153 of volatiles were expressed as peak area percentage of total chromatogram area  
154 (Budryn, Zaczyńska, & Oracz, 2016).

155

#### 156 *2.5 Gas exchange measurements*

157 CO<sub>2</sub> gas exchange was determined using the LI-6400XT portable gas exchange system  
158 (LI-COR, Lincoln, NE, USA) equipped with a large chamber (6400-05, LI-COR,  
159 Lincoln, NE, USA). Measurements were performed on five individual fruits per  
160 treatment and time point, at CO<sub>2</sub> concentration of 400 μmol mol<sup>-1</sup>, air temperature of  
161 20°C and relative humidity of 45-55%. Chamber was maintained under dark condition  
162 and fruits were allowed to adapt to the above conditions within the chamber for about  
163 15-20 min for adjustment and stabilization of the gas exchange parameters.

164

#### 165 *2.6 Statistical analysis*

166 The differences in VOCs and CO<sub>2</sub> emission between apples with and without edible  
167 coating, for each time of storage, were determined with one-way ANOVA for means  
168 comparison, by using JMP software (SAS Institute, Inc., Cary, NC).

169 Least square means were compared according to HSD Tukey test and the values of least  
170 square means were considered statistically significant when  $P \leq 0.05$ .

171 Data of VOCs were also subjected to principal components analysis (PCA) to visualize  
172 all the data set information and possible relationships among samples groups and  
173 variables.

174

### 175 **3. Results and discussion**

#### 176 *3.1 CO<sub>2</sub> gas exchange*

177 CO<sub>2</sub> emission was significantly lower in gelatin-coated fruit (**Figure 1**), the decrease  
178 ranging between 36 % (after 7 days) and 68 % (after 1 and 21 days of storage). The  
179 presence of coating acts as a barrier to the gas diffusion, leading to CO<sub>2</sub> accumulation in  
180 the tissues (Zhou et al., 2008). High levels of CO<sub>2</sub> inhibit succinic dehydrogenase activi-  
181 ty and induce the accumulation of succinic acid, in turn inhibiting the Krebs cycle  
182 (Knee, 1973). Although no direct measurement of fruit respiration was performed in  
183 this experiment, the CO<sub>2</sub> emission measured in coated fruits suggests a possible reduc-  
184 tion of respiration rate.

185 The reduced gas exchanges can lead to conditions similar to storage under modified  
186 controlled atmosphere, and are known to promote the beneficial effects of ECs on the  
187 produce conservation (Kader, Zagory, & Kerbel, 1989). Besides on the kind of biopol-  
188 ymer, the gas barrier effect depends on concentration and thickness of EC, morphology,  
189 density, chemical structure, polymeric orientation and relative humidity (Cisneros-  
190 Zevallos & Krochta, 2003). In accordance with our results, an approximately 50%  
191 lower respiration rate was observed by Lima et al. (2010) in apples coated with 0.05 g/L  
192 of galactomannan and 0.15 g/L of collagen during 60 hours of measurement. The same  
193 authors report a decrease in CO<sub>2</sub> production also by coated mangoes, even if such a de-  
194 crease was only 11% lower than control. Application of gelatin-starch coating resulted  
195 in a marked decrease of CO<sub>2</sub> emission by avocado fruit stored at 20°C and in a delayed

196 respiration climacteric peak of about 3 days (Aguilar-Méndez et al., 2008), indicating  
197 that coating effectively delayed the fruit ripening.

198

### 199 *3.2 Gelatin coating influences the apple VOCs*

200 Analysis of VOCs compounds from apples with and without the edible coating led to  
201 the identification of 78 molecules belonging to different chemical groups, comprising  
202 41 esters, 14 alcohols, 8 carbonyl compounds, 5 terpenes, 7 acids and 3 other  
203 compounds. Because of the high number of volatiles identified, we mainly focused on  
204 those molecules that are considered characteristic of apple fruit, as reported by Dixon  
205 and Hewett (2000) and on other few VOCs that greatly differed between the two  
206 treatments (**Table 1, 2, 3**). The odor descriptors, added for a better explanation, were  
207 taken from Dixon and Hewett (2000), PubChem (Kim et al., 2016) and the Joint  
208 FAO/WHO Expert Committee on Food Additives (JECFA, 2017) databases. Typical  
209 chromatograms of VOCs of coated and control fruit are shown in **Figure 2**.

210

#### 211 *3.2.1 Esters*

212 Esters (**Table 1 and table 1S**) represented the major group of volatiles contributing to  
213 apple aroma, for both coated and uncoated apples. The presence of the coating did not  
214 conspicuously affect total esters production. Observing the behavior of the single  
215 compounds, it is evident that ethyl esters, that generally gives a fruity odor, like ethyl  
216 butanoate and ethyl 2-methylbutanoate, had higher values in coated apples than controls  
217 already after 1 days of storage. This could be related to the high presence of ethanol in  
218 coated apples, as this compound is known to be ethyl esters precursor (Berger &  
219 Drawert, 1984). Also other ethyl esters such as ethyl acetate and ethyl hexanoate were  
220 generally incremented in gelatin-treated apples.



221 Conversely, other VOCs such as *n*-butyl acetate, 2-methyl-1-butyl acetate, pentyl  
222 acetate, butyl butanoate, butyl 2-methylbutanoate, hexyl acetate, hexyl 2-  
223 methylbutanoate, butyl hexanoate and hexyl butanoate exhibited higher peak area  
224 percentage in control fruit. These volatile compounds give overall sensorial notes of  
225 fruity and apple. Acetate esters were generally less present in coated apples, a behavior  
226 coherent with their depressed production observed in low-oxygen conditions (Fellman  
227 & Mattinson, 1993) as those triggered by the gas barrier effect of ECs.

