Original Research Article

PHENOLIC PROFILES AND POSTHARVEST QUALITY CHANGES OF FRESH-CUT RADICCHIO (*CICHORIUM INTYBUS* L.): NUTRIENT VALUE IN FRESH VS STORED LEAVES

Maria Cefola,[†] Virginia Carbone,[‡]* Paola Minasi,[‡] and Bernardo Pace [†]

[†]Institute of Sciences of Food Production, National Research Council (CNR), Via G. Amendola, 122/O, 70126 Bari, Italy

[‡]Proteomic and Biomolecular Mass Spectrometry Center, Institute of Food Sciences, National Research Council (CNR), Via Roma, 64, 83100 Avellino, Italy

*Corresponding Author. Tel.: +39-0825-299211; Fax: +39-0825-781585.

E-mail address: vcarbone@isa.cnr.it

Abstract The quality traits of two "Radicchio di Chioggia" hybrids (Corelli and Botticelli) processed as fresh-cut and stored in unsealed bags or in passive modified atmosphere for 25 days at 5 °C, were measured in two consecutive experiments. Moreover, a detailed structural characterization of polyphenols extracted from fresh and stored radicchio samples was performed by HPLC and electrospray ionization multistage ion trap mass spectrometry (ESI-ITMS^n). Twenty-one compounds were identified and quantified in Corelli extract, while nineteen ones were detected in Botticelli extract. Quercetin-7-*O*-glucoside-3-*O*-(6"-malonyl)-glucoside and isorahmnetin-7-*O*-glucuronide were found only in Corelli. Interestingly, the presence of dihydroflavonol glycosides in "Radicchio di Chioggia" is here reported for the first time. In the second experiment (performed on Botticelli), fresh-cut processing promoted a 2-fold increase in bioactive compounds, quantified in a significant amount (305 mg/100 g fresh weight) also in leaves scored unmarketable. Thus this hybrid, suited for fresh-cut processing, showed to be also an interesting and cheap source of antioxidant phenolics, when was no more acceptable.

Although less suitable for fresh-cut processing, the Corelli fresh-cut radicchio resulted a valuable hybrid for the fresh consumption and a source of polyphenols due to their high content in fresh and stored leaves.

Keywords *Cichorium intybus* L.; fresh-cut storage; ESI-ITMS^n; DPPH assay; polyphenols; valorization of unmarketable leaves, food analysis, food composition, sensory acceptability, nutritional value.

Chemical compounds studied in this article:

5-O-Caffeoylquinic acid (PubChem CID: 5280633); Chicoric acid (PubChem CID: 5281764); Caftaric acid (PubChem CID: 6440397); 5-Feruloylquinic acid (PubChem CID: 15901362); 3,5-Di-caffeoylquinic acid (PubChem CID: 13604687); Quercetin 3-*O*-glucuronide (PubChem CID:

5274585); Quercetin-3-*O*-glucoside (PubChem CID: 5280804); Luteolin-7-*O*-glucuronide (PubChem CID: 25245094); Caffeoylmalic Acid (PubChem CID: 6124299).

Abbreviations: VQ, visual quality; pMA, passive modified atmosphere; fw, fresh weight; HPLC, high performance liquid chromatography; UV, ultraviolet; Vis., visible; ESI-ITMS^n, electrospray ionization multistage ion trap mass spectrometry.

1. Introduction

Cichorium intybus L. consists of many chicory varieties used for fresh or fresh-cut market. Among these, "Radicchio" (*C. intybus* L. group *rubifolium*) includes different typologies denominated "Chioggia", "Treviso" and "Verona" (Pertuzé et al. 2016), which differ in head shape and dimension and are very popular in north-eastern Italy, central Europe and north America (Koukounaras 2014). Radicchio chicories are characterized by a particular bitter taste and red attractive colours, which contribute to the colour variety of fresh-cut mixed salads. The freshness appearance (colour, texture) is highly appreciated by consumers and is the main parameter used to define the radicchio marketability (Vanstreels at al. 2002; Piagentini et al. 2005). Indeed, it was reported that the occurrence of sensory defects in fresh-cut lettuce can be considered as a criterion for consumer acceptability (Piagentini et al. 2005). Besides its very attractive appearance, fresh-cut radicchio salads are very popular for their high content of phytochemicals. In particular, "Chioggia" and "Treviso" radicchio are very rich in phenols: the high concentration of anthocyans (in particular cyanidin) is very important for their antioxidant action (Innocenti et al. 2005; Rossetto et al. 2005).

Fresh-cut processing promotes a series of metabolic alterations, such as the increase in respiration rate, the turgor loss and the oxidation of phenolic compounds and pigments, that affect the sensory quality and consumer acceptability of fresh-cut vegetables (Kader 2002). As consequence, in order to improve the postharvest performance of fresh-cut chicories, it is important to store the products at low temperatures with the beneficial effect of modified atmosphere packaging (Pereira et al. 2014; Cozzolino et al., 2016). It was reported that modified atmosphere packaging plays an important role in delaying respiration rate and senescence of vegetable (Böttcher et al. 2003; Del Nobile et al. 2007). On the other hand, in fresh-cut products, the shredding step can increase the antioxidant capacity associated with wound-induced phenolic compounds (Alarcón-Flores et al. 2014). It has been reported that red leafed varieties of lettuce accumulate phenols during storage at 4 °C, showing sensory loss without browning symptoms (Tavarini et al. 2007). Due to these biochemical aspects,

fresh-cut radicchio could be considered as a possible source of bioactive compounds, also when the product become no more acceptable for the loss of freshness appearance. Similarly, vegetable by-products are valuable sources for the recovery of polyphenols, pectins, and proteins and these compounds may be used as natural antioxidants and functional food ingredients (Kammerer et al. 2014).

Starting from this finding, this paper was aimed to study the postharvest performance of two fresh-cut radicchio hybrids cold stored in air or modified atmosphere packaging and to evaluate the phenolic profile in acceptable leaves from a sensorial point of view, and in unmarketable leaves.

2. Materials and methods

2.1. Plant materials and storage conditions

Two hybrids of "Radicchio di Chioggia" (*C. intybus* L. group *rubifolium*) Corelli and Botticelli (Bejo Italia s.r.l.) were obtained from a farm (Ortomad srl) located in Pontecagnano (southern Italy 40° 36′ N, 14° 53′ E, 60 m above mean sea level) and immediately transported to the laboratory under refrigerated conditions.

Two consecutive experiments were conducted in different periods, using Corelli in the first experiment, and Botticelli in the second one. In each experiment, about five kg of radicchio heads were prepared for further processing by removing and discarding wrapper leaves and the stem with sharp stainless steel knives. Radicchio pieces (3 × 4 cm) were obtained using a vegetable cutter (CL52 Robot Coupe, Vincennes-Cedex, France), and thoroughly pooled and blended, to minimize product heterogeneity. Radicchio pieces were washed in tap water at 4 °C for 4 min. After washing, pieces were dried using a manual centrifuge and about 150 g of product was put in each polypropylene bag (25 × 30 cm, 30 μm, OTR 1100 cm^3/m^2 24h bar, Carton Pack, Rutigliano, Italy). Fifteen bags (three replicates × five storage duration, at 5, 8, 12, 19 and 25 days) were sealed in order to achieve a passive

95 modified atmosphere (pMA), while other 15 bags were left open as control (AIR); all bags were
96 stored at 5 °C.
97 In each experiment, just after harvest (fresh sample) and at each sampling time, samples were firstly
98 scored for sensory visual quality and the respiration rate was measured. Afterwards, the same samples
99 were subjected to chemical analyses as detailed below. Headspace gas composition (O_2 and CO_2)
100 within each package was monitored daily using a gas analyzer (CheckPoint, PBI Dansensor,
101 Ringsted, Denmark).
102
103 *2.2. Chemicals*
104 High Performance Liquid Chromatography (HPLC) grade methanol, formic acid and Folin–Ciocalteu
105 phenol reagent were obtained from Merck (Darmstadt, Germany). Chicoric acid (dicaffeoyl tartaric
106 acid), 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical and 6-Hydroxy-2,5,7,8-tetramethylchroman-2-
107 carboxylic acid (Trolox), used for the antioxidant assay, were purchased from Sigma-Aldrich (St.
108 Louis, MO, USA). Isoquercitrin (quercetin-3-*O*-glucoside) was obtained from Fluka (Buchs SG,
109 Switzerlandand). Chlorogenic acid and cyanidin-3-*O*-glucoside chloride were obtained from
110 Extrasynthese (Genay, France). HPLC grade water (18.2 mΩ) was prepared using a Millipore Milli-
111 Q purification system (Millipore Corp., Bedford, MA, USA). All other chemicals used were of the
112 highest purity grade.
113
114 *2.3. Sensory visual quality and respiration rate*
115 Radicchio pieces were examined by a group of 8 trained researchers to assess leaf acceptability in
116 terms of overall visual quality (VQ), considered as criterion of consumer acceptability for minimally
117 processed lettuce (Lavelli et al. 2009).
118 As reference, a colour photographic scale associated with a brief description of freshness, colour
119 uniformity, and brightness, was used. Coded (3 digits) samples were presented to the judges

individually, to enable them to make independent evaluations. Visual quality was evaluated on a 5-point rating scale, where VQ = 5: excellent, fresh appearance, full sensory acceptability; VQ = 4: good, product acceptable from a sensory point of view; VQ = 3: limit of sensory acceptability (5-10% unacceptable leaves); VQ = 2: product has notable visual defects (10-30% unacceptable leaves); VQ = 1: severe visual defects (> 50% unacceptable leaves). Samples scored below 3 were considered unmarketable for the loss of the overall sensory VQ (loss of turgor and brightness accompanied by softening and browning of leaf tissues).

