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

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Keywords: Malus × domestica Borkh., GC/MS, CO2 emission, storage, volatile organic compounds

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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 metioned 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_2 emission
- Esters responsible of the Fuji varietal aroma were identified also in coated apples

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 CO_2 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.

Keywords

Malus × *domestica* Borkh., GC/MS, CO_2 emission, storage, volatile organic compounds

1. Introduction

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

Among the film-forming proteins, gelatin, obtained by hydrolysis of collagen, has effective barrier properties against oxygen and carbon dioxide (Jiang, Liu, Du, & Wang, 2010). Gelatin-starch coatings prolonged the postharvest shelf life of avocado (Aguilar-Méndez, San Martín-Martínez, Tomás, Cruz-Orea, & Jaime-Fonseca, 2008) and 10% gelatin coating, besides delaying ripening of mango fruit by suppressing the activity of softening enzymes, allowed the retention of higher ascorbic acid and phenolic content as compared with control (Gol & Ramana Rao, 2013). In the last few years, many scientific publications focused on physical and organoleptic changes in fruits covered with ECs, but at the best of our knowledge, only very few papers deal with aroma profile of coated apple fruit (Maya-Meraz et al., 2014; Sepulveda & Olivas, 2016; Olivas, Mattinson, & Barbosa-Cánovas, 2007)), and no paper assessed possible changes in volatile organic compounds (VOCs) induced by gelatin coating.

VOCs contributing to apples aroma are over 300 and they belong to different chemical classes including carboxylic esters, alcohols, aldehydes, ketones, acids, terpenes and ethers (Ferreira, Perestrelo, Caldeira, & Câmara, 2009). However, only about 20 of them are character impact compounds (Dixon & Hewett, 2000) and each individual molecule has its own odor threshold and concentration. Fruit aroma is cultivar-specific (Dixon & Hewett, 2000) but it depends also from pre- and post-harvest conditions like seasonal variation (López, Lavilla, Riba, & Vendrell, 1998) or harvest date and storage technology (Echeverría, Fuentes, Graell, Lara, & López, 2004b). The major volatiles in apples are esters, which are synthesized by esterification of alcohols and acyl-coA from fatty acids or amino acids-pathways; another important group is represented by alcohols, derived from fatty acids and amino acids metabolism and lipoxygenase (LOX) activity, this latter also producing aldehyde volatiles (Dixon & Hewett, 2000).

From a quality point of view, it is extremely important that the aroma profile of produce treated with EC is preserved to ensure good palatability. Therefore, this work aimed to

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

2. Material and methods

2.1 Plant material and experimental design

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

2.2 Edible coating

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

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

The HS-SPME procedure was done according to Ferreira et al. (2009), with some modifications. Briefly, each apple was weighed and, after removing core but keeping pulp and peel, it was cut into small pieces within a beaker filled with saturated calcium chloride (1/1 apple weight/$CaCl_2$ volume) to inhibit enzyme activity and homogenized with a mixer. Two grams of the mixture were put into a glass vial and closed with aluminum cap provided with a PTFE-septum. The vial was placed in a water bath at 50° C for 15 minutes. VOCs were collected by using a divinylbenzene/carboxen/polydimethysiloxane (DVB/Carboxen/PDMS) Stable Flex SPME fiber (50/30 μm; 2-cm long) (Supelco, Bellefonte, PA, USA). The SPME fiber was first preconditioned for 15 min in the GC injection port at 250°C and then exposed to headspace for 30 min, after which the fiber was retracted prior removal from the sample and then inserted into the GC system. Before every sampling, the fiber was preconditioned and blank runs were done in-between to check the absence of volatile residues on it.

2.4 GC/MS analysis

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

carried out by electron impact, using the Full Scan (TIC) mode. Three replicates (n = 3) per sample were run. For the determination of LRI a C_8-C_{20} series was used (Sigma-Aldrich).