228 In accordance with a previous report on aromatic profile of Fuji apples (Echeverria,  
229 Graell, López, & Lara, 2004a), the VOCs most contributing to the specific varietal  
230 aroma are ethyl 2-methylbutanoate, 2-methylbutyl acetate and hexyl acetate. These  
231 compounds undergo ripening-dependent variation: ethyl 2-methylbutanoate declines  
232 while the other two molecules augment with maturity stage (Echeverria et al., 2004a). In  
233 our study, in coated apples, ethyl 2-methylbutanoate was 7-fold higher than in control  
234 fruit already after 1 day of storage, while 2-methylbutyl acetate and hexyl acetate had  
235 significant lower values than controls after 1 day (-78 %) and 7 days (-73 %),  
236 respectively. One of the recognized effects of ECs is the capacity to delay the ripening  
237 process. The profile of these three VOCs indicates a probable slowing down of ripening  
238 in coated apples, as suggested also by the reduced CO<sub>2</sub> emission.

239 Moreover, the fact that these three specific volatiles, mostly contributing to varietal  
240 aroma, were found also in gelatin-coated apples, indicates that the presence of the edible  
241 coating did not conspicuously alter the typical aroma of Fuji fruit.

242

### 243 3.2.2 Alcohols

244 Alcohol volatiles are other prominent compounds contributing to apple aroma. As  
245 reported in **Table 2**, total peak area percentage is higher in EC-treated apples starting  
246 from 7 days of storage (1.3-fold) till the end of the storage (2-fold), in respect to

247 controls. This increment is mostly due to ethanol, which tends to rise its proportion  
248 already after 7 days.

249 Looking at the single compounds (**Table 2**), it is evident that coating affected the profile  
250 of alcohol volatiles: some alcohols were predominant in gelatin-treated apples (ethanol,  
251 4-hexen-1-ol, 6-methylhept-5-en-2-ol, octanol and decanol) while others were  
252 significantly predominant in control fruits, like 1-hexanol, 1-butanol and 2-methylbutan-  
253 1-ol.

254 In particular, the peak area percentage of ethanol, which increased during the storage  
255 period, was noticeably higher in coated apples as compared to uncoated fruit at any time  
256 considered, the increase ranging from 38-fold (21 days) to 154-fold (7 days) (**Table 2**).  
257 However, it should be remembered that the contribute of any volatile to the fruit aroma  
258 is also related to the odor threshold, that for ethanol is  $100,000 \mu\text{g l}^{-1}$  (Flath, Black,  
259 Guadagni, McFadden, & Schultz, 1967).

260 Ethanol is strictly related to anaerobic metabolism: when oxygen level decreases, fruit  
261 respiration decreases as well and glycolysis replaces the tricarboxylic acid cycle.  
262 Pyruvate is converted to  $\text{CO}_2$  and acetaldehyde, this latter being then reduced to ethanol  
263 (Dixon & Hewett, 2000). This switch of metabolism seems to be linked to the low gas  
264 permeability induced by protein EC; as reported by Yang & Paulson (2000) protein-  
265 based films are excellent barrier to oxygen. In the present experiment, the gas-barrier  
266 effect played by gelatin coating reduced  $\text{CO}_2$  emission (**Figure 1**), and probably  
267 triggered a partially anaerobic metabolism. However, the reduced respiration, at the  
268 same time, can produce a positive effect, slowing down the ripening process, as  
269 suggested by the behavior of acetate esters, which normally tend to increase during  
270 maturation, and which were less concentrated in apples covered with gelatin.

271

272 *3.2.3 Carbonyl compounds*

273 Carbonyl compounds identified in this study were 7 aldehydes and 1 ketone (**Table 2**).  
274 Total aldehydes showed significantly lower values in gelatin-coated apples in  
275 comparison to control ones (between -45 and -82 % from 7 to 21 days). Volatile  
276 molecules like hexanal, 2-hexenal-(E) and 2-hexenal-(Z), which are responsible for  
277 green odors, were generally lower in apple covered with EC, even if not significantly.  
278 Other volatiles like octanal, 2-heptenal and 2,4-hexadienal-(E,E) have been detected  
279 only in control apples.

280 The lower aldehydes proportion detected in coated apples may be explained because of  
281 the lower availability of oxygen, as a consequence of the gas-barrier action of EC. It is  
282 important to note that, despite hypoxic conditions are reported to enhance both  
283 acetaldehyde and ethanol (Dixon & Hewett, 2000), acetaldehyde, which gives an  
284 unpleasant (piquancy) aroma, was not detected in coated apples (nor in control fruits).

285

#### 286 *3.2.4 Acids, terpenes and other compounds*

287 Total acid volatiles (**Table 3**) were significantly affected by the coating, being reduced  
288 by the treatment of about 35 and 37 % at 14 and 21 days, respectively. This result may  
289 be due to a lower oxidation rate of aldehydes, for example from hexanal to hexanoic  
290 acid, in coated apples, a phenomenon correlated with the gas-barrier property of protein  
291 coating, as discussed above.

292 Terpenes were not significantly affected by the coating treatment (**Table 3**). In both  
293 coated and control apples,  $\alpha$ -farnesene was the predominant terpene volatile, accounting  
294 for about 90 % of total terpenes.

295 Three other volatiles, not belonging to the chemical classes before described, were also  
296 detected: 2-ethyl furan, found only in controls, a methoxybenzene and a not identified  
297 (NI) molecule, detected only in gelatin-coated apples (**Table 3**).

298

### 299 3.2.5 Multivariate analysis

300 By the application of PCA to the analytical variables (all the VOCs identified in apples  
301 with and without gelatin-based edible coating during 21 days of storage), three principal  
302 components (PCs) were extracted, explaining 57.33 % of the total variance. In  
303 particular, PC1 explains 36.7 %, PC2 10.7 % and PC3 9.93 % of the total variance. The  
304 projections of the samples along the three PCs are reported in **Figure 3**, where PC1 is  
305 plotted against PC2 and PC3. The PC1-PC2 score plot showed a clear separation of the  
306 samples projections: the gelatin-coated samples were situated along the negative part of  
307 the axis, while the controls were distributed along the positive side.