The respiration rate was measured using a closed system as reported by Kader (2002). About 100 g of radicchio pieces for each replicate (n=3) were put into 6 L sealed plastic jars (one jar per replicate) where CO_2 was allowed to accumulate up to 0.1%. The time taken to reach this value was calculated, by taking CO_2 measurements at regular time intervals. For CO_2 analysis, 1 mL gas sample was taken from the head space of the plastic jars through a rubber septum and injected into a gas chromatograph (p200 micro GC, Agilent, Santa Clara, CA, USA) equipped with dual columns and a thermal conductivity detector. CO_2 was analyzed with a retention time of 16 s and a total run time of 120 s on a 10 m porous polymer (PPU) column (Agilent, Santa Clara, CA, USA) at a constant temperature of 70 °C. Respiration rate was expressed as mL CO_2/kg·h.

2.4. Ammonium content and electrolyte leakage

The method reported by Pace et al. (2014) was used for monitoring the ammonium production considered as a senescence indicator. Briefly, 5 g of chopped sample was extracted in distilled water, and, after the reaction with nitroprusside reagent and alkaline hypochlorite solution, colour development was determined after incubation at 37 °C for 20 min, monitoring the absorbance at 635 nm, by means of a spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan). The concentration of NH_4^+ was expressed as μmole NH_4^+/g, using ammonium sulfate as standard (0-10 μg/mL, R^2= 0.99).

144 The procedure described by Cefola and Pace (2015) was used with slight modifications to determine
145 electrolyte leakage, an indirect measure of plant cell membrane integrity. Radicchio disks measuring
146 8 mm (about 2.5 g per replicate), obtained using a cork borer, were immersed in 25 mL of distilled
147 water. After 30 min of storage at 5 °C, the conductivity of the solution was measured using a
148 conductivity meter (Model CM35, Crison, Barcelona, Spain). The tubes containing the vegetable
149 portion were then frozen. After 7 days, samples were thawed and the conductivity was measured and
150 considered as total conductivity. Electrolyte leakage was calculated as the percentage ratio of initial
151 over total conductivity.
152
153 *2.5. Extraction and analysis of antioxidant activity and total phenols*
154 The following extraction procedure was used for DPPH assay and Folin-Ciocalteu Reducing Capacity
155 analysis. Specifically, 5 g of chopped sample for each replicate was homogenized in a methanol :
156 water solution (80:20 v/v) for 1 min, and then centrifuged at 5 °C at 6440 *xg* for 5 min. For DPPH
157 assay, the extract diluted in water (50 µL) was pipetted into 0.95 mL of DPPH solution to initiate the
158 reaction. The absorbance at 515 nm was measured after about 15 min. Results were expressed as
159 Trolox equivalents (mg Trolox/100 g fw) using a Trolox calibration curve (82-625 µM; R^2=0.99).
160 Folin-Ciocalteu Reducing Capacity analysis was carried out according to the method reported by
161 Heimler et al. (2007). Results were expressed as milligrams of gallic acid equivalent (GAE) per 100
162 g of fw. The calibration curve of gallic acid was prepared with five points, from 50 to 500 mg/L with
163 R^2 = 0.99.
164
165 *2.6. Polyphenols Extraction and HPLC-UV/Vis analysis*
166 A detailed structural study of the phenolic composition of the Corelli and Botticelli extracts was
167 conducted on fresh radicchio samples (just after harvest) and on fresh-cut products stored both in AIR
168 and pMA. Corelli was analyzed after 8 and 12 days of storage, whereas Botticelli was analyzed after

19 and 25 days of storage. The extraction procedure was carried out according to the method of Lavelli (2008) with slight modifications. In particular, for each sample, radicchio pieces were finely chopped and 5 g were extracted with 50 mL of 4% formic acid in methanol, at room temperature, on a horizontal shaker. After 2 h, 50 mL of 4% formic acid were added and the extraction was carried out for further 2 h under the same conditions. Samples were filtered through filter paper and extracts were then dried in a rotary evaporator (LaboRota 4000/HB Efficient, Heidolph, Schwabach, Germany) and stored at -20°C until used.

Extracts were reconstituted in 1% formic acid and analyzed by HPLC-UV/Vis using a HP 1110 Series HPLC (Agilent, Palo Alto, CA, USA) equipped with a binary pump (G-1312A) and an UV detector (G-1314A). Individual phenols were separated using an "ad hoc" developed method. The chromatographic analysis was carried out on a XBridge BEH C18 column (130 Å, 5 µm, 4.6 mm × 150 mm) (Waters, Milford, MA, USA) at a flow rate of 1 mL/min; solvent A was 1% formic acid and solvent B was 1% formic acid in methanol and water (50:50, v/v). After a 5 min hold at 20% solvent B, elution was performed according to the following conditions: from 20% (B) to 65% (B) in 22 min, from 65% (B) to 80% (B) in 8 min, isocratic elution (80% B) for 10 min, from 80% (B) to 95% (B) in 5 min. Polyphenols were monitored at 340 nm and at 520 nm.

Standard curves for each pure polyphenol compound were prepared over a concentration range of 0.5–40 µg/mL with six different concentration levels and duplicate injections at each level. Peak area of each polyphenol standard was calculated and plotted against the corresponding concentration using weighed linear regression to generate standard curves. Anthocyanins quantification was performed with external calibration curves generated by repeated injections of a fixed volume of standard solutions of cyanidine-3-*O*-glucoside over a concentration range of 0.5–100 µg/mL with six different concentration levels and duplicate injections at each level. Each replicate was prepared and analyzed in duplicate. Total phenolic contents were calculated by the addition of the individual phenolic content as obtained by HPLC-UV/Vis analyses. Results were expressed as mg/100 g of fw.

194

195 *2.7. Electrospray Ionization multistage Ion Trap Mass Spectrometry (ESI-ITMS^n) analysis*

196 Identification of phenolic compounds present in the different HPLC separated fractions was carried
197 out by ESI-ITMS^n using a Finnigan LCQ DECA XP Max ion trap mass spectrometer (Thermo
198 Finnigan, San Jose, CA, USA) equipped with Xcalibur system manager data acquisition software
199 (Thermo Finnigan). Experimental conditions for the mass spectrometric analyses were optimized
200 using selected standards. Mass spectra were recorded from mass-to-charge ratio (m/z) 80 to 1500
201 both in negative and in positive ionization mode. The capillary voltage was set at -11 V; the spray
202 voltage was at 3 kV; the tube lens offset was at 10 V in negative ion mode, while the capillary voltage
203 was set at 33 V; the spray voltage was at 3 kV and the tube lens offset was at -10 V in positive ion
204 mode. The capillary temperature was 275 °C. Data were acquired in MS, MS/MS and MS^n scanning
205 mode.

206

207 *2.8. Statistical analysis*

208 For each experiment, a multifactor ANOVA for $P \leq 0.05$, was performed with the aim of evaluating
209 the effect of packaging condition (AIR or pMA), storage duration (5, 8, 12, 19 and 25 days) and their
210 interaction on postharvest quality parameters. In addition, for each experiment, individual and total
211 phenolic contents measured by HPLC-UV/Vis were processed performing a one-way ANOVA for
212 $P \leq 0.05$, for fresh and stored samples in AIR or pMA at different sampling times, with data means
213 arranged in a completely randomized design. The mean values (n=3) for individual and total
214 polyphenols (determined by HPLC), were separated using the Student–Newman–Keuls (SNK) test
215 ($P \leq 0.05$). Correlation analysis was performed and Pearson coefficient (r) was calculated ($P \leq 0.05$).
216 Statistica software (version 6.0, StatSoft, Inc, Tulsa, OK, USA) was used for all statistical analyses.

