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Title: CHARACTERIZATION OF FRESH-CUT "RADICCHIO DI CHIOGGIA" STORED IN AIR OR MODIFIED ATMOSPHERE BY SPME GC-MS AND SENSORY ANALYSIS

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Abstract: The volatile profile of two hybrids of "Radicchio di Chioggia", Corelli and Botticelli, packaged in air or modified atmosphere (MAP) during 12 days of cold storage, was monitored by Solid Phase Micro-Extraction (SPME) GC-MS. Botticelli samples were also subjected to sensory analysis. Totally, 61 volatile organic compounds (VOCs) were identified in the headspace of radicchio samples. Principal component analysis (PCA) showed that fresh product presented a metabolic content similar to that of the MAP samples after 5 and 8 days of storage. Projection to Latent Structures (PLS) regression analysis showed a different behaviour of the volatiles amount of the samples depending only on the packaging conditions. Specifically, 12 metabolites describing the time evolution and explaining the effects of the different storage conditions were highlighted. Also, the shelf-life was investigated on the basis of the metabolic content. Finally, a PCA analysis revealed that specific VOCs significantly correlated with sensory attributes.



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Dear Editor,

On behalf of my co-authors and myself, I am pleased to submit the manuscript entitled "Characterization of fresh-cut "Radicchio Di Chioggia" stored in Air or Modified Atmosphere by SPME GC-MS and Sensory Analysis" to Food Chemistry for consideration for publication.

The objective of this study was the evaluation of the VOCs profile by SPME GC/MS of two radicchio hybrids, Corelli and Botticelli, depending on the packaging conditions and storage time and the identification, by multivariate analysis, of putative volatile markers explaining the effects of the different storage conditions. In addition, a sensory analysis of "Botticelli" hybrid samples was defined and a principal component analysis was performed with the aim to investigate the correlation between VOCs and sensory attributes.

We confirm that neither the manuscript nor any part of it has been published or is under consideration for publication elsewhere (abstract excluded). Any reference to or use of previously published material protected by copyright is explicitly acknowledged in the manuscript.

All authors have given a substantial contribution to conception and study design, data acquisition analysis and interpretation, handled drafting of article and revised it critically for intellectual content and participated to final approval of the final version to be published.

In my opinion, the result of the present study could be of interest for Food Chemistry's readers and I hope that the paper might be acceptable for publication.

I thank you in advance for your attention to our manuscript.

Looking forward to your reply.

Yours Sincerely,
Rosaria Cozzolino, PhD

Highlights

- ▶ SPME was useful to the evaluation of volatile profile of “Radicchio di Chioggia”.
- ▶ Explore the VOCs as potential biomarkers in different packaging conditions.
- ▶ Comprehensive data analysis based on PLS regression models.
- ▶ Correlation between specific VOCs and sensory attributes was achieved.

1 **CHARACTERIZATION OF FRESH-CUT “RADICCHIO DI CHIOGGIA” STORED IN AIR OR MODIFIED**
2 **ATMOSPHERE BY SPME GC-MS AND SENSORY ANALYSIS**

3

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28 **Abstract**

29 The volatile profile of two hybrids of “Radicchio di Chioggia”, Corelli and Botticelli, stored in air or passive modified
30 atmosphere (MAP) during 12 days of cold storage, was monitored by Solid Phase Micro-Extraction (SPME) GC-MS.
31 Botticelli samples were also subjected to sensory analysis. Totally, 61 volatile organic compounds (VOCs) were
32 identified in the headspace of radicchio samples. Principal component analysis (PCA) showed that fresh product
33 presented a metabolic content similar to that of the MAP samples after 5 and 8 days of storage. Projection to Latent
34 Structures (PLS) regression analysis showed a different behaviour of the volatiles amount of the samples depending
35 only on the packaging conditions. Specifically, 12 metabolites describing the time evolution and explaining the effects
36 of the different storage conditions were highlighted. Also, the shelf-life was investigated on the basis of the metabolic
37 content. Finally, a PCA analysis revealed that specific VOCs significantly correlated with sensory attributes.

38

39 **Keywords:** Radicchio di Chioggia; Volatile Organic Compounds; Solid Phase Micro-Extraction; Sensory Analysis;
40 Partial Least Squares Regression Analysis

41

42 **1. Introduction**

43 The increased demand of fresh ready-to-eat vegetables led the minimally processed vegetables one of the major
44 growing segments in food retail establishments in most countries. “Radicchio di Chioggia” is a red leafy vegetable
45 popular on fresh-cut food markets, including those of central Europe and the United States, which is characterized by a
46 distinctive bitter taste (Carazzone, Mascherpa, Gazzani, & Papetti, 2013). During postharvest storage and
47 commercialization of radicchio, the loss of freshness appearance is the main factor affecting consumer acceptability; in
48 addition other factors, such as loss of the bitter taste and aroma and the formation of off-odours, could also shorten
49 storage life of the product. The development of these physiological disorders can be minimized by adopting adequate
50 storage conditions (Vanstreels, Lammertyn, Verlinden, Gillis, Schenk, & Nicolai, 2002). Low temperature, controlled
51 atmospheres (CA) or modified atmosphere packaging (MAP) are frequently used to slow down the metabolic rate and
52 extend the shelf-life of fresh-cut products (López-Gálvez, Ragaerta, Azizul Haque, Eriksson, van Labeke, &
53 Devlieghere, 2015). For these reasons, increased knowledge of the ways in which low temperature storage, modified
54 atmosphere packaging, as well as in general fresh-cut processing, affect flavour would be valuable to optimize
55 processing and storage strategies.

56 Flavour and aroma analysis has a central role for fruit and vegetable quality, since volatile organic compounds (VOCs)
57 are important parameters in assessing quality and sensory attributes for consumers and should be measured during the
58 shelf life of fresh-cut products (Deza-Durand, & Petersen, 2011). In a complex system, like a fruit or a vegetable, the
59 perceived sensory attributes are the result of a mixture of odorants and hardly related to a single volatile compound (Le
60 Berre, Béno, Ishii, Chabanet, Etiévant, & Thomas-Danguin, 2008). The effects on flavour are particularly important in
61 fresh-cut products, since washing, peeling and chopping cause damage to the vegetables with accompanying enzymatic
62 reactions that allow the formation of volatiles such as alcohols, aldehydes, terpenes, esters and acids (Torri, Sinelli, &
63 Limbo, 2010). Furthermore, some authors have demonstrated that VOCs profiles are directly related to the formation of
64 odour and flavour sensations, which affect consumer acceptability (Karlsen, Aaby, Sivertsen, Baardseth, & Ellekjær,
65 1999; Aprea et al., 2012, Besada, Sanchez, Salvador, & Granell, 2013).

66 The most common instrumental technique used to evaluate the aroma of fruit and vegetables is solid-phase micro-
67 extraction (SPME) sampling followed by GC-MS (Torri et al., 2010). SPME, developed by Pawliszyn (2009) as an
68 alternative technique for fractionation of volatiles from interfering non-volatile matrix compounds, is a pre-
69 concentration technology, which integrates sampling, extraction, concentration and sample introduction into a single
70 solvent-free step, preventing the production of artefacts compared with conventional solvent extraction procedures.

