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Watercore in 'Pomella Genovese' apples: quality characteristics and antioxidants

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Abstract: The study aimed to evaluate apple fruit affected by watercore by a physical and biochemical point of view and, at the same time, to gain an insight into the mechanisms of the watercore-related oxidative stress and browning. Fruit of the cv. Pomella Genovese (*Malus x domestica* Borkh.) were harvested in three different orchards and stored at 1°C (85-90% RH) for 4 months. The following analysis were performed on the fruit flesh: density, mechanical (firmness and stiffness) and acoustic (crispness) parameters, soluble solids content (SSC), titratable acidity, ascorbic acid (AA), dehydroascorbic acid (DHA), total phenols and antioxidant activity (DPPH). In all the three orchards, fruit affected by watercore (W-Fruit) had a higher density and SSC than watercore-free ones (WF-fruit), probably because of the sugar-rich liquid that accumulates in the intercellular spaces. The peel colour of the W-fruit was darker, their flesh was firmer and crispier and the content in total phenols increased with respect to the WF samples. Watercore led to a decrease of AA and to an increase of DHA, probably caused by an imbalance of the ascorbic-glutathione cycle. The altered AA/DHA ratio can indicate an oxidative stress status of the fruit. DPPH was higher in W fruit and was related to the phenol content (r=0.83) but not with AA.

1. Introduction

Watercore is a serious disorder that occurs in apple fruit when still on the tree. It is characterized by water-soaked and translucent areas which are often associated with the vascular bundles of the core line, but in some cases it can affect the entire fruit (Williams, 1966). A very long list of watercore-susceptible varieties is reported by Marlow and Loescher (1984). Some of the most well-known cultivar are 'Fuji', 'Gloster' (Zupan *et al*., 2016) and 'Delicious' but this disorder affects also local "old" cultivars, such as 'Pomella Genovese'.

'Pomella Genovese' is a typical cultivar of the Pavia territory (Italy),

where the production is around 500-1000 q. It is a very rustic scab-resistant variety that, in December 2019, was included in the list of the traditional food products of the Lombardy region (Regione Lombardia, 2019). It has a white, firm, juicy, sweet and very aromatic flesh; it is usually harvested in October and can bestored in air until April. Unfortunately, 'Pomella Genovese' is highly susceptible to watercore, which affects around 20-30% of the total production preventing the diffusion of this cultivar.

The onset of watercore can be affected by many factors (high or very low fruit temperatures, high day/night fluctuation, high nitrogen levels, high source-to-sink ratio, advanced maturity stage) (Marlow and Loescher, 1984) and the disorder is not visible from outside the fruit. For this reason, a high number of fruits are often needed to carry out studies about watercore.

Though watercore was described several years ago (Faust *et al*., 1969) and many studies were carried out on the subject, its origin is still not completely clear. The cause of the disorder is the accumulation of sugar-rich liquid in the intercellular spaces. There have been proposed different theories to explain this phenomenon and the most reliable hypothesis seems to be related to an accumulation of sorbitol, which is the main transport sugar in the plant (Loescher *et al*., 2005). In tissues affected by watercore, sorbitol, that is unloaded from the phloem, is inhibited from being absorbed by the fruit cells (Gao *et al.*, 2005) and accumulates in the intercellular spaces, causing an increase of the osmotic potential that promotes water retention (Williams, 1966).

Watercore can regress in the first months of storage (Brackmann *et al*., 2001; Kasai and Arakawa, 2010; Neuwald *et al*., 2012) but often several disorders, including browning and brown core, have been observed in affected fruits (Argenta *et al*., 2002). 'Delicious' apples can develop brown core while fruit with medium to slight watercore tends to develop flesh browning (Fukuda, 1984). However, the mechanism of browning remains unclear. Lee *et al.* (2012) suggested that the accumulation of amino acids, acetaldehyde and ethanol, which increase under anaerobic conditions, can cause flesh browning. Other authors (Hulme, 1956) indicate that the accumulation of succinic acid, due to the inhibition of the succinate dehydrogenase activity under high CO_2 conditions, might account for the development of brown heart in apples.

Another hypothesis is linked to oxidative stress. Watercored tissue has a lower intercellular air space volume, reduced permeance to gas diffusion and increased internal CO_2 level (Argenta *et al*., 2002). Under low O_2 or high CO_2 pressure, reactive oxygen species (ROS) like H_2O_2 or O_2^- are produced in the fruit. If the ROS production exceeds the scavenging capacity of the system, fruit undergoes oxidative stress, cell membrane are damaged and phenolic compounds oxidized (Zupan, 2016).

The ascorbate-glutathione cycle is one of the main scavenger systems in plants (Arora *et al*., 2002). During the reduction reaction of H_2O_2 to H_2O, ascorbic acid (AA) is oxidized by ascorbate peroxidase (APX) to monodehydroascorbic acid (MDHA) which is an unstable compound and, if not reduced rapidly again to AA (by MDHA reductase), it can be converted to dehydroascorbic acid (DHA). DHA is reduced to AA by DHA reductase using glutathione as electron donor, while the oxidized form of glutathione (GSSG) is reduced back to glutathione by the glutathione reductase (Lum *et al*., 2016). Under stress conditions caused by low oxygen pressure, the ascorbate-glutathione recycling pathway is dysfunctional and AA content decreases while there is an increase in DHA. Kasai and Arakawa (2010), in Fuji apples affected by watercore, observed a decrease in AA and in APX activity but no increase in DHA, and hypothesized that DHA was hydrolysed in the tissue.

This study aimed to characterize watercore-affected fruit through biochemical and physical analyses and, at the same time, to gain an insight into the mechanisms of watercore-related oxidative stress that leads to tissue browning. The study was carried out on 'Pomella Genovese' apples since this cultivar showed a high percentage of fruit strongly affected by watercore, thus providing a suitable material for the analyses. In order to figure out if the properties of watercore-fruit where somewhat affected by the growing conditions or were, instead, mainly due to the onset of the disorder, apples were harvested from three experimental orchards characterized by different pedoclimatic conditions.

Since this disorder can dissipate in the first months after harvest (Neuwald *et al.*, 2012), fruit analysed too early could be only affected by a "temporary" form of watercore. To overcome this problem, the analyses on the fruit were carried out after 4 months of cold storage, when the disorder was in its stable form and browning symptoms were still at an early stage.

2. Materials and Methods

Raw material

Fruit of the cv. Pomella Genovese (*Malus x domestica*, Borkh.) were harvested in the Staffora valley (Pavia-Italy) in three orchards with different characteristics (Table 1). Immediately after harvest, apples were transported to the CREA-IT laboratory in Milan where a sample of 30 fruit from each orchard was analysed for the standard maturity indices (firmness, soluble solids content, acidity) (Table 2).

Apples were then stored at 1°C (90% R.H.) for 4 months and analysed after 1 day at 20°C. Fifty fruit watercore-free (WF) and 50 fruit affected by different levels of watercore symptoms (W) (Fig. 1) per each orchard, were assessed for quality parameters (skin colour, texture, SSC, acidity), antioxidant compounds (total phenols, ascorbic and dehydroascorbic acid) and antioxidant activity.

Fruit were first subjected to skin colour and texture analysis, then equatorially cut and assigned to a treatment (watercore or watercore-free); finally, the fruit flesh was sampled (avoiding core and seeds), rapidly frozen and lyophilized for the subsequent analysis. Quality analyses were carried out on individual fruit while antioxidant compounds were assessed on pools of 3 lyophilized fruit.

Quality analyses

Acidity was assessed according to the AOAC official methods of analysis (AOAC, 1985). Soluble solids content (SSC) was measured by a multiscale automatic refractometer (mod. RFM91, BS, UK) on few drops of apple juice. A spectrophotometer CM-2600-D Minolta was used to perform the colour measurements : L* (lightness) a* (green-red), b* (yellow-blue) were assessed on the peel on 2 opposite sides of each fruit, avoiding the red blush and any external symptom of watercore, if present. The hue (h°) was calculated as arctangent (b*/a*).

The specific weight was calculated on each fruit as the ratio between the fruit weight (g) and its volume (cm^3) measured underwater.

Fruit mechanical and acoustic parameters were measured on two peeled areas of each fruit using a TA-XT plus Texture Analyzer (Stable Micro Systems, Godalming, UK) coupled with an acoustic emission detector (AED, Stable Micro Systems). By means of this system, it was possible to simultaneously evaluate the mechanical force-displacement and the corresponding acoustic response. A puncture test with an 11 mm diameter cylindrical probe at the cross-head speed of 3.33 mm/s to a depth of 8 mm, allowed to obtain the mechanical profile; at the same time, the AED device, with a frequency cut-off set of 3.125 kHz, gave the acoustic response of the sample. The microphone was placed 10 mm far from the apple at the mid-height of the fruit. From the

Table 1 - Characteristics and average climatic data of the growing season (April-October) for the three orchards

Orchard	Altitude (m asl)	Training system	Planting distance (m)	Row orientation	Slope	Rootstock	Plants age (yrs)	Irrigation	Fertilization	Average Temp. (max/min. °C)	Total rainfall (mm)
A	342	Palmette	4 x 2	SW-NE	slight (<15%)	MM106	25	Emergency irrigation	Manure (winter) + NPK (spring)	14/25	533
B	392	Palmette	4 x 2	SW-NE	slight (<15%)	seedling	17	Emergency irrigation	Manure (winter) + NPK (spring)	15/23	610
C	579	Vase	one row	N-S	slight (<15%)	seedling	11	Emergency irrigation	Burial of crop residues	15/24	547

Table 2 - Maturity indices of 'Pomella Genovese' apples at harvest

Orchard	Firmnes (N)	SSC (%)	Acidity (g/l malic acid)
A	91.0 a	12.5 a	2.51 a
B	88.2 a	12.5 a	2.45 a
C	90.6 a	13.6 b	2.86 b

Different letters indicate significant differences (LSD test, P<0.01).

Fig. 1 - Fruit of 'Pomella Genovese' watercore-free (left) or affected by different levels of watercore (right).

combined mechanical/acoustic profile, a total of five parameters were chosen to describe the texture properties of 'Pomella Genovese' apples. Three mechanical parameters (FBK= the force measured at the breaking point of the flesh; St= the stiffness given by the ratio between the force applied and the deformation before the breaking point, Wr= Work, the energy related to flesh penetration up to 8 mm, given by the area under the force/deformation curve) and two acoustic parameters related with the flesh crispness (PK= the number of the acoustic peaks; ΣPK= the sum of all the peak values) were extracted (Fig. 2).

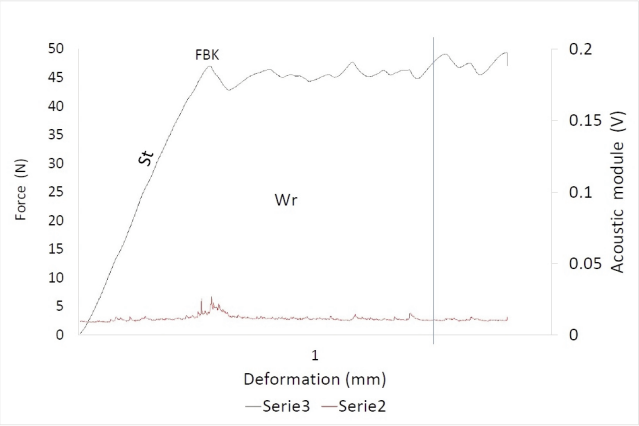


Fig. 2 - Force/deformation curve and acoustic profile of 'Pomella Genovese' apples. FBK= Force at the breaking point; Wr = Work; St= stiffness.

Analysis of antioxidants

Total phenols and antioxidant activity were assessed on the following extract: 50 mg of powder from lyophilized apple flesh were extracted with 1.5 mL of an Ethanol 96%/HCl 0.06N solution (1:1 v/v), vortexed for 30sec and centrifuged (12 min, 4°C, 10000 x *g*).The supernatant was recovered and stored a few days at -20°C until the analyses were carried out.

Total phenols content (TPC) was measured by the Folin-Ciocalteu method, as described by Singleton *et al.* (1999), 100 µL of fruit extract, 3 mL of distilled water and 500 mL Folin-Ciocalteu reagent were incubated for 3 min, followed by the addition of 2 mL 10% sodium carbonate. After 2 h in the dark, the absorbance was measured at 730 nm against blank with a V-630 spectrophotometer (Jasco, Japan). A calibration curve was built with different concentration of Gallic Acid and the polyphenols concentration of the samples was expressed as mg of gallic acid equivalent (GAE) per 100 g of fresh weight (FW).

Samples were also subjected to one of the most used free radical scavenging assays, which is relatively easy and cheap in execution and is strongly correlated with the sample's nutraceutical content: the 2,2-diphenyl-1-pycrilidrazyl ($DPPH^{\cdot}$) quenching (Brand-Williams *et al*., 1995). 600 µL of a 0.5 mM 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) ethanol solution, 2.0 mL ethanol and 200 µL of sample were put in a 1-cm path cuvette, and the absorbance at 517 nm against blank (2.5 mL ethanol and 100 µL of sample) was recorded at 0 s and 180 s. The inhibition percentage of DPPH was calculated as:

$$DPPH\ \% = [(A_{t0} - A_{t180}) / A_{t0}] \times 100$$

A calibration curve was built by using different dilutions of Trolox and the results were expressed as mg Trolox equivalents (TE) in 100 g FW.