217

218 **3. Results and discussion**

3.1. Effect of storage in AIR or pMA on sensory visual quality, respiration rate, ammonium content and electrolyte leakage.

During both experiments, the atmosphere composition inside pMA packages changed, reaching the equilibrium value of 10% O_2 and 7% CO_2 after 5 days in the first experiment and after 7 days in the second one.

Results of the two multifactor ANOVA carried out for the first and the second experiment are reported in Table 1. Sensory VQ was significantly affected only by packaging condition and storage duration in both experiments (Table 1). Regarding packaging condition, in both experiments, samples stored in pMA showed mean scores significantly higher than fresh-cut radicchio stored in AIR throughout the whole storage (Table 2). As for storage duration, in both experiments, a significant reduction of mean scores was observed, regardless packaging condition (Table 2). This positive effect of modified atmosphere packaging on these fresh-cut radicchio hybrids, was also demonstrated by studying their volatile profile (Cozzolino et al. 2016). As consequence of the effect of packaging condition, Corelli reached the sensory acceptability limit (VQ=3) after about 4 days in AIR and 8 days in pMA. On the other hand, Botticelli resulted acceptable from a sensory point of view, for about 11 and 19 days in AIR and in pMA, respectively. Based on these results, the experiment carried out on Corelli was stopped after 12 days, when samples stored in AIR were scored completely unmarketable (VQ=1). The main symptoms involved in the loss of the VQ, which make the leaves unmarketable, were the loss of turgor and brightness accompanied by softening and browning of leaf tissues. Worth noting, limited symptoms of browning were scored in Botticelli; in agreement with this finding, red lettuce varieties have already been reported to be resistant to browning under packaging and storage conditions similar to those utilised in this study (Tavarini et al. 2007; Lavelli et al. 2009). In addition, in both fresh-cut radicchio hybrids no visual decay symptoms, related to microbial spoilage, were observed during the whole storage period.

243 Results from the sensory evaluation of VQ were confirmed by physiological parameters (respiration
244 rate, ammonium production and electrolyte leakage). As regards respiration rate, results obtained
245 from the two multifactor ANOVA showed that all factors (packaging condition, storage duration and
246 their interaction) resulted to be significant in both experiments (Table 1). Just after harvest, Corelli
247 and Botticelli showed a very high respiration rate (Kader, 2002) in the range 40-60 mL CO_2/kg h
248 (Fig. 1A and Fig. 2). In the first experiment (on Corelli), a reduction in respiration rate was measured
249 during the storage in pMA, reaching a 4-fold fall respect to fresh samples after 12 days (Fig. 1A).
250 Furthermore, samples stored in AIR showed a decrease until the 8^{th} day (2-fold respect to fresh
251 samples); and an increase to the initial values in samples stored for 12 days and considered
252 unmarketable from a sensory point of view (Table 2). Regards to the second experiment, the
253 respiration rate of Botticelli fresh-cut radicchio (Fig. 2) decreased until the 8^{th} day respect to fresh
254 sample (regardless packaging condition); after, it remained almost constant until the end of the
255 storage, showing higher mean values for AIR than pMA samples (Fig. 2). These results confirm the
256 beneficial effect of modified atmosphere in slowing the respiration rate of fresh-cut vegetables (Kader
257 2002; Böttcher et al. 2003; Del Nobile et al. 2007).
258 In the first experiment (on Corelli), ammonium production and electrolyte leakage were affected by
259 all factors (packaging, storage and their interaction) (Table 1). In the second experiments (on
260 Botticelli), ammonium was affected only by storage duration, while no factors influenced electrolyte
261 leakage (Table 1). In particular, in Corelli, ammonium accumulated during storage (Fig. 1B), starting
262 from the limits of sensory acceptability (after 5 and 8 days for AIR and pMA samples, respectively),
263 to values significantly higher in AIR than in pMA samples. As for Botticelli, storage duration caused
264 a significant increase in ammonium mean content at the end of storage (Table 2). These findings
265 support the hypothesis that ammonium, which comes from protein catabolism (Chandra et al. 2006),
266 can be considered as an objective indicator of product quality and marketability as recently assessed
267 (Tudela et al. 2013; Pace et al. 2014).

A behaviour similar to that described for ammonium, was also observed for the electrolyte leakage in Corelli (Table 1, Fig. 1C). During storage, samples stored in AIR showed higher leakages of ions than pMA ones. Since the electrolyte leakage can be considered an indirect measure of plant cell membrane integrity (Martínez-Sánchez et al. 2011), results indicate a positive effect of modified atmosphere packaging on preserving leaf structure.

3.2. Effect of storage in AIR or pMA on antioxidant activity and total phenol content

In the first experiment (on Corelli), the antioxidant activity and total phenol content were not affected by the different factors considered (packaging, storage and their interaction). In the second experiment (on Botticelli), the antioxidant activity and total phenols were affected only by storage duration (Table 1). After harvest, Corelli showed an antioxidant activity and total phenol content of 300.0 (± 18.3) mg Trolox/100 g fw and 178.5 (± 36.1) mg_{GAE}/100 g fw, respectively. In the second experiment, carried out on Botticelli, the same initial amounts of antioxidant activity (about 140 mg Trolox/100 g fw) and total phenol content (about 140 mg $_{GAE}$/100 g fw) were found. These values are in agreement with the antioxidant values found by other Authors in radicchio (Lavelli et al. 2009; D'evoli et al. 2013).

In the second experiment, Botticelli samples stored for 12, 19 and 25 days (whether in AIR or pMA) showed higher mean values in antioxidant activity than fresh-cut leaves stored for 5 and 8 days (whether in AIR or pMA) (Table 2). Similarly, significant higher total phenols mean values were measured in Botticelli samples (regardless packaging condition) stored for 19 and 25 days than samples stored for 12 days and the ones preserved for 5 and 8 days (Table 2). In addition, a significant positive linear relationship was found between the antioxidant activity and total phenol content in Botticelli ($r = 0.87$, $p<0.0001$), whereas no correlation was found in Corelli. Similarly, Lavelli (2008) found a positive significant relationship between antioxidant activity and total phenol content in fresh-

292 cut red chicory. This result confirms that phenols are the most representative compounds affecting
293 the antioxidant activity, as already reported for many fruits and vegetable (Sulaiman et al. 2011).
294
295 *3.3. Identification and quantification of polyphenols in two fresh-cut "Radicchio di Chioggia"*
296 *hybrids*
297 The chemical differences in polyphenols content between the two hybrids of radicchio were finely
298 achieved through the analysis and characterization of the two extracts by HPLC-UV/Vis and ESI–
299 $ITMS^n$. The identification was based on m/z values of molecular and characteristic fragment ions.
300 Comparison with analytical features of authentic standards was performed whenever possible. HPLC-
301 UV/Vis chromatograms of the two extracts of Corelli and Botticelli hybrids at the fresh state are
302 shown in Fig. 3. Twenty-one compounds were identified and quantified in the Corelli hybrid extract,
303 while nineteen were identified and quantified in Botticelli hybrid extract (Table 3 and 4). ESI-$ITMS^n$
304 identification of individual phenolics in the two extracts confirmed the presence of hydroxycinnamic
305 acid derivatives (caftaric acid, 5-caffeoylquinic acid, 5-*O*-feruloylquinic acid, chicoric acid, 3,5-di-
306 caffeoylquinic acid), flavonol derivatives (quercetin-3-*O*-glucuronide, quercetin-3-*O*-glucoside,
307 quercetin-3-*O*-(6"-*O*-malonyl)-glucoside, kaempferol-3-*O*-(6"-*O*-malonyl)-glucoside and
308 isorhamnetin-7-*O*-(6"-*O*-malonyl)-glucoside), and luteolin-7-*O*-glucuronide, in agreement with data
309 already reported in previous studies on "Radicchio di Chioggia" (Lavelli 2008; Carazzone et al.
310 2013). Other two flavonol derivatives were found only in the Corelli hybrid extract: quercetin-7-*O*-
311 glucoside-3-*O*-(6"-malonyl)-glucoside, previously characterized in the red *Lactuca sativa* L. (Lollo
312 rosso), and isorahmnetin-7-*O*-glucuronide, already described in "Radicchio di Chioggia" (Ferreres et
313 al. 1997; Llorach et al. 2008; Carazzone et al. 2013). In both extracts, quercetin-7-*O*-glucuronide-3-
314 *O*-(6"-malonyl)-glucoside and caffeoylmalic acid, previously reported in the red *Lactuca sativa* L.
315 (Lollo rosso) (Ferreres et al. 1997; Llorach et al. 2008) and apigenin glucuronide, previously
316 identified in the cultivar Spadona of *Cichorium intybus* L. (Heimler et al. 2007), were also found. The