308 In the PC1-PC2 loading plot (**Figure 4**) we highlighted VOCs characterizing apple  
309 aroma or molecules undergoing the most striking changes. PCA analysis showed that  
310 volatile molecules with negative values for PC1 are ethyl acetate, ethanol, ethyl  
311 propanoate, ethyl butanoate, ethyl 2-methylbutanoate, diethyl carbonate, ethyl  
312 pentanoate, ethyl (E)-but-2-enoate, ethyl hexanoate, ethyl (E)-2-methylbut-2-enoate,  
313 ethyl trans-2-pentenoate, butyl ethyl carbonate, ethyl heptanoate, methoxy benzene, 4-  
314 hexen-1-ol, ethyl octanoate, 1-heptanol, 6-methylhept-5-en-2-ol, acetic acid, ethyl 3-  
315 hydroxybutanoate,  $\beta$ -Linalool, octan-1-ol, nonanol, diethyl succinate, ethyl 3-  
316 hydroxyhexanoate, NI, decanol and ethyl 4-methoxybenzoate. Their presence is  
317 strongly associated to apples covered with the gelatin coating. The great proportion of  
318 ethyl esters in coated apples was likely due to the huge production of ethanol, which  
319 acts as available precursor for the biosynthesis of ethyl esters, whose production is  
320 known to be stimulated by ethanol (Kollmannsberger & Berger, 1992; Dixon & Hewett,  
321 2000).

322 Conversely, other compounds have positive values for PC1; these are 2-ethyl-furan, *n*-  
323 propylacetate, 2-methylpropyl acetate, propyl propanoate, *n*-butyl acetate, hexanal, 2-  
324 methyl-1-butyl-acetate, hexyl 2-methylbutanoate, propyl 2-methylbutanoate, 1-butanol,

325 pentyl acetate, methyl hexanoate, 2-methylbutan-1-ol, 2-hexenal-(E), butyl butanoate,  
326 2-hexenal- (Z), butyl 2-methylbutanoate, pentyl acetate, methyl hexanoate, ethyl  
327 hexanoate, ethyl (E)-2-methylbut-2-enoate, 1-pentanol, 2-methylbutyl butanoate, *n*-  
328 hexyl acetate, 2-methylbutyl 2-methylbutanoate, octanal, propyl hexanoate, pentyl 2-  
329 methylbutanoate, 2-heptenal, hexyl propanoate, 6-methylhept-5-en-2-one, 1-hexanol,  
330 nonanal, 2,4-hexadienal-(E,E), butyl hexanoate, hexyl butanoate, 2-methyl butanoic  
331 acid,  $\alpha$ -farnesene, hexanoic acid, decanoic acid and dodecanoic acid. These molecules  
332 were strongly associated to uncoated control apples.

333 The projections along PC2 of the PC1-PC2 score plot (**Figure 3**) highlighted separation  
334 of 1 day of storage from the other storage times, with positive values for this  
335 component; moreover, looking at PC1-PC3 score plot, and observing the projections  
336 along PC3, the separation of samples after 21 days treated with edible coating was  
337 clearly evident. PC1-PC3 loading plot (**Figure 4**) displayed association of volatiles like  
338 diethyl carbonate, ethyl pentanoate, ethyl (E)-but-2-enoate, ethyl trans-2-pentenoate,  
339 butyl ethyl carbonate, acetic acid, ethyl 3-hydroxybutanoate, diethyl succinate, nonanol,  
340 decanol with coated apples at the end of the storage (21 days). All these compounds  
341 were present only in coated apples and most of them were produced in the later stage  
342 periods (**Table 1**).

343

#### 344 **4. Conclusions**

345 At the best of our knowledge, this is the first report on the influence of a gelatin-based  
346 coating on VOCs profile of apple fruit. Data collected during 3 weeks of storage at  
347 room temperature highlighted decreased proportions of acids, aldehydes and terpenes in  
348 coated apples, that instead showed higher proportions of esters and alcohols. Acetate es-  
349 ters, which usually increase with maturity, were less concentrated in coated apples, as a  
350 probable consequence of the ripening-delaying effect of gelatin coating.

351 Particularly evident was the marked increase in ethanol following gelatin application,  
352 suggesting the onset of partial anaerobiosis, as confirmed by the significantly lower CO<sub>2</sub>  
353 emission. Our data indicate that, although the concentration used strongly limits fruit  
354 respiration, gelatin ECs preserves the overall aroma profile of apples. Indeed, ethyl es-  
355 ters, which are the most significant contributors to apple aroma profile, were strongly  
356 associated to coated samples, and the three esters responsible of the varietal aroma of  
357 Fuji were present in both control and coated fruits, suggesting that gelatin did not modi-  
358 fy the typical aroma extensively.

359 Being aroma an important determinant of food quality which can affect the consumer  
360 acceptance of the produce, further investigation is needed to understand the influence of  
361 gelatin and other ECs on this food character. Attention should be paid therefore to  
362 choose EC concentration and/ or composition able to limit gas exchanges without in-  
363 ducing anaerobiosis and marked production of ethanol.

364

365

366

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373

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468 **Figure captions**

469 **Figure 1.** CO<sub>2</sub> gas exchange of Fuji apples with (solid line) and without (dotted line)  
470 edible coating after 1, 7, 14 and 21 days of storage. Data represent the mean of 5 repli-  
471 cates ± SE. Different letters correspond to statistically significant differences according  
472 to one-way ANOVA followed by Tuckey post hoc test ( $P \leq 0.05$ ).

473 **Figure 2.** Typical GC/MS chromatogram of organic volatile compounds of Fuji apples  
474 after 1 day of storage: A, with gelatin as edible coating; B, without gelatin as edible  
475 coating.

476 **Figure 3.** Score plot for PC1, PC2, PC3. Full symbols, coated apples; empty symbols,  
477 controls; circle, 1 day; triangle, 7 days; rectangle, 14 days; square, 21 days.