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

2.5 Gas exchange measurements

CO_2 gas exchange was determined using the LI-6400XT portable gas exchange system (LI-COR, Lincoln, NE, USA) equipped with a large chamber (6400-05, LI-COR, Lincoln, NE, USA). Measurements were performed on five individual fruits per treatment and time point, at CO_2 concentration of 400 μmol mol^{-1}, air temperature of 20°C and relative humidity of 45-55%. Chamber was maintained under dark condition and fruits were allowed to adapt to the above conditions within the chamber for about 15-20 min for adjustment and stabilization of the gas exchange parameters.

2.6 Statistical analysis

The differences in VOCs and CO_2 emission between apples with and without edible coating, for each time of storage, were determined with one-way ANOVA for means comparison, by using JMP software (SAS Institute, Inc., Cary, NC).

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

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

3. Results and discussion

3.1 CO_2 gas exchange

CO_2 emission was significantly lower in gelatin-coated fruit (**Figure 1**), the decrease ranging between 36 % (after 7 days) and 68 % (after 1 and 21 days of storage). The presence of coating acts as a barrier to the gas diffusion, leading to CO_2 accumulation in the tissues (Zhou et al., 2008). High levels of CO_2 inhibit succinic dehydrogenase activity and induce the accumulation of succinic acid, in turn inhibiting the Krebs cycle (Knee, 1973). Although no direct measurement of fruit respiration was performed in this experiment, the CO_2 emission measured in coated fruits suggests a possible reduction of respiration rate.

The reduced gas exchanges can lead to conditions similar to storage under modified controlled atmosphere, and are known to promote the beneficial effects of ECs on the produce conservation (Kader, Zagory, & Kerbel, 1989). Besides on the kind of biopolymer, the gas barrier effect depends on concentration and thickness of EC, morphology, density, chemical structure, polymeric orientation and relative humidity (Cisneros-Zevallos & Krochta, 2003). In accordance with our results, an approximatively 50% lower respiration rate was observed by Lima et al. (2010) in apples coated with 0.05 g/L of galactomannan and 0.15 g/L of collagen during 60 hours of measurement. The same authors report a decrease in CO_2 production also by coated mangoes, even if such a decrease was only 11% lower than control. Application of gelatin-starch coating resulted in a marked decrease of CO_2 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.

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

In accordance with a previous report on aromatic profile of Fuji apples (Echeverria, Graell, López, & Lara, 2004a), the VOCs most contributing to the specific varietal aroma are ethyl 2-methylbutanoate, 2-methylbutyl acetate and hexyl acetate. These compounds undergo ripening-dependent variation: ethyl 2-methylbutanoate declines while the other two molecules augment with maturity stage (Echeverria et al., 2004a). In our study, in coated apples, ethyl 2-methylbutanoate was 7-fold higher than in control fruit already after 1 day of storage, while 2-methylbutyl acetate and hexyl acetate had significant lower values than controls after 1 day (-78 %) and 7 days (-73 %), respectively. One of the recognized effects of ECs is the capacity to delay the ripening process. The profile of these three VOCs indicates a probable slowing down of ripening in coated apples, as suggested also by the reduced CO_2 emission.

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

3.2.2 Alcohols

Alcohol volatiles are other prominent compounds contributing to apple aroma. As reported in **Table 2**, total peak area percentage is higher in EC-treated apples starting 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 μg 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 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 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*

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

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

3.2.4 Acids, terpenes and other compounds

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

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

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

3.2.5 Multivariate analysis

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

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

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

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

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

4. Conclusions

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

Particularly evident was the marked increase in ethanol following gelatin application, suggesting the onset of partial anaerobiosis, as confirmed by the significantly lower CO_2 emission. Our data indicate that, although the concentration used strongly limits fruit respiration, gelatin ECs preserves the overall aroma profile of apples. Indeed, ethyl esters, which are the most significant contributors to apple aroma profile, were strongly associated to coated samples, and the three esters responsible of the varietal aroma of Fuji were present in both control and coated fruits, suggesting that gelatin did not modify the typical aroma extensively.