Ascorbic (AA) and dehydroascorbic acid (DHA) were determined by HPLC according to Lo Scalzo *et al.* (2007) and Wechtersbach and Cigić (2007). Briefly, 50 mg of lyophilized powder were extracted with 1 ml of 3% metaphosphoric acid, vortexed for 30 s, centrifuged at 12,000 rpm for 20 min at 4°C and immediately analysed for AA content. For the determination of total AA (AA + DHA), the extracts were added with 100 mmol L^{-1} in HCl 1M of Tris-carboxyethyl phosphine (TCEP) as a reducing agent for DHA. 400 µL of extracts were, then, diluted 1:2.5 in a 0.02M ortophosphoric acid solution. AA was determined using a Jasco (Tokyo, Japan) HPLC system consisting of a PU-980 liquid chromatographic pump, a model AS 1055-10 auto sampler and an UV-Vis 15770 detector set at 254 nm, coupled to an Inertsil ODS-3 column (4.6 mm i.d. × 250 mm length, particle diameter 5 µm, GL Science) at the temperature of 30°C, and was eluted with 0.02 M orthophosphoric acid at a flow rate of 0.7 mL min-1.AA was estimated from a standard curve of L-ascorbic acid in 3% MPA. DHA content was calculated by subtracting the AA content from the total AA content. Data were expressed as mg per 100 g of fresh weight (mg AA or DHA 100 g FW^{-1}).

Statistical analysis

Statistical analyses were carried out with the Statgraphics software v.5.1 package (Manugistics, Rockwell MD). Data were submitted to multifactor ANOVA evaluating the main effects of the factors "watercore" and "orchard" as well as their interaction. Differences were determined by the Least

Significant Distance (LSD) test.

3. Results

Watercore-free and watercore-affected fruit revealed different quality characteristics. W-fruit always showed a darker (lower L*) and less yellow (lower b*) peel colour with respect to WF samples, while the parameters a* and h° were mostly affected by the orchard (Table 3). Fruit harvested in the orchard C had a less green (lower a*) and more yellow (lower h°) peel colour.

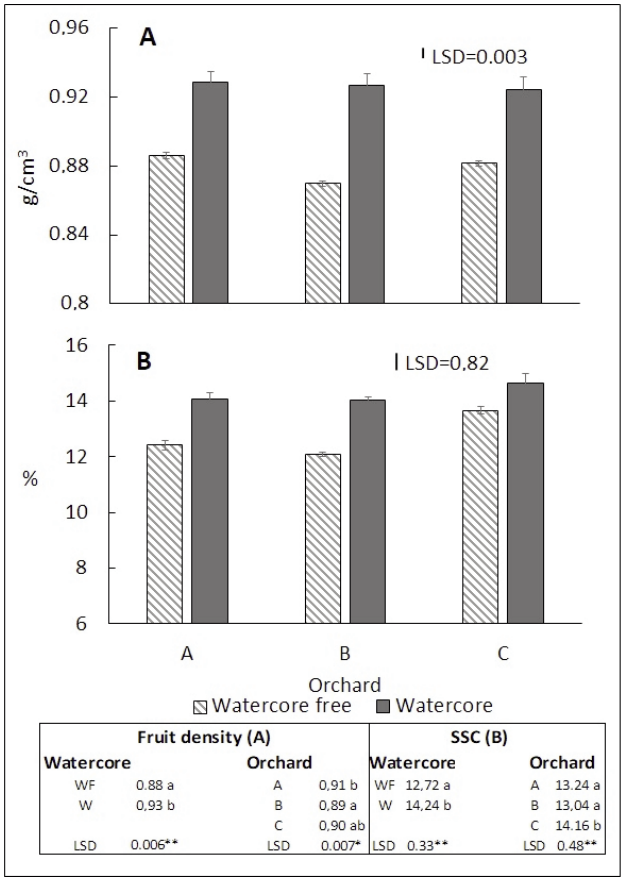
In all the three orchards, fruit density (Fig. 3A) was higher in the W-fruit than in the WF ones. Fruit from the orchard A showed, in average, the highest value, followed by those harvested in the orchard C and B.

This difference was mainly due to the variation in the fruit density among the WF fruit, while the values of W-samples were similar among each other.

The soluble solids content (Fig. 3B) was always higher in the W-fruit that showed, on average, an increase of 12% with respect to WF apples. Fruit harvested in the orchard C had the highest SSC, while those of A and B showed lower values without significant differences among each other. No differences among treatments were found for acidity (data not shown).

The mechanical parameters of the fruit were significantly affected by watercore (Table 4). W-fruit needed more energy (Wr) to penetrate the fruit flesh up to 8 mm and showed a higher value of FBK without any significant difference among fruit harvested in the three orchards.

Stiffness, which measure the "rigidity" of the flesh, was markedly higher in W fruit than in the WF ones. The acoustic parameters related to the flesh



Fruit density (A)				SSC (B)			
Watercore		Orchard		Watercore		Orchard	
WF	0.88 a	A	0,91 b	WF	12,72 a	A	13.24 a
W	0,93 b	B	0,89 a	W	14,24 b	B	13,04 a
		C	0,90 ab			C	14.16 b
LSD	0.006**	LSD	0.007*	LSD	0.33**	LSD	0.48**

Fig. 3 - Fruit density (A) and soluble solids content (B) of watercore-free (WF) and watercore (W) fruit of 'Pomella Genovese' from three different orchards. Average ± standard error. LSD is reported in each figure (P>0.01). In the box at the bottom the average and LSD values are indicated for each of the main factors (*=P<0.05; **=P<0.01).

crispness also showed important differences among the treatments. W-fruit always had more acoustic peaks and a higher sum of the peak value without any differences among orchards.

In fruit affected by watercore, the concentration of some antioxidant compounds changed. Regardless of the orchard, TPC increased, in average, 11.5% in W

Table 3 - Color parameters of watercore-free (WF) and watercore (W) fruit of 'Pomella Genovese'

Orchard	L*		a*		b*		Hue (*h**)	
	WF	W	WF	W	WF	W	WF	W
A	70.8	63.7	-10.9	-9.1	44.7	38.9	103.1	102.7
B	70.6	64.1	-13.7	-12.3	46.2	40.8	106.3	106.8
C	67.7	64.7	-8.1	-9.9	44.4	41.6	97.6	102.1
Mean	*69.7*	*64.2*	*-10.9*	*-10.4*	*45.1*	*40.4*	*102.4*	*103.9*
Main factors								
Watercore	1.41 **		NS		1.23 **		NS	
Orchard	NS		2.22 **		NS		3.43 **	
Orchard x watercore	2.94 **		3.99 **		2.93 **		5.11 **	

LSD values for the two main factors and their interaction are reported at the bottom (**=P<0.01).

Table 4 - Mechanical and acoustic parameters of watercore-free (WF) and watercore (W) fruit of 'Pomella Genovese'

Orchard	Mechanic						Acoustic			
	FBK (N)		WR (N x mm)		St (N/mm)		PK (n*)		∑PK (mV)	
	WF	W	WF	W	WF	W	WF	W	WF	W
A	44.3	57.4	79.6	109.6	59.7	72.8	2.8	5.4	0.09	0.21
B	42.6	60.3	76.6	113.2	61.2	77.9	2.4	5.1	0.08	0.19
C	46.0	65.7	83.6	125.3	61.5	79.0	3.0	5.4	0.09	0.18
Mean	*44.3*	*61.1*	*79.9*	*116.0*	*60.8*	*76.6*	*2.7*	*5.3*	*0.08*	*0.19*
Main factors										
Watercore	3.1 **		7.2 **		6.6 **		0.75 **		0.03 **	
Orchard	NS		NS		NS		NS		NS	
Orchard x watercore	6.5 **		19.4		3.3 **		1.56 **		0.06 **	

FBK= force at the breaking point; Wr= work; St= stiffness; PK= peak number; ΣPK= sum of peak values.
LSD values for the two main factors and their interaction are reported at the bottom (*=P<0.05; **=P<0.01).

fruit with respect to the WF samples (Fig. 4A). Fruit from the orchard C showed the highest value while no difference was found between A and B.

The average AA content (Fig. 5A) decreased by 30% in W-fruit with respect to WF ones. This effect was found, above all, in fruit from orchards A and B, while the decrease was not significant in the orchard C. The DHA content (Fig. 5B) increased markedly in fruit affected by watercore. W-fruit showed, on average, an increase in dehydroascorbic acid of about 40% with respect to WF-fruit. The ratio between ascorbic and dehydroascorbic acid was, on average, 1.2 for the WF-fruit and decreased by half (0.6) in the fruit affected by watercore.

Antioxidant activity (Fig. 4B) was always higher in watercore-affected fruit, regardless of the orchard. A high correlation coefficient was found between antioxidant activity and total polyphenol content (r=0.83) while no relationship was observed between antioxidant activity and ascorbic acid content.

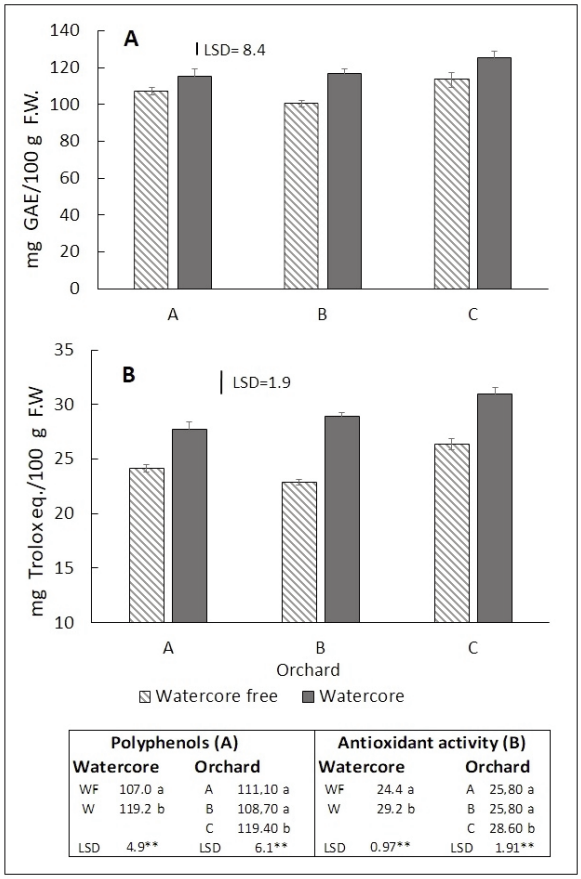


Fig. 4 - Total phenols content (A) and antioxidant activity (B) of watercore-free (WF) and watercore (W) fruit of 'Pomella Genovese'from three different orchards. Average ± standard error. LSD is reported in each figure (P>0.01). In the box at the bottom the averages and LSD values are indicated for each of the main factors (*=P<0.05; **=P<0.01).

4. Discussion and Conclusion

Despite the different field characteristics and plants training systems, W-fruit had always a lower lightness and were less yellow than WF apples, even if the measurement were taken avoiding any external symptom of watercore on the peel. Fruit from plants grown in the field C, had a more yellowish colour, probably because of the change in the light interception inside the canopy due to the different training systems and planting distances (Table 1). However, also in this case, peel colour (h° and b*) was significantly different between WF and W-fruit. According to the results of Watkins *et al*. (1993) on Fuji apples, background peel colour could be considered a possible indicator for watercore anyway, further studies are needed to confirm this hypothesis.

Fruit density of 'Pomella Genovese' W-samples was increased by the fluid accumulation in the intercellular spaces, which is considered a distinctive fea-

ture of watercore fruit (Bowen and Watkins, 1997; Suzuki *et al*., 2002; Zupan *et al*., 2016). The fruit density is usually cultivar-dependent (Yamada and Kobayashi, 1999) but, in this case, it was also dependent on the orchard. The variability in the fruit density makes it difficult to use some watercore detection methods which have been developed based on this fruit parameter (Cavalieri *et al*., 1998).

The liquid that accumulates in the intercellular spaces of watercore fruit is rich in sugars, above all sorbitol, which is the primary transport carbohydrate in apple (Loescher *et al*., 2005). Sorbitol is actively unloaded from the phloem, but a decreased ability to transport sugars into the fruit can lead to sorbitol accumulation in the intercellular spaces (Gao *et al*., 2005) that promotes water retention (Williams, 1966). Yamada and Kobayashi (1999) found an increase in sorbitol, as well as glucose, fructose and SSC in any cellular compartment (vacuole, cytoplasm, free spaces) of watercore-affected fruit, while other authors (Zupan *et al*., 2016) reported only an increase in sorbitol and a decrease in other sugars and in soluble solids content. In 'Pomella Genovese' apples, the SSC of W-fruit was well above the value of the WF samples and this may indicate an increase in all the main sugars of the fruit. Fruit from the orchard C had, on average, higher soluble solids content than A and B, probably because of the different training systems and planting distances that allowed a higher light interception efficiency. In any case, the higher SSC value shown by W-fruit with respect to WF-treatments was not influenced by the orchard characteristics.