478 **Figure 4.** Loading plot for PC1, PC2, PC3. VOCs characterizing apple aroma or  
479 molecules undergoing the most striking changes were highlighted. Green labels,  
480 characteristic volatiles of Fuji apples (ethyl 2-methylbutanoate, *n*-hexyl acetate, 2-  
481 methyl-1-butyl -acetate); blue label, ethanol.

482

**Table 1.** Most important ester volatiles (peak area percentage) detected in Fuji apples coated with gelatin (T) and controls (C) during 21 days of storage.

Compounds <i>Esters</i>	RT <sup>c</sup>	ID <sup>d</sup>	Coating treatment	Days of storage			
				1	7	14	21
Ethyl acetate	7.86	W/L	C	0.09 ± 0.09 <sup>b</sup>	0.15 ± 0.04 <sup>b</sup>	0.72 ± 0.28 <sup>b</sup>	1.16 ± 0.58 <sup>b</sup>
			T	1.91 ± 0.37 <sup>a</sup>	11.53 ± 2.84 <sup>a</sup>	10.96 ± 1.58 <sup>a</sup>	8.89 ± 1.84 <sup>a</sup>
Ethyl propanoate	9.80	W/L	C	nd <sup>b</sup>	nd	0.28 ± 0.14	0.38 ± 0.05
			T	0.24 ± 0.05 <sup>a</sup>	0.17 ± 0.17	0.22 ± 0.22	0.57 ± 0.29
<i>n</i> -Propylacetate	10.33	W/L/S	C	0.096 ± 0.10	0.18 ± 0.12	0.45 ± 0.16 <sup>a</sup>	0.31 ± 0.20
			T	nd	nd	nd <sup>b</sup>	nd
2-Methylpropyl acetate	11.60	W/L	C	0.04 ± 0.02	0.15 ± 0.01 <sup>a</sup>	0.12 ± 0.00 <sup>a</sup>	0.16 ± 0.04 <sup>a</sup>
			T	nd	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>
Ethyl butanoate	12.51	W/L/S	C	0.49 ± 0.29 <sup>b</sup>	0.81 ± 0.24 <sup>b</sup>	2.90 ± 1.15	1.92 ± 0.76 <sup>b</sup>
			T	5.10 ± 0.64 <sup>a</sup>	6.17 ± 0.71 <sup>a</sup>	5.65 ± 0.29	6.26 ± 0.41 <sup>a</sup>
Ethyl 2-methylbutanoate	13.06	W/L	C	0.18 ± 0.15 <sup>b</sup>	0.15 ± 0.08 <sup>b</sup>	1.06 ± 0.49	1.32 ± 0.23 <sup>b</sup>
			T	1.49 ± 0.43 <sup>a</sup>	3.53 ± .099 <sup>a</sup>	3.27 ± 1.55	2.31 ± 0.17 <sup>a</sup>
<i>n</i> -Butyl acetate	13.91	W/L/S	C	4.52 ± .109 <sup>a</sup>	5.38 ± 0.63 <sup>a</sup>	5.67 ± 0.33 <sup>a</sup>	3.20 ± 0.93 <sup>a</sup>
			T	1.33 ± 0.47 <sup>b</sup>	0.60 ± 0.05 <sup>b</sup>	0.19 ± 0.06 <sup>b</sup>	0.07 ± 0.01 <sup>b</sup>
2-Methyl-1-butyl-acetate	15.96	W/L	C	17.22 ± 0.03 <sup>a</sup>	12.76 ± 0.48 <sup>a</sup>	10.10 ± 1.67 <sup>a</sup>	13.21 ± 3.02 <sup>a</sup>
			T	3.78 ± 0.41 <sup>b</sup>	3.69 ± 1.10 <sup>b</sup>	0.88 ± 0.41 <sup>b</sup>	0.17 ± 0.02 <sup>b</sup>
Pentyl acetate	18.26	W/L/S	C	0.64 ± 0.05 <sup>a</sup>	0.92 ± 0.09 <sup>a</sup>	0.83 ± 0.12 <sup>a</sup>	0.55 ± 0.14 <sup>a</sup>
			T	0.27 ± 0.04 <sup>b</sup>	0.27 ± 0.03 <sup>b</sup>	0.12 ± 0.06 <sup>b</sup>	nd <sup>b</sup>
Butyl butanoate	20.26	W/L	C	1.09 ± 0.29	0.96 ± 0.22 <sup>a</sup>	0.59 ± 0.05 <sup>a</sup>	0.30 ± 0.15
			T	0.37 ± 0.11	nd <sup>b</sup>	nd <sup>b</sup>	nd
Butyl 2-methylbutanoate	20.84	W/L	C	1.11 ± 0.07	1.13 ± 0.07 <sup>a</sup>	0.79 ± 0.15 <sup>a</sup>	0.66 ± 0.33
			T	0.38 ± 0.29	nd <sup>b</sup>	nd <sup>b</sup>	nd
Ethyl hexanoate	20.96	W/L/S	C	0.76 ± 0.48 <sup>b</sup>	0.67 ± 0.17 <sup>b</sup>	3.23 ± 0.17	0.86 ± 0.28 <sup>b</sup>
			T	6.30 ± 0.29 <sup>a</sup>	7.24 ± 0.60 <sup>a</sup>	4.07 ± 0.23	4.32 ± 0.42 <sup>a</sup>
<i>n</i> -Hexyl acetate	22.74	W/L/S	C	4.34 ± 0.34	9.36 ± 1.95 <sup>a</sup>	9.76 ± 1.77 <sup>a</sup>	4.14 ± 1.29 <sup>a</sup>
			T	3.21 ± 0.96	2.58 ± 0.02 <sup>b</sup>	1.31 ± 0.28 <sup>b</sup>	0.32 ± 0.03 <sup>b</sup>
Hexyl propanoate	25.70	W/L	C	0.18 ± 0.03 <sup>a</sup>	0.22 ± 0.14	0.13 ± 0.13	0.03 ± 0.04
			T	nd <sup>b</sup>	nd	nd	nd
Hexyl 2-methylbutanoate	28.99	W/L/S	C	2.95 ± 0.97	2.22 ± 0.26 <sup>a</sup>	1.09 ± 0.27 <sup>a</sup>	0.98 ± 0.38
			T	2.27 ± 0.50	0.54 ± 0.09 <sup>b</sup>	0.26 ± 0.04 <sup>b</sup>	0.45 ± 0.23