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

Aknowledgements

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468 **Figure captions**

469 **Figure 1**. CO_2 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

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	RT[c]	ID[d]	Coating treatment	Days of storage			
Esters				1	7	14	21
Ethyl acetate	7.86	W/L	C	0.09 ± 0.09 [b]	0.15 ± 0. 04 [b]	0.72 ± 0.28 [b]	1.16 ± 0.58 [b]
			T	1.91 ± 0.37 [a]	11.53 ± 2.84 [a]	10.96 ± 1.58 [a]	8.89 ± 1.84 [a]
Ethyl propanoate	9.80	W/L	C	nd [b]	nd	0.28 ± 0.14	0.38 ± 0.05
			T	0.24 ± 0.05 [a]	0.17 ± 0.17	0.22 ± 0.22	0.57 ± 0.29
n-Propylacetate	10.33	W/L/S	C	0.096 ± 0.10	0.18 ± 0.12	0.45 ± 0.16 [a]	0.31 ± 0.20
			T	nd	nd	nd [b]	nd
2-Methylpropyl acetate	11.60	W/L	C	0.04 ± 0.02	0.15 ± 0.01 [a]	0.12 ± 0.00 [a]	0.16 ± 0.04 [a]
			T	nd	nd [b]	nd [b]	nd [b]
Ethyl butanoate	12.51	W/L/S	C	0.49 ± 0.29 [b]	0.81 ± 0.24 [b]	2.90 ± 1.15	1.92 ± 0.76 [b]
			T	5.10 ± 0.64 [a]	6.17 ± 0.71 [a]	5.65 ± 0.29	6.26 ± 0.41 [a]
Ethyl 2-methylbutanoate	13.06	W/L	C	0.18 ± 0.15 [b]	0.15 ± 0.08 [b]	1.06 ± 0.49	1.32 ± 0.23 [b]
			T	1.49 ± 0.43 [a]	3.53 ± .099 [a]	3.27 ± 1.55	2.31 ± 0.17 [a]
n-Butyl acetate	13.91	W/L/S	C	4.52 ± .109 [a]	5.38 ± 0.63 [a]	5.67 ± 0.33 [a]	3.20 ± 0.93 [a]
			T	1.33 ± 0.47 [b]	0.60 ± 0.05 [b]	0.19 ± 0.06 [b]	0.07 ± 0.01 [b]
2-Methyl-1-butyl-acetate	15.96	W/L	C	17.22 ± 0.03 [a]	12.76 ± 0.48 [a]	10.10 ± 1.67 [a]	13.21 ± 3.02 [a]
			T	3.78 ± 0.41 [b]	3.69 ±1.10 [b]	0.88 ± 0.41 [b]	0.17 ± 0.02 [b]
Pentyl acetate	18.26	W/L/S	C	0.64 ± 0.05 [a]	0.92 ±0.09 [a]	0.83 ± 0.12 [a]	0.55 ± 0.14 [a]
			T	0.27 ± 0.04 [b]	0.27 ± 0.03 [b]	0.12 ± 0.06 [b]	nd [b]
Butyl butanoate	20.26	W/L	C	1.09 ± 0.29	0.96 ± 0.22 [a]	0.59 ± 0.05 [a]	0.30 ± 0.15
			T	0.37 ±0.11	nd [b]	nd [b]	nd
Butyl 2-methylbutanoate	20.84	W/L	C	1.11 ± 0.07	1.13 ± 0.07 [a]	0.79 ± 0.15 [a]	0.66 ± 0.33
			T	0.38 ± 0.29	nd [b]	nd [b]	nd
Ethyl hexanoate	20.96	W/L/S	C	0.76 ± 0.48 [b]	0.67 ± 0.17 [b]	3.23 ± 0.17	0.86 ± 0.28 [b]
			T	6.30 ± 0.29 [a]	7.24 ± 0.60 [a]	4.07 ± 0.23	4.32 ± 0.42 [a]
n-Hexyl acetate	22.74	W/L/S	C	4.34 ± 0.34	9.36 ± 1.95 [a]	9.76 ± 1.77 [a]	4.14 ± 1.29 [a]
			T	3.21 ± 0.96	2.58 ± 0.02 [b]	1.31 ± 0.28 [b]	0.32 ± 0.03 [b]
Hexyl propanoate	25.70	W/L	C	0.18 ± 0.03 [a]	0.22 ± 0.14	0.13 ± 0.13	0.03 ± 0.04
			T	nd [b]	nd	nd	nd
Hexyl 2-methylbutanoate	28.99	W/L/S	C	2.95 ± 0.97	2.22 ± 0.26 [a]	1.09 ± 0.27 [a]	0.98 ± 0.38
			T	2.27 ± 0.50	0.54 ± 0.09 [b]	0.26 ± 0.04 [b]	0.45 ± 0.23

