Manuscript Details

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Title	Postharvest application of oxalic acid to preserve overall appearance and nutritional quality of fresh –cut green and purple asparagus during cold storage: a combined electrochemical and mass-spectrometry analysis approach
Article type	Research Paper

Abstract

The effect of oxalic acid (OA) treatment on visual properties and bioactive compounds of two green and one purple cultivars of fresh asparagus was investigated during twelve storage days at 5 °C. Cold storage and OA treatment positively affected the overall appearance of the investigated cultivars. Cut-end dehydration increased, all along the storage period, in all cultivars but, the negative effect of the storage, clear on control samples, was mitigated by OA. The most represented compounds in Grande and Vegalim cultivars were: quercetin rutinoside, feruloyl quinic acid and cumaroyl quinic acid. Cyanidin glucosyl rutinoside, cyaniding rutinoside and peonidin rutinoside were identified in Purple Passion cultivar. The bioactive compounds seemed to be affected by storage but not by OA treatment. The sensor-biosensor system indicated that the antioxidant activity is negatively affected by storage but not by OA. The decrease of antioxidant activity coincided with the reduction of ascorbic acid levels in all the cultivars.

Keywords	Asparagus, oxalic acid, cold storage, sensor-biosensor system, LCMS phenol characterization

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Suggested reviewers	Yueming Jiang, Maria Serrano, Xuewu Duan

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Figure 1.TIF [Figure]

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Maria Isabel Gil
Associate Editor
Postharvest Biology and Technology

Dear Dr. Maria Isabel Gil,

Enclosed please find the revised version of the manuscript POSTEC_2018_771 entitled "Postharvest application of oxalic acid to preserve overall appearance and nutritional quality of fresh –cut green and purple asparagus during cold storage: a combined electrochemical and mass-spectrometry analysis approach"
The authors would like to thank the editor for giving them the opportunity to revise the manuscript. Please find a point by point response to the reviewer suggestions and comments.

Yours sincerely,

Antonio Barberis

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-Reviewer 1

The work concerning postharvest application of oxalic acid to preserve overall appearance and nutritional quality of fresh–cut green and purple asparagus during cold storage might be of an interest. The study indicated that cold storage and OA treatment affected the overall appearance of the investigated cultivars. The sensor-biosensor system indicated that the antioxidant activity was negatively affected by storage but not by OA. The decrease of antioxidant activity coincided with the reduction of ascorbic acid levels in all cultivars. The paper contains some interesting data and enhances the previous reports in this research area. The authors should consider these following points before the paper is considered for publication.

1. In the materials and methods section, sampling and replication for each analysis are very unclear. The authors should clarify them.
In agreement with the reviewer, the paragraph 2.3 "Oxalic acid treatment and storage" (please see pag. 5 lines 114-130) of Materials and Methods section was re-written to clarify the sampling schedule. The authors are available for further changes if necessary.

2. In this study, cut-end dehydration increased, from harvest to the end of the cold storage, in all cultivars, and was observed by storage and treatment. It could be considered to respiration rate in relation to the dehydration in the discussion.
The authors would like to thank the reviewer for the valuable suggestion. The discussion in the paragraph "*3.2. Respiration rate and ammonium content"* was improved accordingly. Please check page 12 lines 304-306.

3. In general, the paper is well written, but a few descriptions of English in this manuscript could be improved further.
We tried to improve and clarify all the sentences or descriptions pointed out by all the reviewers. We hope that now the manuscript is more readable.

-Reviewer2

In the manuscript POSTEC-2018-771 authors evaluated the effects of oxalic acid (OA) treatment on visual properties and bioactive compounds of two green and one purple cultivars of fresh asparagus during twelve storage days at 5 °C. The manuscript provides new and interesting information since no previous reports are available in the literature regarding oxalic acid treatment of asparagus. Thus, the manuscript could be suitable for publication after taking into account the following comments:

- The effects of OA treatment on asparagus quality properties should be addressed in highlights.
The first highlight was changed as follows: "Oxalic acid treatments affect the quality properties of green and purple asparagus";

- In Abstract section it should be also pointed out the effect of OA treatment.
The positive effect of OA treatment has been explained in the lines 32 and 33 of the abstract of the original manuscript but, according to the reviewer suggestion, we added the word "positively" in line 31 of the abstract of the revised manuscript to emphasize it.

- Sampling schedule is not clear. If one replicate was a bag with 150 g of asparagus (line 120) not enough material was available to take 100 g for each sampling date (lines 122). Check and clarify. How many spears were used for each analytical determination for each replicate, treatment, and sampling date?
In agreement with the reviewer, the paragraph 2.3 "Oxalic acid treatment and storage" (please see page 5 lines 114-130 of the revised manuscript) of Materials and Methods section was re-written to clarify the sampling schedule. The authors are available for further changes if necessary.

- Line 195: In table 3 results are expressed as mg/kg. Check and correct properly (see also comments below regarding to total phenolic concentrations).
The reviewer observation is correct and we apologize for this. We changed the values in table 3 (please check table 3_revised) using mg kg^{-1} as unit of measure.

- Lines 271-273: This is not clear. Spears firmness does not decrease during storage, but on the contrary, it increases due to dehydration and lignification processes.
The sentence at lines 271-273 cites the paper of Ruíz-Jiménez et al. 2014 on artichoke which reports a firmness decrease during storage. In the paragraph "3.2. Artichoke quality parameters and bioactive compounds"and in table 1 of the paper, the authors observed a clear decrease of the firmness during storage. In the samples treated with OA this decrease was slightly lower. In our paper firmness was not taken into consideration. We agree with the reviewer that dehydration and lignification should increase the firmness and that, such a consideration, deserve further experiments to be clarified. For this reason we prefer to delete the sentence from the manuscript.

- Line 324: It should be Table 3 instead of Fig. 5.
The quantification of the 13 compounds over the LOD refers both to Table 3 and Figure 5, so we add both into brackets. Please check line 324 page 13 of the revised manuscript.

- Line 325: At day 6 of storage, higher levels were found in 3 mM OA treated spears than in controls and 1 mM treated ones.
The reviewer indicated line 325 and we hypothesized that he/she probably suggested to add the sentence "At day 6 of storage, higher levels were found in 3 mM OA treated spears than in controls and 1 mM treated ones" to the manuscript. Unfortunately, this sentence does not match with the content of line 325 of the manuscript, probably an incorrect "copy and paste". We supposed that he/she was referring to line 335 (please, the reviewer should confirm) and we added the sentence to the manuscript. Please check lines 338-339 page 14 of the revised manuscript.

- Line 325: Also increase in Vegalen cultivar.
The reviewer indicated line 325 to the manuscript. Unfortunately, his/her sentence does not match with the content of line 325 of the manuscript, probably an incorrect "copy and paste". In this case we were not able to understand which sentence he/she was reffering to.

- Line 370: This statement is not true because no changes in ascorbic acid are observed during storage in control samplers or in OA treated ones for any of the cultivars.
In this case we do not agree with the reviewer comment. In cv Grande, the AA content did not significantly decrease after 6 days of storage (from 316.1 at the moment of treatment, to 305.6 after

6 d) but it significantly decreased after 12 d (285.6 mg kg^{-1}) d. Similar trends were observed also in *cv* Vegalim and in *cv* Purple passion (please see Table 4). Even if standard deviation was not reported, the statistical analysis was provided, and the differences among means are indicated by different letters as indicated at the bottom of table 4.

- Line 402: It should be Table 4 instead of Table 5.
We agree with the reviewer and we changed the table number in the revised manuscript. Please check line 400 page 16 of the revised manuscript.

- Line 409: According to data on Table 4, it seems that, in general, no significant changes occurred during storage, although statistical analysis for this issue are nor addressed.
In this case we do not agree with the reviewer comment. The antioxidant activity followed the trend of ascorbic acid: even though the values at the moment of treatment and after 6 d did not statistically differ, a significant decrease was observed after 12 d. Standard deviation was not reported but the statistical analysis was provided, and the differences among means are indicated by different letters as indicated at the bottom of table 4.

- Values for total phenolic content are very low (8-12 mg/kg f.w., Table 1) as compared with previous reports. For instance, values of 1.4 g/kg f.w. have been reported in green spears of the cv. 'Grande' (Wang et al., 2017, ScientiaHorticulturae, 225, 788-794), 10-12 g/kg d.w. (ca. 1-1.2 g/kg f.w) in spears of cv. 'Atlas' (Toscano et al., 2018, Postharvest Biology and Technology, 140, 34-41), 12-14 mg/g d.w. (ca. 1.2-1.4 g/kg f.w.) in spears of cv. 'UC157' depending on cultivation system (Ku et al., 2018, Food Chemistry, 244, 349-358), etc. Thus, these huge differences between the present results and the previous ones should be justified.
The reviewer observation is correct and we apologize for this. We corrected the values in table 1 (please check table 1_revised) using mg kg^{-1} as unit of measure.

- Values for ascorbic acid concentration, measured for both methods, around 3-4.5 mg/kg f.w. (Table 4) are also very low as compared with previous reports. For instance, values of 7.1-7.4 mg/g d.w. (ca. 710-740 mg/kg f.w.) has been reported in spears of cv. 'UC157' depending on cultivation system (Ku et al., 2018, Food Chemistry, 244, 349-358), 40 and 55 mg/kg in the base and top, respectively, of green spears of one unknown cultivar (Techavuthiporn, C., Boonyaritthongchai, P., Postharvest Biology and Technology, 117, pp. 64-70), etc.
The reviewer observation is correct and we apologize for this. We corrected the values in table 4 (please check table 4_revised) using mg kg^{-1} as unit of measure.

- References do not follow the format of the journal.
We checked the Guide for Authors of PBT which reported that "there are no strict requirements on reference formatting at submission". It seems to us that references in the manuscript like:
An, J., Zhang, M., Lu, Q., Zhang, Z., 2006. Effect of a prestorage treatment with 6-benzylaminopurine and modified atmosphere packaging storage on the respiration and quality of green asparagus spears. J. Food Eng. 77, 951-957. https://doi.org/10.1016/j.jfoodeng.2005.08.024
are in accordance with the following example found in the list of references to a journal publication
Van der Geer, J., Hanraads, J.A.J., Lupton, R.A., 2010. The art of writing a scientific article. J. Sci. Commun. 163, 51–59. https://doi.org/10.1016/j.Sc.2010.00372.

Highlights

- Oxalic acid treatments affect the quality properties of green and purple asparagus;
- Cold storage and OA affected the overall appearance and the cut end-dehydration;
- Rutin, feruloyl and cumaroyl quinic acids were detected in Grande and Vegalim spears;
- Cyanidin rutinoside was the most represented anthocyanin in *Purple Passion* cultivar;
- A sensor-biosensor system was used to measure antioxidant activity and ascorbic acid.