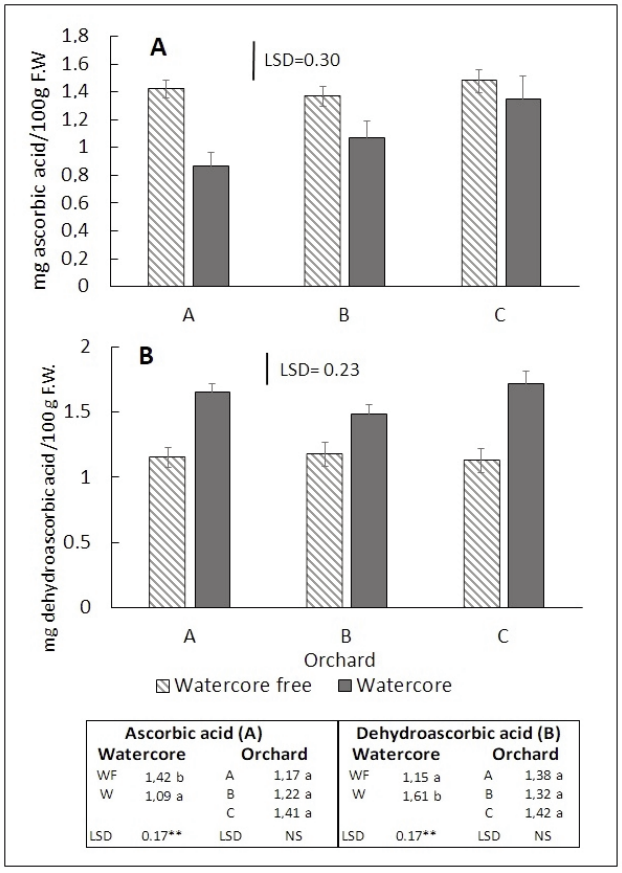


Fig. 5 - Ascorbic (A) and dehydroascorbic (B) acid content of watercore-free (WF) and watercore (W) fruit of 'Pomella Genovese' apple from three different orchards. Average ± standard error. LSD is reported in each figure (P>0.01). In the box at the bottom the average and LSD values are indicated for each of the main factors (*=P<0.05; **=P<0.01).

The flooding of the intercellular spaces affected the texture properties of watercore fruit. Since the standard method to assess firmness (usually the maximum force tested by a penetrometer), is limited to a data/point measurement and can be considered inappropriate to describe the complex texture of an apple (Ioannides *et al*., 2007), we included in the analysis different mechanical and acoustic parameters related with fruit firmness, stiffness and crispness. The results of the mechanical/acoustic profile indicate that the watercore fruit had a firmer texture and were crispier than the WF-fruit. Apple texture properties depend on the morphological features of the cells, with a firmer tissue having smaller and compact intercellular spaces than a softer one (Ting *et al*., 2013). The higher flesh firmness of the W-apples could, then, be due to the greater turgidity of the fruit and to the increased density and compactness of the tissue, associated with fluid-filled instead of air-filled intercellular spaces (Bowen and Watkins, 1997). Different authors reported this watercore effect on "Fuji" and "Himerami" apples (Bowen and Watkins, 1997; Yamada and Kobayashi, 1999).

In the plant cells, the electron transport chain (ETC) is the major producer of reactive oxygen species (ROS). In apples (cv. Fuji) affected by watercore, the lower intercellular air volume reduces the permeance to gas diffusion, and causes an increase of the CO_2 pressure inside the fruit, while the O_2 concentration decreases (Argenta *et al*., 2002; Kasai and Arakawa, 2010). The anoxia in watercore-fruit led to the inactivation of the two terminal oxidases of the ETC (cytochrome C oxidase and alternative oxidases), causing an increase of ROS, as well as of other enzymes involved in the oxidation process (Kasai and Arakawa, 2010; Zupan *et al*., 2016). This increased ROS level in the mitochondria can act as a stress signal, affecting the nuclear gene expression and modi-

fying the level of some stress-related compounds as, for example, ascorbic acid or phenols.

Phenols are plant secondary metabolites and are synthesized through the shikimic acid pathway. The key enzyme in the biosynthesis of phenols is the phenylalanine ammonia lyase (PAL) which catalyses the reaction converting L-phenylalanine to ammonia and trans-cinnamic acid (Tomás-Barberán and Espín, 2001). Through the regulation of PAL activity, the plant can modulate all the phenylpropanoid biosynthesis. The activity of phenylalanine ammonia lyase can be stimulated by a variety of environmental stresses, including tissue wounding, UV irradiation, low temperatures (Zhang and Liu, 2015). Tissue exposure at high levels of ethylene can cause an increase in PAL activity (Tomás-Barberán and Espín, 2001) and in 'Himerami' and 'Fuji' apples severely affected by watercore an internal accumulation of ethylene was reported (Bowen and Watkins, 1997; Yamada and Kobayashi, 1999), because of the osmotic stress and of the reduction of permeance to ethylene (Argenta *et al*., 2002).

In all the three orchards assessed in this study, fruit affected by watercore had a higher amount of polyphenols with respect of the watercore-free samples. An increase of ROS and ethylene production, caused by the reduced gas permeability inside the fruit, could have enhanced PAL activity and, consequently, induced the increase of the polyphenol content in the watercored fruit.

Some authors (Zupan *et al.*, 2016) found a lower phenols content in watercore fruit of the cultivars 'Delicious', 'Gloster' and 'Fuji', which were analysed immediately after harvest. The different results observed in 'Pomella Genovese' apples could be due to a different cultivar behavior but also to the different watercore intensity of the fruit. Pomella Genovese fruit were analyzed after 4 months of storage, when the "temporary watercore" had already dissipated (Neuwald *et al*., 2012). The fruit analysed were, then, only those severely affected by watercore, in which the higher ethylene content (Bowen and Watkins, 1997; Yamada and Kobayashi, 1999) could have induced the increase in polyphenol content.

In plants, the ascorbate-glutathione cycle operates to detoxify the hydrogen peroxide in order to avoid its reaction with the superoxide anion that produces the highly reactive hydroxyl radical (Hodges *et al*., 2004). Ascorbate acts as a scavenger of peroxide and it is oxidized by the ascorbate peroxidase (APX) to dehydroascorbic acid, which is recycled to ascorbate by means of the dehydroascorbate reductase (Mittler, 2002). However, when the production of ROS exceeds the scavenger capacity of the system, the ascorbic acid starts to decrease, so that this compound is often used to assess oxidative stress in postharvest studies (Hodges *et al*., 2004).

In our study, we found, on average, lower ascorbic acid and higher dehydroascorbic acid content in W-fruit. The higher ROS production due to the hypoxia caused by the flooding of intercellular spaces can affect the ascorbic-glutathione cycle. Kasai and Arakawa (2010) found lower ascorbic acid content and higher APX activity in watercore-affected 'Fuji' apples, probably caused by the higher H_2O_2 levels in the fruit flesh. The decrease of the ascorbic/dehydroascorbic ratio in our experiment can indicate a shift in the reduction state of ascorbate under oxygen deprivation (Blokhina *et al.*, 2003) and could be considered a signal of an oxidative stress status.

Total antioxidant activity of the fruit followed the trend of polyphenol content and seems to be not related to ascorbic acid. As reported by different authors (Miller and Rice-Evans, 1997; Szeto *et al*., 2002), ascorbic acid activity represents, in fact, a minimal fraction of the total antioxidant activity of apple fruit while some phenolic compounds, as the hydroxycinnamate chlorogenic acid, are the major contributors.

In conclusion, in this work different aspects of watercore-affected apples have been studied. The flooding of intercellular spaces influenced different quality characteristics of watercore-apples. Fruit density and SSC increased, the texture was firmer and crispier, and the peel background colour was darker. The anoxic conditions caused by watercore lead to oxidative stress, as shown by the decrease in the AA/DHA ratio in the watercore-fruit. On the other hand, the probable increase in ethylene content in fruit severely affected by watercore could have enhanced the activity of the PAL enzyme, leading to an increase in the polyphenol content and antioxidant activity.

Antioxidant compounds showed roughly the same differences between watercore and watercore free-fruit regardless to the orchard, suggesting that these compounds were mostly affected by the onset of the disorder, while they were only slightly influenced by the growing conditions.

The findings of this work support the hypothesis that, in watercore-affected fruit, browning disorders

could be due to oxidative stress that causes the losing of membrane integrity and disrupt cell compartmentation. Polyphenols are then oxidized by polyphenol oxidase to mono-diphenolic compounds which impart a brown colour to the fruit.

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Postharvest aptitude of *Begonia semperflorens* and *Viola cornuta* edible flowers

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Abstract: The edible flowers are sold as pot plants or fresh cut produce and are attracting interest recently thanks not only to their organoleptic characteristics but also to their content in bioactive molecules. However, there is little information about the variations that these characteristics undergo during postharvest. In this study, the productivity and longevity of *Begonia x semperflorens-cultorum* Hort. and *Viola cornuta* L. pot plants were evaluated in an interior environment simulating the house conditions. Besides, the effect of cold storage (4°C) was evaluated on the aesthetic quality and the bioactive compounds content (total polyphenols, total anthocyanins, antioxidant activity through FRAP assay) of *B. semperflorens* and *V. cornuta* fresh cut flowers, using two different packaging, modelling a plastic box or a flowpack. The results suggest that *V. cornuta* could be a better choice for retailers because of its longer shelf life and better maintenance of its content in bioactive compounds, especially in the flowpack packaging. Conversely, *B. semperflorens* could be more suitable as pot plant, showing more adaptability and flower production in a domestic environment.

1. Introduction

Numerous flowers have been used in culinary arts since ancient times both in Europe, including *Rosa* L. spp., *Calendula officinalis* L., *Viola* spp., and *Taraxacum officinale* F.H. Wigg (Mlcek and Rop, 2011; Grzeszczuk *et al*., 2016; Fernandes *et al.*, 2017; Scariot *et al*., 2018), and in Asia and South-America, such as *Begonia* spp. (Laferrière, 1992; Basurto-Peña *et al*., 2003; Zheng *et al*., 2018). Nowadays, edible flowers are horticultural niche products, sold as pot plant or as fresh cut flowers, with increasing appeal for the food industry due to their organoleptic and healthy properties (Kaisoon *et al.*, 2012; Grzeszczuk *et al*., 2016; Lu *et al.*, 2016).

Edible flowers improve the sensorial qualities of food by adding colour, fragrance, flavour and visual appeal to culinary preparations (Kelley *et al*., 2001a; Mlcek and Rop, 2011; Koike *et al.*, 2015).

In the third millennium, several studies revealed the chemical composition of many wild and cultivated flowers, highlighting the presence of

important bioactive compounds, such as carotenoids and phenolics (Lu *et al.*, 2016). These phytochemicals with antioxidant activity are very important for plants, since they inhibit their natural senescence process, mainly caused by the presence of reactive oxygen species (ROS) (Mlcek and Rop, 2011). During metabolism ROS and other free radicals are produced in human body too, normally inactivated by an endogenous antioxidant system (Mlcek and Rop, 2011; Loizzo *et al.*, 2016). However, under stress conditions, in high load situations, because of lifestyle or pathological situations, these free radicals can accumulate, generating oxidative stress (Loizzo *et al.*, 2016) by reacting and damaging all types of biomolecules such as lipids, proteins, carbohydrates, and DNA (Kaisoon *et al.*, 2012). If damaged DNA is left unrepaired, it may become cancerous (Mlcek and Rop, 2011; Kaisoon *et al.*, 2012; Li *et al.*, 2014). Thus, a diet rich in antioxidants, which can scavenge free radicals, can reduce the oxidative stress and may be a strategy to prevent some chronic conditions (Kaisoon *et al.*, 2012; Loizzo *et al.*, 2016; Lu *et al*., 2016). Epidemiological data showed that dietary patterns were significantly associated with the prevention of these chronic diseases, especially when rich in antioxidants (Kaisoon *et al.*, 2012), including carotenoids and phenolics (Koike *et al*., 2015; Grzeszczuk *et al*., 2016).

As a result of the increased knowledge of the edible flowers' properties, the consumers' demand of this kind of product is increasing worldwide (Fernandes *et al*., 2017; Pires *et al*., 2019; Falla *et al*., 2020), thanks to the increased attention to the quality of foodstuffs and to the content of individual compounds (Rop *et al.*, 2012; Lu *et al*., 2016).

However, edible flowers are highly perishable and have a short shelf life (petal abscission and discoloration, flower wilt, dehydration, and tissue browning start to appear 2-5 days after harvest), which limits their marketability (Koike *et al.*, 2015; Fernandes *et al.*, 2018). Fresh cut edible flowers are typically packaged in small, rigid, plastic boxes, in order to protect them from desiccation and to preserve their frail structure (Kelley *et al*., 2003; Kou *et al.*, 2012).

It is noteworthy that consumers eat with their eyes well before they taste with their mouths, thus it is important to maintain the visual appeal of a flower on market; quality is essential: consumers want more varieties of top quality plants with a longer shelf life (Kelley *et al.*, 2001 b). Nevertheless, edible flower's postproduction technology still receives less attention than that of other horticultural products, such as vegetables and fruits, because edible flowers' production is still low and it is a niche market (Fernandes *et al.*, 2018).