Butyl hexanoate	29.11	W/L/S	C	0.59 ± 0.22	0.44 ± 0.19	0.33 ± 0.10 [a]	0.17 ± 0.0 5[a]
			T	0.16 ± 0.13	nd	nd [b]	nd [b]
Hexyl butanoate	29.57	W/L	C	2.53 ± 1.37	2.32 ± 0.32 [a]	1.63 ± 0.33 [a]	0.66 ± 0.38
			T	1.05 ± 0.22	0.50 ± 0.16 [b]	0.30 ± 0.01 [b]	0.06 ± 0.03
Hexyl hexanoate	37.28	W/L/S	C	1.07 ± 0.51	0.64 ± 0.08 [a]	0.40 ± 0.22	0.15 ± 0.09
			T	0.72 ± 0.24	0.22 ± 0.08 [b]	0.15 ± 0.02	0.57 ± 0.51
Total Esters[e]			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

[c] RT, retention time; [d] ID, identification based on: W, Wiley; S, standard, L, literature; [e]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

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[c]	ID[d]	Coating treatment	Days of storage			
				1	7	14	21
Alcohols							
Ethanol	9.03	W/L/S	C	0.09 ± 0.06 [b]	0.10 ± 0.02 [b]	0.48 ± 0.28 [b]	0.81 ± 0.44 [b]
			T	3.86 ± 0.52 [a]	15.52 ± 1.79 [a]	26.39 ± 3.05 [a]	31.20 ± 0.90 [a]
1-Butanol	16.82	W/L/S	C	3.03 ± 0.56	1.75 ± 0.27 [a]	2.51 ± 0.42 [a]	2.20 ± 0.91
			T	1.49 ± 0.42	0.73 ± 0.00 [b]	0.58 ± 0.12 [b]	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 [a]	5.10 ± 0.22 [a]
			T	2.69 ± 0.41	1.99 ± 0.48	1.06 ± 0.22 [b]	1.51 ± 0.36 [b]
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 [a]	6.30 ± 1.08 [a]	4.36 ± 2.17
			T	8.45 ± 1.61	3.65 ± 0.34 [b]	2.80 ± 0.69 [b]	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 [b]	nd [b]	nd	nd
			T	0.30 ± 0.06 [a]	0.55 ± 0.11 [a]	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 [b]	0.02 ± 0.02
			T	0.06 ± 0.03	0.05 ± 0.03	0.12 ± 0.01 [a]	0.09 ± 0.05
6-Methylhept-5-en-2-ol	31.03	W/L	C	nd	nd [b]	nd [b]	nd
			T	nd	0.47 ± 0.21 [a]	1.13 ± 0.37 [a]	0.15 ± 0.16
2-Ethylhexan-1-ol	32.19	W/L/S	C	0.02 ± 0.03	0.13 ± 0.02	0.03 ± 0.03b	0.01 ± 0.02
			T	0.02 ± 0.02	0.19 ± 0.09	0.18 ± 0.02a	nd
Octan-1-ol	35.05	W/L	C	0.12 ± 0.03	0.11 ± 0.02 [b]	0.11 ± 0.02 [b]	0.05 ± 0.03 [b]
			T	0.32 ± 0.07	0.25 ± 0.01 [a]	0.67 ± 0.13 [a]	0.8 ± 0.13 [a]
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 [b]
			T	nd	nd	0.11 ± 0.06	0.16 ± 0.03 [a]
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 [b]	12.38 ± 7.15 [b]	13.10 ± 7.57 [b]
			T	18.27 ± 10.43	23.79 ± 13.73 [a]	33.91 ± 19.58 [a]	39.81 ± 22.98 [a]