Postharvest application of oxalic acid to preserve overall appearance and nutritional quality of fresh –cut green and purple asparagus during cold storage: a combined electrochemical and mass-spectrometry analysis approach

Running title: oxalic acid affects the quality of fresh-cut asparagus during cold storage

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Antonio Barberis, Maria Cefola and Bernardo Pace equally contributed to this work.

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ABSTRACT

The effect of oxalic acid (OA) treatment on visual properties and bioactive compounds of two green and one purple cultivars of fresh asparagus was investigated during twelve storage days at 5 °C. Cold storage and OA treatment positively affected the overall appearance of the investigated cultivars. Cut-end dehydration increased, all along the storage period, in all cultivars but, the negative effect of the storage, clear on control samples, was mitigated by OA. The most represented compounds in Grande and Vegalim cultivars were: quercetin rutinoside, feruloyl quinic acid and cumaroyl quinic acid. Cyanidin glucosyl rutinoside, cyaniding rutinoside and peonidin rutinoside were identified in Purple Passion cultivar. The bioactive compounds seemed to be affected by storage but not by OA treatment. The sensor-biosensor system indicated that the antioxidant activity is negatively affected by storage but not by OA. The decrease of antioxidant activity coincided with the reduction of ascorbic acid levels in all the cultivars.

Key words: Asparagus, oxalic acid, cold storage, sensor-biosensor system, LCMS phenol characterization.

1. Introduction

Asparagus (*Asparagus officinalis* L.) is a herbaceous perennial plant, marketed worldwide as fresh or fresh-cut, very appreciated by consumers for its structural and sensory characteristic and for its nutritional properties (Dawid and Hofmann, 2012a, 2012b; Nikaido, 2014). Once the asparagus buds start to open, the shoots quickly turn woody, so only young shoots are eaten. Furthermore, fresh spears deteriorate rapidly after harvest, and the high respiration rate is their main limiting factor (Simón and Gonzalez-Fandos, 2011). The refrigeration, in association with modified atmosphere packaging (MAP), is able to retard visual and nutritional quality loss by slowing many of the deteriorative processes (Huyskens-Keil and Herppich, 2013; Kitazawaet al. 2011; Simón and Gonzalez-Fandos, 2011; Sothornvit and Kiatchanapaibul, 2009).

Recently, different methods have been proposed to preserve quality and extend storage. Treatments with 6-benzyaloaminopurine combined with MAP improved the quality of green asparagus spears (An et al., 2006), whereas short term (30s or 90s) washing in 50% ethanol solution at 10 °C reduced toughening of spears (Herppich et al., 2015). The use of oxalic acid (OA) have also been considered but, whereas a fair amount of papers dealt with the effects of OA preharvest treatments (Li et al., 2014; Martínez-Esplá et al., 2014; Martínez-Esplá et al., 2017), only a limited number of publications focused on the OA postharvest application (Valero et al., 2011; Zheng and Tian, 2006; Zheng et al., 2007) and, as far as we know, no one on postharvest of asparagus. Postharvest treatments with OA have been used, alone or in association with low storage temperatures, to preserve fruit or vegetable freshness (Feliziani et al., 2016; Ruíz-Jiménez et al.,2014). The application of OA on mango and peach reduced the respiratory activity and ethylene production, thus delaying ripening and senescence processes and extending the shelf life (Felizianiet al., 2016; Zheng et al., 2007). Analogous effects were observed on artichokes, where OA induced an extension of the shelf-life by reducing the respiration activity, the color changes, chlorophyll degradation and the development of the microbial flora (Ruíz-Jiménez et al., 2014). Similarly, it was demonstrated that OA can be used to extend the shelf-life of wild rocket and baby spinach leaves, with positive effects on quality parameters (Cefola

and Pace, 2015). The interest in the asparagus consumption is also due to its content of bioactive and antioxidant compounds. The antioxidant capacity, the phenolic content and composition (Fuentes-Alventosa et al., 2008) of green asparagus, in comparison with other fresh green vegetables, have been carefully studied. Even though it is not always possible to establish a good correlation between phenolics and antioxidant capacity, it was observed that the evolution of phenolic content and antioxidant activity of asparagus increased during the first days of cold storage and was stable afterwards (Kevers et al., 2007).

The aim of this work was to study the effects of postharvest treatments with OA, on the shoots of two green and one purple asparagus cultivars, during cold storage. Variation of the overall appearance, sensory characteristics, respiration rate and, particularly, the changes of antioxidant properties and phenolic composition during cold storage, were investigated by electrochemical and mass-spectrometry analysis approach.

2. Materials and methods

2.1. Reagents

All reagents and solvents were of analytical grade unless otherwise specified and used without further purification. 2,2-Diphenyl-1-picryhydrazyl radical (DPPH) was purchased from Alfa Aesar (London, UK), methanol, L-ascorbic acid (AA), gallic acid (GA; 3,4,5-trihydroxybenzoic acid), oxalic acid (OA), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and fullerene-C_{60} (FC_{60}) were purchased from Sigma–Aldrich (Milan, Italy). Ascorbate oxidase from *Cucurbita sp*. (EC 1.10.3.3), from Sigma Aldrich, was dissolved in the BSA solution: 1 U AOx will oxidize 1.0 μmol AA to dehydroascorbic acid (DHAA) per min at 25 °C and pH 5.6. Water was purified with a Milli-Q system from Millipore (Millipore Corporation, Billerica, MA, USA).

105 *2.2. Plant material*

106 The spears of three asparagus (*Asparagus officinalis* L.) cultivars, two green (*cv* Grande and *cv*
107 Vegalim) and one purple (*cv* Purple Passion) were provided by POA agricultural farm (Foggia,
108 Southern Italy). The spears were delivered to the postharvest laboratory, under refrigeration, within
109 two hours of the harvest. Asparagus with defects, such as bruising or discoloration, were removed.
110 Healthy spears were cut with a steel knife at 15 cm from head to include the entire edible portion, not
111 only the apical part exposed to light. About 500 g of spears of each cultivar were selected to be
112 analyzed at harvest.

113

114 *2.3. Oxalic acid treatment and storage*

115 Uniform spears of each cultivar were randomly divided into three lots: the first lot was used for
116 control (not treated samples); the second lot was treated with 1 mM OA, and the third was treated
117 with 3 mM OA.

118 OA treatment was performed by dipping the spears for 5 min in a 10°C tap water solution (pH=8)
119 containing 0 (control) or 1 mM OA (pH=6) or 3 mM OA (pH=2.9). After dipping, the spears were
120 rinsed for 1 min in 10 °C tap water and manually dried.

121 From each lot, 3 open unsealed polyethylene bags were filled with 150 g of spears (one bag per
122 replicate) and stored for 6 d at 5 °C, while 3 bags of 150 g each were prepared to be stored for 12 d
123 at 5 °C. Separately, three replicates of 100 g each were used to assess respiration rate.

124 The analytical determinations were carried out at harvest, after 6 d and 12 d of storage. Fifty grams
125 of asparagus spears from each replicate, were used to evaluate sensory characteristics, overall
126 appearance, cut-end dehydration, and color of spears. Hundred grams were chopped in order to obtain
127 a homogeneous sample used for chemical analyses: ammonium content, total phenolic compounds,
128 antiradical activity by DPPH, ascorbic acid content and antioxidant activity of all samples through a
129 sensor-biosensor system. A phenolic characterization was also performed for each cultivar, OA
130 treatment and storage time.

131

132 *2.4. Evaluation of overall appearance and cut-end dehydration*

133 The asparagus spears of each sample were examined by a group of eight trained researchers to assess
134 their sensory characteristics, and a score was assigned. Coded (3 digits) samples were presented to
135 the judges individually, to enable them to make independent evaluations. The overall appearance was
136 evaluated on a 5-point rating scale according to Fadda et al. (2016), where 5 = excellent (fresh
137 appearance); 4 = good; 3 = fair (limit of marketability); 2 = poor (just below the limit of
138 marketability); 1 = very bad (unmarketable). Cut-end dehydration was also evaluated on the basis of
139 a 1 to 5 scale, as described by Kitazawa et al. (2011) with minor modifications, where 1 = none, just
140 after cut; 2 = slight; 3 = moderate; 4 = severe; 5 = extreme.

141

142 *2.5. Evaluation of color changes of spears*

143 The color parameters L*, a*, b* (L* for lightness and a* and b* for the color components green–red
144 and blue–yellow respectively) were measured on three random points on the surface of 5 asparagus
145 spears per replicate. A colorimeter (CR-400, Konica Minolta, Osaka, Japan) equipped with a D65
146 illuminant in the reflectance mode and in the CIE L* a* b* color scale was used. The colorimeter was
147 calibrated with a standard reference having values of L*, a*, b* corresponding to 97.55, 1.32 and
148 1.41, respectively.

149

150 *2.6. Respiration rate and ammonium content*

151 The respiration rate of asparagus spears was measured at 5 °C, using a closed system. One hundred
152 grams of spears per replicate were put into 6 L sealed plastic jars where CO_2 was allowed to
153 accumulate. The time needed to reach 0.1% CO_2 was calculated by monitoring it at regular time
154 intervals. One milliliter of gas sample was taken, through a rubber septum, from the head space of
155 the plastic jars and injected into a gas chromatograph (p200 micro GC, Agilent, Santa Clara, CA)
156 equipped with dual columns and thermal conductivity detector. Carbon dioxide was analyzed with a

157 retention time of 16 s and total run time of 120 s on a 10 m porous polymer (PPU) column at a constant
158 temperature of 70 °C. Respiration rate was expressed as μmol $kg^{-1}s^{-1}$ CO_2.
159 Ammonium production was evaluated according to Fadda et al. (2016). Five grams of chopped
160 asparagus spears were extracted with 20 mL of distilled water. The obtained extract was added with
161 nitroprusside reagent in an alkaline 5% hypochlorite solution. Then, color development was
162 determined after incubation at 37 °C for 20 min, reading the absorbance by spectrophotometer (UV-
163 1800, Shimadzu, Kyoto, Japan) at 635 nm. Results are expressed on a fresh weight basis and the
164 concentration of NH_4^+ was reported as μmol kg^{-1} using ammonium sulfate as standard (0–10 mgL^{-1},
165 $R^2 = 0.96$).
166
167 *2.7. Extract preparation for phenolic composition and characterization, and for antioxidant capacity*
168 *evaluation*
169 Five grams of asparagus were extracted in a 10 mL methanol/water solution (80% MeOH) by
170 homogenizing spears at 5.489 g for 1 min (Ultra-Turrax, T25 Basic IKA, Germany). The
171 homogenates were centrifuged at 4.629 g for 10 min (A.L.C.-4227R, A.L.C. s.r.l. Milano, Italy), then
172 the organic extract was filtered with n. 4 Wathman filter paper. All the samples were stored in ultra-
173 freezer at -80 °C and lyophilized. All freeze-dried samples were rehydrated before *in vitro* calibrations
174 and chemical analyses (total phenolic content, phenolic characterization, antiradical activity,
175 simultaneous ascorbic acid and antioxidant activity determination through a sensor-biosensor system)
176 (Fadda et al., 2018).
177
178 *2.7.1. Total phenolic content*
179 Total phenolic concentration was determined with the Folin-Ciocalteu method according to Fadda et
180 al. (2014). The diluted extracts (0.4 mL) were mixed with 2 mL of water, 0.4 mL of the Folin-
181 Ciocalteu reagent and after three minutes with 4 mL of a sodium carbonate (Na_2CO_3) solution (75 g
182 L^{-1}). The mixture was shaken and adjusted to a final volume of 10 mL with water. Samples were