317 analysis of peak 6 (Fig. 3) showed a pseudo-molecular ion ($[M-H]^-$) at m/z 551 in the mass spectrum
318 and a prominent fragment ion at m/z 507 in the MS^2 spectrum, probably originating from the
319 decarboxylation of the malonic acid moiety. In the MS^3 experiment, an ion at m/z 303 was observed,
320 corresponding to the aglycone and due to the release of the hexose-ketene moiety (204 Da).
321 Assignment of the aglycone to dihydroquercetin was based on MS^4 fragmentation. In fact, the
322 fragment ion at m/z 285, generated through the neutral loss of 18 Da from the parent ion at m/z 303,
323 indicated its flavanonol type, while the ion at m/z 243, generated through the neutral loss of 42 Da
324 (C_2H_2O), suggested the presence of 4'–OH, as reported in literature (Ye et al. 2012). Therefore, the
325 compound eluted in peak 6 was tentatively identified as dihydroquercetin-malonylhexoside.
326 Similarly, the analysis of peak 4 (Fig. 3) showed a $[M-H]^-$ at m/z 799 in the mass spectrum and
327 fragment ions at m/z 755 and m/z 711 in the MS^2 spectrum, probably originating from the
328 decarboxylation of two malonic acid moieties. In the MS^3 fragmentation spectrum, two ions at m/z
329 507 and m/z 303 were observed due to the subsequent release of two hexose-ketene moieties (204
330 Da). Therefore, the compound eluted in peak 4 was tentatively identified as dihydroquercetin-di-
331 malonylhexoside. The presence of dihydroflavonol glycosides in "Radicchio di Chioggia" is here
332 reported for the first time. Their occurrence could be justified as the dihydroflavonols are
333 intermediates in the biosynthesis of flavonols and anthocyanins, compounds present in the radicchio
334 extracts (Davies et al. 2003).
335 As to anthocyanins, both Corelli and Botticelli hybrids contained cyanidin-3-*O*-glucoside, cyanidin-
336 3,5-di-*O*-(6"-*O*-malonyl)-glucoside and cyanidin-3-*O*-(6"-*O*-malonyl)-glucoside (Table 3 and 4),
337 which were known to be present in "Radicchio di Chioggia" (Carazzone et al. 2013; Ferioli et al.,
338 2015).
339
340 *3.4. Effect of storage in AIR or pMA on the polyphenols composition*

Individual and total phenolic contents of both hybrids, were measured by HPLC-UV/Vis in the fresh samples (just after harvest) and during storage: after 8 and 12 days on Corelli and after 19 and 25 days on Botticelli both preserved in AIR or pMA. The samples analyzed by HPLC-UV/Vis were selected on the basis of sensory VQ assessment (as described in detail in paragraph 3.1).

Table 4 reported the results of the two one-way ANOVA carried out on the first and second experiment (Corelli or Botticelli) for fresh samples and samples stored in AIR or pMA at different sampling times.

Fresh-cut Corelli showed an initial mean content of total polyphenols of 248 (±10.9) mg/100 g fw, which remained almost constant during storage in both packaging condition, also in unmarketable leaves (i.e. sample stored for 12 days) (Table 4). Moreover, in Botticelli an initial content of total polyphenols of 143 (±21.1) mg/100 g fw was measured, which, increased significantly during storage (until the 25th day, when samples were scored unmarketable) in both packaging conditions (AIR or pMA) . This finding is well correlated with the data previously observed, that indicated an increase of the antioxidant activity of Botticelli extract at the end of the storage. The increase of the total polyphenols content could be associated with wound-induced stimulation of the enzyme phenylalanine ammonia lyase (PAL) (Lopez-Galvez et al. 1996), a plant enzyme that converts L-phenylalanine into trans-cinnamic acid, which in turn is the precursor of various phenylpropanoids, such as lignins, flavonoids, and coumarins (Hanson and Havir 1978; Hahlbrock and Scheel 1989). Ferreres et al. (1997) previously highlighted that the wound-induced biosynthesis of phenylpropanoids in lettuce, was much lower in tissues richer in pre-existing phenols. In fact, in their study on Lollo rosso, they described a higher increase of polyphenols in the lettuce midribs compared to the green and red tissues. In addition, Tomás-Barberán et al. (1997a) reported similar results on stems of Iceberg lettuce. Probably, in a similar manner, in fresh-cut Corelli, showing a higher content of polyphenols, the wound-induced phenylpropanoids biosynthesis could be reduced in comparison to fresh-cut Botticelli, exhibiting, in contrast, a lower content of polyphenols. It is well known that

366 caffeic acid is produced from phenylalanine via cinnamate and p-coumarate and conjugated with
367 tartaric acid to form caffeoyl tartaric (caftaric) and dicaffeoyl tartaric (chicoric) acids (Tomás-
368 Barberán et al. 1997b). The results showed that these two phenolic compounds accumulated in both
369 Corelli and Botticelli during cold storage. In particular, caftaric acid content increased in both Corelli
370 and Botticelli during cold storage, while chicoric acid content increased in Corelli in both AIR and
371 pMA conditions after 8 days, while it decreased after 12 days in AIR. In addition, fresh-cut processing
372 promotes a 3-fold increase in chicoric acid in Botticelli hybrid; this significant amount remained
373 almost constant until the end of the storage (both in AIR and in pMA) and was also detected in
374 unmarketable leaves (samples stored for 25 days). Similarly, 3,5-di-caffeoylquinic acid and
375 Quercetin-3-*O*-glucuronide increased significantly in fresh-cut leaves of Botticelli hybrid, and their
376 total content remains high (about 20 mg/100 g fw) also in unmarketable leaves (leaves stored for 25
377 days). Apigenin glucuronide also accumulated in both radicchio hybrids during cold storage (AIR
378 and pMA conditions), while the dihydroquercetin-di-malonylhexoside content increased significantly
379 in Botticelli (stored in AIR or pMA) and in Corelli after 8 days in AIR. The trend of isorahmnetin-7-
380 *O*-glucuronide was quite peculiar, as, although it was detected only in Corelli samples at the fresh
381 state, it accumulated in Botticelli during cold storage, increasing its content during the time, especially
382 under pMA conditions. The increase of anthocyanins content during cold storage was significant only
383 in Botticelli, in both AIR and pMA conditions. Among anthocyanins, Cyanidin-3-*O*-(6"-*O*-malonyl)-
384 glucoside, increased of 3-fold after fresh-cut processing and was found in high amount (about 50
385 mg/100 g fw) also in unmarketable leaves (Table 4). The synthesis of anthocyanins in several plant
386 tissues has also been associated with increased PAL activity (Tan 1979).
387 Moreover, the higher perishability of Corelli, as highlighted above, can be explained by the higher
388 initial content of polyphenols of this hybrid. Indeed, vegetables having a higher content of
389 polyphenols are subject to a more rapid enzymatic browning compared to those with a lower content
390 (Altunkaya and Gökmen 2008). On the contrary, the *de novo* biosynthesis of polyphenols, as found

391 in Botticelli, is thus considered to be a limiting factor for enzymatic browning (Hisaminato et al.
392 2001).
393
394 **4. Conclusions**
395 In Corelli and Botticelli hybrids, fourteen phenolic compounds already reported in previous studies
396 on “Radicchio di Chioggia” and three compounds found in other plants belonging to the Asteraceae
397 family were detected, while quercetin-7-*O*-glucoside-3-*O*-(6"-malonyl)-glucoside and isorahmnetin-
398 7-*O*-glucuronide were found only in the Corelli hybrid. Furthermore, to the best of our knowledge,
399 this is the first report describing the presence of dihydroflavonol glycosides in “Radicchio di
400 Chioggia”. The beneficial effect of modified atmosphere in preserving quality of fresh-cut radicchio
401 was demonstrated in both experiments. Botticelli radicchio hybrid resulted particularly suited for
402 fresh-cut processing on the basis of the main sensory and physiological parameters. In addition, fresh-
403 cut processing promoted the increase of bioactive compounds, which in many cases were quantified
404 in a significant amount also in fresh-cut leaves no more acceptable for the loss of freshness
405 appearance. Among these, chicoric acid, and cyanidin-3-*O*-(6"-*O*-malonyl)-glucoside were found in
406 high concentration (about 50 mg /100 g fw) in unmarketable leaves. This suggests the possibility to
407 use unmarketable leaves of Botticelli hybrid as a cheap source of antioxidant phenols, that could be
408 used as natural antioxidants or to functionalize foods. Corelli, even if less suitable for fresh-cut
409 processing, resulted a valuable hybrid for the fresh consumption and a source of polyphenols for their
410 high content in fresh and stored leaves.
411

Acknowledgments This work was supported by the PON4a2_Be&-Save project 2007-2013 SMART CITIES AND COMMUNITIES AND SOCIAL INNOVATION from the Italian Ministry of Education, Universities and Research (MIUR)

References

Alarcón-Flores, M. I., Romero-González, R., Vidal, J. L. M., González, F. J. E., Frenich, A. G. (2014). Monitoring of phytochemicals in fresh and fresh-cut vegetables: A comparison. Food Chem. 42, 392–399.