Butyl hexanoate	29.11	W/L/S	C	0.59 ± 0.22	0.44 ± 0.19	0.33 ± 0.10 <sup>a</sup>	0.17 ± 0.05 <sup>a</sup>
			T	0.16 ± 0.13	nd	nd <sup>b</sup>	nd <sup>b</sup>
Hexyl butanoate	29.57	W/L	C	2.53 ± 1.37	2.32 ± 0.32 <sup>a</sup>	1.63 ± 0.33 <sup>a</sup>	0.66 ± 0.38
			T	1.05 ± 0.22	0.50 ± 0.16 <sup>b</sup>	0.30 ± 0.01 <sup>b</sup>	0.06 ± 0.03
Hexyl hexanoate	37.28	W/L/S	C	1.07 ± 0.51	0.64 ± 0.08 <sup>a</sup>	0.40 ± 0.22	0.15 ± 0.09
			T	0.72 ± 0.24	0.22 ± 0.08 <sup>b</sup>	0.15 ± 0.02	0.57 ± 0.51
Total Esters <sup>e</sup>			C	39.66 ± 1.41	40.17 ± 3.49	42.42 ± 6.41	32.17 ± 2.29
			T	40.30 ± 6.42	43.92 ± 2.83	36.75 ± 2.98	38.16 ± 2.24

<sup>c</sup> RT, retention time; <sup>d</sup> ID, identification based on: W, Wiley; S, standard, L, literature; <sup>e</sup>Total esters, calculated on the basis of all esters identified (see Supplementary table 1S). nd, not detected.

Data represent the mean of 3 replicates ± SE. At any time point, different letters correspond to statistically significant differences between control and coated samples according to one-way ANOVA followed by Tuckey post hoc test ( $P \leq 0.05$ ).

**Table 2.** Alcohols, aldehydes and ketones volatiles (peak area percentage) detected in Fuji apples coated with gelatin (T) and controls (C) during 21 days of storage.

Compounds	RT <sup>c</sup>	ID <sup>d</sup>	Coating treatment	Days of storage			
				1	7	14	21
<i>Alcohols</i>							
Ethanol	9.03	W/L/S	C	0.09 ± 0.06 <sup>b</sup>	0.10 ± 0.02 <sup>b</sup>	0.48 ± 0.28 <sup>b</sup>	0.81 ± 0.44 <sup>b</sup>
			T	3.86 ± 0.52 <sup>a</sup>	15.52 ± 1.79 <sup>a</sup>	26.39 ± 3.05 <sup>a</sup>	31.20 ± 0.90 <sup>a</sup>
1-Butanol	16.82	W/L/S	C	3.03 ± 0.56	1.75 ± 0.27 <sup>a</sup>	2.51 ± 0.42 <sup>a</sup>	2.20 ± 0.91
			T	1.49 ± 0.42	0.73 ± 0.00 <sup>b</sup>	0.58 ± 0.12 <sup>b</sup>	0.72 ± 0.14
2-Methylbutan-1-ol	19.59	W/L/S	C	3.38 ± 1.18	2.23 ± 0.59	2.53 ± 0.17 <sup>a</sup>	5.10 ± 0.22 <sup>a</sup>
			T	2.69 ± 0.41	1.99 ± 0.48	1.06 ± 0.22 <sup>b</sup>	1.51 ± 0.36 <sup>b</sup>
1-Pentanol	21.56	W/L	C	0.24 ± 0.02	0.16 ± 0.03	0.19 ± 0.05	0.17 ± 0.11
			T	0.20 ± 0.02	0.10 ± 0.01	0.07 ± 0.04	0.14 ± 0.02
1-Hexanol	26.22	W/L/S	C	8.81 ± 1.05	5.32 ± 0.15 <sup>a</sup>	6.30 ± 1.08 <sup>a</sup>	4.36 ± 2.17
			T	8.45 ± 1.61	3.65 ± 0.34 <sup>b</sup>	2.80 ± 0.69 <sup>b</sup>	3.97 ± 1.03
(E)-2-Hexen-1-ol	28.64	W/L	C	0.29 ± 0.07	0.03 ± 0.03	nd	0.05 ± 0.03
			T	0.29 ± 0.15	nd	0.07 ± 0.04	nd
4-Hexen-1-ol	28.87	W/L	C	nd <sup>b</sup>	nd <sup>b</sup>	nd	nd
			T	0.30 ± 0.06 <sup>a</sup>	0.55 ± 0.11 <sup>a</sup>	0.33 ± 0.20	0.34 ± 0.10
1-Heptanol	30.71	W/L/S	C	0.03 ± 0.03	0.06 ± 0.03	0.03 ± 0.04 <sup>b</sup>	0.02 ± 0.02
			T	0.06 ± 0.03	0.05 ± 0.03	0.12 ± 0.01 <sup>a</sup>	0.09 ± 0.05
6-Methylhept-5-en-2-ol	31.03	W/L	C	nd	nd <sup>b</sup>	nd <sup>b</sup>	nd
			T	nd	0.47 ± 0.21 <sup>a</sup>	1.13 ± 0.37 <sup>a</sup>	0.15 ± 0.16
2-Ethylhexan-1-ol	32.19	W/L/S	C	0.02 ± 0.03	0.13 ± 0.02	0.03 ± 0.03 <sup>b</sup>	0.01 ± 0.02
			T	0.02 ± 0.02	0.19 ± 0.09	0.18 ± 0.02 <sup>a</sup>	nd
Octan-1-ol	35.05	W/L	C	0.12 ± 0.03	0.11 ± 0.02 <sup>b</sup>	0.11 ± 0.02 <sup>b</sup>	0.05 ± 0.03 <sup>b</sup>
			T	0.32 ± 0.07	0.25 ± 0.01 <sup>a</sup>	0.67 ± 0.13 <sup>a</sup>	0.8 ± 0.13 <sup>a</sup>
Nonanol	39.17	W/L	C	nd	nd	nd	nd
			T	nd	nd	nd	0.35 ± 0.17
Decanol	43.11	W/L	C	nd	nd	nd	nd <sup>b</sup>
			T	nd	nd	0.11 ± 0.06	0.16 ± 0.03 <sup>a</sup>
1-Undecanol	50.46	W/L	C	0.36 ± 0.03	0.35 ± 0.06	0.18 ± 0.10	0.31 ± 0.10
			T	0.37 ± 0.04	0.29 ± 0.02	0.38 ± 0.03	0.35 ± 0.04
Total alcohols			C	16.39 ± 9.46	10.27 ± 5.93 <sup>b</sup>	12.38 ± 7.15 <sup>b</sup>	13.10 ± 7.57 <sup>b</sup>
			T	18.27 ± 10.43	23.79 ± 13.73 <sup>a</sup>	33.91 ± 19.58 <sup>a</sup>	39.81 ± 22.98 <sup>a</sup>