183 stored in the dark at room temperature for 2 h, then the absorbance was read at 750 nm with an Agilent
184 8453 UV-Vis spectroscopy system. Results are expressed on a fresh weight basis and reported as
185 gallic acid (GA) equivalents (mg kg^{-1} GA), using gallic acid as external standard (1-10 mg L^{-1}, R^2 =
186 0.99).
187
188 *2.7.2. Characterization of phenolic compounds*
189 *2.7.2.1 HPLC-UV Analysis*
190 Asparagus extracts were filtered through a 0.2 μm RC membrane syringe filters (Phenomenex,
191 Torrence, CA., USA). Phenolic compounds and anthocyanins were analyzed by LCMS system
192 according to previously described conditions (Barberis et al., 2015). A DAD detector at 280, 320 and
193 520 nm was used for quantitative analyses. The quantification of feruloylquinic acid isomers,
194 quercetin glucosyl rutinoside, quercetin rutinoside, kaempferol rutinoside, isorhamnetin rutinoside,
195 dioscin, quercetin glucoside, cyaniding glucosyl rutinoside, cyaniding rutinoside and peonidin
196 rutinoside was performed using the external calibration curves according to their commercial
197 standards; the coumaroyl quinic acid content was expressed on a fresh weight basis and reported as
198 caffeoyl quinic acid equivalents (g kg^{-1} CA) calculated using a caffeoyl quinic acid standard curve.
199
200
201 *2.7.2.2. LC–HRMS Analysis*
202 High Resolution MS analyses were performed on QExactive Orbitrap (Thermo Scientific, Bremen,
203 Germany) coupled to 1200 series HPLC (Agilent Technologies, USA) equipped with a quaternary
204 pump, a thermostated autosampler and a column oven set to 37°C.
205 To investigate secondary metabolite profile, the QExactive was equipped with heated electrospray
206 ionization source (HESI) operating in both positive and negative ion mode. The HESI parameters
207 were: spray voltage, 2.80 kV; sheath gas flow rate 30 (arbitrary units); auxiliary gas, 10 (arbitrary
208 units); sweep gas, 2 (arbitrary units); and capillary temperature at 300 °C. Full MS acquisition was

209 performed with resolution power 70000 FWHM for parent ions and 17500 for the fragment ions with
210 mass accuracy of 5 ppm. The MS parameters were: AGC target $3e^6$, maximum injection time (IT)
211 200 ms, and scan range 100–1200 *m*/*z*. The Xcalibur™ 3.1.66 software (Thermo Scientific, Bremen,
212 Germany) was used to control the instruments and to process the data.
213 A Phenomenex Kinetex EVO C18 (100mm × 2.1 mm, 5 µm, 100 A°) was used for the
214 chromatographic separation. The flow rate was 0.2 mL min^{-1} during a 55 min period with an injection
215 volume of 5 µL. A linear gradient elution of solvent acetic acid 0.2% (A) and acetonitrile (B) was
216 applied with the following program: 0 min, 10% B; 0–20 min, 10–20% B; 20–40 min, 20–40% B;
217 40–50 min, 40–70% B. The column was equilibrated for 8 min prior to each analysis. These
218 conditions were adapted from our previous study with some modifications (Barberis et al., 2015).
219 Peaks were identified on the basis of their retention time relative to external standards (t_R), UV-Vis
220 spectra (200 – 650nm), high resolution mass spectra, phytochemicals library and reference literature.
221 Quantification of the single phenolic compound was performed using calibration curves of the
222 respective reference compounds. When reference compounds were not available, the calibration was
223 based on structurally related molecules.
224
225 *2.7.3. Antiradical activity*
226 The radical scavenging activity was determined spectrophotometrically with the DPPH method
227 according to Fadda et al. (2014) with some modifications. Diluted methanol extracts (1.9 mL) were
228 mixed with 100 µL of a DPPH solution (1 mM in methanol). The mixture was stored in the dark at
229 room temperature for one hour and UV-Vis readings were carried out with a spectrophotometer
230 Agilent 8453 at 517 nm. The antiradical activity was expressed on a fresh weight basis and reported
231 as TEAC units (mmol kg^{-1} Trolox) using a Trolox calibration curve (2 – 20 µM, $R^2 = 0.99$).
232
233 *2.7.4. Amperometric determination of ascorbic acid content and antioxidant activity*

234 A sensor-biosensor system (SB), based on FC_{60} and on an ascorbate oxidase biosensor, specifically
235 developed to distinguish between AA and phenolic compounds contribution to the antioxidant
236 capacity, was used for an amperometric determination of AA content and antioxidant activity. A
237 complete description of the SB and of its working principle is reported in Barberis et al. (2014 and
238 2015). The SB system works at a potential of +500 mV. This value, according to Buratti et al. (2008),
239 would enable to determine the antioxidant capacity of samples containing different classes of
240 compounds (phenolic compounds, sugars, organic acids) and, in general, every antioxidant molecule
241 which can be oxidized at this specific potential, thus excluding any other molecule with a redox peak
242 potential higher than +500 mV (Barberis et al., 2014 and 2015; Buratti et al., 2008). Results relative
243 to AA content obtained with the SB were validated by a standard titrimetric method (Ting and
244 Roussef, 1986). Ascorbic acid content was expressed on a fresh weight basis and reported as mg kg^{-1}.
245 The antioxidant activity was expressed on a fresh weight basis too, and reported as mmol kg^{-1} AA
246 equivalents.
247
248 *2.8. Statistical analysis*
249 All analytical determinations were carried out in triplicate. A two-way ANOVA was performed, for
250 each cultivar, to evaluate the effect of treatment (control or 1mM OA or 3mM OA) and storage time
251 (6 d and 12 d) on quality parameters. Moreover, at each storage time, a one-way Anova was performed
252 to highlight significant differences among treatments. The Student-Newman-Keuls (SNK) test was
253 used to separate the mean value ($P \leq 0.05$). Mean values ± standard deviation (SD) are reported in
254 figures.
255
256 3. **Results and discussion**
257 *3.1. Overall appearance, cut-end dehydration and color*
258 Cold storage and OA treatment affected the overall appearance of the investigated cultivars (Fig. 1
259 and 2). According to judges' opinion, the score of all samples, treated and not treated, decreased from

260 “excellent” to “good” after 6 d of storage. A further reduction of overall appearance was detected
261 after 12 d, but a positive effect of OA treatment, at 1 mM and 3mM on *cv* Grande, and at 3 mM on
262 Vegalim and Purple Passion, was observed.
263 Cut-end dehydration increased, from harvest to the end of the cold storage, in all cultivars, and was
264 affected by storage and treatment (Fig. 3). The dehydration, negative effect of the storage, very clear
265 on the control samples, was mitigated by OA treatment: after 6 d, the samples of all cultivars treated
266 with OA showed significantly lower scores than the control and, in *cv* Grande, the 3 mM OA
267 treatment was more effective than 1 mM. At the end of storage, all treated samples received scores
268 lower than the control, with the exception of *cv* Purple Passion, where the 1mM OA treatment was
269 ineffective. Even though the mechanism of action of OA is not completely clear, its positive effect
270 on preserving the visual quality was previously showed on rocket and baby spinach stored under
271 refrigerated condition (Cefola and Pace, 2015). Feliziani et al. (2016) reported that the action of OA
272 could be attributed to its lipid peroxidation inhibitory activity, which was able to preserve quality and
273 improve shelf-life of different fruit and vegetable. Furthermore, even though its mechanism of action
274 is not completely clear to us, an important role could also be played by pH of the dipping solution
275 and its lowering due to OA treatment (OA 1 mM and OA 3 mM have pH = 6 and pH = 2.9
276 respectively). None of the cultivars exhibited any macroscopic outward damage, probably because
277 the time of treatment was only 5 min. Previous works on artichoke, which reported of a positive effect
278 of OA treatment, did not describe any issue after a 10 min dipping in a low pH solution (Ruíz-Jiménez
279 et al., 2014), although the outer bracts of artichoke ensure a much better protection than the soft
280 epidermis of asparagus spears. Neither storage nor OA treatments affected the color of Grande and
281 Vegalim spears (data not shown). In *cv* Purple passion, where anthocyanins are highly represented,
282 the spears treated with the 3 mM OA solution appeared brighter than those treated with 1 mM OA
283 and the control. Indeed, the L* values recorded by colorimeter indicated that, after 12 d of storage,
284 the lightness of spears was reduced of 31.0 %, 25.1 % and only 13.0 % in the control, 1 mM OA and
285 3 mM OA respectively, confirming that the low pH, tends to confer a more intensely and more stable

286 red color to vegetables containing anthocyanins. As for the other color parameters (a* and b*), no
287 significant effect was measured in *cv* Purple Passion due to storage or treatment (data not shown).
288
289 *3.2. Respiration rate and ammonium content*
290 The respiration rate of *cv* Grande and Vegalim was significantly affected by storage and treatment
291 while it appeared more stable in *cv* Purple Passion (Fig. 4). Results showed that, at harvest, the green
292 cultivars had a higher respiration rate (both about 65 µmol kg^{-1} s^{-1} CO_2) than the purple one (about 20
293 µmol kg^{-1} s^{-1} CO_2). Similar values were previously reported for green asparagus spears (Zhang et al.,
294 2008). During storage, the respiration rate of *cv* Grande decreased at about 40 µmol kg^{-1} s^{-1} CO_2 at
295 the sixth day, then it remained almost stable. Differently, the *cv* Vegalim showed a progressive
296 reduction of the respiration rate during all the storage period and, at the end of storage, values 75 %
297 lower than those measured at harvest, were recorded in treated and not treated samples. Focusing on
298 the effect of treatment, the samples of *cv* Grande treated with 3 mM OA showed significantly lower
299 respiration rate than 1 mM OA and control, both after 6 d and 12 d of storage. The positive effect of
300 3 mM OA on respiration rate was also observed after 6 d of storage on Vegalim spears and, at the end
301 of storage, on Purple Passion. Our results on asparagus spears are in accordance with previous works
302 on fruit (Huang et al., 2013; Sayyari et al., 2010) and vegetables (Cefola and Pace, 2015; Ruíz-
303 Jiménez et al., 2014) treated with OA, which linked the low respiration rate to a reduced metabolic
304 activity. In our study, the decrease of the respiration rate throughout storage could also be related to
305 the increase of cut end dehydration that, according to Lewicki et al. (2001), lowers the water
306 availability for biological reactions.
307 The ammonium content, which is an indicator of senescence (Pace et al., 2014), was 1.55 ± 0.25 µmol
308 kg^{-1} and 1.58 ± 0.03 µmol kg^{-1} NH_4^+ in *cv* Grande and *cv* Vegalim respectively, at harvest. It was only
309 affected by storage, increasing 3- or 5-fold respectively after 12 d, whereas OA treatment seemed to
310 be ineffective (data not shown). Differently, in Purple Passion, the ammonium content value (1.1
311 µmol kg^{-1}) measured at harvest, remained almost unvaried.