Temperature is usually the most important environmental factor limiting shelf life of horticultural products (Kelley *et al*., 2003): both respiration and transpiration processes are considered as the major causes of postharvest losses and poor quality in produce. Thereby, controlling temperature of storage is very important since these factors directly influence the two metabolic processes mentioned above, extending the product's shelf life (Flores-López *et al.*, 2016).

A flower's short shelf life may cause not only a rapid decrease in visual quality, but also a rapid loss of its nutraceutical compounds, however very few articles reported the effects of storage on quality of edible flowers, and even less investigated these effects on their nutraceutical compounds (Landi *et al.*, 2015). Therefore, it would be interesting to deepen the knowledge on whether the loss of nutraceutical compounds in edible flowers during storage occurs more or less quickly than the loss of visual quality. This information could promote the consumption of edible flowers at visual quality levels less than perfect, with minor flaws (Kelley *et al.*, 2001 b).

Thus, the aim of this work was to evaluate two common edible flowers' species (*Begonia x semperflorens-cultorum* Hort., commonly referred to as *Begonia semperflorens*, and *Viola cornuta* L.) as 1) pot plants, by evaluating the productivity and longevity in an interior environment simulating the domestic conditions; and 2) fresh cut flowers by evaluating the shelf life and the content in biologically-active compounds (total polyphenols, anthocyanins) and antioxidant activity, when stored at 4°C, testing two types of packaging (a plastic box closed with its own lid or closed with a plastic film in a flowpack).

2. Materials and Methods

Pot plant postproduction

The potted flowering plants of *Begonia semperflorens* (10 plants) and *Viola cornuta* (36 plants) were obtained from the nursery Fratelli Gramaglia (Collegno, Italy; 45°05'22.4'' N, 7°34'26.4'' E, 302 m a.s.l.).

Plants were kept at room temperature (about 20°C), in a peat-based substrate, hand-watered when needed, throughout the harvest period: 30 days for begonias and 11 days for violas. Every two days

opened flowers were harvested and weighed to evaluate the flowering longevity and productivity. For each plant, the number of flowers produced was counted and the weight of flowers suitable to be consumed (opened flowers in good visual conditions) was calculated: namely each plant's productivity.

Fresh cut flower postharvest

Fresh flowers harvested when fully open and in good visual conditions were put into plastic packages (Ondipack 250 cc, 123x114x50 mm, polypropylene, Plemet, France), 5 g of flowers for each box (Fig. 1).

Two packaging methods were assessed, for both species:

- Plastic box closed with its own plastic lid (abbr. PP; 8.96 g);
- Plastic box without its lid, inserted into a bi-oriented polypropylene plastic film (abbr. BOPP; 6.04 g).

The plastic films were folded and closed on three sides through heat-sealing (FR400, Ferplast, Cuneo, Italy), modelling a flowpack.

Fig. 1 - Flowers of *V. cornuta* (A) and *B. semperflorens* (B) freshly harvested and put in the plastic package.

Flowers were stored at 4°C, in refrigerators with a glass door [Fiocchetti fridge, Luzzara (RE), Italy], simulating markets' shelves conditions, with four repetitions per packaging type.

Every two days, each package was weighed, in order to obtain data about the flowers' weight variation.

The visual appeal of flowers was scored on a 9-point scale based on visual observation of the degree of decay (Aquino-Bolaños *et al.*, 2013; Landi *et al*., 2018), where 9 was assigned to flowers without imperfections, 5 was the limit of marketability of the product (the limit of acceptability for the consumer), while 1 was the value of a decomposing flower, at the end of its life cycle.

Ultrasound extraction

At the beginning of the trial, so that the day of harvest (t0) could be represented, about 5 g of flowers of both *B. semperflorens* and *V. cornuta* were collected from pot plants; the same was done after storage, when the flowers reached grade 5 of the visual scale. One of the four packages stored per method was taken and the 5 g of flowers contained in it were stored at -80°C until analysis. Flower samples were grinded with liquid nitrogen, then 0.5 g of grinded plant material were put into a glass tube, to which 25 ml of a 50% aqueous MeOH (methanol) solution were added. Three repetitions were carried out for each sample. The tubes were put into the ultrasound extractor (23 kHz, Reussarl, Drap, France) for 15 minutes at room temperature. The obtained phyto extract was filtered with paper filters (Whatman filter papers No. 1, Whatman, Maidstone, UK) and the obtained solution was stored at -20°C for further analysis.

Total polyphenols

The total phenolic content was determined following the Folin-Ciocalteu method (Singleton *et al*., 1999).

The analysis was performed as follows: 750 µl of diluted 1:10 Folin reagent were mixed with 150 µl of phytoextract and 600 µl of Na_2CO_3 (7.5%) in each plastic tube. Samples were left in the dark at room temperature for 30 minutes. Absorbance was measured at 765 nm by means of a spectrophotometer (Agilent Technologies, Cary 60 UV-Vis, Santa Clara, CA, United States), and the results were expressed in milligrams of gallic acid equivalents per 100 g of fresh weight (mg GAE/100 g FW).

Total anthocyanins

The total anthocyanin content in the extracts was determined through the pH-differential method as indicated by Lee *et al*. (2005) and Giusti and Wrolstad (2005).

The analysis was performed as follows: 1 ml of phytoextract was put into a 10 ml flask, and then made up to volume with an aqueous buffer solution at pH 1 (KCl and HCl). The same was made in a second flask with an aqueous buffer solution at pH 4.5 ($C_2H_3NaO_2$and $C_2H_4O_2$). Samples were put in the dark at room temperature for 20 minutes. Absorbance of both flasks was measured at 515 nm and 700 nm by means of a spectrophotometer (Agilent Technologies, Cary 60 UV-Vis, Santa Clara, CA, United States), and the results were expressed in milligrams of cyanidin-3-O-glucoside per 100 grams of fresh weight (mg C3G/100 g FW).

Antioxidant activity - FRAP assay

The method used to evaluate the antioxidant activity is the FRAP (Ferric ion Reducing Antioxidant Power) assay as indicated by Benzie and Strain (1996).

The antioxidant activity was determined mixing 30 µl of phytoextract with 90 µl of deionised water and 900 µl of FRAP reagent. The samples were then placed at 37°C for 30 minutes. Absorbance was measured at 595 nm by means of a spectrophotometer (Agilent Technologies, Cary 60 UV-Vis, Santa Clara, CA, United States). Results were expressed as millimoles of ferrous iron equivalents per kilogram of fresh weight (mmol Fe^{2+}/kg FW).

Statistical analysis

All data were subjected to the statistical analysis for the homogeneity of variance (Levene test).

Weight variations were compared using a one-way ANOVA test.

Mean comparisons between data obtained from the two different packages during postharvest were performed using an independent samples t-test, by means of the SPSS 25 software (version 25.0; SPSS Inc., Chicago. Illinois).

3. Results

Pot plant productivity

Begonia semperflorens and *V. cornuta* pot plants showed differences in the number of flowers produced over time. On average, from the day of arrival at the laboratory, plants of *B. semperflorens* produced flowers for 30 days (with an initial production of about 38 flowers per plant, and a final production of 1-2 flowers per plant) (Fig. 2), with an average total productivity of 11 flowers per plant per day. Pot plants of *V. cornuta* bloomed for 11 days (starting with an average of 21 flowers per plant up to 3-4 flowers per plant) (Fig. 2), with an average total productivity of 9 flowers per plant per day.

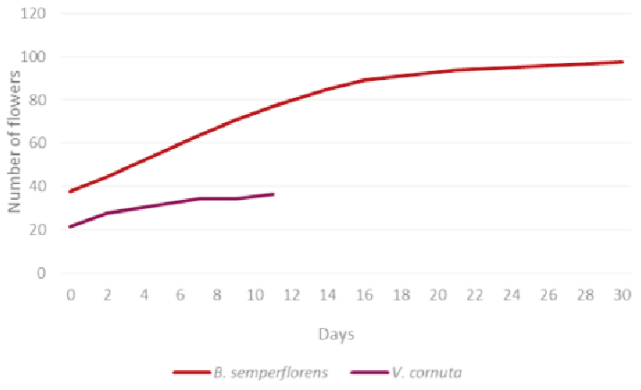

Fig. 2 - Flower production of *B. semperflorens* (red) and *V. cornuta* (purple). Data are shown in a cumulative curve.

Cut flower shelf life

As shown in figure 3, the shelf life of *B. semperflorens* and *V. cornuta* assessed at 4°C is quite different: the first species reached the limit of marketability (grade 5) after 9 days (both in PP and in BOPP) (Fig. 3, red lines), while the viola flowers remained acceptable for the consumer up to two weeks (both for PP and for BOPP) (Fig. 3, purple lines).

During storage up to grade 5, the flowers did not show significant weight variations in both packaging type (Table 1).

Bioactive compounds

The total polyphenol and anthocyanin content, and the antioxidant activity (FRAP) of *B. semperflorens* and *V. cornuta*'s flowers are reported in Table 2. Values are referred to flowers freshly picked, corresponding to grade 9 (t0), and after storage at 4°C, when they reached grade 5 of the visual scale (i.e. limit of marketability), that corresponded to 9 days for begonias and 16 days for violas (Fig. 3).

Comparisons aimed to highlight flower differences between the two species and along time within the same packaging and between the two types of packaging (Table 2). Variations are visualized in figure 4. Concerning the pot plants, *V. cornuta* flowers at t0 showed higher values of both polyphenols ($p<0.001$) and antioxidant activity ($p<0.001$) than *B. semperflorens*, while this latter showed a higher content in anthocyanins ($p<0.05$) than *V. cornuta*.

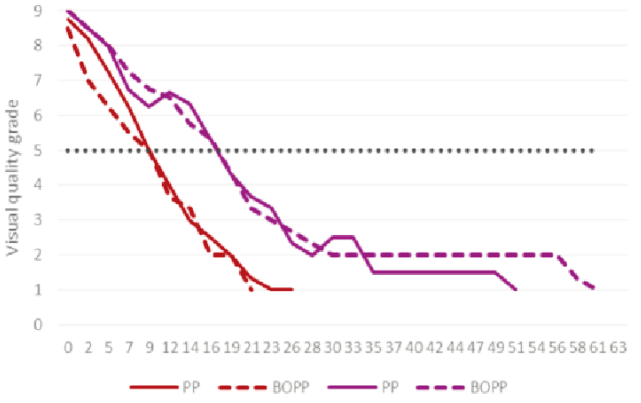

Fig. 3 - Trend of visual quality during storage for *B. semperflorens* (red) and *V. cornuta* (purple). Data are shown as mean values. The intersection of the curves with the dotted horizontal line corresponds to the marketability limit (grade 5). PP= plastic box + lid; BOPP= Plastic box without its lid, inserted into a bi-oriented polypropylene plastic film.

Regarding the bioactive compound's evaluation during the post-harvest, begonia flowers kept in PP encountered a decrease of all parameters (polyphenols: -45.78%; anthocyanins: -85.33%; antioxidant activity: -52.35%) while in BOPP only the anthocyanins decreased (-99.44%). Viola flowers kept in PP encountered an increase in anthocyanins (+202.5%) and a decrease of antioxidant activity (-34.21%) while total polyphenols were constant (Table 2). In BOPP all the parameters decreased (phenolic content -76.51%, antioxidant activity -88.05%, anthocyanins -32.52%).

Table 1 - Flower weight variation during cold storage (4°C) up to the grade of marketability

Flower species	Day	Weight (g) PP	Weight (g) BOPP
Begonia semperflorens	0	5.25	5.04
	2	5.25	5.04
	4	5.25	5.03
	7	5.24	5.18
	9	5.23	5.01
	p	NS	NS
Viola cornuta	0	5.18	5.43
	2	5.18	5.43
	4	5.14	5.44
	7	5.16	5.47
	9	5.12	5.41
	11	5.10	5.40
	14	5.17	5.38
	16	5.17	5.37
	p	NS	NS

PP= plastic box + lid; BOPP= Plastic box without its lid, inserted into a bi-oriented polypropylene plastic film). Data are shown as mean values. Comparisons between data were performed using a one-way ANOVA analysis.