Altunkaya, A., Gökmen, V. (2008). Effect of various inhibitors on enzymatic browning, antioxidant activity and total phenol content of fresh lettuce (*Lactuca sativa*). Food Chem. 107, 1173–1179.

Böttcher, H., Günther, I., Kabelitz, L. (2003). Physiological postharvest responses of Common Saint-John's wort herbs (Hypericum perforatum L.). Postharvest Biol. Technol. 29, 342-350.

Carazzone, C., Mascherpa, D., Gazzani, G., Papetti, A. (2013). Identification of phenolic constituents in red chicory salads (*Cichorium intybus*) by high-performance liquid chromatography with diode array detection and electrospray ionisation tandem mass spectrometry. Food Chem. 138, 1062–107.

Cefola, M., Pace, B. (2015). Application of oxalic acid to preserve the overall quality of rocket and baby spinach leaves during storage. J. Food Process. Preserv. 39, 2523-2532.

Chandra, D., Matsui, T., Suzuki, H., Kosugi, Y. (2006). Postharvest changes in some physiological traits and activities of ammonia-assimilating enzymes in lettuce during storage. Asian J. Plant Sci. 5, 378–384.

Cozzolino, R., Martignetti, A., Pellicano, M. P., Stocchero, M., Cefola, M., Pace, B., De Giulio, B. (2016). Characterization of volatiles profile and sensory analysis of fresh-cut “Radicchio di Chioggia” stored in air or modified atmosphere. Food Chem. 192, 603–611.

Davies, K. M., Schwinn, K. E., Deroles, S. C., Manson, D. G., Lewis, D. H., Bloor, S. J., Bradley, J. M. (2003). Enhancing anthocyanin production by altering competition for substrate between flavonol synthase and dihydroflavonol 4-reductase. Euphytica 131, 259–268.

Del Nobile, M.A., Licciardello, F., Scrocco, C., Muratore, G. and Zappa, M. (2007). Design of plastic packages for minimally processed fruits. J. Food Engin. 79, 217-224.

D'evoli, L., Morroni, F., Lombardi-Boccia, G., Lucarini, M., Hrelia, P., Cantelli-Forti, G., Tarozzi, A. (2013). Red chicory (*Cichorium intybus* L. *cultivar*) as a potential source of antioxidant anthocyanins for intestinal health. Oxid. Med. Cell Longev. 2013.

Ferioli, F., Manco, M. A., D'Antuono, L. F. (2015). Variation of sesquiterpene lactones and phenolics in chicory and endive germplasm. J. Food Comp Anal. 39, 77–86.

Ferreres, F., Gil, M. I., Castañer, M., Tomás-Barberán, F. A. (1997). Phenolic metabolites in red pigmented lettuce (*Lactuca sativa*). Changes with minimal processing and cold storage. J. Agric. Food Chem. 45, 4249–4254.

Hahlbrock, K., Scheel, D. (1989). Physiology and molecular biology of phenylpropanoid metabolism. Annu. Rev. Plant Phys. Plant Mol. Biol. 40, 347–369.

Hanson, K. R., Havir, E. A. (1978). An introduction to the enzymology of phenylpropanoid biosynthesis. Rec. Adv. Phytochem. 12. 91–137.

Heimler, D., Isolani, L., Vignolini, P., Tombelli, S., Romani, A. (2007). Polyphenol content and antioxidative activity in some species of freshly consumed salads. J. Agric. Food Chem. 55, 1724–1729.

Hisaminato, H., Murata, M., Homma, S. (2001). Relationship between enzymatic browning and phenylalanine ammonia-lyase activity of cut lettuce, and the prevention of browning by inhibitors of polyphenol biosynthesis. Biosci. Biotech. Bioch. 65, 1016–1021.

Innocenti, M., Gallori, S., Giaccherini, C., Ieri, F., Vincieri, F. F., Mulinacci, N. (2005). Evaluation of the phenolic content in the aerial parts of different varieties of *Cichorium intybus* L. J. Agric. Food Chem .53, 6497–6502.

Kader, A. A. (Ed.) (2002) Postharvest technology of horticultural crops (Vol. 3311) UCANR Publications.

465 Kammerer, D. R., Kammerer, J., Valet, R., Carle, R. (2014). Recovery of polyphenols from the by-
466 products of plant food processing and application as valuable food ingredients. Food Res. Int.
467 65, 2–12.
468 Koukounaras, A. (2014). Yield and quality parameters of two Radicchio (*Cichorium intybus* L.)
469 cultivars as affected by growth season. Eur. J. Hort. Sci. 79, 283–287.
470 Lavelli, V. (2008). Antioxidant activity of minimally processed red chicory (*Cichorium intybus* L.)
471 evaluated in xanthine oxidase-, myeloperoxidase-, and diaphorase-catalyzed reactions. J. Agric.
472 Food Chem. 56, 7194–7200.
473 Lavelli, V., Pagliarini, E., Ambrosoli, R., Zanoni, B. (2009). Quality of minimally processed red
474 chicory (*Cichorium intybus L.*) evaluated by anthocyanin content, radical scavenging activity,
475 sensory descriptors and microbial indices. Int. J. Food Sci. Tech. 44, 994–1001.
476 Llorach, R., Martínez-Sánchez, A., Tomás-Barberán, F. A., Gil, M. I., Ferreres, F. (2008).
477 Characterisation of polyphenols and antioxidant properties of five lettuce varieties and escarole.
478 Food Chem. 108, 1028–1038.
479 Lopez-Galvez, G., Saltveit, M. E., Cantwell, M. I. (1996). Wound-induced phenylalanine ammonia
480 lyase activity: factors affecting its induction and correlation with the quality of minimally
481 processed lettuce. Postharvest Biol. Tec. 9, 223–233.
482 Martínez-Sánchez, A., Tudela, J. A., Luna, C., Allende, A., Gil, M. I. (2011). Low oxygen levels and
483 light exposure affect quality of fresh-cut Romaine lettuce. Postharvest Biol. Tec. 59, 34–42.
484 Pace, B., Cefola, M., Renna, F., Da Pelo, P., Attolico, G. (2014). Non-destructive evaluation of
485 quality and ammonia content in whole and fresh-cut lettuce by computer vision system. Food
486 Res. Int. 64, 647–655.
487 Pereira, G. D. M., Kothe, C. I., Machado, C. C., Lopes, S. M., Flôres, S. H., Rios, A. D. O. (2014).
488 Effect of modified atmosphere applied to minimally processed radicchio (*Cichorium intybus*
489 *L.*) submitted to different sanitizing treatments. Food Sci. Technol. (Campinas) 34, 513–521.