312

313 *3.3. Phenolic content*

314 *3.3.1. Characterization at harvest*

315 The total phenolic concentration, at harvest, of 836, 991 and 700 mg kg^{-1} GA in Grande, Vegalim and
316 Purple Passion cultivars respectively (Table 1), is in agreement with previous studies on green (Wang
317 et al., 2017) and purple asparagus (Maeda et al., 2005). The different behavior found among cultivars
318 could be attributed to some reasons: genetic material has been reported to be the most relevant factor
319 affecting the phenolic content, while the length and the color of spear tips and the apical spear
320 portions, where the largest amount of phenolic compounds is mainly located, imply that exposure to
321 light is essential for its accumulation.

322 Thirty-nine phenolic compounds were identified at harvest in the investigated cultivars and a
323 complete list of them is provided in Table 2. Among these, thirteen molecules (all the phenolic
324 compounds over the LOD of LCMS system) were quantified (Table 3 and Fig. 5). The most
325 represented compounds in the Grande and Vegalim cultivars were flavonoids and hydroxyl cinnamic
326 acids: quercetin rutinoside (rutin), and two isomers offeruloyl quinic acid in *cv* Grande; quercetin
327 rutinoside, two isomers offeruloyl quinic acid and cumaroyl quinic acid in *cv* Vegalim. Quercetin
328 rutinoside and the isomer II of feruloylquinic acid are the main flavonoids found in the *cv* Purple
329 Passion, while the anthocyanins cyaniding glucosyl rutinoside, cyaniding rutinoside and peonidin
330 rutinoside, were identified only in this purple cultivar, in accordance with the study of Sakaguchi et
331 al. (2008). Similar composition has recently been observed on green and purple asparagus of different
332 origin (Slatnar, 2018). The role of rutin, its loss and the consequent reduction of the antioxidant
333 capacity in asparagus spears were previously emphasized (Sunet al., 2007).

334

335 *3.3.2 Changes during storage*

336 Table 1 reports the total phenolic concentration of OA treated asparagus spears over storage. After 6
337 d of storage at 5 °C, the total phenolic compounds of control spears increased in all cultivars, as

338 similarly reported for asparagus spears stored at 2 °C and 10 °C (Palma et al., 2015). Higher levels
339 of phenols were found in 3 mM OA treated spears than in controls and 1 mM treated ones. After 12
340 d, the combined effect of storage and OA treatment was different and *cv* dependent. An enhancement
341 of total phenolic compounds was monitored in *cv* Grande, while a reduction was observed in *cv*
342 Vegalim. No considerable treatment's effect was observed in the *cv* Purple Passion either after 6 d
343 nor after 12 d of storage. It was previously observed that the phenolic content of asparagus increased
344 during the first days of storage at 4 °C and was stable afterward (Kevers et al., 2007). A direct role of
345 OA on phenolic content can be also hypothesized since it preserves the natural antioxidant
346 compounds present in vegetable tissues (Cefola and Pace, 2015; Zheng et al., 2007). In peach fruit,
347 OA treatments caused an increase of the activity of antioxidant enzymes and reduced the production
348 of active oxygen species such as superoxide and hydrogen peroxide, while in fruit of pomegranates
349 held for 84 d at 2 °C, OA dipping led to a lower loss of phenolic compounds than in not treated fruit
350 (Sayyari et al., 2010).
351 The evolution during storage and after OA treatment of each phenolic compound is reported in Table
352 3. Quercetin rutinoside clearly increased in *cv* Grande due to storage but it was not affected by OA
353 treatment; it was almost unvaried in *cv* Vegalim, and grew in *cv* Purple Passion being influenced by
354 storage and treatment. The isomer I of feruloyl quinic acid increased in *cv* Grande, decreased in *cv*
355 Vegalim and was under the LOD in *cv* Purple Passion; the isomer II increased in *cv* Grande and *cv*
356 Purple Passion, while was almost unvaried in *cv* Vegalim. Less represented was the isomer II of
357 cumaroyl quinic acid (the highest concentration at harvest was 107.5 mg kg^{-1} detected in *cv* Vegalim
358 *vs* 44.9 mg kg^{-1} and 42.0 mg kg^{-1} in *cv* Grande and *cv* Purple Passion respectively) that increased in
359 all the varieties and was affected by storage and OA treatment. In *cv* Purple Passion, an important
360 role was played by three anthocyanins: the cyanidin rutinoside (774.2 mg kg^{-1} at harvest) slightly
361 decreased during storage and due to OA treatments too; the cyaniding glucosyl rutinoside (125.5 mg
362 kg^{-1} at harvest) increased during storage; the peonidin rutinoside (84.8 mg kg^{-1} at harvest) was almost
363 unvaried during the 12 d of storage. The three mentioned anthocyanins have already been studied in

364 asparagus, because they have red pigments very appealing for consumers' acceptability and since
365 they are effective scavengers against oxidative stress (Sakaguchi et al., 2008).
366
367 *3.4. Determination of the ascorbic acid content*
368 The AA content of asparagus spears was not high in all the studied cultivars but still in accordance
369 with the literature, that indicate that there are significant differences during the harvest season (An et
370 al., 2008). AA values were negatively affected by storage, as expected (Barberis et al., 2012), but not
371 by OA treatment (Table 4). The values recorded, by the SB system at harvest were 326.5, 394.8 and
372 308.8 mg kg^{-1} for Grande, Vegalim and Purple Passion respectively and are in accordance with the
373 titrimetric method.
374
375 *3.5. Determination of the antioxidant and radical scavenging activities*
376 The antioxidant activity, measured with the sensor system, of the three asparagus varieties is reported
377 together with the radical scavenging activity measured with the DPPH method (Table 4). Even though
378 both the methods give a measure of the antioxidant capacity, they did not provide the same
379 information: the sensor system measured only the antioxidant activity of the phenolic compounds, or
380 of any other molecule, which can be oxidized at the applied potential of +500 mV. This threshold,
381 from an electrochemical point of view, is an indicator of the reaction time of an antioxidant pool of
382 molecules and, more extensively, of the efficiency of a defensive system, against oxidative damage.
383 The lower the potential, the easier the oxidation, the higher the antioxidant capacity and so, the faster
384 the plant response to oxidative stress (Barroso et al., 2011). This is the reason why ascorbic acid is
385 considered the best natural antioxidant, it can be oxidized at very low potential, lower than every
386 other antioxidant in plants (Barberis et al., 2010). So, all the phenolic compounds with an oxidation
387 peak higher than +500 mV did not give any contribution to this measure. Differently, the radical
388 scavenging activity measured the capacity of all the antioxidants to react against free radicals,
389 including the phenolic compounds with an oxidation potential higher than +500 mV.

390 In this work, the SB highlighted a trend common to all the cultivars, indicating that the antioxidant
391 activity is negatively affected by storage. Differently, OA treatment did not affect the antioxidant
392 activity thus validating previous studies on OA treated artichokes, stored at 20°C (Ruíz-Jiménez et
393 al., 2014). The highest values were recorded at harvest (3.45, 4.68 and 3.66 mmol kg^{-1} AA equivalents
394 in Grande, Vegalim and Purple Passion respectively), they remained almost unvaried for the first six
395 days and then decreased to the lower values after twelve storage days. The reduction of antioxidant
396 activity coincided with the reduction of AA levels in all the cultivars, supporting the theory that
397 ascribe to AA a main role against oxidative stress (Barberis et al., 2014; Gardner et al.,2000).
398 The radical scavenging activity provided results similar to those of SB. The values measured at
399 harvest were 2.63, 2.68 and 3.73 mmol kg^{-1}trolox equivalents in *cv* Grande, Vegalim and Purple
400 Passion respectively (Table 4) in accordance with literature (Maeda et al., 2005). Little differences
401 between methods were expected since the spectrophotometric analysis consider the whole pool of
402 phenolic compounds independently from the specific antioxidant capacity of each molecule. Also in
403 this case the antioxidant activity was mainly affected by storage and, only for *cv* Vegalim, by OA
404 treatment.
405 The SB, distinguishing between the AA and phenolic compounds contribution to antioxidant activity,
406 could also provide information on interactions among antioxidants. According to our results the
407 progressive consumption of AA during storage could be the main responsible for the decrease of
408 antioxidant activity in all the cultivars, due to a redox potential lower than +100 mV (Barberis et al.,
409 2010). Unfortunately, a decreasing of antioxidant activity due to phenolic compounds and a
410 simultaneous increase of quercetin rutinoside and other phenolic compounds during storage were
411 monitored. These results seem clashing, but they are not if we considered the interactions among
412 antioxidants (Choe and Min, 2009): we can hypothesize a synergism where AA, the antioxidant with
413 the lowest redox potential, regenerate quercetin rutinoside and other phenolic compounds with higher
414 redox potential, thus allowing them to carry out their scavenging role and many other complex
415 functions in plants, all along the storage period.

The SB system also highlighted the role of phenolic compounds with low redox potential, those which act first as radical scavengers. A difference in antioxidant capacity cannot be simply attributed to a difference of the total amount of phenolic compounds, but should be ascribed to the quality of phenolic compounds. Green and purple varieties have deeply different phenolic composition (Table 3). At harvest, Grande and Vegalim have a prevalence of flavanoids and hydroxyl cinnamic acids while about 60 % of phenolic compounds of *cv* Purple Passion is represented by anthocyanins. The most represented flavanoid, common to all the cultivars, is the quercetin rutinoside that, according to previous results have redox potentials of about + 280 mV, lower than the most of other flavanoids and hydroxyl cinnamic acids (Barberis et al., 2015). In *cv* Purple Passion, the amount of AA and quercetin rutinoside is lower than in the green cultivars, but the high content of cyaniding rutinoside and cyaniding glucosyl rutinoside, which have a low redox potential (+280 mV and +270 mV respectively) according to Barberis et al. (2015), ensured an effective protection against free radicals.