71 In this study, SPME GC-MS was used to investigate the VOCs profile of two fresh-cut cold stored "Radicchio di
72 Chioggia" hybrids (Corelli and Botticelli), stored in unsealed bags (AIR) or packed in passive modified atmosphere
73 (MAP) during 12 days of cold storage. The first aim was the evaluation of the volatile metabolic content of the samples
74 depending on the packaging conditions and storage time and the identification of putative volatile markers by
75 multivariate analysis. In addition, a sensory analysis of "Botticelli" hybrid samples was defined by using a specially
76 trained panel of 8 judges. Furthermore, a principal component analysis (PCA) was also performed with the aim to
77 investigate the correlation between VOCs and sensory attributes.

78

79 **2. Materials and Methods**

80 *2.1. Chemicals and reagents*

81 Ethanol was purchased from Romil, while ultra-pure water from a Milli-Q system (Millipore, Bedford, MA, USA) with
82 a conductivity of 18M Ω was used throughout. Sodium chloride (NaCl) and potassium carbonate (Na₂SO₄) were from
83 Sigma-Aldrich, while hydrogen chloride (HCl) was from Carlo Erba. Helium at a purity of 99.999% (Rivoira, Milan)
84 was utilized as the GC carrier gas. The SPME fibres and the glass vials were purchased from Supelco (Bellofonte, PA,
85 USA); the capillary GC-MS column HP-Innowax (30m \times 0.25 mm \times 0.5 μ m) was obtained from Agilent J&W (Agilent
86 Technologies Inc.). The SPME fibres were conditioned as recommended by the manufacturer, but below the maximum
87 recommended temperature prior to their first use. Before the initially daily analysis, the fibres were conditioned for 5
88 min at the operating temperature of the GC injector port and the blank level was checked. Triplicate analyses were
89 performed.

90 *2.2. Plant material preparation*

91 Two hybrids of "Radicchio di Chioggia" (*C. intybus* L. group *rubrifolium*), Corelli and Botticelli (Bejo Italia s.r.l.),
92 were used in the present study. The two hybrids were obtained from a farm (Ortomad srl) located in Pontecagnano
93 (Southern Italy). Two experiments were consecutively conducted: the first one on Corelli and the second one on
94 Botticelli. In both experiments radicchio heads were prepared for further processing by removing and discarding
95 wrapper leaves and the stem with sharp stainless steel knives using a vegetable cutter (CL52 Robot Coupe, Vincennes-
96 Cedex, France). Subsequently, radicchio pieces (5 \times 7 cm) were pooled and blended, to minimize product heterogeneity,
97 and washed in tap water at 4 °C for 4 min. Afterwards, radicchio pieces were dried using a manual centrifuge and about
98 150 g of pieces were stored in passive modified atmosphere (MAP: 20/0.0/80%–O₂/CO₂/N₂) or in unsealed (AIR)
99 polypropylene bags (25 \times 30 cm, 30 μ m, Carton Pack, Rutigliano, Italy). In MAP samples, after roughly 5 days an

100 equilibrium gas concentration, measured by a gas analyser (CheckPoint, PBI Dansensor, Ringsted, Denmark), of about
101 10% O₂ and 7% CO₂ was established due to product respiration and film permeability.

102 In the first experiment, for each packaging condition (MAP or AIR), 3 bags for each storage time (5, 8 and 12 days)
103 were prepared and stored at 5°C. Whereas, in the second experiment, for each packaging condition (MAP or AIR), 2
104 bags for each storage time (5, 8 and 12 days) were prepared and stored at 5 °C.

105 After harvest, and at each sampling day, samples were analysed for volatile metabolites, as reported in 2.3 paragraph. In
106 addition, on Botticelli hybrid a sensory descriptive analysis was also performed, as detailed in 2.5 paragraph.

107

108 *2.3. Sample preparation and SPME procedure*

109 The optimization of SPME extraction and desorption conditions were carried out by analysing commercial “Radicchio
110 di Chioggia” samples purchased at a local supermarket.

111 The nature of the adsorptive phase, the sampling temperature and the extraction time required to achieve equilibration
112 between the analytes and the fibre were investigated by applying a univariate experimental design. The optimization of
113 the different parameters involved in SPME, performed by choosing the conditions that achieved the maximum response
114 in terms of metabolite peak area, number of detected metabolites and reproducibility, were reached using the
115 DVB/CAR/PDMS (50/30 mm) fibre, the extraction temperature of 40°C and the extraction time of 20 min.

116 The sample preparation procedure was the following: 5 mg of fresh “Radicchio di Chioggia” leaves were mixed into a
117 20 ml screw-on cap HS vial (Supelco, Bellefonte, PA, USA) to 5 ml of 5% Ethanol and 0.5 mg of Na₂SO₄. After
118 stirring, vials were sealed with a Teflon (PTFE) septum and an aluminium cap (Chromacol, Hertfordshire, UK) for the
119 production of headspace and the consecutive analysis.

120 The sample vial was put in the instrument dry block-heater and held at 40°C for 20 min to equilibrate the system. The
121 extraction and injection processes were automatically performed using an autosampler MPS 2 (Gerstel, Mülheim,
122 Germany). The fibre was, then, automatically inserted into the vial’s septum for 10 min, to allow the volatile
123 compounds adsorption onto the SPME fibre surface. Each SPME fibre was conditioned before its first use, as
124 recommended by the manufacturer.

125 *2.4. Gas chromatography–quadrupole mass spectrometry analysis (GC–qMS)*

126 In order to desorb the volatile metabolites, the SPME fibre was introduced into the injector port of the gas
127 chromatograph device, model GC 7890A, Agilent (Agilent Technologies, Santa Clara, USA) coupled with a mass
128 spectrometer 5975 C (Agilent), wherein the metabolites were thermally desorbed and transferred directly to a capillary
129 column HP-Innowax (30m×0.25 mm×0.5µm Agilent J&W) and analyzed.

130 The oven temperature program was initially set at 40°C for 5 min, ramped to 240°C at 4°C min⁻¹ and held at 240°C for
131 5 min.

132 The temperature of the ion source and the quadrupole were kept at 230°C and 150°C, respectively; helium was used as
133 carrier gas with a flow of 1.5 ml/min; injector temperature was held at 240 °C and the pulsed splitless mode was used
134 for the analysis.

135 The fibre was maintained in the injector for 10 min. Mass Spectra were acquired at an ionization energy of 70 eV and
136 volatile components were detected by mass selective detector. The detector operated in a mass range between 30 and
137 300 u with a scan rate of 2.7 scans/s. Each sample was analysed in triplicate.

138 Blanks, related to analyses of coating fibres not submitted to any extraction procedure, were run between sets of three
139 analyses.