490 Pertuzé, R., Bravo, R., Silva, P. (2016). Radicchio (*Cichorium intybus* L.) variety selection for the
491 Chilean central area. Sci. Hortic-Amsterdam, 198, 197–206.
492 Piagentini, A. M., Mendez, J. C., Guemes, D. R., Pirovani, M. E. (2005). Modeling changes of
493 sensory attributes for individual and mixed fresh-cut leafy vegetables. Postharvest Biol. Tec.
494 38, 202–212.
495 Rossetto, M., Lante, A., Vanzani, P., Spettoli, P., Scarpa, M., Rigo, A. (2005). Red chicories as potent
496 scavengers of highly reactive radicals: a study on their phenolic composition and peroxyl
497 radical trapping capacity and efficiency. J Agric. Food Chem. 53, 8169–8175.
498 Sulaiman, S. F., Sajak, A. A. B., Ooi, K. L., Seow, E. M. (2011). Effect of solvents in extracting
499 polyphenols and antioxidants of selected raw vegetables. J. Food Compos. Anal. 24, 506–515.
500 Tan, S. C. (1979). Relationships and interactions between phenylalanine ammonia-lyase,
501 phenylalanine ammonia-lyase inactivating system, and anthocyanin in apples. J. Am. Soc. Hort.
502 Sci. 104, 581–586.
503 Tavarini, S., Degl'Innocenti, E., Paradossi, A., Guidi, L. (2007). Biochemical aspects in two
504 minimally processed lettuces upon storage. Int. J. Food Sci. Tech. 42, 214–219.
505 Tomás-Barberán, F. A., Gil, M. I., Castañer, M., Artés, F., Saltveit, M. E. (1997a). Effect of selected
506 browning inhibitors on phenolic metabolism in stem tissue of harvested lettuce. J. Agric. Food
507 Chem. 45, 583–589.
508 Tomás-Barberán, F. A., Loaiza-Velarde, J., Bonfanti, A., Saltveit, M. E. (1997b). Early wound- and
509 ethylene-induced changes in phenylpropanoid metabolism in harvested lettuce. J. Am. Soc.
510 Hort. Sci. 122, 399–404.
511 Tudela, J. A., Marín, A., Garrido, Y., Cantwell, M., Medina-Martínez, M. S., Gil, M. I. (2013). Off-
512 odour development in modified atmosphere packaged baby spinach is an unresolved problem.
513 Postharvest Biol. Tec. 75,75–85.

514 Vanstreels, E., Lammertyn, J., Verlinden, B. E., Gillis, N., Schenk, A., Nicolaï, B. M. (2002). Red
515 discoloration of chicory under controlled atmosphere conditions. Postharvest Biol. Tec. 26,
516 313–322.
517 Ye, M., Yang, W. Z., Liu, K. D., Qiao, X., Li, B. J., Cheng, J., Feng, J., Guo, D. A., Zhao, Y. Y.
518 (2012). Characterization of flavonoids in *Millettia nitida var. hirsutissima* by HPLC/DAD/ESI-
519 MS^n. J. Pharm. Anal. 2, 35–42.
520

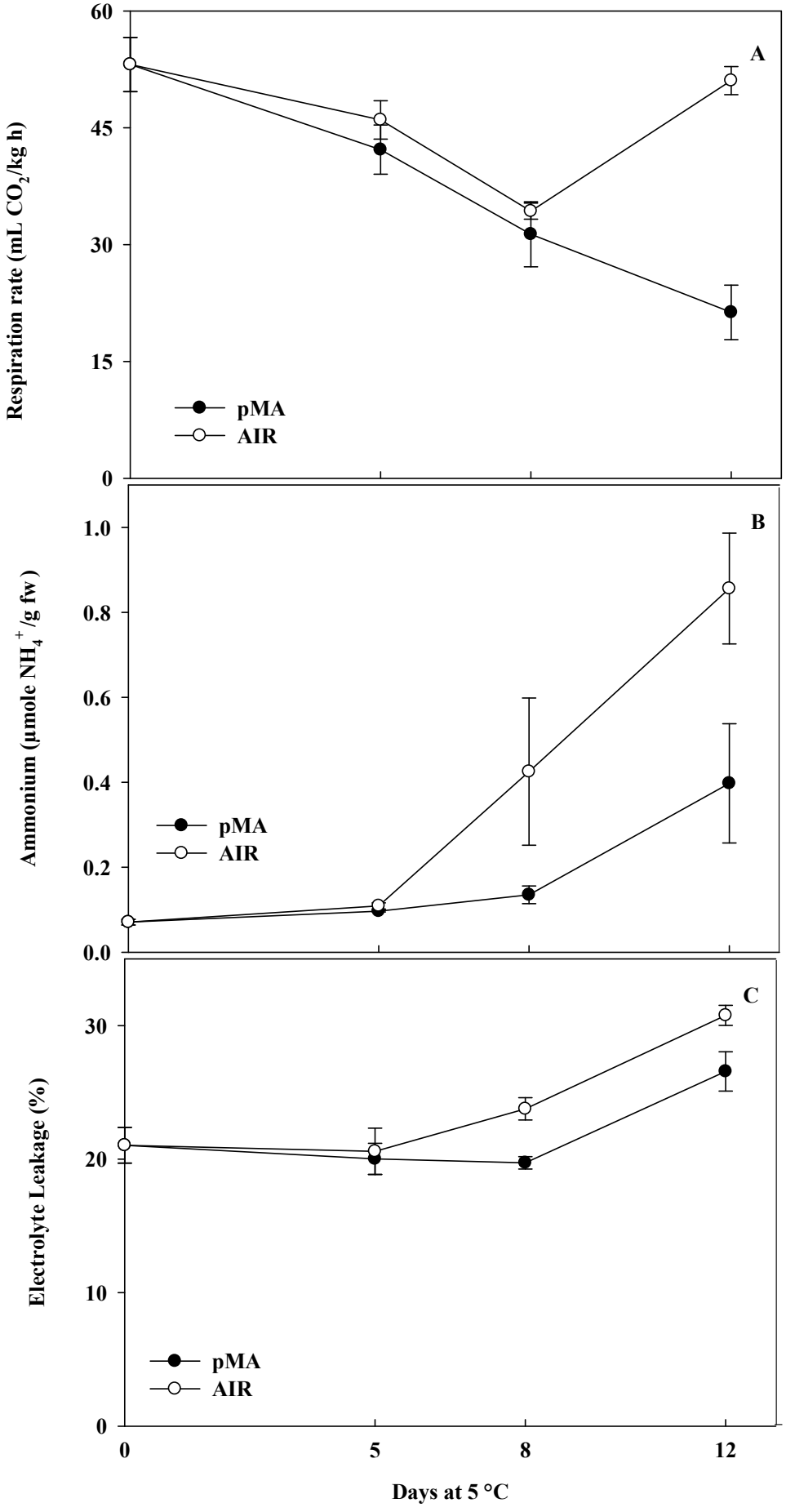
FIGURE CAPTIONS

Fig. 1 Changes in respiration rate (A), ammonium content (B) and electrolyte leakage (C) of Corelli fresh-cut "Radicchio di Chioggia" hybrid during cold storage in unsealed bags (AIR) or passive modified atmosphere (pMA). Mean data (n=3) ± standard deviation (bars) are reported.

Fig. 2 Changes in respiration rate of Botticelli fresh-cut "Radicchio di Chioggia" hybrid during cold storage in unsealed bags (AIR) or passive modified atmosphere (pMA). Mean data (n=3) ± standard deviation (bars) are reported.

Fig. 3 HPLC-UV/Vis chromatograms of fresh samples (just after harvest) of the two fresh-cut "Radicchio di Chioggia" hybrids extracts: Corelli hybrid extract (first experiment) recorded at 340 (A) and at 520 (B) nm; Botticelli hybrid extract (second experiment) recorded at 340 (C) and at 520 (D) nm. For peak assignments, see Table 3.