4. Conclusions

The combined effect of cold storage and oxalic acid treatment resulted a valid and sustainable solution to preserve the visual quality of green and purple asparagus spears. OA treatment, especially at 3 mM, improved the overall appearance of spears of all varieties, all along the storage period, and the use of OA solution with low pH tends to confer a more intensely, more stable and more appealing red color to spears of purple cultivar. OA treatments also help to reduce the respiration rate thus minimizing the effect of storage. Differently, the bioactive compounds content seemed to be affected by storage but not by OA treatment. The overview of the quality data together with the quantity and the antioxidant activity of phenolic compounds, allowed us to express an opinion on each investigated variety. The *cv* Grande has a low content of AA and quercetin rutinoside and, as a possible consequence, a low antioxidant capacity. Moreover, the high respiration rate and ammonium content make this *cv* highly perishable. The *cv* Vegalim owns excellent nutraceutical properties due to the highest antioxidant activity, the highest content of Ascorbic acid and quercetin rutinoside. The high

442 amount of red pigments characterizes the *cv* Purple Passion which results particularly appealing for
443 consumers. The most of these pigments are anthocyanins with low redox potential that confer a high
444 nutraceutical value to this cultivar.
445 Finally, the combined use of new electrochemical systems based on biosensors, together with
446 traditional methods like mass spectrometry and DPPH, could help the understanding of the interaction
447 between antioxidants in the studied asparagus cultivars, and could be a valuable approach for other
448 species too.
449

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615 FIGURE CAPTIONS

616

617 Figure 1. Effect of 1 mM and 3mM oxalic acid (OA) treatment on overall appearance of asparagus
618 spears cultivars (Grande, Vegalim and Purple Passion) stored at 5 °C. Mean ± standard deviation
619 (SD). Overall appearance score was attributed on a rating scale: 5 = excellent, fresh appearance, 4 =
620 good, 3 = fair (limit of marketability), 2 = poor (just below the limit of marketability), 1 = very bad,
621 unusable. Unlike letters differ statistically by the Student-Newman-Keuls (SNK) at $P \leq 0.05$.

622

623 Figure 2. Cut-end surface appearance of asparagus spears of Grande, Vegalim and Purple Passion
624 cultivars, at harvest (just after cut), and after 12 d of storage at 5 °C (control and spears treated with
625 3 mM oxalic acid, OA).

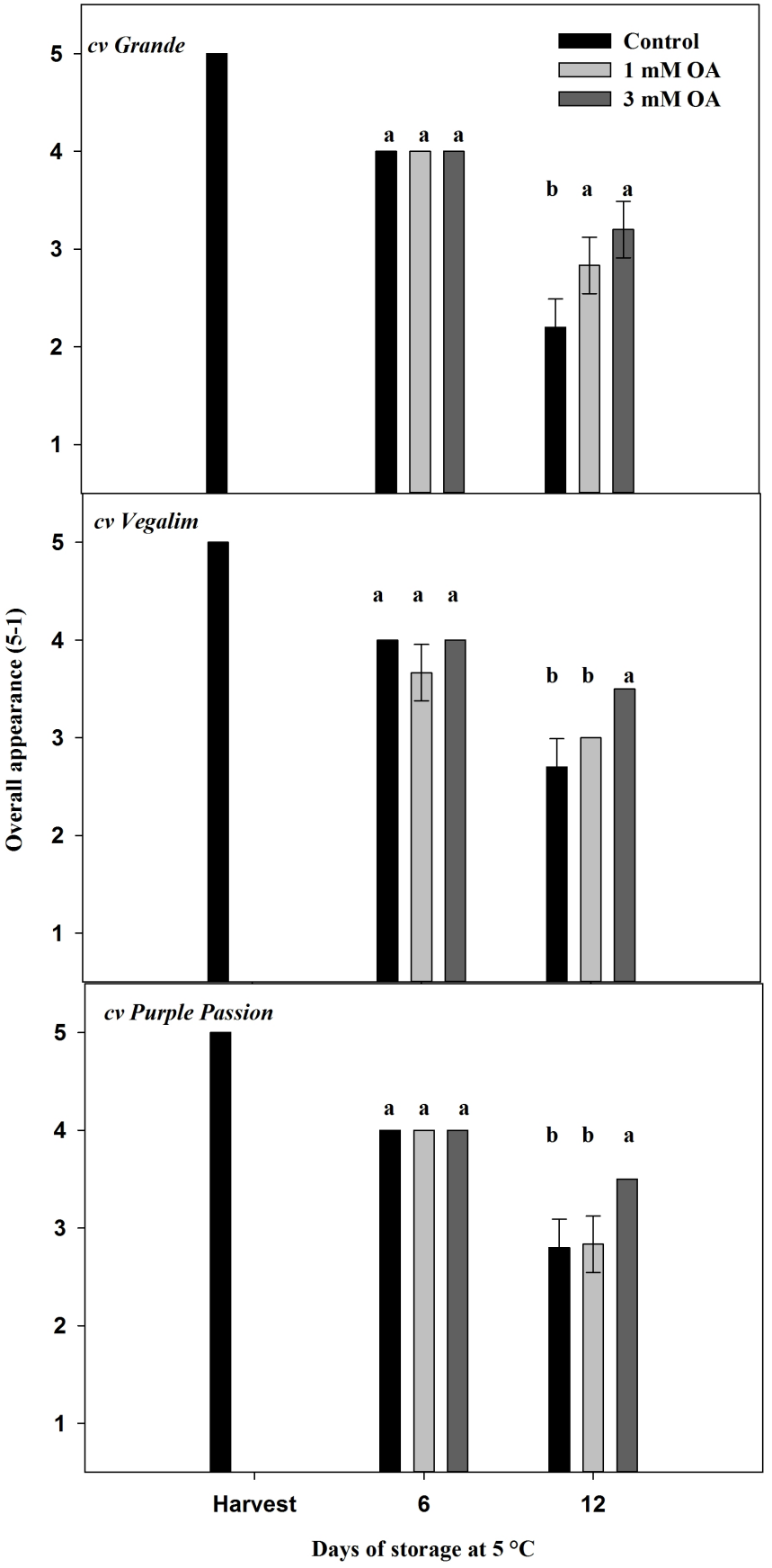
626

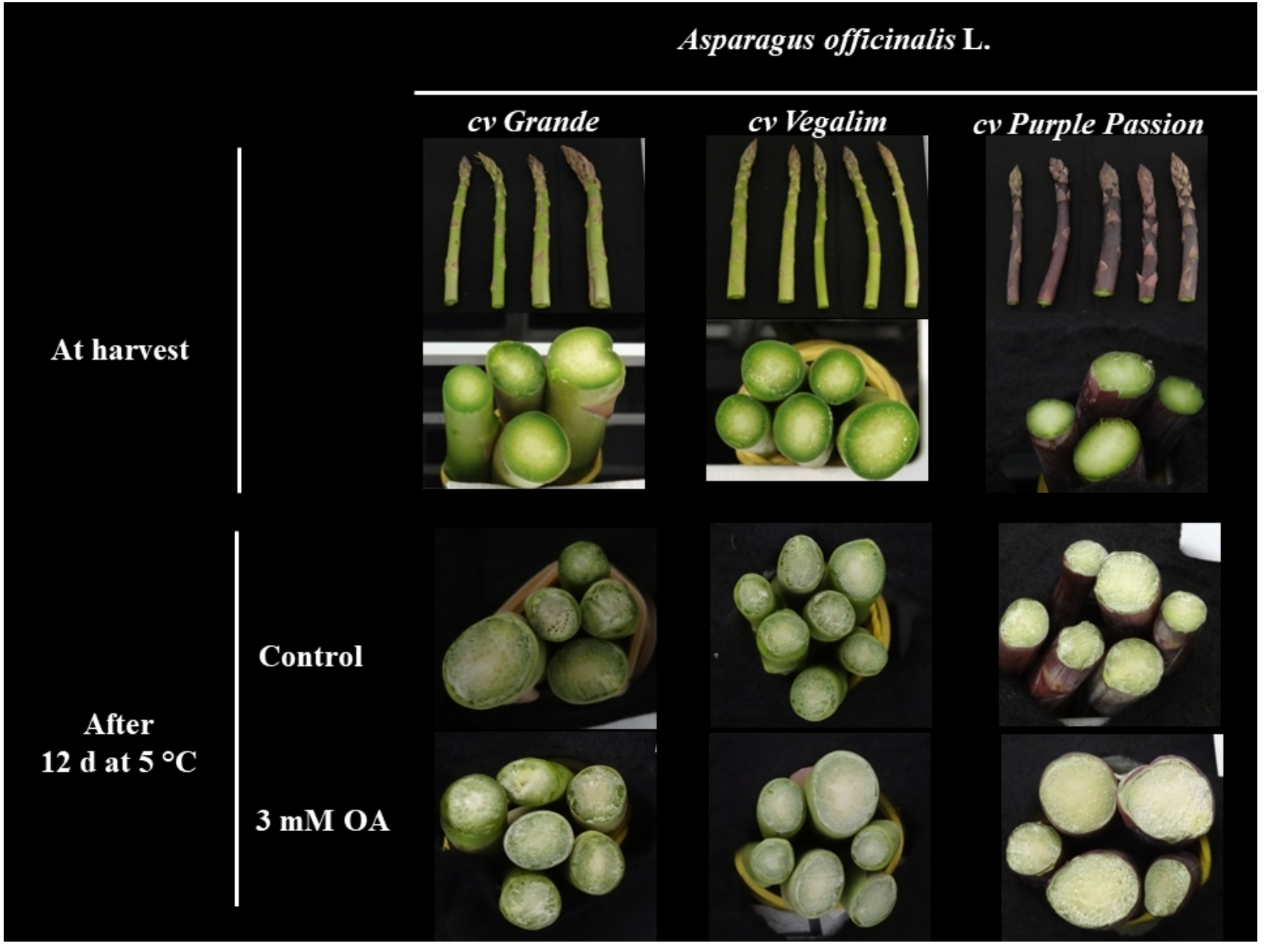
627 Figure 3. Effect of 1 mM and 3 mM oxalic acid (OA) treatment on cut end dehydration of asparagus
628 spears cultivars (Grande, Vegalim and Purple Passion) stored at 5 °C. Mean ± standard deviation
629 (SD). Cut-end dehydration was attributed on a rating scale where 1 = none, just after cut; 2 = slight;
630 3 = moderate; 4 = severe; 5 = extreme. Unlike letters differ statistically by the Student-Newman-
631 Keuls (SNK) at $P \leq 0.05$.