140 Metabolites were identified by searching mass spectra in the available database (NIST, version 2005; Wiley, version
141 2007), by the comparison of their retention times with an in-house developed retention time library based on reference
142 standard samples and by matching their retention indices (RI), determined relative to the retention time of a C₈-C₂₀ n-
143 alkanes series with linear interpolation, with those of authentic compounds or literature data.

144 Semi-quantitative data (percent ratio of the respective peak area relative to the total peak area, RPA%) were directly
145 calculated from total ion current (TIC) peak areas, assuming no differences in response factor for all volatiles
146 quantified.

147 *2.5. Sensory analysis*

148 The sensory profiles of Botticelli hybrid were obtained by applying quantitative descriptive analysis (QDA) (Stone,
149 Sidel, Oliver, Woolsey & Singleton, 1974; Lawless & Heymann, 1998). To define the sensory qualities a specially
150 trained panel of 8 judges was used. The methodology includes the detailed description of the organoleptic qualities of
151 the products: visual, olfactory, gustatory-tactile and retro-olfactory. After the round-table discussion, the 16 most
152 frequently recognized attributes (indicated in figure S1) were selected by all the members of the panel. These were
153 included in a score sheet for the quantitative evaluation, with scale value from 0 to 10. All the samples, coded with
154 three-digit number, were evaluated twice in different sessions. The data from the same type of sample were pooled
155 together, acquired and processed with the software FIZZ Forms (Biosystemes, Couternon, France). The sensory
156 evaluation area was equipped with 8 booths, lit with white light at 700 Lux, air conditioned at 20 ± 2 °C and 50 ± 5%
157 HR (Humidity Relative).

158 *2.6. Statistical data analysis*

159 SPME GC-MS data were elaborated performing a one-way Anova for packaging condition at different sampling times;
160 mean values were separated using the Student-Newman-Keuls test.

161 SPME GC-MS data were also submitted to a multivariate analysis. Collected data were normalized by Median Fold
162 Change normalization, log-transformed and autoscaled. Data modelling was performed by applying multivariate
163 techniques based on projection. Specifically, Principal Component Analysis (PCA) (Jackson 1991) was used for
164 exploratory data analysis and Projection to Latent Structures by partial least squares regression (PLS) (Wold, Sjöström
165 & Eriksson, 2001), to investigate the relationships between the metabolic content of the samples and the time of storage
166 under different storage condition (AIR and MAP). In our study, a longitudinal design was implemented. Then, to
167 perform an efficacy data modelling able to extract the whole information contained into the collected data, the design
168 matrix of our study was explicitly considered and PLS was used to relate the volatile metabolites with the design
169 matrix. An interaction model including time of storage, storage conditions and time of storage x storage conditions was
170 used to define the design matrix. The volatile compounds and the design matrix were used as X-block and Y-block,
171 respectively, in the regression. The significance of the effects included in the design matrix was estimated by
172 permutation test. To better interpret the obtained model, a new method for rotating the PLS model, called post-
173 transformation of PLS2, was applied. It is able to decompose the structured variation of the X-block discovered by PLS
174 into two main blocks, corresponding to the variations correlated and orthogonal to the Y-block (Dall'Acqua et al.,
175 2014). The first block will be called parallel part of the model, while the second orthogonal part. Post-transformation of
176 PLS2 is a three steps approach. In the first step, a PLS regression model is built on the data, while in the second step,
177 the weight matrix of the model is rotated in order to obtain a new weight matrix able to focus the structured variation of
178 the X-block in the two blocks of interest. In the third step, a regression model is rebuilt by using the same framework of
179 the PLS algorithm, but the new weight matrix as the weight for projecting the data. The relationships between X-block
180 and Y-block can be discovered by exploring only the parallel part of the model. As a result, the model obtained by post-
181 transformation of PLS2 can be more easily interpreted than the unrotated PLS model, because the number of
182 components useful for interpreting the model are usually reduced. On the other hand, post-transformed model maintains
183 the same power in prediction and regression coefficients of the unrotated PLS model.

184 The analysis of the correlation loadings plot for the parallel part of the model allowed the identification of a small set of
185 metabolites, able to describe the time evolution of the samples and explain the effects of the different atmospheres used
186 in the package. Thus, Multiple Linear Regression (MLR) was applied to better characterize the effects of the time of
187 storage and the type of storage on the concentration of the selected metabolites. Specifically, for each particular
188 metabolite, an interaction model was built and the effects of each factor estimated based on the regression coefficients.

189 Following good practice for model validation, *N*-fold full cross-validation with different values of *N* (*N*=6, 7, 8) and
190 permutation test on the response (500 random permutations) were performed, in order to avoid over-fitting and prove
191 the robustness of the obtained models.

192 PCA and PLS were accomplished by SIMCA 13 (Umetrics, Umea, Sweden), MLR by MODDE 10 (Umetrics, Umea,
193 Sweden) while the platform R 3.0.2 (R Foundation for Statistical Computing) was used for post-transforming the PLS
194 model.

195 Sensory data were elaborated performing a two-way ANOVA packaging condition x storage time to test the effects on
196 sensory attributes intensity over time. One-way ANOVA on fresh and preserved “Radicchio di Chioggia” and Fisher's
197 Least Significant Difference (LSD) method for the determination of the minimum significant difference were used to
198 test the sensory attributes intensity among all the samples.

199 In order to correlate sensory attributes with the VOCs profile, a PCA analysis was carried out on data of the second
200 experiment related to Botticelli hybrid. Two PCA were performed on autoscaled data prior to analysis. In the first PCA,
201 a data matrix of 65 variables and 7 cases was used (mean of the two replicates). In particular, the 65 variable were
202 composed by the volatiles, Esters (20), Terpenes (12), Alcohols (14), Hydrocarbons (2), Aldehydes (7), Ketones (2),
203 Furans (3), and the five sensory attributes, *olfactive intensity*, *herbaceous smell*, *sweet*, *bitter* and *bitter after taste*. The
204 cases considered were: Fresh (fresh sample at 0 day), Air-5d, Air-8d, Air-12d (samples stored in unsealed bags after 5, 8
205 and 12 days, respectively), MAP-5d, MAP -8d, MAP -12d (samples stored in MAP after 5, 8 and 12 days, respectively).
206 In the second PCA, the variables were reduced to 39 by the elimination of compounds belonging to Terpenes and
207 Alcohols that from the first PCA resulted not correlated with sensory attributes.

208

209 **3. Results and Discussion**

210 *3.1. Determination of VOCs profile of two hybrids of fresh-cut “Radicchio di Chioggia” during storage in AIR or* 211 *MAP*

212 Sixty-one volatile compounds were identified in total in the SPME GS-MS analysis of the radicchio samples stored in
213 AIR or MAP and at different time of storage. The identification of each metabolite was achieved by: (i) comparison of
214 the GC retention time and mass spectra with those, when available, of the pure standard compounds; by (ii) comparison
215 between the MS spectra for each putative compound with those of the data system libraries (NIST 2005 and Wiley
216 2007) and by (iii) the determination of the Kovats index using a C₈–C₂₀ n-alkanes series and matching the experimental
217 values with those reported in the literature for similar chromatographic columns. The identified metabolites included a
218 variety of chemical structures: esters (20), alcohols (14), terpenes (12), aldehydes (7), furans (3), hydrocarbons (3) and

219 ketones (2). The identified 61 volatiles, the abbreviation code, the experimental and literature reported Kovats index and
220 the identification methods are listed in Table 1.