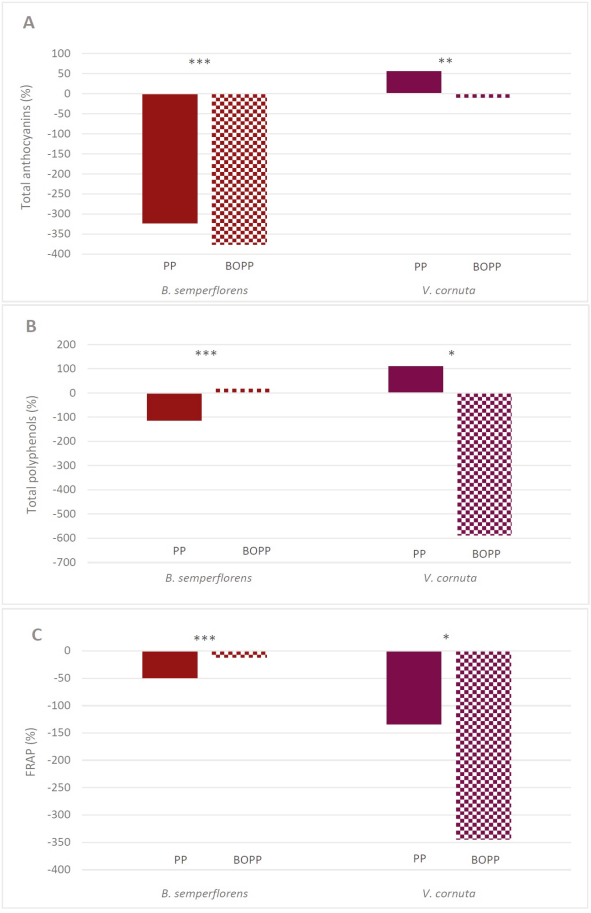

Fig. 4 - Percentage variation of the content of A) total anthocyanins, B) total polyphenols, C) antioxidant activity (FRAP) in *B. semperflorens* (red) and *V. cornuta* (purple), up to grade 5 of visual quality scale, depending on the type of packaging (stored at 4°C). The statistical analyses were made separately on *B. semperflorens* and *V. cornuta* values. Mean comparisons between data were performed using an independent samples T-test. * p≤0.05. ** p≤0.01. ***p≤0.001. Axis 0 uses the t0 value as a reference. PP= plastic box + lid; BOPP= Plastic box without its lid, inserted into a bi-oriented polypropylene plastic film.

Table 2 - Total polyphenols, total anthocyanins, and antioxidant activity (FRAP) at grade 9 (day of harvest) and grade 5 (i.e. limit of marketability, corresponding to 9 days for begonias and 14 days for violas) of visual quality scale of *B. semperflorens* and *V. cornuta* flowers stored at 4°C in two different packaging

Flower species	Packaging	Total polyphenols (mg GAE/100 g FW)			Total anthocyanins (mg C3G/100 g FW)			Antioxidant activity FRAP (mmol Fe^{2+}/kg FW)		
		Grade 9	Grade 5	*p*	Grade 9	Grade 5	*p*	Grade 9	Grade 5	*p*
Begonia semperflorens	PP	246.71	133.75	**	378.67	55.57	***	95.23	45.38	**
	BOPP	246.71	264.77	NS	378.67	2.12	***	95.23	83.49	NS
	p	-	***		-	***		-	***	
Viola cornuta	PP	767.26	877.01	NS	27.76	83.99	**	391.89	257.84	*
	BOPP	767.26	180.26	***	27.76	18.74	*	391.89	46.83	*
	p	-	*		-	**		-	*	

PP= plastic box with its own lid; BOPP= flowpack. Data are shown as mean values. * p≤0.05. ** p≤0.01. *** p≤0.001. Mean comparisons between data were performed using an independent samples T-test.

4. Discussion and Conclusions

This study aimed to evaluate the aptitude of two common edible flower species, i.e. *B. semperflorens* and *V. cornuta*, to be sold as pot plants or fresh cut flowers.

The growing conditions adopted in this study (i.e. 18-20°C and low lighting) were useful to simulate the maintenance of pot plants in a domestic environment, so to give information on productivity to the final consumer. These conditions are unlikely to be fully appropriate, especially for violas. Indeed, *V. cornuta* plants are more productive at temperatures from 4 to 10°C (Ball, 1991; Nau, 1998). Cooper and Watson (1952) noticed that flowers sizes are also bigger when plants grow at night-time temperatures of 10°C. A frequent harvest could concur to cause a general reduction in productivity and in flowers size too. In this study, where flowers were picked every 2-3 days, a change in flowers weight was observed. Begonia flowers weighted 0.5-0.6 g at the beginning of the experiment and 0.3-0.4 g at the end, while viola flower varied from 0.2 g to 0.1 g. Begonias flowers, moreover, showed petals discoloration during the last days of harvest.

Few data are available in literature about *B. semperflorens* and *V. cornuta* so that comparisons with results of other studies are difficult. Some information could be found in other congener species. Low temperature (4°C) and natural lighting during storage mimed the retailer conditions. Data about *B. semperflorens* agreed with those assessed by Friedman *et al*. (2007) in flowers of *Begonia elatior* and *B. semperflorens* that were stored in plastic trays for about ten days at 2-5°C. *Viola cornuta* shelf life was in accordance with the data found by Kelley *et al.* (2003) in *Viola wittrockiana,* that was considered marketable after two weeks of storage at 5°C. Regarding the phytochemical content, results were partially discordant with those found by Benvenuti *et al.* (2016) in *V. wittrockiana* that showed a higher antioxidant activity but also a higher anthocyanins content than *B. semperflorens*.

Results of works that evaluated the content of edible flower phytochemicals during cold storage are sometimes conflicting. Aquino-Bolaños *et al*. (2013) observed a reduction in the nutraceutical values of squash (*Cucurbita pepo* L.) edible flowers, conversely Friedman *et al*. (2007) found no differences in anthocyanins content in *B. semperflorens* flowers. Landi *et al*. (2018) analysed *B. semperflorens* flowers too, finding a general constancy in the nutraceutical values during storage. Data obtained in this study showed that the phytochemical content of *B. semperflorens* decreased during storage in the plastic box closed with its own lid, while in the flow pack the total phenolic content, and the antioxidant activity, remained constant. The best way to store flowers of *B. semperflorens* could be therefore the flowpack, preferably a perforated one to prevent condensation of vapours on their inner surface (Mlcek and Rop, 2011). Conversely, *V. cornuta* flowers seemed to better preserve its characteristics in the plastic box closed with its own lid, showing a certain constancy in polyphenols and antioxidant activity, and a significantly increased level of anthocyanins, while in the flow-pack showed a significant reduction in all three parameters.

In conclusion, our data confirm that *B. semperflorens* and *V. cornuta* are suitable for edible flower production both in term of shelf life and phytochemical characteristics. *Begonia semperflorens* seems preferably marketable as pot plants, thanks to its better adaptability to grow in the domestic environment and longer flowering. Conversely, *V. cornuta* flowers resulted more suitable as fresh cut produce, showing a longer shelf life and preserving better the phytochemical characteristics during storage at 4°C.

New technological approaches (ethylene inhibitors, modified atmosphere packaging, edible film coatings, high hydrostatic pressure, irradiation, etc.) could further improve the distribution and marketing efficiency of edible flowers, contributing to their success in the market (Fernandes *et al.*, 2018).

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Modified atmosphere packaging to improve the shelf-life of Goji berries during cold storage

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Key words: *Lycium barbarum* L., marketability, respiration rate, visual quality, wolfberries.

Abbreviation: AA= Antioxidant activity; AIR= Control in air; CIE= Commission Internationale de l'Eclairage; EtOH= Ethanol; FW= fresh weight; MAP= modified atmosphere packaging; MeOH= Methanol; pMAP= passive modified atmosphere packaging; RR= Respiration rate; TP= Total phenols; TSS= total soluble solids; VQ= visual quality.

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All relevant data are within the paper and its Supporting Information files.

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The authors declare no competing interests.

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Abstract: This study was carried out to evaluate the effect of modified atmosphere packaging on the quality parameters and the shelf-life of fresh goji berries. Fruits, placed in trays, were closed in passive modified atmosphere packaging (pMAP) using polypropylene bags or kept in open polyethylene bags (AIR) as control. Samples were analyzed just after harvest and during storage (5, 13 days) at 7°C for visual quality (VQ), color parameters, weight loss, dry weight, total soluble solids (TSS), antioxidant activity (AA) and total phenols (TP), while respiration rate (RR) was evaluated only after 5 days. Changes in gas composition in pMAP samples was measured daily. The use of pMAP allowed to reduce the RR of about 26% compared to fresh sample, to preserve the berries weight loss during storage and their marketability until 13 day at 7°C, while AIR samples were not edible after 5 days due to mold growth on the berries surfaces. No changes of color parameters, dry weight, TSS, AA and TP were observed during storage comparing treatments. In conclusion, the use of pMAP was able to extend the shelf-life of goji berries for 13 days at 7°C, 8 days more than berries stored in AIR.

1. Introduction

The goji (*Lycium barbarum* L.) berries, also known as wolfberries, are considered "superfruits" for their high nutritional value, richness in nutrients, antioxidants and bioactive compounds of which the health promoting properties are known (Sidhu and Zafar, 2012; Jatoi *et al.*, 2017; Niro *et al.*, 2017). The berries are mostly grown for dry fruit, but nowadays the

goji market is significantly expanding, focus also on the fresh fruit. However, due to the tender peel and high-water content, fresh goji berries are easy to damage and rot, so their transport and storage are difficult (Fan *et al.*, 2019). For these reasons the use of postharvest handling to preserve the storability of this perishable fruit are required. Very few studies are been conducted on the storage of fresh goji berries. Jatoi *et al.* (2018) evaluated the postharvest quality of fresh goji berries stored at different temperature, from -2°C to 20°C, concluding that the optimum storage temperature to preserve phytochemical and sensory attributes was 0°C. The application of the lecithin (Jatoi *et al.*, 2017) or edible coating based on lotus leaf extract (Fan *et al.*, 2019) were studied to improve the shelf-life of fresh goji berries. Ban *et al.* (2015) reported that a combination of heat treatment at 40°C for 30 min followed by chitosan coating protect goji berries from decay, extending their postharvest life up to 28 days of storage at 2°C. The use of additives or coatings, even though are natural, is often undesirable from consumers that are more attracted to fresh products without any additional ingredients. From this point of view, the use of modified atmosphere packaging (MAP) during storage, consisting in a reduction of O_2 and/or an increase in CO_2 levels, can be a valid tool, in addition to the proper temperature, in order to improve the shelf-life of goji berries. Modified atmosphere act reducing respiration rate and weight loss, delaying ripening and softening, thus minimizing the incidence of some physiological disorders and decay (Kader, 2002 a). Kafkaletou *et al.* (2017) tested the effectiveness of a short-term treatments with different atmospheres enriched in CO_2 to prevent fungal decay in fresh goji berries, concluding that atmospheres with high CO_2, from 15 to 20%, were able to reduce fungal decay incidence in goji berries stored for 14 days at 1°C. To the best of our knowledge, no further studies on the application of modified atmosphere on goji berries are available, so the present study is aimed to evaluate the application of MAP technology to extend the shelf-life of fresh goji berries.

2. Materials and Methods

Reagents

Extraction solvents (MeOH, EtOH), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) and all standards used in the experiments were obtained from Sigma-Aldrich (St. Louis, Mo., USA). Folin-Ciocalteu's phenol reagent was purchased from Merck (Germany).

Plant material and experimental set-up

Fresh goji berries (*Lycium barbarum* L.), about 2 kg, were provided from Favella group located in the South of Italy (Corigliano Calabro, Italy), and transported in cold condition to the Postharvest Laboratory of CNR-ISPA to be processed. After elimination of damaged fruits, 3 replicates of about 80 grams berries were used for the initial determination, while the remaining samples were placed in polyethylene terephthalate trays (model C250/50 Carton Pack®, Italy), 80 grams per trays, and sealed in passive modified atmosphere packaging (pMAP) using polypropylene bags (dimension 200 x 150 mm, 30 μm thickness), or in unsealed polyethylene bags (AIR) as control. For each packaging condition (pMAP or AIR), 6 bags (3 replicates × 2 storage times) were stored at 7°C (±1) and analysed initially and after 5 and 13 days for visual quality, color parameters, weight loss, dry weight, total soluble solids, antioxidant activity and total phenols, while respiration rate was evaluated initially and after 5 days, because of mould development in AIR samples after 13 days. In addition, changes in gas composition in pMAP samples was monitored daily using a gas analyser (CheckPoint, PBI Dansensor, Ringsted, Denmark).

Respiration rate

The respiration rate of goji berries was measured at 7°C using a closed system as reported by Kader (2002 b). About 80 grams of berries for each replicate were put into 6 L sealed plastic jars (one jar for replicate) where CO_2 was allowed to accumulate until the value of 0.1%. The time needed to reach this value was calculated, making CO_2 measurement at regular time intervals. For the CO_2 analysis, 1 mL gas sample was taken from the head space of the plastic jars through a rubber septum and injected into the gas chromatograph (p200 micro GC, Agilent, Santa Clara, CA) equipped with dual columns and thermal conductivity detector. CO_2 was analyzed with a retention time of 16 s and total run time of 120 s on a 10 m porous polymer (PPU) column at a constant temperature of 70°C. Respiration rate was expressed as mL CO_2 kg^{-1} h^{-1}. After the respiration rate evaluation, berries were used for the following analysis.

Visual quality and color analysis

Visual quality was evaluated by a group of ten

trained people, on a subjective 5 to 1 scale, with 5= excellent, no defects; 4= very good, minor defects; 3= fair, moderate defects; 2= poor, major defects; 1= inedible. A score of 3 was considered to be the limit of marketability, while a score of 2 represented the limit of edibility.

Color parameters (*L**, *a** and *b**) were measured, for each replicate, on 3 random points on peel surface of 5 goji berries using a colorimeter (CR-400, Konica Minolta, Osaka, Japan) in the reflectance mode and in the CIE *L* a* b** color scale. Colorimeter was calibrated with a standard reference having values of *L**, *a** and *b** corresponding to 97.55, 1.32 and 1.41, respectively. Hue angle ($h°=\arctan b^*/a^*$) and saturation ($Chroma=\sqrt{a^{*2}+b^{*2}}$) were then calculated from primary *L**, *a** and *b** readings.