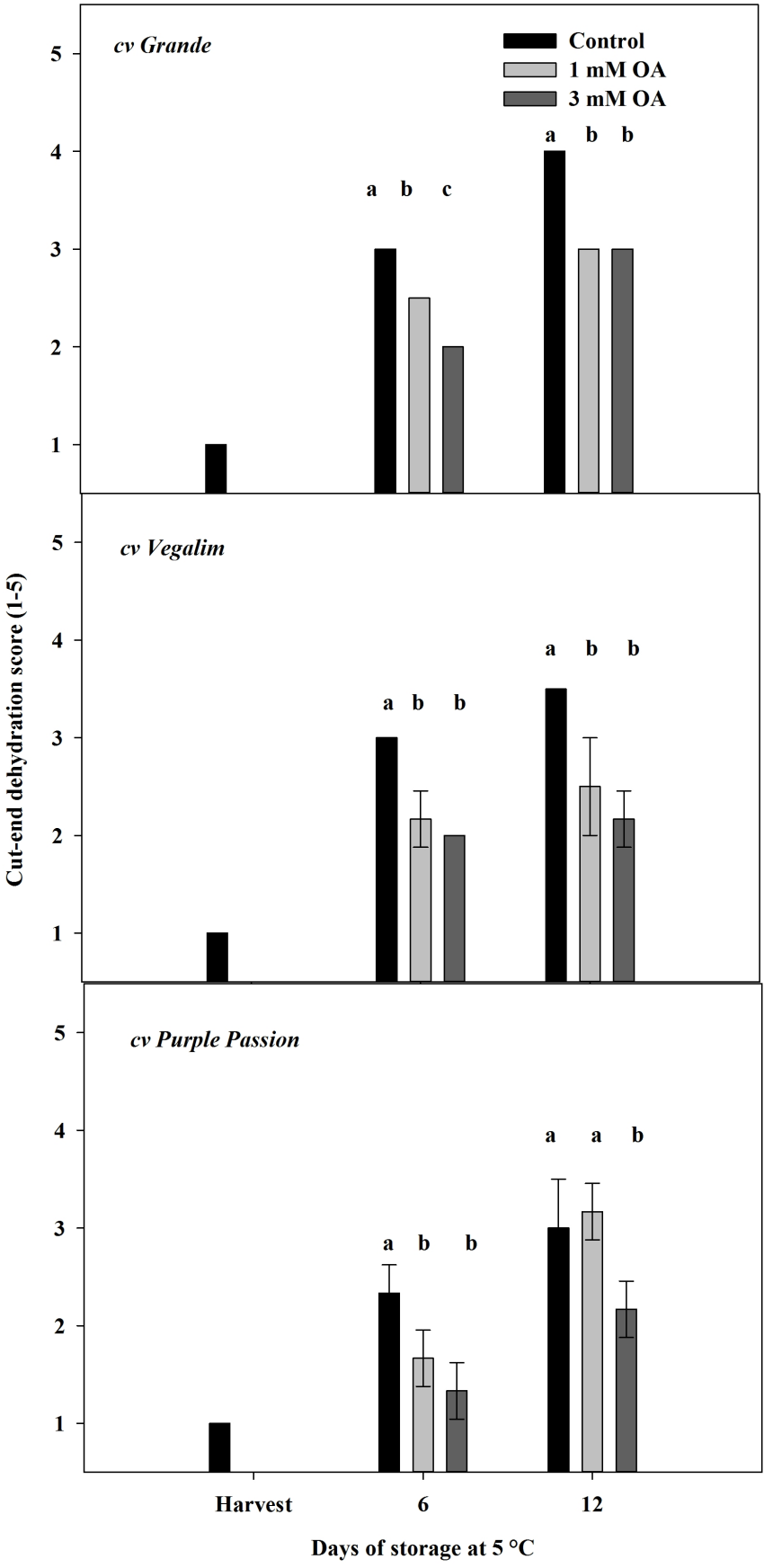
632

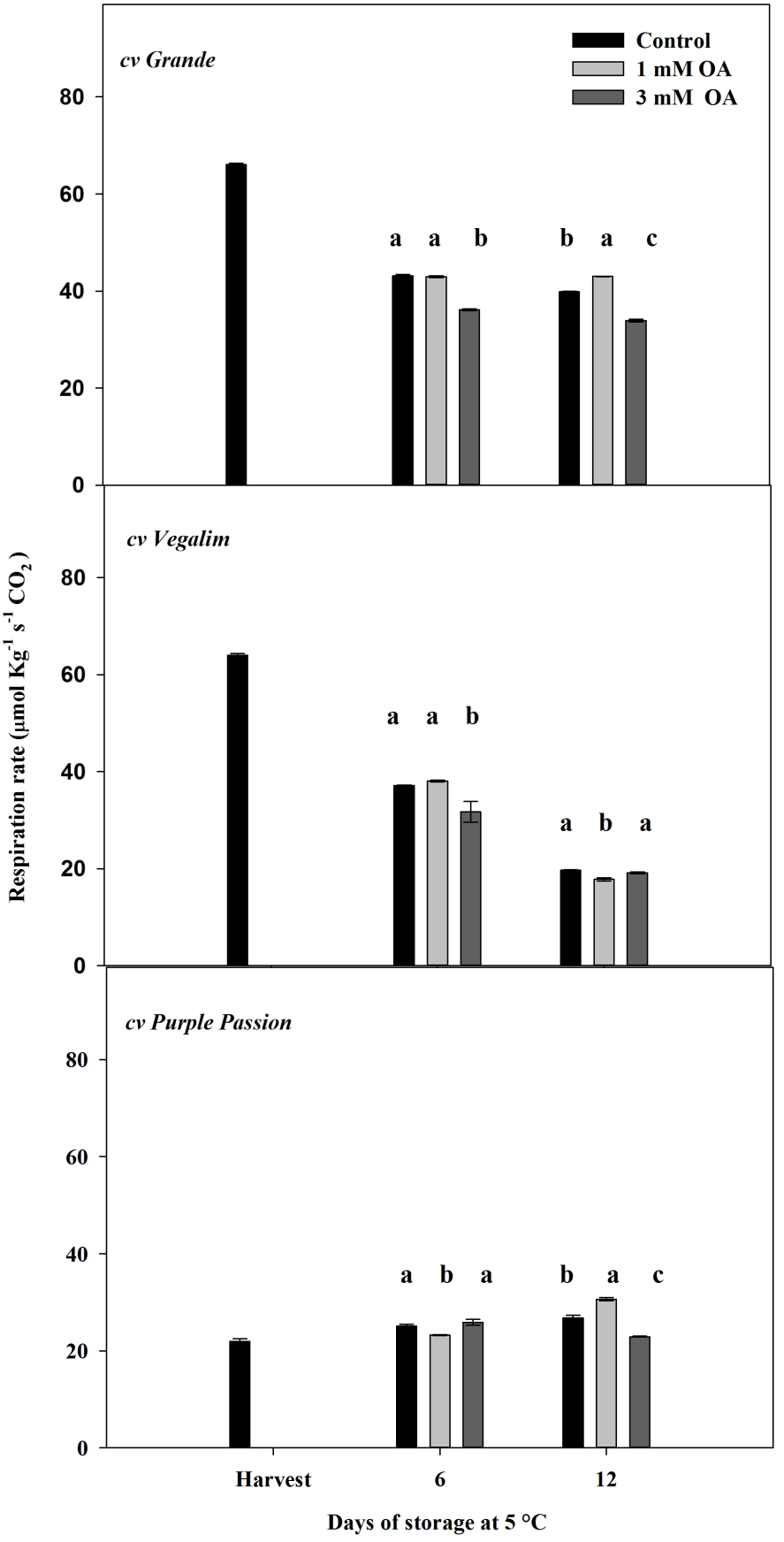
633 Figure 4. Effect of 1 mM and 3mM oxalic acid (OA) treatment on respiration rate of asparagus spears
634 of Grande, Vegalim and Purple Passion cultivars stored at 5 °C. Mean ± standard deviation (SD).
635 Unlike letters differ statistically by the Student-Newman-Keuls (SNK) at $P \leq 0.05$.

Figure 5. HPLC-DAD chromatograms of phenolic compounds detected, at harvest, at 320 nm on Grande, Vegalim and Purple Passion I asparagus spears, and of anthocyanin compounds detected at 520 nm on Purple passion II asparagus spears. Feruloylquinic acid Isomer I (A); Cumaroylquinic acid Isomer II (B); Feruloylquinic acid Isomer II (C); Feruloylquinic acid Isomer III (D); Cyanidinglucosylrutinoside (E); Cyanidinrutinoside (F); Peonidinrutinoside (G); Quercetin glucosylrutinoside (H); Quercetin rutinoside (I); Kaempferolrutinoside (L); Isorhamnetinrutinoside (M); Dioscin (N); Quercetin glucoside (O).


cv Grande
Control
1 mM OA
3 mM OA
a a a
b a a
cv Vegalim
a a a
b b a
cv Purple Passion
a a a
b b a
Overall appearance (5-1)
5
4
3
2
1
Harvest
6
12
Days of storage at 5 °C


Asparagus officinalis L.
cv Grande
cv Vegalim
cv Purple Passion
At harvest
After
12 d at 5 °C
Control
3 mM OA


cv Grande
Control
1 mM OA
3 mM OA
a b c
a b b
cv Vegalim
a b b
a b b
cv Purple Passion
a b b
a a b
Cut-end dehydration score (1-5)
1
2
3
4
5
Harvest
6
12
Days of storage at 5 °C


cv Grande
Control
1 mM OA
3 mM OA
a a b
b a c
cv Vegalim
a a b
a b a
cv Purple Passion
a b a
b a c
Respiration rate (µmol Kg-1 s-1 CO2)
0
20
40
60
80
Harvest
6
12
Days of storage at 5 °C