<i>Aldehydes</i>							
Hexanal	14.44	W/L/S	C	4.25 ± 0.55	10.59 ± 1.08	7.68 ± 2.10	10.39 ± 2.41 <sup>a</sup>
			T	3.10 ± 1.76	6.02 ± 2.22	5.68 ± 1.36	1.97 ± 0.79 <sup>b</sup>
2-Hexenal (E)	19.80	W/L	C	0.59 ± 0.06	0.70 ± 0.09 <sup>a</sup>	0.64 ± 0.16 <sup>a</sup>	0.75 ± 0.12 <sup>a</sup>
			T	0.39 ± 0.18	0.26 ± 0.07 <sup>b</sup>	0.24 ± 0.04 <sup>b</sup>	0.05 ± 0.06 <sup>b</sup>
2-Hexenal (Z)	20.62	W/L	C	18.31 ± 1.38	20.65 ± 1.51 <sup>a</sup>	19.86 ± 4.61 <sup>a</sup>	17.82 ± 4.58 <sup>a</sup>
			T	14.92 ± 6.73	11.43 ± 2.78 <sup>b</sup>	6.49 ± 0.64 <sup>b</sup>	3.05 ± 1.54 <sup>b</sup>
Octanal	23.65	W/L	C	0.06 ± 0.00 <sup>a</sup>	0.02 ± 0.02	0.01 ± 0.01	nd
			T	nd <sup>b</sup>	nd	nd	nd
2-Heptenal	25.43	W/L	C	0.13 ± 0.00 <sup>a</sup>	0.16 ± 0.02 <sup>a</sup>	0.08 ± 0.04	0.14 ± 0.01 <sup>a</sup>
			T	nd <sup>b</sup>	nd <sup>b</sup>	nd	nd <sup>b</sup>
Nonanal	28.36	W/L/S	C	0.21 ± 0.02	0.20 ± 0.02	0.16 ± 0.02	0.17 ± 0.01
			T	0.14 ± 0.03	0.13 ± 0.02	0.18 ± 0.02	0.10 ± 0.05
2,4-Hexadienal (E.E)	28.83	W/L	C	0.30 ± 0.03 <sup>a</sup>	0.35 ± 0.09 <sup>a</sup>	0.36 ± 0.06 <sup>a</sup>	0.34 ± 0.04 <sup>a</sup>
			T	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>
Total aldehydes			C	23.88 ± 1.81	32.68 ± 0.84 <sup>a</sup>	28.80 ± 6.62 <sup>a</sup>	29.61 ± 6.83 <sup>a</sup>
			T	18.56 ± 8.71	17.87 ± 5.08 <sup>b</sup>	12.59 ± 1.68 <sup>b</sup>	5.17 ± 2.34 <sup>b</sup>
<i>Ketones</i>							
6-Methylhept-5-en-2-one	25.87	W/L	C	0.36 ± 0.05	0.25 ± 0.01	0.20 ± 0.03	0.23 ± 0.04
			T	0.28 ± 0.06	0.19 ± 0.05	0.31 ± 0.11	0.16 ± 0.04

<sup>c</sup> RT, retention time; <sup>d</sup> ID, identification based on: W, Wiley; S, standard, L, literature. nd, not detected.

Data represent the mean of 3 replicates ± SE. At any time point, different letters correspond to statistically significant differences between control and coated samples according to one-way ANOVA followed by Tukey post hoc test ( $P \leq 0.05$ ).

**Table 3.** Acids, terpenes and other volatiles (peak area percentage) detected in Fuji apples coated with gelatin (T) and controls (C) during 21 days of storage.