Aldehydes							
Hexanal	14.44	W/L/S	C	4.25 ± 0.55	10.59 ± 1.08	7.68 ± 2.10	10.39 ± 2.41 [a]
			T	3.10 ± 1.76	6.02 ± 2.22	5.68 ± 1.36	1.97 ± 0.79 [b]
2-Hexenal (E)	19.80	W/L	C	0.59 ± 0.06	0.70 ± 0.09 [a]	0.64 ± 0.16 [a]	0.75 ± 0.12 [a]
			T	0.39 ± 0.18	0.26 ± 0.07 [b]	0.24 ± 0.04 [b]	0.05 ± 0.06 [b]
2-Hexenal (Z)	20.62	W/L	C	18.31± 1.38	20.65 ± 1.51 [a]	19.86 ± 4.61 [a]	17.82 ± 4.58 [a]
			T	14.92 ± 6.73	11.43 ± 2.78 [b]	6.49 ± 0.64 [b]	3.05 ± 1.54 [b]
Octanal	23.65	W/L	C	0.06 ± 0.00 [a]	0.02 ± 0.02	0.01 ± 0.01	nd
			T	nd [b]	nd	nd	nd
2-Heptenal	25.43	W/L	C	0.13 ± 0.00 [a]	0.16 ± 0.02 [a]	0.08 ± 0.04	0.14 ± 0.01 [a]
			T	nd [b]	nd [b]	nd	nd [b]
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 ± 003 [a]	0.35 ± 0.09 [a]	0.36 ± 0.06 [a]	0.34 ± 0.04 [a]
			T	nd [b]	nd [b]	nd [b]	nd [b]
Total aldehydes			C	23.88 ± 1.81	32.68 ± 0.84 [a]	28.80 ± 6.62 [a]	29.61 ± 6.83 [a]
			T	18.56 ± 8.71	17.87 ± 5.08 [b]	12.59 ± 1.68 [b]	5.17 ± 2.34 [b]
Ketones							
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

[c] RT, retention time; [d] 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 ($P \leq 0.05$).

Table 3

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[c]	ID[d]	Coating treatment	Days of storage			
				1	7	14	21
Acids							
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 [a]	0.68 ± 0.36	1.94 ± 0.72 [a]
			T	nd	nd [b]	nd	nd [b]
Hexanoic acid	46.53	W/L/S	C	0.29 ± 0.05 [a]	0.30 ± 0.02 [a]	0.24 ± 0.13	0.32 ± 0.07
			T	0.04 ± 0.04 [b]	0.14 ± 0.06 [b]	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 [a]	1.22 ± 0.30 [a]	0.71 ± 0.30
			T	0.95 ± 0.03	0.48 ± 0.03 [b]	0.52 ± 0.07 [b]	0.55 ± 0.01
Total acids			C	6.02 ± 0.44	6.24 ± 1.04	6.67 ± 0.69 [a]	6.36 ± 0.56 [a]
			T	4.90 ± 0.43	3.87 ± 0.92	4.29 ± 0.35 [b]	4.00 ±0.55 [b]
Terpenes							
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