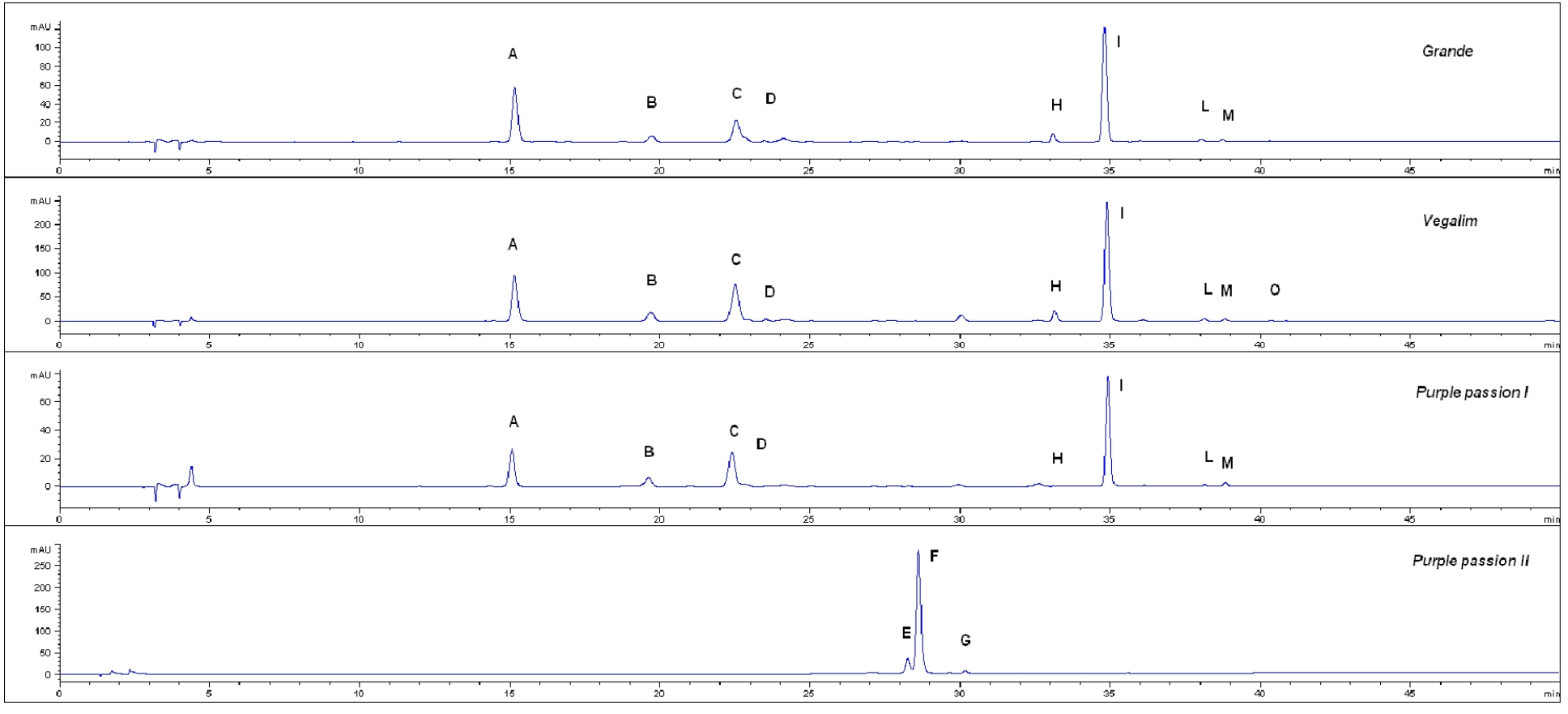

Grande
A
B
C
D
H
I
L
M
Vegalim
A
B
C
D
H
I
L
M
O
Purple passion I
A
B
C
D
H
I
L
M
Purple passion II
E
F
G
mAU
min

Table 1. Total phenolic content in fresh and cold stored asparagus spears of *cv* Grande, Vegalim and Purple Passion treated with oxalic acid (OA). Results are expressed as mg kg^{-1} GA on a fresh weight basis.

Storage time	OA treatment	Total phenols (mg kg^{-1} GA)		
		Grande	*Vegalim*	*Purple Passion*
Harvest		836 ± 50	991 ± 10	700± 60
6 d	Control	878 ± 30 b	1161 ± 50 a	937± 40
	1 mM	921 ± 110 b	1019 ± 40 b	833 ± 10
	3 mM	1256 ± 70 a	846 ± 100 c	855 ± 80
		*	*	n.s.
12 d	Control	1214 ± 90	824 ± 30	954 ± 120
	1 mM	1232 ± 110	851 ± 20	795 ± 40
	3 mM	1138 ± 20	856 ± 60	826 ± 20
		n.s.	n.s.	n.s.

Significance is related to the differences among treatments within each storage time. * = significant at $P \leq 0.05$. Values are mean of 3 replicates ± standard deviation. Means separation was performed by Newman-Keuls (SNK) test.

Table 2. ESI HRMS data for phenolic compounds characterized on Grande, Vegalim, and Purple passion asparagus spears cultivars.

Peak	RT (min)	Molecular formula	m/z calculated	m/z experimental	Δ (ppm)	Species	Cultivars	Compound
1	8,15	$C_{16}H_{18}O_8$	337,0929	337,0934	-1,3349	$[M-H]^-$	all	Coumaroylquinic acid isomer I
2	9,49	$C_{16}H_{18}O_9$	353,0878	353,0875	0,8496	$[M-H]^-$	all	5-Caffeoylquinic acid
3	9,99	$C_{33}H_{40}O_{21}$	771,1989	771,1993	-0,5705	$[M-H]^-$	all	Quercetin galattosyl rutinoside
4	10,1	$C_{33}H_{41}O_{21}$	773,2135	773,2141	-0,8018	$[M+H]^+$	Purple	Cyanidin 3-sophoroside-5-glucoside
5	10,36	$C_{17}H_{20}O_9$	367,1034	367,1032	0,5448	$[M-H]^-$	all	Feruloylquinic acid isomer I
6	10,55	$C_{33}H_{41}O_{20}$	757,2186	757,2177	1,2546	$[M+H]^+$	Purple	Cyanidin 3-O-glucosyl-rutinoside
7	11,02	$C_{27}H_{31}O_{15}$	595,1736	595,1698	6,2839	$[M+H]^+$	Purple	Cyanidin 3-O-rutinoside
8	12,9	$C_{16}H_{18}O_8$	337,0929	337,0932	-0,7416	$[M-H]^-$	all	Coumaroylquinic acid isomer II
9	12,91	$C_{22}H_{23}O_{11}$	464,1314	464,1293	4,4169	$[M+H]^+$	Purple	Peonidin 3-O glucoside
10	13,73	$C_{28}H_{33}O_{15}$	610,1892	610,1862	4,9329	$[M+H]^+$	Purple	Peonidin 3-O rutinoside
11	14,65	$C_{16}H_{18}O_9$	337,0929	337,0938	-2,6699	$[M-H]^-$	all	Coumaroylquinic acid isomer III
12	15,38	$C_{17}H_{20}O_9$	367,1034	367,1033	0,2724	$[M-H]^-$	all	Feruloylquinic acid isomer III
13	15,89	$C_{16}H_{18}O_9$	353,0878	353,0880	-0,5664	$[M-H]^-$	all	3- Caffeoylquinic acid
14	16,85	$C_{17}H_{20}O_9$	367,1034	367,1039	-1,3620	$[M-H]^-$	all	Feruloylquinic acid isomer III
15	20,89	$C_{17}H_{20}O_9$	367,1034	367,1037	-0,8172	$[M-H]^-$	all	Feruloylquinic acid isomer IV
16	21,51	$C_{25}H_{24}O_{12}$	515,1195	515,1213	-3,4943	$[M-H]^-$	all	3,5-O Dicaffeoylquinic acid
17	22,17	$C_{25}H_{24}O_{12}$	515,1195	515,1209	-2,7178	$[M-H]^-$	all	1,3-O-Dicaffeoylquinic acid
18	23,12	$C_{33}H_{40}O_{21}$	771,1989	771,2005	-2,0747	$[M-H]^-$	all	Quercetin glucosyl rutinoside
19	24,19	$C_{27}H_{30}O_{16}$	609,1461	609,1450	1,8058	$[M-H]^-$	all	Quercetin 3-O galattosyl rhamnoside
20	25,4	$C_{27}H_{30}O_{16}$	609,1461	609,1456	0,8208	$[M-H]^-$	all	Quercetin 3-O rutinoside

21	25,96	$C_{50}H_{84}O_{23}$	1051,5331	1051,5270	5,8011	$[M-H]^-$	all	Asparanina B9
22	26,88	$C_{21}H_{20}O_{12}$	463,0882	463,0885	-0,6478	$[M-H]^-$	all	Quercetin 3- O glucoside
23	27,96	$C_{34}H_{42}O_{21}$	785,2145	785,2152	-0,8915	$[M-H]^-$	all	Isorhamnetin glucosyl rutinoside
24	29,36	$C_{27}H_{30}O_{15}$	593,1512	593,1517	-0,8430	$[M-H]^-$	all	Kaempferol 3-O glucoside
25	30,18	$C_{28}H_{32}O_{16}$	623,1618	623,1621	-0,4814	$[M-H]^-$	all	Isorhamnetin rutinoside
26	31,41	$C_{45}H_{72}O_{16}$	867,4747	867,4699	5,5333	$[M-H]^-$	all	Dioscin
27	31,74	$C_{33}H_{56}O_{14}$	675,3597	675,3608	-1,6288	$[M-H]^-$	all	Ginger glicolipid A
28	35,46	$C_{51}H_{90}O_7$	815,6569	815,6532	4,5362	$[M-H]^-$	all	ß Sitosteryl-glucoside 6- palmitate
29	36,21	$C_{45}H_{76}O_{18}$	903,4958	903,4902	6,1981	$[M-H]^-$	all	ASP V
30	39,32	$C_{51}H_{82}O_{21}$	1029,5381	1029,5426	-4,3709	$[M-H]^-$	all	Pseudo-protodioscin
31	43,94	$C_{18}H_{32}O_5$	327,2177	327,2172	1,5280	$[M-H]^-$	all	Trihydroxy-octadecadienoic acid isomer
32	44,3	$C_{18}H_{32}O_5$	329,2233	329,2274	-12,3624	$[M-H]^-$	all	Trihydroxy-octadecaenoic acid isomer
33	44,7	$C_{18}H_{34}O_5$	327,2177	327,2174	0,9168	$[M-H]^-$	all	Trihydroxy-octadecadienoic acid isomer
34	45,09	$C_{18}H_{32}O_5$	327,2177	327,2177	0,0000	$[M-H]^-$	all	Trihydroxy-octadecadienoic acid isomer
35	46,42	$C_{18}H_{34}O_5$	329,2233	329,2229	1,2150	$[M-H]^-$	all	Trihydroxy-octadecaenoic acid isomer
36	46,94	$C_{18}H_{34}O_5$	329,2233	329,2228	1,5187	$[M-H]^-$	all	Trihydroxy-octadecaenoic acid isomer
37	49,58	$C_{18}H_{34}O_5$	329,2233	329,2232	0,3037	$[M-H]^-$	all	Trihydroxy-octadecaenoic acid isomer
38	52,5	$C_{18}H_{32}O_4$	311,2228	311,0000	715,8859	$[M-H]^-$	all	13- Hydroperoxy-octadecadienoic acid isomer
39	53,88	$C_{18}H_{34}O_4$	313,2384	313,0000	761,0817	$[M-H]^-$	all	12,13- Dihydroperoxy-octadecadienoic acid isomer

Table 3. Evolution of quantified phenols (mg kg^{-1}) of the three asparagus cultivars (Grande, Vegalim and Purple Passion) during storage (0 d, 6 d and 12 d at 5 °C) and after oxalic acid (OA) treatment (1 mM or 3 mM). Results are expressed as mg kg^{-1} on a fresh weight basis.