Weight loss, dry weight and total soluble solid content

Goji weight loss was calculated at each storage time as percentage of variation from the initial fresh weight. To measure dry weight, goji berries were maintained in a forced-draft oven at 65°C until constant weight was reached. Total soluble solid content, expressed in °Brix, was measured using a digital refractometer (model DBR35, XS Instruments, Carpi, Italy) on a liquid extract obtained by whisking in a blender (1 min; 14,000 rev. min^{-1}) 10 goji berries from each replicate and then filtering the juice.

Antioxidant activity and total phenols

To determine both antioxidant activity and total phenol contents, the extraction procedure reported by Cefola *et al.* (2012) was followed. In detail, 5 grams samples were homogenized (Ultraturrax T-25, IKA Staufen Germany) in a MeOH:water (80:20) solution for 1 min, and then centrifuged at 5°C at 6440 × *g* for 5 min. The supernatant was therefore used for the assays. The antioxidant activity assay was performed following the procedure described by Brand-Williams *et al.* (1995) with minor modifications. Briefly, the supernatant, proper diluted, was pipetted into 0.95 mL of DPPH solution to start the reaction. The absorbance was read after about 30 min at 515 nm. Trolox was used as a standard and the antioxidant activity was expressed in milligrams of Trolox per 100 g of fresh weight (fw) (mg Trolox 100 g^{-1} fw). The total phenol content was determined according to the method of Singleton and Rossi (1965). Each extract (100 µL), proper diluted, was mixed with 1.58 mL water, 100 µL of Folin-Ciocalteu reagent and 300 µL of sodium carbonate solution (200 g L^{-1}). The absorbance was read after 2 h at 765 nm. Total phenol content was calculated on the basis of the calibration curve of gallic acid and expressed as milligrams of gallic acid per 100 g of fresh weight (mg gallic acid 100 g^{-1} fw).

Statistical analysis

In order to evaluate the effect of packaging condition (pMAP or AIR) on quality parameters of goji berries, a one way ANOVA was performed at each storage time (5 and 13 days), and mean values were separated applying Least Significant Difference (LSD) Multiple Range Test with significant difference when $P \leq 0.05$.

3. Results

At harvest, the respiration rate of goji berries was 23.6 (±3.5) mL CO_2 kg^{-1} h^{-1} at 7°C. After 5 days of storage, respiration rate in AIR samples slightly increased, while the use of pMAP allowed to reduce the rate of respiration of about 26% compared to fresh sample (Table 1).

In figure 1 changes in gas composition inside bags in pMAP samples were reported. Starting from air composition (21% O_2 and 0.03% CO_2), oxygen was gradually consumed by the product, due to the respiration process, with a consequent accumulation of CO_2. At the first sampling time, after 5 days at 7°C, O_2

Table 1 - Respiration rate of goji berries at harvest and after 5 days at 7°C (±1) in pMAP or AIR

	Respiration rate (mL CO_2 kg^{-1} h^{-1})
At harvest	
Fresh	23.6 ± 3.5
After 5 days at 7°C	
pMAP	17.3 ± 0.2 b
AIR	28.4 ± 1.9 a

Values are means of three replicates ± standard deviation. Different letters indicate statistical differences for $P \leq 0.05$, according to LSD test.

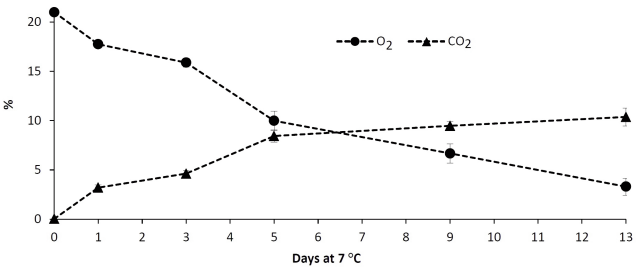

Fig. 1 - Changes in gas composition of goji berries stored in pMAP for 13 days at 7°C (±1). Values are means of three replicates for each storage time ± standard deviation.

and CO_2 values were 10.0% (± 1.0) and 8.4% (± 0.6), respectively. Then, the consumption of oxygen was slowdown, reaching the concentration of 3.3% (± 0.9) after 13 days at 7°C, while CO_2 was 10.4% (± 0.9) (Fig. 1).

The visual quality of the product at harvest was not optimal, in fact panelists gave an initial score of 4 (good) (Fig. 2). At each storage time, significant differences between treatments were observed. In particular, after 5 days at 7°C goji berries stored in AIR were not edible, mainly due to mold growth on the berries surfaces, while pMAP samples were scored as more than acceptable, keeping their marketability until the end of the storage (Fig. 2).

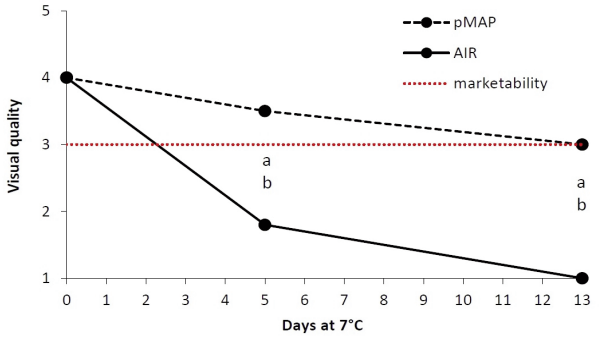

Fig. 2 - Changes in visual quality of goji berries stored in pMAP or AIR for 13 days at 7°C (±1). Values are means of three replicates for each packaging condition at each storage time. Within the same storage time, different letters indicate statistical differences, P≤0.05. Visual quality score: 5=excellent, no defects; 4=very good, minor defects; 3=fair, moderate defects; 2=poor, major defects; 1=inedible.

The weight loss of goji berries stored in pMAP was almost constant for all the storage period while fruits stored in AIR lost about 17.5% of the initial weight after 13 days at 7°C (Fig. 3).

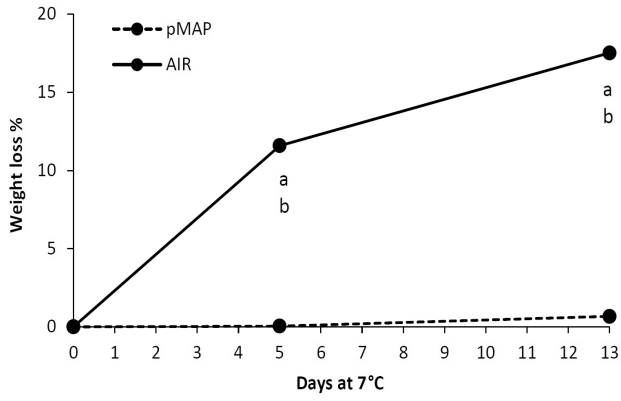

Fig. 3 - Weight loss of goji berries stored in pMAP or AIR for 13 days at 7°C (±1). Values are means of three replicates for each packaging condition at each storage time. Within the same storage time, different letters indicate statistical differences, P≤0.05.

Regard the other physical (color, dry weight) and chemical (total soluble solid content, antioxidant activity, total phenols) parameters, the values measured at harvest are reported in Table 2; however, no significant changes were observed during storage and comparing treatments (at the end of the storage dry weight, TSS, AA and TP had mean values of 23.9%±0.6, 21.7±0.4°Brix, 82.1±2.4 mg Trolox 100 g^{-1} FW and 216.1±1.3 mg gallic acid 100 g^{-1} FW, respectively), except for all the color parameters that slightly decreased during storage (at the end of the storage, *L**, *a**, *b**, *h°* and *Chroma* mean values were 46.8±0.7, 38.4±0.1, 37.3±0.1, 14.0±0.3, 4.8±1.3, respectively).

Table 2 - Color parameters, dry weight, total soluble solid content, antioxidant activity and total phenols evaluated in goji berries at harvest

Evaluated parameters	Data
Color parameters	
*L**	49.1±0.6
*a**	46.9±2.4
*b**	48.2±2.6
h°	45.1±0.3
Chroma	67.2±3.5
Dry weight (%)	22.8±0.3
Total soluble solid content (° Brix)	21.4±0.5
Antioxidant activity (mg Trolox 100 g^{-1} fw)	85.8±1.5
Total phenols (mg gallic acid 100 g^{-1} fw)	211.1±2.4

Values are means of three replicates ± standard deviation.

4. Discussion and Conclusions

Respiration rate represents one of the most important parameters that should be taken into account for the study of the postharvest performance of fresh produce since it is inversely correlated with shelf-life and thus, it can be used as an indicator of perishability. Considering the initial respiration rate of goji berries (23.6±3.5 mL CO_2 kg^{-1} h^{-1} at 7°C), these fruits have a high respiratory metabolism, according to the classification reported by Kader (2002 c). In the proposed pMAP, the O_2 reduction and CO_2 accumulation, due to the high berries respiration rate and the permeability properties of the packaging, slowed down the rate of respiration of goji berries. This positive effect of MAP was previously reported on different fruit by Sandhya (2010). Similar results on goji berries treated with different atmospheres of low oxygen and high CO_2 were

reported by Kakfaletou *et al*. (2017). The positive effect of the application of pMAP was reported also on the weight loss that was significant lower in pMAP compared to AIR samples. This because the use of packaging represents a barrier to vapor diffusion which allows to maintain an adequate relative humidity within the package, so tissue dehydration is limited (Zagory and Kader, 1988).

The presence of fungal decay was the main factor that influenced the loss of marketability of fresh goji berries during storage. The CO_2 accumulation (until 10%) inside the packages was able to inhibit the mold growth, as previously observed (Kafkaletou *et al*., 2017). In particular these Authors reported that an atmosphere with 15-20% of CO_2 applied as a short-term treatment for 2 days at 1°C was able to reduce fungal decay incidence in goji berries stored for 14 days at 1°C.

The red color of goji berries was not influenced by packaging condition (pMAP or AIR), whereas a slight decrease in all the color parameters measured were observed during storage. Similar results were reported on goji berries by Kafkaletou *et al*. (2017) and Jatoi *et al*. (2017). Regarding to dry weight, our data (22.8%±0.3) are in accordance with data reported by Niro *et al*. (2017) that found a moisture of 77.4% on fresh goji berries, means 22.6% dry weight. Also, the presented data of total soluble solids at harvest (21.4±0.5°Brix) are similar to that reported by Kafkaletou *et al*. (2017) (from 21 to 25°Brix) and Fan *et al*. (2019) (about 22°Brix). Goji berries is considered a "superfruit" for their antioxidant activity due to the high content of bioactive compounds and vitamin C (Sidhu and Zafar, 2012; Jatoi *et al*., 2017; Niro *et al*., 2017). In the present research paper, the value of total phenols (211.1±2.4 mg gallic acid 100 g^{-1} fw) at harvest, is quite similar to that reported in literature on fresh goji berries by Donno *et al*. (2016) (from 199.5 to 240.3 mg gallic acid 100 g^{-1} fw, depending on region of cultivation) and Jatoi *et al*. (2017) (about 223 mg gallic acid 100 g^{-1} fw). As for antioxidant activity, our data at harvest were similar (85.8±1.5 mg Trolox 100 g^{-1} fw) to data reported by Jatoi *et al*. (2017) on the same fruit. Both total phenols and antioxidant activity remain unchanged during storage in pMAP and in AIR samples, as previously reported by Jatoi *et al*. (2017) for total phenols.

Fresh goji berries are highly perishable fruits, with a high respiration rate, if stored in air. The loss of marketability is very fast, and it is mainly due to the development of molds on the peel surface. In addition, the high transpiration of the peel causes a rapid weight loss, with the consequence depreciation of the product. On the other hand, the application of a modified atmosphere packaging resulted a valid tool to delay the loss of quality of goji berries, prolonging their shelf-life. Results of the present study demonstrated that the use of pMAP allowed to reduce the respiration rate, preserved the berries weight loss and the heath properties, and control the mould development. As consequence berries goji stored in pMAP showed a shelf-life of 13 days at 7°C, 8 days more than berries stored in AIR.

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Short-term low temperature treatments of harvested wine grapes (cv. Vermentino) affect the volatile organic compound profile of the berries

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Key words: aroma, post-harvest, temperature conditioning, terpenoids, *Vitis vinifera.*

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Abstract: In the recent years, due to the climate change and the effects of greenhouse gases average temperatures are increasing. Grapes cultivated in Mediterranean areas are exposed to high temperatures especially during the late growing season and at harvest. This may induce undesirable biochemical processes (e.g. aroma losses and oxidative reactions) with negative effects on the berry composition and specific quality traits of the resulting wine. In the present study the effects of short-term low temperature treatments on harvested grapes before vinification have been evaluated. Bunches of wine grapes cv. Vermentino have been hand-harvested and then refrigerated at 4°C and 10°C for 24 and 48 hours, while 22°C has been applied as control temperature. Grapes were analysed in terms of technological parameters (weight loss, total soluble solids, titratable acidity, pH and total polyphenols) and volatile organic compound profile by HS-SPME GC-MS. Low-temperature post-harvest treatments affect total polyphenols content of the berries and appear to reduce the heat-related aroma loss, increase the content of four volatile terpenoids and decrease the accumulation of ethyl acetate.