221 For each hybrid, the quali/quantitative VOCs profile, obtained from the SPME GC-MS experiments of the fresh
222 radicchio samples, was compared with those gained from samples stored in the two different packaging conditions (AIR
223 or MAP) at different storage time. At this regard, SPME GC-MS quantitative data were subject to a one-way Anova
224 analysis in order to investigate the effect of packaging condition (AIR or MAP) during storage on the identified VOCs.
225 Tables S1 and S2 report the Anova analyses for the two different hybrids where the identified 61 volatiles and their
226 relative composition, calculated as the percent ratio of the respective peak area relative to the total peak area (RPA%)
227 gained by SPME GC-MS analysis, are presented. Generally, the identified VOCs showed a different behaviour during
228 storage in AIR or MAP in the two radicchio hybrids. Thus, data were subjected to a multivariate analysis with the aim
229 to build predictive models of radicchio shelf-life on the basis of the metabolic content (independently from genotype)
230 and to correlate VOCs with the sensory attributes.

231

232 *3.2. Multivariate statistical analysis of the SPME GC-MS data of fresh-cut "Radicchio di Chioggia" during storage* 233 *in AIR or MAP*

234 Exploratory data analysis performed by PCA produced the score scatter plot of Figure 1, where fresh products show a
235 metabolic content similar to that of the MAP samples after 5 and 8 days of storage. To better investigate the effects of
236 storage time and packaging condition (AIR or MAP) on the quantified metabolites obtained from the two hybrids, a
237 multivariate analysis was performed. As a result, a post-transformed PLS2 model having one orthogonal and three
238 parallel components was obtained. All the effects included in the design matrix resulted to be significant at the level of
239 95%. Specifically, for the storage time the model showed $R^2=0.81$ and $Q^2=0.61$, for the packaging condition $R^2=0.89$
240 and $Q^2=0.72$ and for time of storage x storage conditions $R^2=0.90$ and $Q^2=0.72$. Figure 2 reports the score scatter plot of
241 the model, where it is possible to observe a different time evolution of the metabolic content of the samples depending
242 only on the packaging conditions. The MAP and the AIR samples show a similar behaviour within each group,
243 independently of the sampling time. Indeed, samples collected a different time do not form clusters or present specific
244 patterns within the groups defined based on the packaging condition.

245 Further, to prove possible behaviour differences respect to the time of storage of the two groups of samples under
246 investigation, independent PLS regression models for each group were built, in order to estimate the time of storage or
247 shelf-life on the basis of the metabolic content. For the MAP group a model with A=2 components, $R^2=0.89$ and
248 $Q^2=0.84$, was obtained, while the AIR group presented a model with A=2 components, $R^2=0.93$ and $Q^2=0.84$. If the

249 models are used to predict the time of storage for the other group not included in the training set wrong predictions are
250 obtained, as it can be seen in Figure 3.

251 The analysis of the correlation loading plot of the post-transformed PLS2 model allowed to select 12 putative markers,
252 listed in Table 2, which seems to characterize the differences in the time evolution of the radicchio samples kept in Air
253 or MAP. Among the 12 metabolites of interest, 3-nonanol, 4-nonanol, α -selinene, 2-ethyl-furan, octane, 3-methyl-ethyl
254 pentanoate and ethyl tiglate show higher levels at each sampling time in radicchio samples preserved in AIR compared
255 to the MAP samples. On the other hand, nonanal and tetradecane present higher concentrations at each storage time in
256 radicchio samples preserved in MAP compared to the AIR samples. Ethyl benzoate, 3-methyl-butanal and phenylethyl
257 alcohol are not affected by packaging condition (Table 2).

258

259 *3.3. Analysis of sensory profile of fresh-cut "Radicchio di Chioggia" during storage in AIR or MAP*

260 Statistical analysis of the data obtained by the panel on the Botticelli hybrid samples was preceded by the evaluation of
261 the performance of judges for each attribute, using analysis of variance. The analysis showed that there were no
262 significant differences between the opinions expressed.

263 Sensory profiles of Botticelli radicchio over 12 days period is shown in Figure S1. A series of two-way factorial
264 ANOVAs packaging condition x storage time on sensory profile attributes demonstrated significant main effects for
265 packaging condition and storage time for *Stains*, *Olfactive intensity*, *Herbaceous smell* and *Sweet* attributes: for
266 packaging condition ($F = 66.16, 17.68, 40.40$ and 12.36 ; d.f. = 1/188 for all; $p < 0.0001, < 0.004, < 0.0001$ and < 0.01 ,
267 respectively) and for storage time ($F = 155.9, 14.2, 6.86$, and 14.5 ; d.f. = 2/188 for all; $p < 0.0001, < 0.001, < 0.03$ and
268 < 0.001 , respectively). For *Bitter* and *Bitter after-taste* attributes the analysis yielded main effect for packaging condition
269 only ($F = 56.02$ and 93.58 ; d.f. = 1/188 for both; $p < 0.0001$ for both).

270 The average values of *Stains*, *Olfactive intensity*, *Herbaceous smell*, *Sweet*, *Bitter* and *Bitter after-taste* attributes of
271 fresh and stored samples in AIR or MAP over 12 days period is shown in Figure S2. A series of one way ANOVA,
272 using the products as factor, showed significant differences for the *Stains*, *Olfactive intensity*, *Herbaceous smell*, *Sweet*,
273 *Bitter* and *Bitter after-taste attributes* ($F = 85.05, 14.05, 17.15, 14.29, 16.13$ and 22.95 , respectively; d.f. = 6/217 for all;
274 $p < 0.0001$ for all). A multiple comparison procedure was used to determine which means were significantly different
275 (Figure S2).

276

277 *3.4. Correlation between sensory attributes and VOCs profile of fresh-cut "Radicchio di Chioggia" during storage* 278 *in AIR or MAP*