Compounds	RT <sup>c</sup>	ID <sup>d</sup>	Coating treatment	Days of storage			
				1	7	14	21
<i>Acids</i>							
Acetic acid	31.19	W/L/S	C	0.17 ± 0.01	0.13 ± 0.08	0.29 ± 0.08	0.30 ± 0.04
			T	0.14 ± 0.01	0.17 ± 0.09	0.24 ± 0.02	0.64 ± 0.20
2-Methyl butanoic acid	39.99	W/L	C	0.61 ± 0.40	1.29 ± 0.41 <sup>a</sup>	0.68 ± 0.36	1.94 ± 0.72 <sup>a</sup>
			T	nd	nd <sup>b</sup>	nd	nd <sup>b</sup>
Hexanoic acid	46.53	W/L/S	C	0.29 ± 0.05 <sup>a</sup>	0.30 ± 0.02 <sup>a</sup>	0.24 ± 0.13	0.32 ± 0.07
			T	0.04 ± 0.04 <sup>b</sup>	0.14 ± 0.06 <sup>b</sup>	0.08 ± 0.08	0.18 ± 0.03
Octanoic acid	53.84	W/L/S	C	0.62 ± 0.07	0.54 ± 0.14	0.75 ± 0.09	0.56 ± 0.15
			T	0.82 ± 0.13	0.79 ± 0.28	0.82 ± 0.05	0.66 ± 0.14
Nonanoic acid	57.25	W/L/S	C	2.12 ± 0.31	1.79 ± 0.43	2.59 ± 0.26	1.89 ± 0.49
			T	1.95 ± 0.19	1.73 ± 0.43	2.02 ± 0.22	1.50 ± 0.18
Decanoic acid	60.5	W/L/S	C	0.93 ± 0.12	0.84 ± 0.17	0.90 ± 0.12	0.62 ± 0.23
			T	0.99 ± 0.12	0.55 ± 0.09	0.61 ± 0.08	0.47 ± 0.03
Dodecanoic acid	68.51	W/L	C	1.28 ± 0.18	1.35 ± 0.28 <sup>a</sup>	1.22 ± 0.30 <sup>a</sup>	0.71 ± 0.30
			T	0.95 ± 0.03	0.48 ± 0.03 <sup>b</sup>	0.52 ± 0.07 <sup>b</sup>	0.55 ± 0.01
Total acids			C	6.02 ± 0.44	6.24 ± 1.04	6.67 ± 0.69 <sup>a</sup>	6.36 ± 0.56 <sup>a</sup>
			T	4.90 ± 0.43	3.87 ± 0.92	4.29 ± 0.35 <sup>b</sup>	4.00 ± 0.55 <sup>b</sup>
<i>Terpenes</i>							
β-Linalool	34.64	W/L	C	nd	nd	nd	nd
			T	nd	nd	0.11 ± 0.06	0.03 ± 0.03
Z-β-Farnesene	41.85	W/L	C	0.30 ± 0.18	0.23 ± 0.12	0.35 ± 0.11	0.50 ± 0.13
			T	0.45 ± 0.08	0.18 ± 0.12	0.29 ± 0.09	0.23 ± 0.14
α-Farnesene	42.78	W/L	C	12.32 ± 5.28	7.40 ± 0.67	8.88 ± 2.65	13.10 ± 4.01
			T	11.21 ± 1.14	5.00 ± 2.83	5.19 ± 2.46	6.67 ± 2.46
β-Damascenone	45.93	W/L	C	0.44 ± 0.04	0.50 ± 0.11	0.29 ± 0.15	0.37 ± 0.05
			T	0.36 ± 0.19	0.63 ± 0.13	0.49 ± 0.10	0.21 ± 0.05
trans-Geranylacetone	46.84	W/L	C	0.12 ± 0.12	0.19 ± 0.09	nd	nd
			T	0.08 ± 0.08	0.06 ± 0.07	nd	nd
Total terpenes			C	13.17 ± 5.16	8.32 ± 0.69	9.52 ± 2.766	13.98 ± 4.09
			T	12.12 ± 0.99	5.88 ± 2.80	6.09 ± 2.53	7.15 ± 2.56

<i>Others</i>							
2-Ethyl-furan	9.72	W/L	C	0.24 ± 0.01 <sup>a</sup>	0.11 ± 0.11	0.08 ± 0.099	nd
			T	nd <sup>b</sup>	nd	nd	nd
Methoxy benzene	26.37	W/L	C	nd	nd	nd	nd
			T	nd	nd	0.19 ± 0.10	0.05 ± 0.06
NI (not identified)	40.69	W	C	nd	nd <sup>b</sup>	nd <sup>b</sup>	nd
			T	0.19 ± 0.19	0.77 ± 0.08 <sup>a</sup>	1.60 ± 0.32 <sup>a</sup>	0.34 ± 0.34
Total others			C	0.25 ± 0.01	0.11 ± 0.11 <sup>b</sup>	0.09 ± 0.09 <sup>b</sup>	nd
			T	0.19 ± 0.19	0.77 ± 0.08 <sup>a</sup>	1.79 ± 0.49 <sup>a</sup>	0.40 ± 0.32

<sup>c</sup> RT, retention time; <sup>d</sup> ID, identification based on: W, Wiley; S, standard, L, literature. nd, not detected.

Data represent the mean of 3 replicates ± SE. At any time point, different letters correspond to statistically significant differences between control and coated samples according to one-way ANOVA followed by Tuckey post hoc test ( $P \leq 0.05$ ).