1. Introduction

Several challenges characterize the wine industry and have a marked impact on the production chain, final quality of the wines and consumer acceptance. One major problem, in particular in warm-temperate climates, is represented by the increase of the average temperatures, mainly due to the accumulation of greenhouse gases, that often leads to different pheno/physiological processes in grape berry and strongly affects berry development. High temperatures induce anticipated and unbalanced ripening and, at harvest, undesirable biochemical changes such as aroma losses and oxidative processes (Ribéreau-Gayon *et al*., 2006). This negatively affects grape composition and wine quality. Hence, it is crucial to find and develop effective strategies for mitigating these negative effects. One option is represented by the application of postharvest cool-

ing treatments of the bunches.

Postharvest protocols based on controlling (lowering) temperature are used for the management and storage of fresh horticultural crops with the main goal of prolonging commercial life and freshness (Tonutti, 2013). The effects of low temperature on harvested fruits are diverse and depend on a number of factors including the fruit type, pre-harvest factors, ripening stage, the applied temperature and the duration of the treatment (Kader, 1999). Both primary and secondary metabolisms are affected (Brizzolara *et al*., 2020), with changes in the composition and quality parameters, including those related to polyphenols and volatile organic compounds (VOCs) (Valenzuela *et al*., 2017, Brizzolara *et al.*, 2018). This is also the case of table grapes that, when stored at 0°C to prolong commercial life, show changes in the VOC profiles and related-metabolic pathways, resulting in altered overall flavours (Maoz *et al*., 2019).

With other goals, post-harvest treatments can also be applied on specific crops undergoing processing. This is the case of wine grapes on which techniques such as controlled dehydration, high carbon dioxide, ozone, ethylene and pre-cooling treatments have been applied or studied to modulate the composition of the harvested berries and the style of the resulting wines (Mencarelli and Tonutti, 2013; Becatti *et al*., 2014; Mencarelli and Bellincontro, 2018). Maintaining harvested wine grapes at low temperature is a practice that is already applied in certain production areas and for specific enological purposes. Low temperature treatments prior to vinification appear to have a positive effect on the aromatic profile of the wines, especially when white-skinned berries are processed. This empirical approach has, so far, very little scientific evidence and, differently from table grapes, just few studies report the effects of such treatments on technological parameters and secondary metabolism (including aroma compounds) of wine grapes. Marais (2003) showed that keeping grapes (cv. Pinotage) overnight at 10°C and then maintaining the same temperature during skin contact with the must prior to fermentation resulted in the production of the most typical and highest quality Pinotage wines, compared to the same treatments carried out at 15°C. This effect appears to be related to changes in ester metabolism occurring in the berries. Mencarelli and Bellincontro (2018) reported that following a 10°C treatment applied on wine grapes during post-harvest partial dehydration (a practice used to produce special wines, such as the "passiti") an up regulation of genes involved in the phenylpropanoid pathway occurs together with a slight increase of stilbenes and a decrease of polyphenol oxidase activity. The present study aimed at evaluating the effect of a short-term low temperature conditioning on harvested wine grapes cv. Vermentino in terms of technological parameters and VOCs profile.

2. Materials and Methods

Grapes samples and cooling treatments

Bunches of white-skinned wine grapes (*Vitis vinifer*a L.) cv. Vermentino were hand harvested in 2018 in correspondence of an average total soluble solid (TSS) value of 21°Brix. The grapes were collected from a commercial vineyard (Lodolina) located in the hills of Candia (Massa province, Tuscany, Italy. 44°02'197.6" N, 10°11'265.9" E). The vines are trained at simple Guyot, and all agronomic practices follow the disciplinary of production for the Appellation of Controlled Origin (DOC) Candia dei Colli Apuani. After harvest, grapes were immediately transported to the laboratory and selected based on absence of evident defects or diseases. Grapes were randomly distributed into six lots (of 5 kg each) and subjected to post-harvest low temperature treatments as follow: two lots were cooled at 4°C (±0.5) for 24 (4°C 24 h) and 48 (4°C 48 h) h; two other lots were cooled at 10°C (±0.5) for 24 (10°C 24 h) and 48 (48°C 48 h) h. The last two lots were used as a control and kept at 22°C (±0.5) for 24 (22°C 24 h) and 48 (22°C 48 h) h. Immediately after harvest (T0) and at the end of each treatment,30 berries per biological replicate (three biological replicates per lot) were collected and immediately analyzed for technological parameters. For VOCs analysis 30 berries per biological replicate (five biological replicates for each treatment) were homogenized and a NaCl buffer solution (1 M) has been added (1:1) by using an UltraTurrax (Mod. T25, IKA),immediately frozen in liquid nitrogen and stored at -80°C.

Technological parameters

The weight loss (WL) of 5 bunches from each lot was measured by using a technical balance. These 5 bunches were tagged and weighed at T0 and at the end of each treatment. For each of the three biological replicates a total of 30 berries were manually pressed and the obtained must was centrifugated

(8,000 rpm, 5 min, 22°C), filtered with syringe filters (0.22 µm pore size, 33 mm diameter, Sigma-Aldrich, Italy) and used for the following analyses: pH, using a pH meter (pH-metro GLP21; Crison Instruments); TSS employing an optical refractometer; titratable acidity (TA), titrating 7.5 mL of filtered must with 0.1 N sodium hydroxide (NaOH), expressed in g/L of tartaric acid equivalent. For each of the three biological replicates, 30 berries were powdered with liquid nitrogen and total polyphenols were then extracted from 250 mg of berries powder with 1.25 mL of 80 per cent methanol and then centrifugated at 4°C, 10,000 rpm for 15 min. The total polyphenols content (TPC) was then measured using the Folin-Ciocalteau method (Singleton and Rossi, 1965), expressed as mg of gallic acid equivalents (GAE) x 100 g^{-1} fresh weight.

HS-SPME GC-MS analysis

The pre-homogenized (as described above) samples were thawed and 10 g were weighed in a 20 mL glass crimpvial for headspace analysis (Cat. No. SU860049, Sigma-Aldrich, Italy) sealed with silicone septa for SPME (Cat. No. 27362, Sigma-Aldrich, Italy). The grape samples were incubated under agitation for 30 minutes at 40°C. VOCs were sampled at the same temperature for 30 min using an SPME fiber (50/30 µm, DVB/CAR/PDMS, 1 cm long; Supelco, Bellefonte, PA, USA). The fiber was desorbed into the injector of the GC set at 250°C for 5 min (splitless mode). A Clarus 680 Gas Chromatograph equipped with a split/splitless injector (PerkinElmer®, Waltham, Massachusetts) was used for the analysis. Volatiles were separated on a fused-silica capillary column (DB-Wax, 60 m, 0.32 mm ID, 0.25 µm film thickness; Restek, Bellefonte, PA). Helium was used as carrier gas with a flow rate of 1 mL min^{-1}. The GC-MS settings employed were the same adopted by Genova and Montanaro (2012). For the identification of the compounds, a mass spectrometer (Clarus 500 Mass spectrometer, PerkinElmer®, Waltham, Massachusetts) coupled to the GC was used. Each chromatogram was deconvoluted using AMDIS software (National Institute of Standards, Gaithersburg, MD, USA). Each peak was identified by comparing the experimental spectra with those of the National Institute for Standards and Technology (NIST98, Version 2.0, USA) data bank including only compounds with 75 per cent of identity or more. The peaks were quantified using TurboMass software (TurboMass®, Version 5.4.2 PerkinElmer Inc., USA, 2008), by integration of the peak's areas. The area of each peak was normalized on the sum of the areas of all peaks detected in the same chromatogram to eliminate variations in fiber adsorption. The efficiency of the fiber was monitored by running on daily bases a quality check (QC) sample, calculating the percent of variance in the total area of the QC chromatograms. For each sampling time and treatment five biological replicates were analyzed.

Statistical analysis

Each set of replicates was tested to detect outliers performing principal component analysis (PCA) employing Metaboanalyst online tool (Chong *et al*., 2019).

One-way ANOVA was performed on technological parameter and GC-MS data following a post hoc Tukey's honestly significant difference (HSD) test (with $p = \leq 0.05$) for multiple comparation using GraphPad Prism version 7 (GraphPad Software, La Jolla California USA).VOCs revealing statistically significant differences between treatments were then analyzed by means of partial least square discriminant analysis (PLS-DA) using Metaboanalyst online too (Chong *et al*., 2019).

3. Results

Considering technological parameters, as expected all samples lost weight after 24 and 48 h, following both cooling treatments (4 and 10°C) and control conditions (22°C) (Table 1). The WL percentage was higher in the control, which showed the highest value after 48 h. The lowest WL value was recorded for grapes cooled at 4°C for 24 h. The WL of grapes cooled at 10°C for 24 h was not significantly different from samples kept at 4 and 22°C for the same time. Both cooled samples at 48h showed significantly lower WL values than the respective control. Compared to T0 samples the pH values were slightly lower in all samples except for grapes kept at 22°C for 48 h, while TA values significantly decreased only in berries kept for 24h at 4°C and in the 48 h control sample (Table 1). With the exception of this latter sample, compared to T0 a general reduction of TSS values was observed in comparison with T0 sample. TPC was significantly lower in comparison to T0 in control berries kept at 22°C for 24 and 48 h (Table 1). Low temperature treatments induced variable effects on this parameter with increases in 10°C 24 h and decreases in 10°C 48 h samples.

The grapes VOCs profile was acquired by HS-SPME GC-MS. A total of 35 VOCs has been detected. Among

Table 1 - Technological parameters in Vermentino wine grapes at harvest (T0) and after post-harvest treatments at 4°C for 24 h (4°C 24 h), 10°C for 24 h (10°C 24 h), 4°C for 48 h (4°C 48 h) and 10°C for 48 h (10°C 48 h). 22°C is the temperature of the control samples

Technological parameters	T0	4°C 24 h	10°C 24 h	22°C 24 h	4°C 48 h	10°C 48 h	22°C 48 h
Weight loss (%)	-	1.4±1.5 c	2.3±0.6 bc	4.5±1.9 b	2.8±1.5 bc	4.5±1.5 b	9.3±2.1 a
pH	3.46±0.02 b	3.40±0.0 c	3.33±0.0 d	3.34±0.0 d	3.39±0.0 c	3.36±0.01 d	3.50±0.0 a
Titratable acidity (g/L^{-1})	4.6±0.2 ab	4.2±0.0 c	5±0.1 a	4.5±0.0 b	5±0.1 a	4.7±0.0 a	3.7±0.0 c
Total soluble solid (° Brix)	21±0.0 a	19±0.0 b	16.5±0.4 d	19±0.0 b	17.6±0.3 c	18±0.0 c	20.4±0.2 a
Total polyphenols content (GAE/100 gr FW)	613.6±60.4 b	550±47.3 bc	722.7±84.5 a	560.4±9 c	555.6±83 bc	492.1±77.2 c	363.3±46.7 d

Different letters indicate statistically significant differences at p≤0.05 according to the results of the Tukey's HSD test. Values are the mean of three biological replicates +/- SD.

them, 14 terpenes, 7 esters, 4 alcohols, 3 aldehydes, 2 alkanes, 2 benzene derivates, 1 ether, 1 alkane and 1 phenol were identified. One-way ANOVA test was run on the whole VOCs dataset: a total of 11 compounds resulted significantly different (p≤0.05) between treatments (data not shown). Among the 11 statistically significant VOCs, 5 compounds of interest, known for their impact on grapes and wine aroma, were present and so used for a PLS-DA analysis. These compounds were three sesquiterpenes (cadinene, cubebene and isoledene) and the monoterpene dihydro-citronellol, which are generally associated with floral and spicy notes, and ethyl acetate, which is considered an off-flavor and associated with the anaerobic metabolism. The PLS-DA was carried out separately for the two sampling times. Cadinene, cubebene and isoledene, dihydro-citronellol and ethyl acetate levels were used as predictor variables, while the different treatments and T0 were used as response variable. The effect of the 24 h treatment is reported in figure 1. After 24 h of treatment, the model explained 51.6 per cent of the variability present in the dataset and in this projection the different treatments and T0 samples partially overlaps (Fig. 1A). Figure 1B reports the VIP scores for the employed features. The highest score is attributed to cubebene, which seems to be strongly accumulated in berries held at 10°C. Noticeably, the level of all the terpenoids considered is higher in the cooled grapes, regardless the temperature, with the only exception of the monoterpene dihydro-citronellol which showed the lowest level in 4°C sample. Interestingly, the most marked variation for the three sesquiterpenes (cubebene, cadinene and isoledene) is observed when comparing cooled with T0 samples. On the other hand, control grapes kept at 22°C are characterized by an accumulation of ethyl acetate. A slight accumulation of this compound is found also in grapes held at 10°C (Fig. 1B).The effect of 48 h treatment is reported in figure 2. The model explains 55.3 per cent of the variability with still an overlapping of the treatments (Fig. 2A). Fig. 2B reports the VIP scores for the employed features. The highest score is attributed to isoledene, which is markedly accumulating in berries kept at 10°C. Furthermore, cooled grapes showed again a higher content of terpenoids comparing with the T0. As far as ethyl acetate is concerned, this compound shows the highest level in the control grapes and the lowest in T0 samples.