279 Statistical analysis on sensory profiles of Botticelli hybrid over storage period allowed to detect significant differences
280 on *Stains*, *Olfactive intensity*, *Herbaceous smell*, *Sweet*, *Bitter* and *Bitter after-taste* attributes. In order to correlate these
281 sensory data (with the exception of *Stains*) with the volatile metabolites identified by SPME GC-MS, two PCA analyses
282 were accomplished. The first PCA, performed using all metabolites reported in Table 1, with the exception of two
283 Alcohols (4-nonanol, A9; and 3-nonanol, A11) and one hydrocarbon (tetradecane, H2), eliminated by the analysis for the
284 absence of variance, allowed to select the volatile compounds significantly correlated with the sensory descriptors and
285 to eliminate all terpenes and alcohols, as they did not show significant correlation. Thus, a second PCA, reported in
286 Figure 4, was performed, which described 76.4% of the total variance in the data. In particular, the first component,
287 PC1, explained the differences between samples stored in AIR and in MAP placed in the right and left part of the score
288 plot, respectively (Figure 4A). Furthermore, according to PCA of Figure 1, MAP-5d and MAP-8d samples are located
289 near to Fresh product. The second component, PC2, allowed to differentiate storage time, with samples evaluated after
290 12 days (MAP-12d and Air-12d) positioned in the upper part of the score plot (Figure 4A). Examining the variables
291 distribution (loading plot, Figure 4B) on the PC1, the *Olfactive intensity*, *Herbaceous smell*, *Bitter* and *Bitter after-taste*
292 attributes are found in the left part of the plot, showing to be more associated to the fresh product and the MAP samples
293 stored for 5 and 8 days. Whereas, *Sweet*, placed in the right part of the loading plot, resulted to be more associated to
294 samples stored in AIR (in particular, AIR-12d), which have lost freshness (Figure 4B). The correlation matrix obtained
295 by the PCA permitted to highlight a significantly relationship between the sensory descriptors and the volatile
296 metabolites. In Table 3 the metabolites significantly correlated with each sensory trait are reported. Specifically, the
297 sensory attributes associated to freshness (*Olfactive intensity*, *Herbaceous smell*, *Bitter* and *Bitter after-taste*) resulted
298 inversely correlated with 2-methyl-ethyl propionate (E2), 3-methyl-ethyl butyrate (E7), ethyl pentanoate (E8), 2-ethyl-
299 furan (F2) and octane (H1) (with the exception of *Bitter*). The same metabolites resulted significantly and positively
300 correlated with the *Sweet* attribute and are present in higher concentration in samples stored in AIR, which have lost
301 their freshness.

302 These findings confirm the data obtained by the PLS2 model related to 2-ethyl-furan (F2) and octane (H1), considered
303 as putative markers of AIR samples. 2-ethyl-furan, characterized by a chemical-like odor, showed an increasing
304 concentration in AIR preserved samples during the storage. This behaviour can be due to the degradation reactions of
305 linolenate hydroperoxides, through still unclear mechanisms of formation (Vichi, Pizzale, Conte, Buxaderas, & Lopez-
306 Tamames, 2003). Together with 2-ethyl-furan, octane have been indicated as markers of the degree of oxidation (Vichi
307 et al., 2003).

308 Similarly, 3-methyl-ethyl pentanoate (E10) showed a negative correlation with *Bitter* and *Bitter after-taste* traits
309 according to the PLS2 model, which indicated it as a marker of AIR samples. On the other hand, hexanal (Ald-3), 2-
310 hexenal (Ald-5) and benzene acetaldehyde (Ald-7) resulted inversely related to *Sweet* and positively correlated to
311 sensory traits associated to freshness (*Olfactive intensity, Herbaceous smell, Bitter and Bitter after-taste*). Hexanal,
312 characterised by green and grassy odor notes, is reported to be associated with sensory perception of freshness in green
313 leaves (Hatanaka, 1996). The fresh “green odor” of green leaves arises from eight volatile compounds, C6-aldehydes
314 and C6-alcohols, which include leaf aldehyde, (2E)-hexenal and leaf alcohol, (3Z)-hexenol. These volatile compounds
315 are biosynthesized in green leaves from α -linolenic and linoleic acids via their respective hydroperoxides (Hatanaka,
316 1996). Benzene acetaldehyde is a phenylalanine-derived product which has a flavour described as green, floral and
317 fruity (Flament, 2002).

318

319 **4. Conclusions**

320 Sixty-one volatile compounds were identified in total in the SPME GS-MS analysis of two fresh-cut radicchio hybrids
321 (Corelli and Botticelli) stored in AIR or MAP for 12 days. Two independent PLS regression models for samples stored
322 in AIR or in MAP were built, able to estimate the shelf-life on the basis of the metabolic content ($R^2=0.89$ for MAP
323 model; $R^2=0.93$ for AIR model). In addition, a post-transformed PLS2 model allowed the identification of 12 putative
324 markers of samples stored in AIR (3-nonanol, 4-nonanol, α -selinene, 2-ethyl-furan, octane, 3-methyl-ethyl pentanoate
325 and ethyl tiglate) or MAP (nonanal and tetradecane). PCA performed on volatile metabolites and descriptive sensory
326 data revealed that specific VOCs significantly correlated with sensory attributes. In particular, 2-ethyl-furan, octane and
327 3-methyl-ethyl pentanoate, selected as markers of AIR samples by PLS2, showed a negative correlation with *Bitter* and
328 *Bitter after-taste* traits (descriptors associated to fresh or MAP samples) and a positive correlation with *Sweet*
329 (descriptors associated to AIR samples). Otherwise, hexanal, 2-hexenal and benzene acetaldehyde resulted inversely
330 related to *Sweet* and positively correlated to the sensory traits associated to freshness (*Olfactive intensity, Herbaceous*
331 *smell, Bitter and Bitter after-taste*).

332

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335

336 **Figure Captions**

337 Figure 1. Score scatter plot of the PCA model ($R^2=0.33$). Fresh product (open circles) result similar to MAP samples
338 after 5-8 days of storage (inverted triangles). 1=Corelli hybrid; 2=Botticelli hybrid.

339

340 Figure 2. Score scatter plot of the post-transformed PLS2 model. The metabolic content of the AIR and MAP samples
341 show a different time evolution during storage time.

342

343 Figure 3. Predicted Storage Time vs real Storage Time for the PLS model built by considering the MAP samples. The
344 predicted time of the AIR samples does not accord with the experimental time proving a different time evolution of the
345 AIR samples with respect to MAP samples; similar results were found by predicting the MAP group with the model
346 built on the AIR group (data not shown).

347

348 Figure 4. Principal component analysis (PCA) scores plot (A) and loading plot (B) for fresh-cut “Radicchio di
349 Chioggia” (Botticelli) stored in AIR or MAP for 12 days. The data matrix submitted to PCA was made up of 39
350 variables (VOCs and sensory attributes) and seven cases: Fresh (fresh sample at 0 day), Air-5d, Air-8d, Air-12d
351 (samples stored in unsealed bags after 5, 8 and 12 days respectively), MAP-5d, MAP -8d, MAP -12d (samples stored in
352 MAP after 5, 8 and 12 days respectively).