cv *Grande*													
Storage	Oxalic acid (mM)	Feruloyl quinic acid Isomer I (A)	Cumaroyl quinic acid Isomer II (B)	Feruloyl quinic acid Isomer II (C)	Feruloyl quinic acid Isomer III (D)	Quercetin glucosyl rutinoside (H)	Quercetin rutinoside (I)	Kaempferol rutinoside (L)	Isorhamnetin rutinoside (M)	Dioscin (N)	Anthocyanins		
Harvest		189.6 ± 22	44.9 ± 4	120.2 ± 19	41.6 ± 7	41.9 ± 6	571.7 ± 82.1	37.4 ± 2	34.5 ± 2	0.00 ± 0	-	-	-
6 d	0	227.3 ± 25	56.3 ± 13	224.0 ± 10	49.7 ± 6	44.8 ± 6	1120.0 ± 315	56.6 ± 7	42.7 ± 5	0.00 ± 0	-	-	-
	1 mM	279.3 ± 20	70.5 ± 14	370.4 ± 11	53.3 ± 6	63.9 ± 10	972.4 ± 14,2	53.2 ± 6	61.5 ± 3	31.2 ± 6	-	-	-
	3 mM	485.8 ± 78	119.3 ± 5	729.9 ± 93	62.5 ± 22	27.1 ± 2	2135.4 ± 0,0	88.0 ± 0	93.1 ± 6	10.7 ± 1.9	-	-	-
12 d	0	297.5 ± 61	113.3 ± 2	669.3 ± 15.6	47.0 ± 7	39.1 ± 2,9	1561.1 ± 138	63.8 ± 12	111.6 ± 6	110.9 ± 11.1	-	-	-
	1 mM	253.4 ± 1	97.7 ± 10	868.1 ± 83	49.8 ± 6	62.8 ± 6	1872.3 ± 41	76.6 ± 9	123.8 ± 4	0.00 ± 0	-	-	-
	3 mM	361.9 ± 12	73.9 ± 0	616.9 ± 52	43.9 ± 8	70.2 ± 19	1698.2 ± 135	86.5 ± 9	75.4 ± 6	103.7 ± 18.0	-	-	-

cv *Vegalim*													
Storage	Oxalic acid (mM)	Feruloyl quinic acid Isomer I (A)	Cumaroyl quinic acid Isomer II (B)	Feruloyl quinic acid Isomer II (C)	Feruloyl quinic acid Isomer III (D)	Quercetin glucosyl rutinoside (H)	Quercetin rutinoside (I)	Kaempferol rutinoside (L)	Isorhamnetin rutinoside (M)	Quercetin glucoside (O)	Anthocyanins		
Harvest		283.0 ± 24	107.5 ± 17	231.1 ± 5	32.0 ± 4	99.1 ± 4	1227.4 ± 46	53.2 ± 3	44.5 ± 5	38.1 ± 0.0	-	-	-
											-	-	
6 d	0	211.5 ± 39	290.1 ± 35	295.5 ± 27	38.4 ± 1	122.0 ± 18	1530.6 ± 17	64.8 ± 15	83.2 ± 18	37.2 ± 3	-	-	-
	1 mM	97.2 ± 8	370.4 ± 76	249.9 ± 35	32.4 ± 0	128.1 ± 21	1058.1 ± 5	47.0 ± 4	76.4 ± 4	31.0 ± 2	-	-	-
	3 mM	168.9 ± 4	253.0 ± 1	246.2 ± 7	34.0 ± 2	99.2 ± 9	1208.1 ± 58	57.1 ± 5	75.5 ± 23	32.8 ± 0	-	-	-
											-	-	
12 d	0	70.6 ± 13	311.6 ± 47	163.7 ± 31	35.1 ± 0	113.5 ± 15	1497.3 ± 81	61.5 ± 7	105.9 ± 4	34.4 ± 3	-	-	-
	1 mM	100.0 ± 7	471.2 ± 49	173.1 ± 3	39.2 ± 9	100.4 ± 6	1455.6 ± 9	64.1 ± 4	88.3 ± 12	33.0 ± 3	-	-	-
	3 mM	139.5 ± 3	175.0 ± 3	315.7 ± 9	29.5 ± 3	122.4 ± 15	1169.5 ± 48	56.7 ± 3	66.8 ± 3	32.2 ± 2	-	-	-

cv *Purple Passion*

Storage	Oxalic acid (mM)	Feruloyl quinic acid Isomer I (A)	Cumaroyl quinic acid Isomer II (B)	Feruloyl quinic acid Isomer II (C)	Feruloyl quinic acid Isomer III (D)	Quercetin glucosyl rutinoside (H)	Quercetin rutinoside (I)	Kaempferol rutinoside (L)	Isorhamnetin rutinoside (M)	Quercetin glucoside (O)	Cyanidin glucosyl rutinoside (E)	Cyanidin rutinoside (F)	Peonidin rutinoside (G)
Harvest		-	42.0 ± 6	118.1 ± 21	0.0 ± 0.0	17.7 ± 1	345.1 ± 31	27.0 ± 1	32.3 ± 2	0.0 ± 0.0	125.5 ± 3	774.2 ± 30	84.8 ± 3
		-											
6 d	0	-	47.3 ± 2	232.7 ± 47	14.5 ± 2.5	31.2 ± 4	659.3 ± 1	30.4 ± 5	55.9 ± 1	32.7 ± 8	224.5 ± 3	812.3 ± 40	79.7 ± 2
	1 mM	-	49.6 ± 4	138.2 ± 16	11.6 ± 2	39.7 ± 2	641.9 ± 138	32.3 ± 9	41.8 ± 1	11.6 ± 2	261.2 ± 1	675.1 ± 16	81.7 ± 3
	3 mM	-	53.5 ± 6	216.2 ± 61	0.0 ± 0.0	30.9 ± 5	1040.2 ± 28	32.1 ± 2	55.8 ± 10	9.0 ± 1.6	270.1 ± 2	648.9 ± 15	79.9 ± 4
		-											
12 d	0	-	76.3 ± 8	241.8 ± 36	0.0 ± 0.0	80.7 ± 17	1298.2 ± 38	38.4 ± 3	53.1 ± 4	10.1 ± 1.7	214.9 ± 4	606.8 ± 28	69.5 ± 6
	1 mM	-	55.9 ± 7	342.8 ± 2	0.0 ± 0.0	61.8 ± 2	1244.9 ± 110	36.0 ± 4	70.7 ± 9	19.8 ± 1.7	237.1 ± 5	486.3 ± 26	73.0 ± 5
	3 mM	-	50.5 ± 5	239.5 ± 20	0.0 ± 0.0	81.5 ± 1	1090.3 ± 110	35.0 ± 3	52.5 ± 6	0.0 ± 0.0	286.9 ± 9	511.5 ± 18	76.7 ± 5

Table 4. Ascorbic acid (AA) content (mg kg^{-1}) as determined by titrimetric method (AA^1) and by SB-FC_{60} at +500 mV (AA^2), antioxidant activity (ascorbic acid equivalents) as determined by SB-FC_{60} at +500 mV, and antiradical activity (trolox equivalents) measured in the extracts of Grande, Vegalim and Purple Passion cultivars of asparagus stored at 5 °C. AA and phenols contribution to antioxidant capacity were also provided. Results are expressed on a fresh weight basis.

Table 3a (cv *Grande*)	**AA^1** (mg kg^{-1})	**AA^2** (mg kg^{-1})	**Antioxidant activity** (mmol kg^{-1} AA equivalents)	**Antioxidant activity due to AA**	**Antioxidant activity due to phenols**	**Antiradical activity** (mmol kg^{-1}trolox equivalents)
Harvest	328.1	326.5	3.45	2.36	1.09	2.63
Storage (d)						
0	317.2 a	316.1 a	3.32 a	2.20 a	1.12 a	3.26 a
6	327.3 a	305.6 a	3.25 a	2.15 a	1.10 a	3.18 a
12	277.0 b	285.6 b	2.70 b	1.77 b	0.93 b	2.98 b
Oxalic acid						
Control	314.3 n.s.	312.8 n.s.	3.07 n.s.	2.01 n.s.	1.06 n.s.	3.25 n.s.
1 mM	303.7 n.s.	322.9 n.s.	3.11 n.s.	2.06 n.s.	1.05 n.s.	3.46 n.s.
3 mM	313.5 n.s.	301.6 n.s.	3.09 n.s.	2.05 n.s.	1.04 n.s.	3.40 n.s.

Table 3b (cv *Vegalim*)	**AA^1** (mg kg^{-1})	**AA^2** (mg kg^{-1})	**Antioxidant activity** (mmol kg^{-1}AA equivalents)	**Antioxidant activity due to AA**	**Antioxidant activity due to phenols**	**Antiradical activity** (mmol kg^{-1}trolox equivalents)
Harvest	398.5	394.8	4.68	3.22	1.46	2.68
Storage (d)						
0	397.2 a	394.7 a	4.76 a	3.13 a	1.63 a	2.26 ab
6	384.3 b	401.6 a	4.70 a	3.10 a	1.60 a	2.41 a
12	348.0 c	335.4 b	3.73 b	2.45 b	1.28 b	1.97 b
Oxalic acid						
Control	369.9 n.s.	377.4 n.s.	4.38 n.s.	2.87 n.s.	1.51 n.s.	2.63 a
1 mM	380.0 n.s.	366.8 n.s.	4.40 n.s.	2.89 n.s.	1.52 n.s.	2.32 b
3 mM	368.6 n.s.	367.5 n.s.	4.41 n.s.	2.92 n.s.	1.49 n.s.	2.47 ab

Table 3c (cv *Purple Passion*)	**AA^1** (mg kg^{-1})	**AA^2** (mg kg^{-1})	**Antioxidant activity** (mmol kg^{-1}AA equivalents)	**Antioxidant activity due to AA**	**Antioxidant activity due to phenols**	**Antiradical activity** (mmol kg^{-1} trolox equivalents)
Harvest	-	308.8	3.66	1.30	2.34	3.73
Storage (d)						
0	-	289.7 a	3.69 a	1.25 a	2.44 a	3.67 a
6	-	297.2 a	3.71 a	1.37 a	2.34 a	3.51 b
12	-	248.8 b	2.93 b	1.12 b	1.80 b	3.40 b
Oxalic acid						
Control	-	291.0 n.s.	3.46 n.s.	1.36 n.s.	2.11 n.s.	3.25 n.s.
1 mM	-	302.6 n.s.	3.43 n.s.	1.24 n.s.	2.19 n.s.	3.27 n.s.
3 mM	-	289.1 n.s.	3.42 n.s.	1.15 n.s.	2.28 n.s.	3.05 n.s.

Means followed by unlike letters differ significantly by Newman-Keuls (SNK) test, $P \leq 0.05$.
(*) AA content of cultivar Purple Passion could not be determined by titrimetric method since samples were coloured. The presence of red pigments negatively influenced the results of titrimetric analysis based on colour change of redox indicators (Barberis et al., 2014).