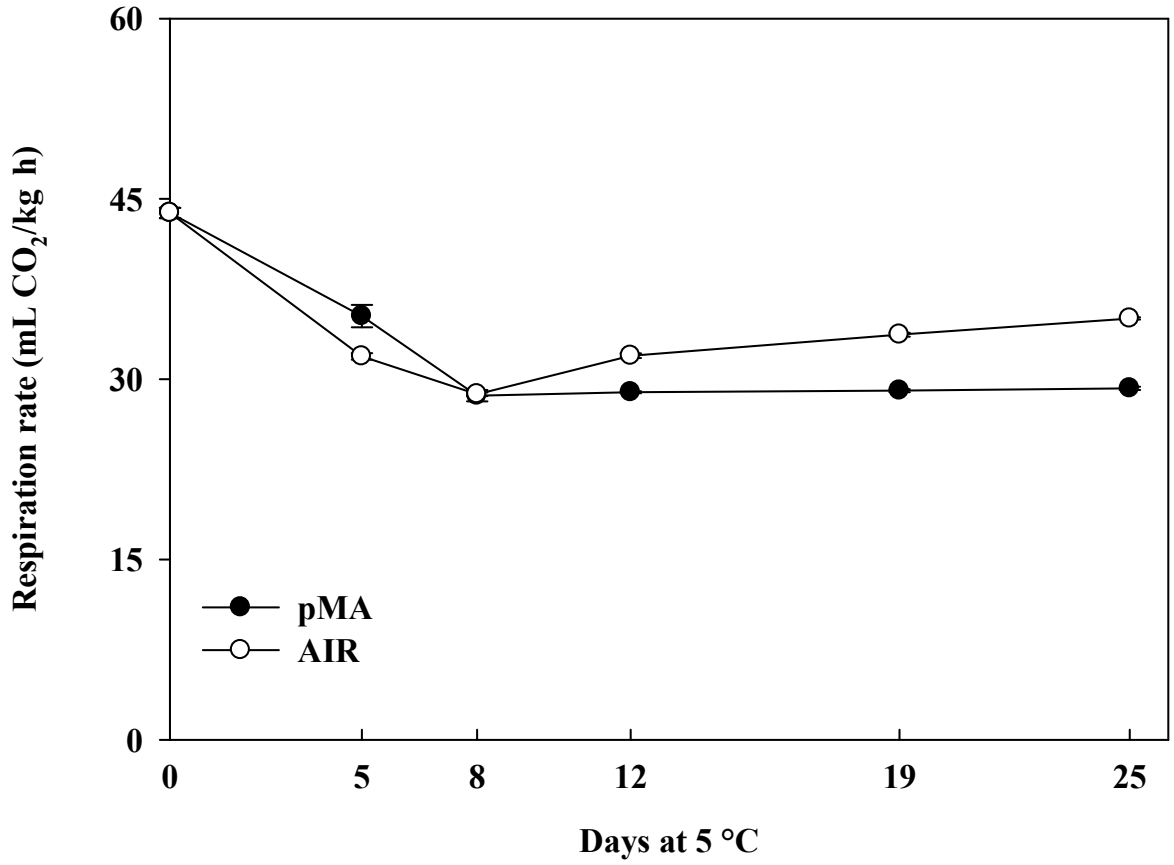
Figure 1


A
Respiration rate (mL CO_2/kg h)
60
45
30
15
0
pMA
AIR
B
Ammonium (µmole NH_4^+/g fw)
1.0
0.8
0.6
0.4
0.2
0.0
pMA
AIR
C
Electrolyte Leakage (%)
30
20
10
0
pMA
AIR
0
5
8
12
Days at 5 °C

532

533 **Figure 2**

534



535

536

537

538 **Figure 3**

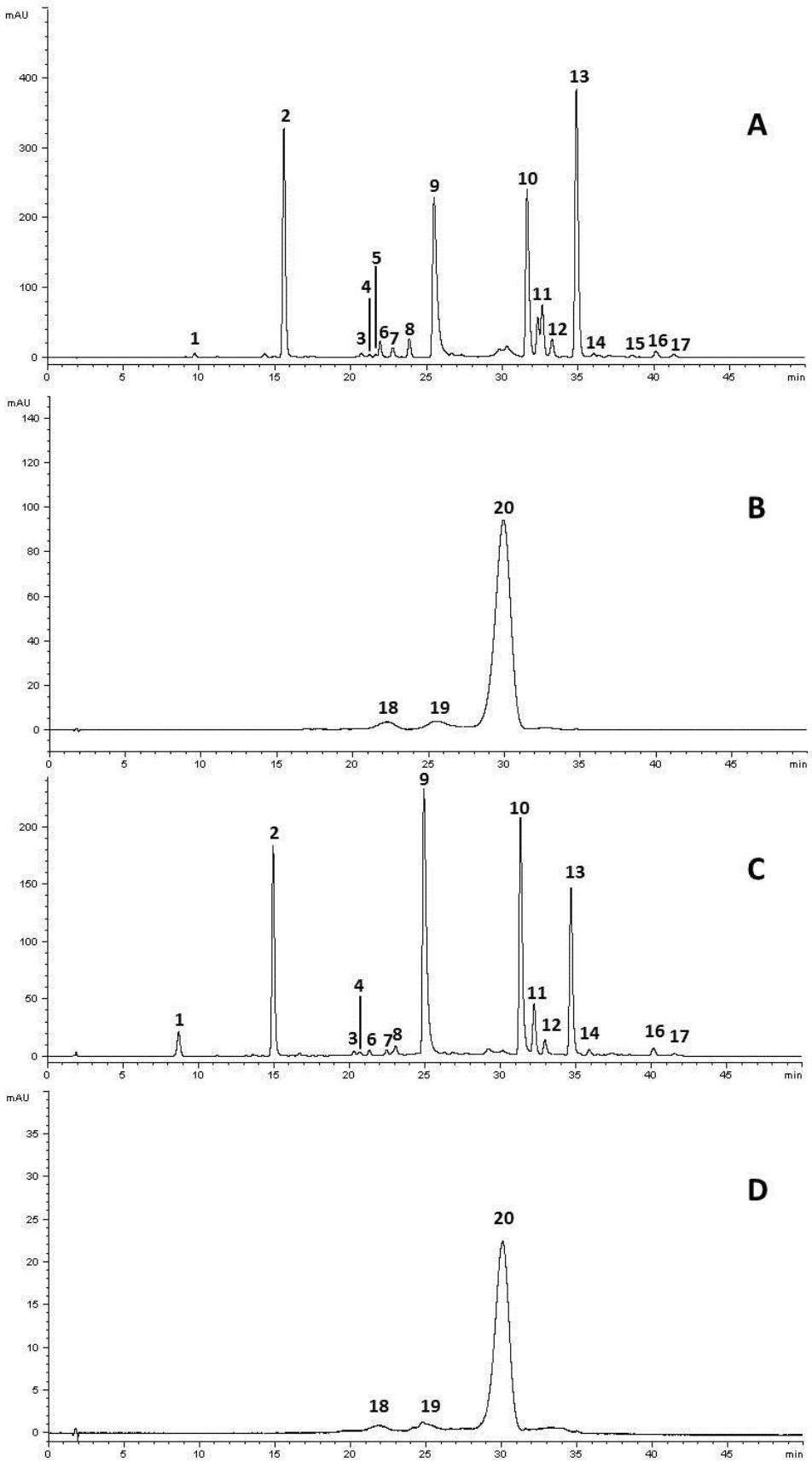

mAU
A
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
min
mAU
B
18
19
20
min
mAU
C
1
2
3
4
6
7
8
9
10
11
12
13
14
16
17
min
mAU
D
18
19
20
min

Table 1 Effects of packaging condition (AIR or passive modified atmosphere, pMA), storage duration (5, 8, 12 days - Experiment 1 or 5, 8, 12, 19, 25 days - Experiment 2) and their interaction on quality parameters of Corelli (experiment 1) and Botticelli (experiment 2) fresh-cut radicchio di Chioggia hybrids.

Parameters	Experiment 1: Corelli Radicchio hybrid			Experiment 2: Botticelli Radicchio hybrid		
	Packaging condition (A) (AIR or pMA)	Storage duration (B) (5, 8, 12 days)	A x B	Packaging condition (A) (AIR or pMA)	Storage duration (B) (5, 8, 12, 19, 25 days)	A x B
Visual Quality score (5-1)	***	***	ns	**	***	ns
Respiration rate (mL CO_2/kg h)	***	***	***	***	***	***
Ammonium (µmole NH_4^+/g fw)	***	***	*	ns	*	ns
Electrolytic leakage (%)	***	***	*	ns	ns	ns
Antioxidant activity (mg Trolox/100 g fw)	ns	ns	ns	ns	***	ns
Total Phenols (mg $_{GAE}$ /100 g fw)	ns	ns	ns	ns	***	ns

ns: not significant; * significant for P≤ 0.05; **significant for P≤ 0.01; *** significant for P≤ 0.001. For packaging condition dataset of 9 samples (experiment 1= 3 replication x 3 storage duration) or 15 samples (experiment 2= 3 replication x 5 storage duration) were used; for storage duration dataset of 6 samples (3 replication x 2 packaging condition in both experiments) were used fw: fresh weight.

Table 2 Main effect of packaging condition (AIR or passive modified atmosphere, pMA) and storage duration (5, 8, 12 days - experiment 1 or 5,8,12,19,25 days - experiment 2) on qualitative parameters of Corelli (experiment 1) and Botticelli (experiment 2) radicchio hybrids.