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Table 1: Volatile metabolites detected in Corelli and Botticelli hybrids of Radicchio “Rosso di Chioggia” and their identification codes

Metabolite	Code	^a RIt/RIsp	^b ID	Metabolite	Code	^a RIt/RIsp	^b ID
Esters				Terpens			
Ethyl propionate	E1	944/937	RI/MS/S	α -pinene	T1	1021/1019	RI/MS/S
2-methyl-ethyl propionate	E2	955/938	RI/MS	β -pinene	T2	1106/1118	RI/MS/S
2-methyl-methyl butyrate	E3	1000/1005	RI/MS	β -myrcene	T3	1165/1156	RI/MS/S
3-methyl-methyl butyrate	E4	1015/1014	RI/MS	D-limonene	T4	1206/1206	RI/MS/S
Ethyl butyrate	E5	1025/1032	RI/MS/S	β -thujone	T5	1143/1438	RI/MS/S
2-methyl-ethyl butyrate	E6	1054/1056	RI/MS	Camphor	T6	1521/1518	RI/MS/S
3-methyl-ethyl butyrate	E7	1060/1055	RI/MS	β -elemene	T7	1591/1596	RI/MS
Ethyl pentanoate	E8	1124/1130	RI/MS/S	Caryophyllene	T8	1603/1604	RI/MS/S
Ethyl tiglate	E9	1234/1241	RI/MS	α -humulene	T9	1679/1672	RI/MS/S
3-methyl ethyl pentanoate	E10	1179/1181	RI/MS	α -terpineol	T10	1707/1706	RI/MS
3-methyl-ethyl-2 butyrate	E11	1216/1230	RI/MS	β -selinene	T11	1729/1727	RI/MS
Ethyl hexanoate	E12	1223/1236	RI/MS/S	α -selinene	T12	1724/1736	RI/MS
Ethyl-3-hexenoate	E13	1301/1308	RI/MS	Hydrocarbons			
Ethyl heptanoate	E14	1321/1339	RI/MS/S	Octane	H1	800/800	RI/MS/S
Ethyl octanoate	E15	1423/1441	RI/MS/S	Tetradecane	H2	1400/1400	RI/MS/S
Ethyl nonanoate	E16	1523/1548	RI/MS/S	Benzene 1,3-bis-(1,1-dimethyl-ethyl)	H3	1433/1436	RI/MS
Ethyl decanoate	E17	1630/1636	RI/MS/S	Aldehydes			
Ethyl benzoate	E18	1647/1676	RI/MS/S	2-methyl-butanal	Ald1	864/863	RI/MS
Ethyl-phenyl-acetate	E19	1773/1783	RI/MS	3-methyl-butanal	Ald2	912/870	RI/MS/S
Ethyl dodecanoate	E20	1826/1828	RI/MS/S	Hexanal	Ald3	1085/1088	RI/MS/S
Alcohols				2-methyl-2-butenal	Ald4	1094/1088	RI/MS/S
Butanol-2-methyl	A1	1203/1208	RI/MS/S	2-hexenal	Ald5	1027/1020	RI/MS/S
Iso amyl alcohol	A2	1184/1211	RI/MS/S	Nonanal	Ald6	1400/1397	RI/MS/S
Pentanol-3-methyl	A3	1297/1228	RI/MS/S	Benzene acetaldehyde	Ald7	1671/1663	RI/MS
1-hexanol *	A4	1316/1318	RI/MS/S	Ketones			
3-hexen-1-ol	A5	1351/1360	RI/MS/S	3-pentanone	K1	980/970	RI/MS/S
2-hexen-1-ol	A6	1410/1411	RI/MS/S	2-methyl-3-pentanone	K2	989/984	RI/MS/S
1-octen-3-ol	A7	1456/1465	RI/MS/S	Furans			
1-heptanol	A8	1419/1430	RI/MS/S	3-methyl-furan	F1	832/842	RI/MS
4-nonanol	A9	1479/1486	RI/MS/S	2-ethyl-furan	F2	936/927	RI/MS
1-hexanol-2-ethyl	A10	1495/1487	RI/MS/S	2-pentyl-furan	F3	1229/1236	RI/MS
3-nonanol	A11	1496/1498	RI/MS				
1-octanol	A12	1519/1565	RI/MS/S				
1-nonanol	A13	1624/1636	RI/MS/S				
Phenyl-ethyl alcohol	A14	1859/1861	RI/MS/S				

^aRI_i: Relative retention indices on polar column reported in literature by www.pherobase.com; www.flavornet.org; www.ChemSpider.com; Jennings & Shibamoto 1980 and Davies, 1990; RI_{sp}: Relative retention indices calculated against n-alkanes (C₈-C₂₀) on HP-Innowax column; ^bIdentification method as indicated by the following: RI: Kovats retention index on a on HP-Innowax column; MS: NIST and Wiley libraries spectra; S: co-injection with authentic standard compounds on the HP-Innowax column

Table 2 *P*-value for the Multiple Linear Regression (MLR) model and the *p*-values for the three effects (storage time, packaging condition and their interaction) taken into account for modelling the selected 12 metabolites (putative markers).

Metabolites	<i>P</i> -values			
	MLR Model	Storage duration	Packaging condition	Storage duration x Packaging condition
3-nonanol	**	ns	*** (>AIR)	ns
4-nonanol	**	ns	*** (>AIR)	ns
α -selinene	***	ns	*** (>AIR)	ns
2-ethyl-furan	***	***	*** (>AIR)	***
nonanal	***	**	* (>MAP)	ns
octane	*	ns	* (>AIR)	ns
3-methyl-ethyl pentanoate	**	ns	** (>AIR)	ns
tetradecane	***	ns	***(>MAP)	ns
ethyl tiglate	***	ns	*** (>AIR)	ns
ethyl benzoate	**	ns	ns	**
3-methyl-butanal	**	**	ns	*
phenylethyl alcohol	**	*	ns	**

Significance : *, **, *** = $p < 0.05, 0.01, 0.001$ respectively

Table 3. Pearson correlation matrix between metabolites and sensory attributes obtained by PCA.

Metabolites	Code	Sensory attributes				
		Olfactive intensity	Erbaceous smell	Sweet	Bitter	Bitter after taste
Ethyl propionate	E1	-0.41 ns	-0.66 ns	0.41 ns	-0.82 *	-0.75 ns
2-Methyl-ethyl propionate	E2	-0.87 *	-0.86 *	0.83 *	-0.81 *	-0.88 **
2-methyl-methyl butyrate	E3	-0.83 *	-0.69 ns	0.78 *	-0.63 ns	-0.71 ns
3-methyl-methyl butyrate	E4	-0.81 *	-0.64 ns	0.75 ns	-0.60 ns	-0.68 ns
2-methyl-ethyl butyrate	E6	-0.72 ns	-0.91 **	0.72 ns	-0.87 *	-0.91 **
3-methyl-ethyl butyrate	E7	-0.92 **	-0.96 **	0.90 **	-0.94 **	-0.98 ***
Ethyl pentanoate	E8	-0.85 *	-0.78 *	0.84 *	-0.87 *	-0.84 *
3-methyl ethyl pentanoate	E10	-0.60 ns	-0.75 ns	0.53 ns	-0.90 **	-0.89 **
Ethyl dodecanoate	E20	0.78 *	0.59 ns	-0.69 ns	0.44 ns	0.57 ns
Hexanal	Ald3	0.84 *	0.82 *	-0.79 *	0.77 *	0.83 *
2-Hexenal	Ald5	0.91 **	0.92 **	-0.94 **	0.75 ns	0.81 *
Benzene Acetaldehyde	Ald7	0.88 **	0.89 **	-0.85 *	0.80 *	0.86 *
2-Methyl-3-pentanone	K2	0.65 ns	0.52 0.23	-0.76 *	0.35 ns	0.36 ns
Furan 2-ethyl	F2	-0.80 *	-0.90 **	0.80 *	-0.74 ns	-0.83 *
Octane	H1	-0.85 *	-0.80 *	0.78 *	-0.66 ns	-0.78 *

Significance: ns, *, **, *** = not significant, $p < 0.05$, 0.01 , 0.001 .