Others							
2-Ethyl-furan	9.72	W/L	C	0.24± 0.01 [a]	0.11 ± 0.11	0.08 ± 0.099	nd
			T	nd [b]	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 [b]	nd [b]	nd
			T	0.19 ± 0.19	0.77 ± 0.08 [a]	1.60 ± 0.32 [a]	0.34 ± 0.34
Total others			C	0.25 ± 0.01	0.11 ± 0.11 [b]	0.09 ± 0.09 [b]	nd
			T	0.19 ± 0.19	0.77 ± 0.08 [a]	1.79 ± 0.49 [a]	0.40 ± 0.32

[c] RT, retention time; [d] 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

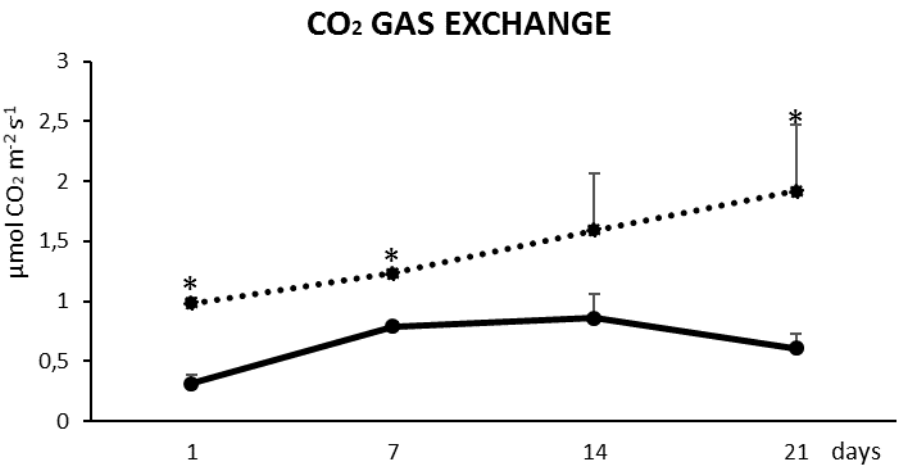

Figure 1

Figure 2

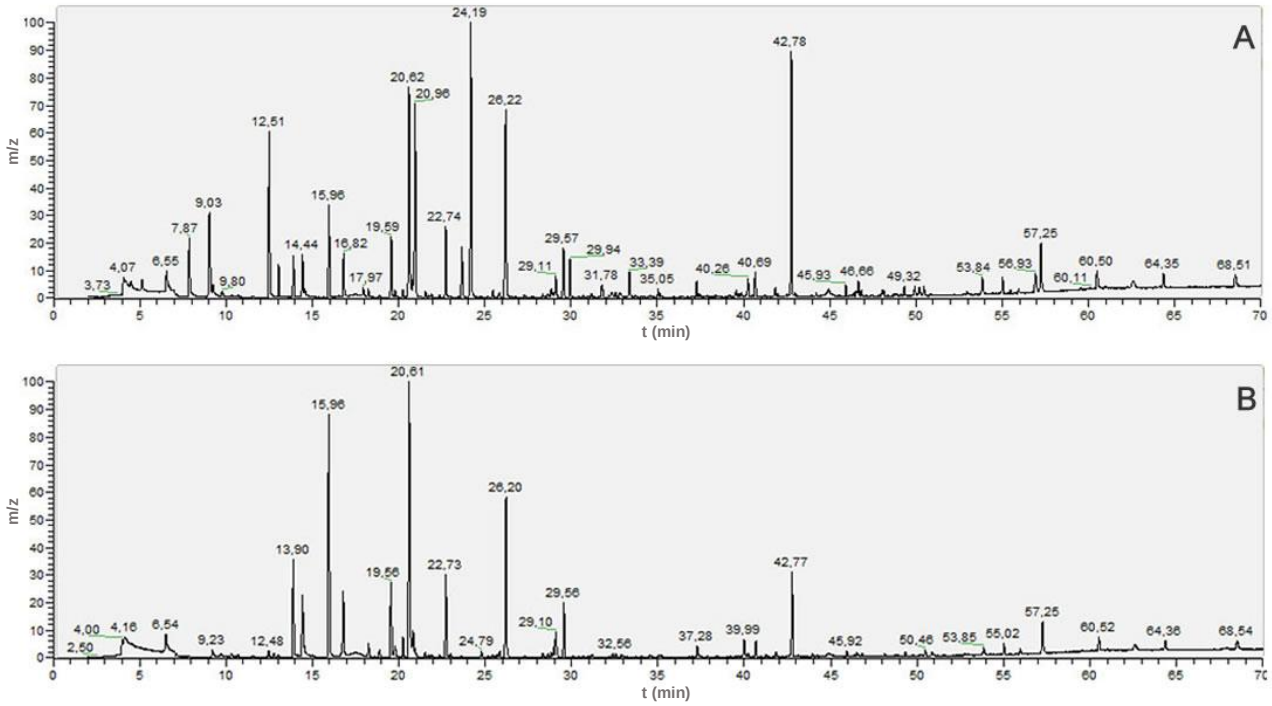

Figure 2

Figure 3

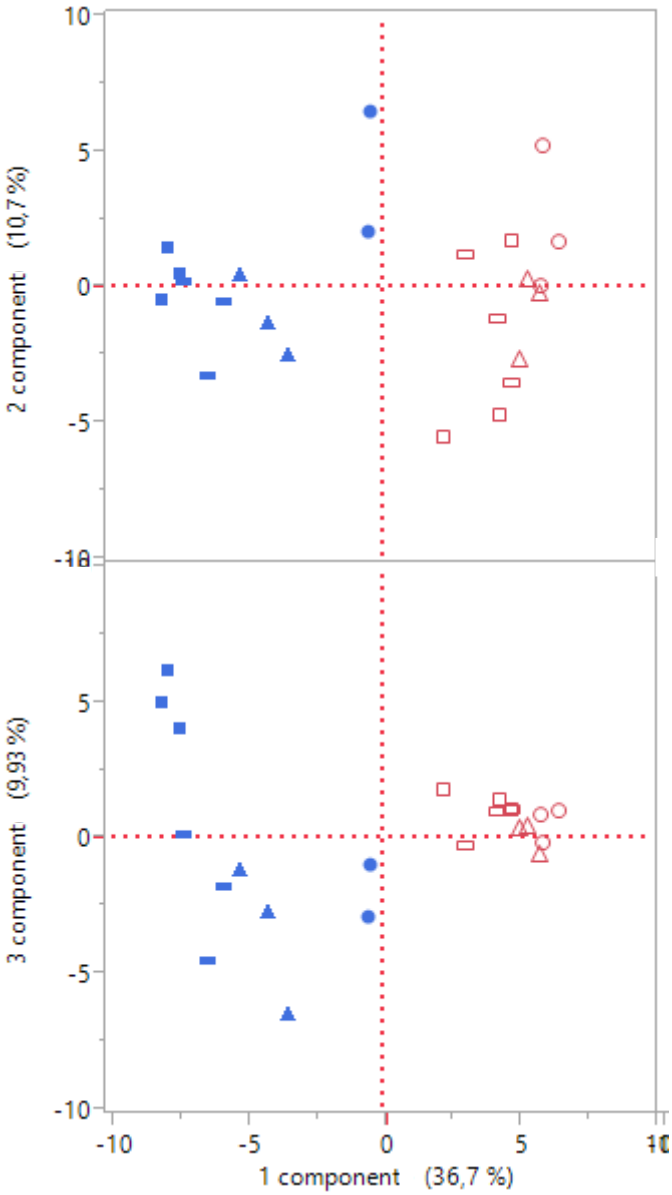

Figure 3

Figure 4

Figure 4

Supplementary Material Table 1S

Click here to download Supplementary Material: Supplementary table-Identified compounds.docx