	Experiment 1: Corelli Radicchio hybrid				
Parameters	**Packaging condition**		**Storage duration (days)**		
	AIR	**pMA**	**5**	**8**	**12**
Visual Quality score (5-1)	1.75 b	3.04 a	3.15 a	2.29 b	1.75 c

	Experiment 2: Botticelli Radicchio hybrid						
Parameters	**Packaging condition**		**Storage duration (days)**				
	AIR	**pMA**	**5**	**8**	**12**	**19**	**25**
Visual Quality score (5-1)	2.75 b	3.55 a	4.25 a	4.00 a	3.00 b	2.50 c	2.00 d
Ammonium (μmole NH_4^+/g fw)	0.27 ns	0.25 ns	0.26 a	0.25 a	0.24 a	0.25 a	0.31 b
Antioxidant activity (mg Trolox/100 g fw)	164 ns	173 ns	133 b	126 b	184 a	204 a	195 a
Total Phenols (mg $_{GAE}$ /100 g fw)	166 ns	160 ns	143 c	141 c	161 b	186 a	183 a

In each experiment, for each factor (packaging condition or storage duration) and for each quality parameter, mean values followed by different letters (a, b, c, d) are significantly different for $P \leq 0.05$; ns: not significant. For packaging condition mean values of 9 samples (experiment 1= 3 replication x 3 storage duration) or 15 samples (experiment 2= 3 replication x 5 storage duration) were used; for storage duration mean values of 6 samples (3 replication x 2 packaging condition in both experiments) were used. fw: fresh weight.

Table 3 List of compounds identified in the two extracts of Corelli and Botticelli hybrids at the fresh state. Specific quasi-molecular ions and fragment ions are reported for each compound.

Peak	$[M-H]^-$ (m/z)	MS^n ions (m/z)	Identification
1	311	179, 149, 135	Caftaric acid
2	353	191, 179	5-caffeoylquinic acid
3	725	681, 505, 463, 300	Quercetin-7-*O*-glucuronide-3-*O*-(6"-malonyl)-glucoside
4	799	755, 711, 507, 303, 285, 241	Dihydroquercetin-di-malonylhexoside [a]
5	711	667, 505, 463, 300	Quercetin-7-*O*-glucoside-3-*O*-(6"-malonyl)-glucoside[b]
6	551	507, 303, 285, 241	Dihydroquercetin-malonylhexoside [a]
7	367	191, 173	5-*O*-feruloylquinic acid
8	295	179, 135, 133	Caffeoylmalic acid
9	473	311, 293, 275, 219, 179, 149	Chicoric acid
10	461	285	Luteolin-7-*O*-glucuronide
11	515	353, 191	3,5-Di-caffeoylquinic acid
	477	301, 179, 151	Quercetin-3-*O*-glucuronide
12	463	301, 179, 151	Quercetin-3-*O*-glucoside (Isoquercitrin)
13	549	505, 463, 301	Quercetin-3-*O*-(6"-*O*-malonyl)-glucoside
14	445	269, 225, 201, 149, 117	Apigenin glucuronide
15	491	315, 300	Isorahmnetin-7-*O*-glucuronide[b]
16	533	489, 447, 285	Kaempferol-3-*O*-(6"-*O*-malonyl)-glucoside
17	563	519, 315	Isorhamnetin-7-*O*-(6"-*O*-malonyl)-glucoside
	$[M]^+$ (m/z)	**MS/MS ions (m/z)**	
18	449	287	Cyanidin-3-*O*-glucoside
19	783	535, 287	Cyanidin-3,5-di-*O*-(6"-*O*-malonyl)-glucoside
20	535	491, 449, 287	Cyanidin-3-*O*-(6"-*O*-malonyl)-glucoside

[a] Tentatively identified compounds
[b] Not found in Botticelli hybrid

Table 4 Concentration of individual and total polyphenols of Corelli (Experiment 1) and Botticelli (Experiment 2) "Radicchio di Chioggia" hybrids determined by HPLC-UV/Vis (mg/100 g fresh weight) in the fresh samples (just after harvest, at 0 days -d-) and in samples stored (for 8 and 12 d in Experiment 1; for 19 and 15 d in Experiment 2) in unsealed bags (AIR) or passive modified atmosphere (pMA).

Peak	Identification	*Experiment 1: Radicchio Corelli*						*Experiment 2: Radicchio Botticelli*					
		Fresh	pMA-8d	AIR-8d	pMA-12d	AIR-12d		**Fresh**	pMA-19d	AIR-19d	pMA-25d	AIR-25d	
1	Caftaric acid	0.47 b	3.05 ab	1.76 ab	4.33 a	2.03 ab	*	1.61 b	7.98 ab	6.06 ab	10.7 a	5.20 ab	*
2	5-caffeoylquinic acid	28.1 ab	24.6 bc	32.3 a	22.9 bc	19.6 c	*	12.3	21.6	24.1	18.8	24.5	ns
3	Quercetin-7-*O*-glucuronide-3-*O*-(6"-malonyl)-glucoside	0.84 c	1.60 a	1.52 a	1.68 a	1.21 b	**	0.62	1.21	1.06	1.09	1.09	ns
4	Dihydroquercetin-di-malonylhexoside	0.67	1.01	1.09	1.18	0.88	ns	0.60 b	1.55 a	1.47 a	1.91 a	1.60 a	*
5	Quercetin-7- *O* -glucoside-3- *O* -(6"-malonyl)-glucoside	0.46 a	0.00[♯] b	0.00[♯] b	0.00[♯] b	0.00[♯] b	***	0.00[♯]	0.00[♯]	0.00[♯]	0.00[♯]	0.00[♯]	ns
6	Dihydroquercetin-malonylhexoside	3.86	4.32	4.85	4.98	4.66	ns	1.02	3.29	4.18	4.26	2.57	ns
7	5- *O* -feruloylquinic acid	1.21	0.94	1.10	0.93	0.70	ns	0.55	0.71	0.93	0.65	0.80	ns
8	Caffeoylmalic acid	3.05	2.69	3.09	2.68	2.54	ns	0.94	1.61	1.75	2.04	2.07	ns
9	Chicoric acid	17.4 c	33.5 ab	34.3 ab	38.2 a	28.5 b	**	17.9 b	49.6 a	51.6 a	52.6 a	53.7 a	*
10	Luteolin-7- *O* -glucuronide	41.2	44.3	43.7	47.6	38.2	ns	42.2	73.4	58.3	85.6	65.4	ns
11	3,5-Di-caffeoylquinic acid + Quercetin-3- *O* -glucuronide	15.8	25.2	24.2	26.7	22.0	ns	5.71 b	26.2 a	19.6 a	23.7 a	17.4 a	*
12	Quercetin-3- *O* -glucoside (Isoquercitrin)	5.59	7.46	7.85	8.57	6.51	ns	4.14	9.20	7.33	10.3	8.37	ns
13	Quercetin-3- *O* -(6"- *O* -malonyl)-glucoside	65.1 b	107 a	109 a	118 a	95.3 a	*	35.8	70.1	54.6	72.1	60.8	ns
14	Apigenin glucuronide	1.02 b	1.33 ab	1.66 a	1.56 ab	1.43 ab	*	0.89 b	1.87 a	2.17 a	2.19 a	1.66 a	*
15	Isorahmnetin-7- *O* -glucuronide	0.47 b	1.19 ab	1.35 ab	1.71 a	1.19 ab	*	0.00[♯] b	1.10 ab	1.47 ab	2.15 a	1.17 ab	*
16	Kaempferol-3- *O* -(6"- *O* -malonyl)-glucoside	2.08 c	3.46 b	4.25 ab	4.81 a	3.88 ab	**	1.84	3.43	3.93	4.34	3.45	ns
17	Isorhamnetin-7- *O* -(6"- *O* -malonyl)-glucoside	0.84 c	1.72 ab	1.79 ab	2.39 a	1.38 bc	*	0.87	2.59	1.83	2.72	2.14	ns
18	Cyanidin-3- *O* -glucoside	2.63	3.15	3.05	3.57	2.54	ns	0.61 b	3.83 ab	3.09 ab	4.76 a	3.78 ab	*
19	Cyanidin-3,5-di-O-(6"- *O* -malonyl)-glucoside	2.98	2.78	2.85	3.30	2.61	ns	0.58 b	2.89 a	2.32 ab	3.23 a	2.33 ab	*
20	Cyanidin-3- *O* -(6"- *O* -malonyl)-glucoside	54.9	61.5	60.7	64.7	55.8	ns	14.4 b	55.1 a	42.8 a	56.9 a	47.2 a	*
	Total Phenolic Contents (HPLC)	248	331	340	360	291	ns	143 b	337 a	289 ab	360 a	305 ab	*

For each experiment (experiment 1 on Corelli or experiment 2 on Botticelli) and for each compound identified, mean values (n=3) followed by different letters (a, b, c) are significantly different for P≤ 0.05. ns: not significant; * significant for P≤ 0.05; **significant for P≤ 0.01; *** significant for P≤ 0.001 Method: 95.0 percent Student-Newman-Keuls.

[♯]=not detected (LOD=0.15 mg 100 g $^{-1}$ fresh weight).