Figure1

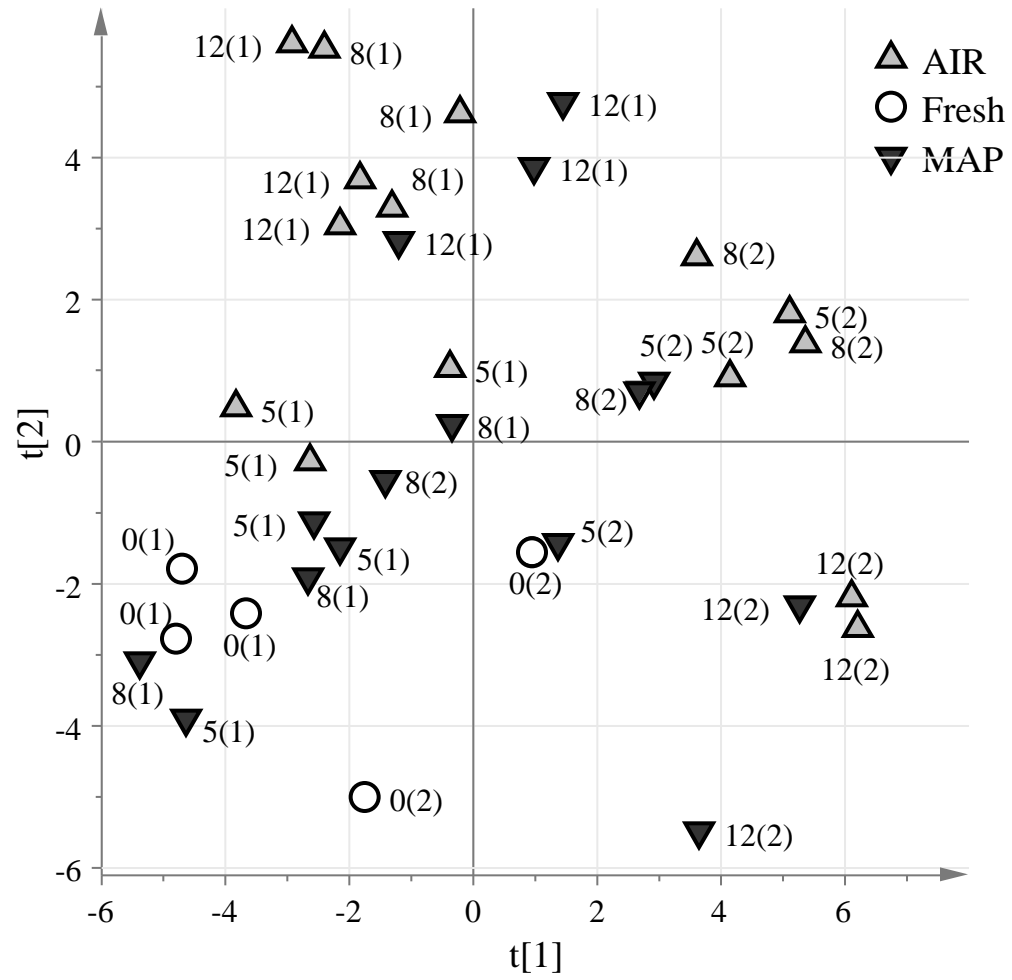


Figure 1

Figure2

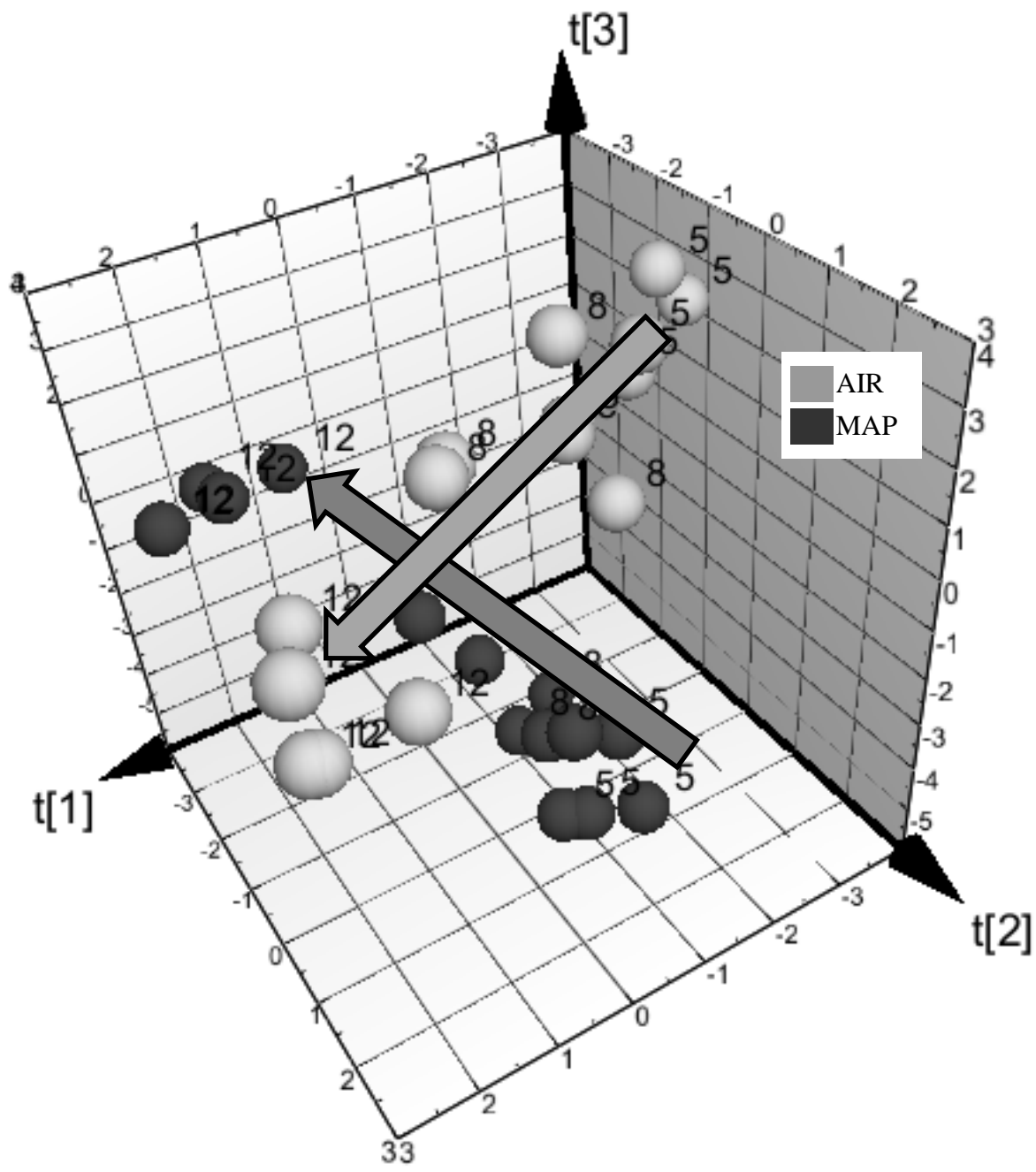


Figure 2

Figure3

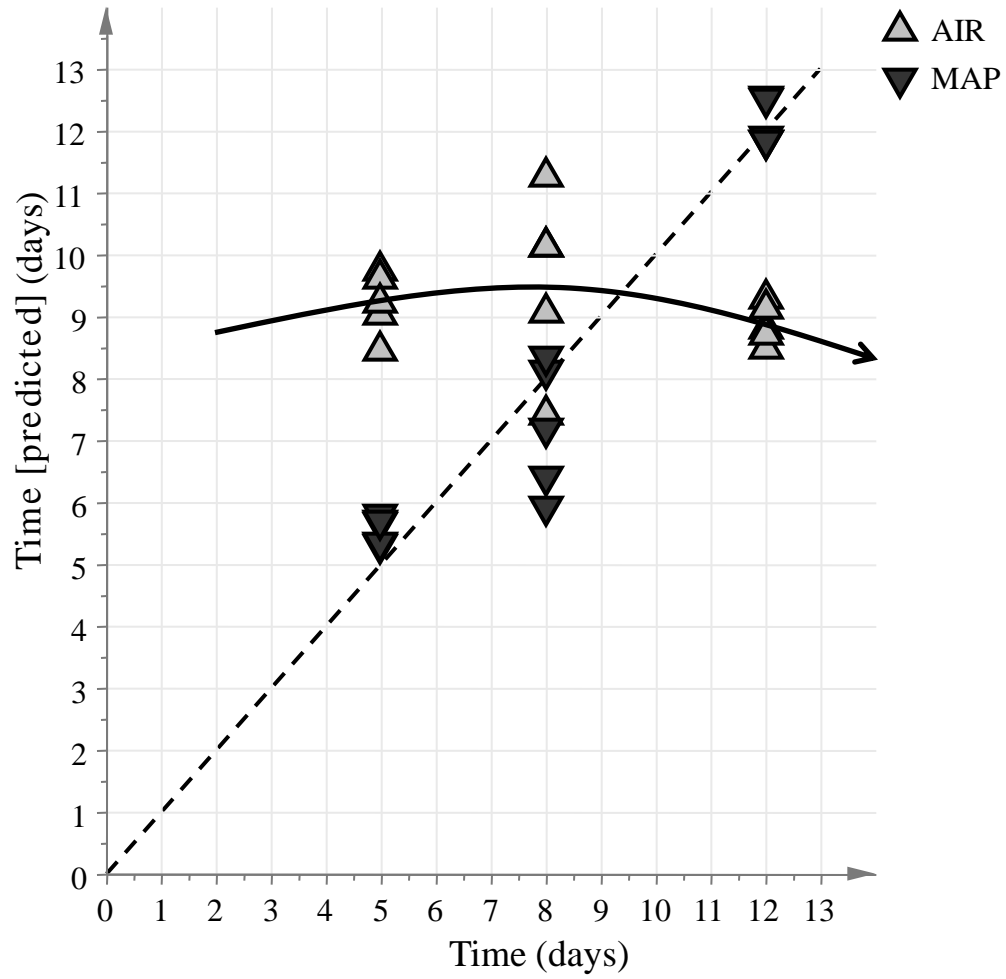


Figure 3

Figure4

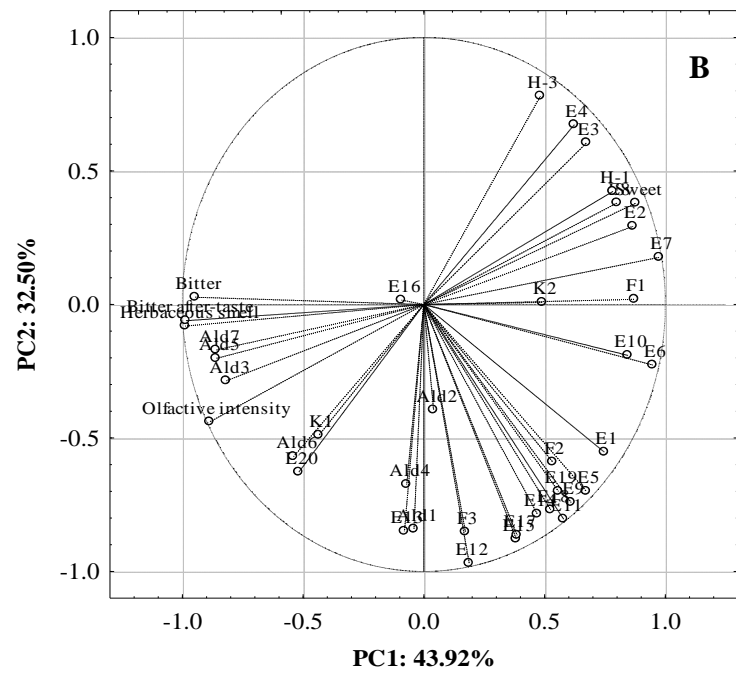