Figure 1

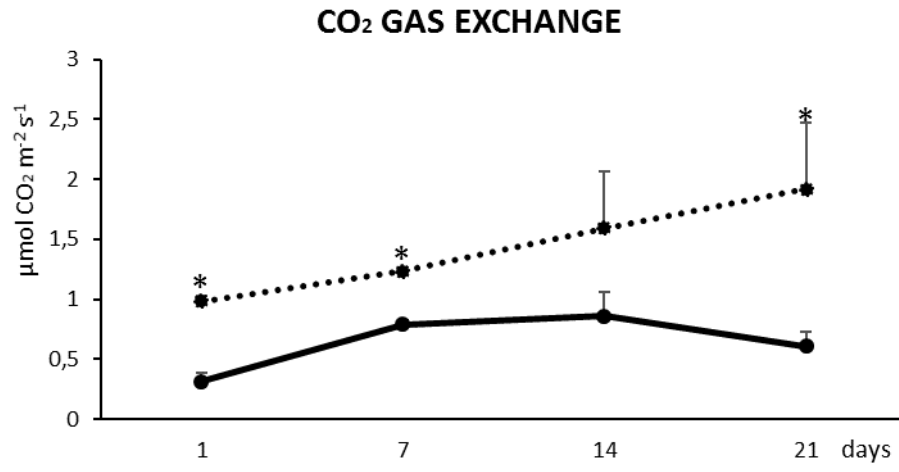


Figure 1

Figure 2

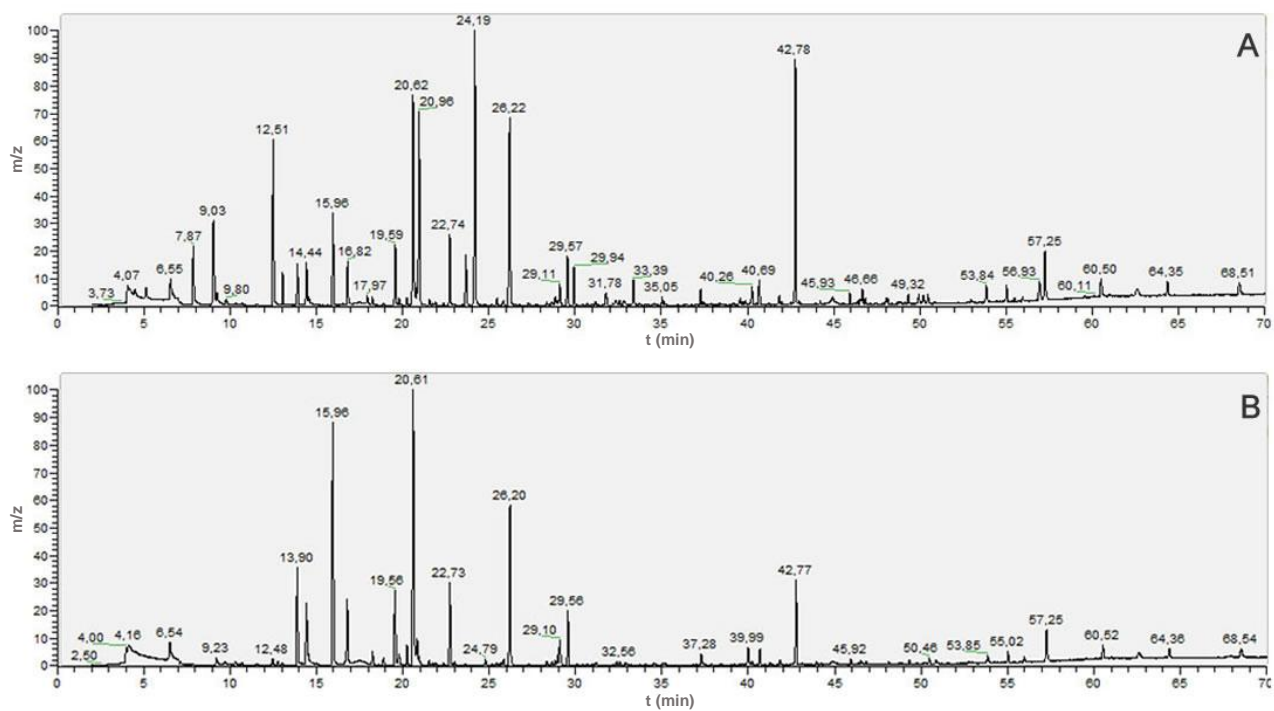


Figure 2

Figure 3

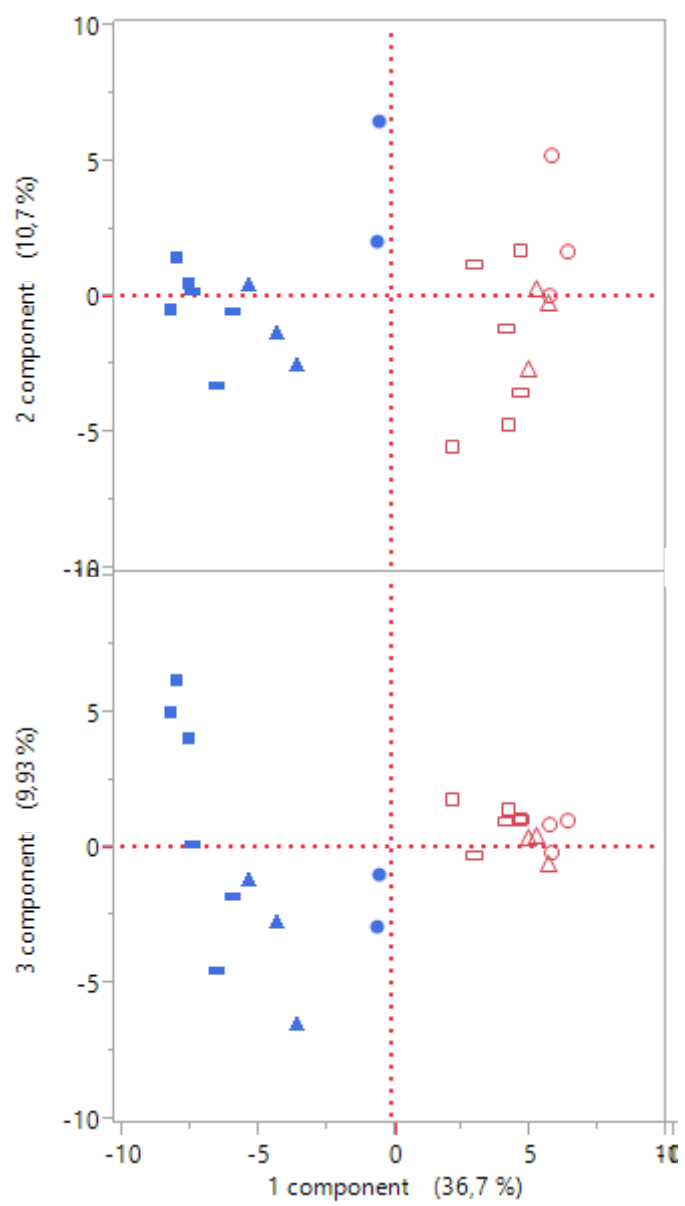


Figure 3

Figure 4



Figure 4

**Supplementary Material Table 1S**

[Click here to download Supplementary Material: Supplementary table-Identified compounds.docx](#)