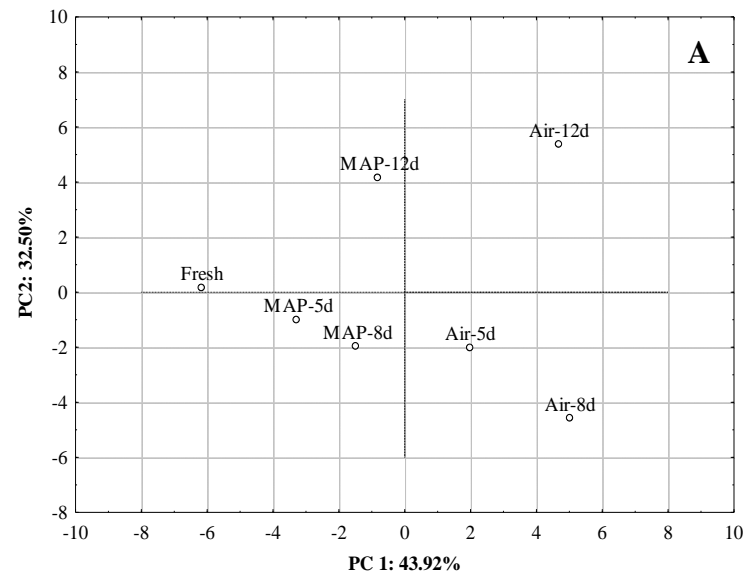


Figure 4

Figure S1

[Click here to download Supplementary Material: Figure S1_Cozzolino et al.ppt](#)

Figure S2

[Click here to download Supplementary Material: Figure S2_Cozzolino et al.ppt](#)