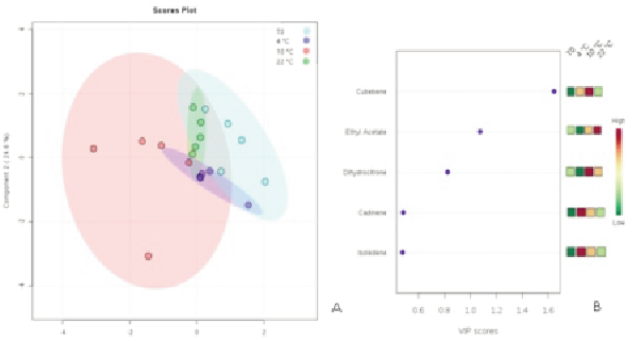

Fig. 1 - A) Partial least squares discriminant analysis (PLS-DA) performed on VOCs detected in Vermentino grapes following cooling treatment at 4 and 10°C for 24 h and control treatment at 22°C. Cadinene, cubebene, dihydrocitronellol, isoledene and ethyl acetate levels were used as predictor variables while the different treatments and T0 were used as response variables. Each color represents different treatment with five replicates. 95% confident intervals are presented in ellipses. B) The variable importance in projection scores of PLS-DA (VIP scores). The coloured boxes on the right indicate the relative concentrations of the corresponding metabolite in each group under study.

4. Discussion and Conclusions

In detached fruits, WL progresses with time and is dependent on the vapour pressure deficit, the evapo-

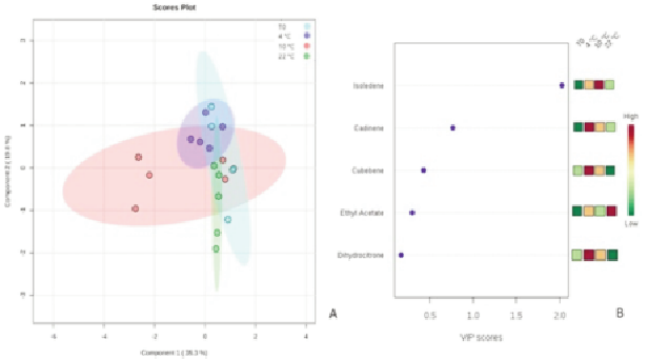

Fig. 2 - A) Partial least squares discriminant analysis (PLS-DA) performed on VOCs detected in Vermentino grapes following cooling treatment at 4 and 10 °C for 48 h and control treatment at 22°C. Cadinene, cubebene, dihydro-citronellol, isoledene and ethyl acetate levels were used as predictor variables while the different treatments and T0 were used as response variables. Each color represents different treatment with five replicates. 95% confident intervals are presented in ellipses. B) The variable importance in projection scores of PLS-DA (VIP scores). The coloured boxes on the right indicate the relative concentrations of the corresponding metabolite in each group under study.

rative driving force for water movement and affected by both temperature and relative humidity (Cirilli *et al*., 2012). Harvested fruits, including grape berries, already react at low WL values with metabolic changes eventually affecting grape composition (Costantini *et al*., 2006; Rizzini *et al*., 2009; Tonutti and Bonghi, 2013). In the present trial, control grapes that showed the highest WL values most likely underwent specific water stress-related reactions more pronounced than those occurring in low temperature samples. Previous studies (Bellincontro *et al*., 2009) have demonstrated that a temperature between 5 and 10°C helps to reduce weight loss and to maintain the cellular structure of the berries, with a general reduction of metabolic events. In both control samples (kept for 24 or 48 h at 22°C), high values of TSS well correlate with the loss of weight and the consequent concentration of solutes. The variability present among samples for this parameter but also for pH and TA might be the consequence of the heterogeneity of the samples, collected in a commercial vineyard.

The effects of cooling grapes before vinification on these technological parameters appear to be more clearly defined after 48 h of treatment. This appears also true concerning TPC that increased in cooled samples after 48 h. The effect of postharvest low temperature on TPC has been reported for table grapes by Maoz *et al.* (2019) who showed that storage at 0°C for 6 weeks, led to an upregulation of several genes involved in the phenylpropanoid pathway and to the accumulation of stilbenes and flavonoids. Maintaining harvested berries at 4-10°C can be considered as mild stress: it is well known that postharvest cold stress induces changes in fruit secondary metabolic pathways and compounds, including phenylpropanoids (Dixon and Paiva, 1995; Ruiz-García and Gómez-Plaza, 2013; Mencarelli and Bellincontro, 2018).

Concerning the volatile compounds, our results indicate that, as general effect, low temperature conditioning of Vermentino grapes has an impact on the volatile terpenoid content of the berries. It is well known that the presence of terpenoids significantly affects the aroma of grapes and wines (D'Onofrio *et al*., 2017), and this is particular important for wines vinificated from neutral variety such as Vermentino. Terpenoids are classified based on the number of carbons present in the chemical structure: monoterpenes (10 carbons), sesquiterpenes (15 carbons), diterpenes (20 carbons), triterpenes (30 carbons), and carotenes (40 carbons) (Yu and Utsumi, 2009; Li *et al.*, 2019). Among the different classes, a significant influence on the aroma of grapes and wine has been attributed to the monoterpene class which, in wine, is generally associated with pleasant floral notes (D'Onofrio, 2011). Along with monoterpenes, sesquiterpenes are another important subclass. To date, there has been limited research on sesquiterpenes since they are considered less volatile and aroma-active than monoterpenes (May and Wüst, 2012; Black *et al*., 2015). However, sesquiterpenes have been recently correlated with significant organoleptic characteristics of grapes (D'Onofrio *et al.*, 2017). Indeed, their concentrations in berries can be crucial for the final wine quality (Luo *et al*., 2019) since sesquiterpenes are more stable than monoterpenes and once extracted from the berry they can be retained in the finished wine (Dunlevy *et al*., 2009). It has been suggested that they provide balsamic, woody and spicy notes (Slaghenaufi and Ugliano, 2018). Based on our preliminary results, it can be hypothesized that low temperature post-harvest treatment is effective in improving specific aromatic traits of Vermentino berries and, possibly, wines. The observed increase of terpenoids could be the result of changes in specific metabolic steps of this chemical class. A key reaction is the conversion of farnesyl pyrophosphate (FPP) to sesquiterpenes, catalyzed by the different members of the terpene synthase (TPS) family (Tholl, 2006; Muhlemann *et al*., 2014). It is well known that TPS activity and so terpenoids

biosynthesis strongly depends on both endogenous and environmental factors (Robinson *et al*., 2014). Specific studies performed on grape berries in the field showed that high temperatures could reduce terpenoids biosynthesis, and also induce the degradation of thermolabile compounds (D'Onofrio, 2011). Concerning specifically post-harvest, TPS have been studied in toon buds by Zhao *et al.* (2019). They found a strong increase of transcripts related to terpenoid biosynthesis under low temperature condition, which resulted with sesquiterpenoid accumulation. Additional studies are so needed to understand if the observed increase of terpenoids in Vermentino berries is due to a low-temperature induced biosynthesis or just a maintenance of the pre-accumulated compounds.

The use of low temperature in post-harvest and food production is widespread. This preliminary study shows that cooling treatment immediately after harvest have significant metabolic effect on wine grapes. As general effect, it seems that cooling treatments are effective in improving the terpenoids-related aroma pool of the neutral variety Vermentino. This effect appears to be strongly dependent on the applied temperature as well as on the treatment duration: the specific effects of cooling and the interplay between these two parameters (temperature x treatment duration) need to be further elucidated. In fact, the time-course of events occurring in detached fruits (progression of senescence and water loss) may amplify/widen, or limit/reduce the metabolic changes induced by low temperature treatments. In addition, these preliminary results obtained on wine grape berries need to be implemented and compared with the technological and organoleptic evaluations of the resulting wines.

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AHS
Advances in Horticultural Science

The residues of fruit and vegetable processing: from "waste" to "resource" of natural phytochemical compounds

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Abstract: The project of Sant'Anna School, in line with the Italian legislation on limiting waste and promoting the redistribution of surpluses and unused goods, aimed to study the potential healthy value of residues obtained from the transformation of fruit and vegetable products that represent a cost, as they must be handled, stored and disposed according to stringent actual regulations. Two "model" species (potato and apple) were considered to test the possibility of using industrial processing waste for food applications. The extracts, obtained with "green" methods from potato and apple peels, were evaluated as natural antioxidants in the preparation of minimally processed fresh-cut apple. Results suggest the possibility to use these novel byproduct extracts as valuable alternative treatments to traditional chemical additives employed for minimally processed apples.

1. Introduction

The Italian law n. 166 (2016) defined as "antisprechi" has been formed with the aim of limiting waste, while promoting the redistribution of surpluses and unused goods. This law relies on two fundamental principles guaranteed by Italian Constitution: subsidiarity and solidarity. For the first time, it formally defines the terms "waste" and "surplus", constituting a reference point for dialogues between different institutions opening up new perspective for investigation on new products recovered from byproducts. In agreement with these regulations, the Italian Ministery of Agriculture (Mipaaft) established financial contributions to support innovative projects, related to research and technological development, in the field of food shelf life and packaging, which ensure a concrete application of the results achieved. The project of Sant'Anna School was one out of 10 selected and financed by Mipaaft in 2017.

The Italian food industry is constantly growing, with an estimated

increase of + 1.5% in 2018 equal to 134 billion euro (there were 132 in 2016). The industrial production of the agri-food sector has grown by more than 5% in the last fifteen years (Caroli *et al*., 2019).

The transformation activities produce large quantities of organic waste whose disposal represents an additional cost for the food industries as they must be moved, stored and disposed. In Italy, the annual residues deriving from the canning industry are estimated at around 1400 kt of d.m. (Balsari *et al*., 2011). Consequently, the reuse of waste is a strategic theme in the search for 'renewability' for the industrial application of raw materials of vegetable origin, also to meet the interest of users towards eco-sustainable products.

Processing waste from the agri-food industry, in particular fruit and vegetables, are generally considered an excellent source of bioactive compounds with antioxidant activity such as vitamins, phenols and carotenoids. Plants produce a wide range of secondary metabolites, mainly phenolics, with different functions: pigmentation, growth, reproduction, resistance to pathogens (Castoria *et al*., 2009). The positive effects on human health of these phytochemicals have been widely documented and concern the antioxidant, anti-inflammatory and anticarcinogenic action (Auger *et al*., 2004; Manach *et al*., 2005; Bitler *et al*., 2007). Phytochemicals extracted from various species such as olive, citrus, tomato, oregano, green tea, grapes and garlic have been shown to have the ability to inhibit lipid oxidation in various model systems. Therefore, the transformation of waste byproducts into synthetic food additives appears particularly promising (Goni and Hervert-Hernández, 2011).

Two widely worldwide consumed fresh or processed foods, such as potatoes and apples, generate a large waste amount. The by-product management of potatoes (*Solanum tuberosum*) is considered an important problem faced by food processing companies, as they cannot be discharged into the environment due to their high polluting potential (Rodriguez Amado *et al*., 2014). Potato is a source of different bioactive compounds such as starch, dietary fiber, amino acids, minerals, vitamins, and phenolics (Akyol *et al*., 2016). The apple is the most widely consumed fruit, with a wide range of varieties with different organoleptic characteristics. Numerous scientific studies demonstrated these fruits possess a wide range of chemical compounds with beneficial effects on human health: dietary fibers and proteins, secondary metabolites such as vitamins, phenols and carotenoids, which have proven anti-inflammatory and anticarcinogenic antioxidant effects (Boyer and Liu, 2004). The main structural classes of apple constituents include hydroxycinnamic acids, di-hydrochalcones, flavonols, catechins and oligomeric procyanidins, as well as anthocyanin in red apples (Jakopic *et al*., 2007; Veberic *et al*., 2007). In particular, considering the antioxidant capacity of compounds present in apple tissues, this fruit may be considered a valid challenge in the chain of ready to eat products. One of most critical points in fresh-cut fruit slices is the appearance of cut surface browning as a consequence of physical stresses imposed on cells during preparation. To avoid the loose of quality, chemical treatments have been used as food additives (Paiva-Martins *et al*., 2007). However, some of the most common synthetic compounds such as BHA (butylated hydroxyanisole) and BHT (butylated hydroxytoluene) are suspected of harmful effects; thus, alternative antioxidant additives or disinfectants would be needed (Chen *et al*., 2016).

The aim of the project was to evaluate, in two fruit and vegetable "model" species (potato and apple), the possibility of using industrial processing waste for food applications. In this work the attention was focused on the effect of extracts obtained from organic potato and apple peels on the preservation of the physicochemical quality parameters of fresh-cut apples.

2. Materials and Methods

Preparation of fresh-cut apples

Fresh-cut apples were prepared from organic undamaged 'Golden Delicious' (*Malus domestica* Borkh) purchased from a local large retailer. The experimental procedure is showed in figure 1, according to safety statements and recommendations for minimally processed apples (Dávila-Aviña *et al*., 2015). Apples were washed in running water, hand-peeled and cored with a ceramic knife, longitudinally cut into cubes (12 per apple) and completely dipped in different preserving solutions for 2 min, manually stirring. Dipping treatments were performed comparing usually used preservative media (1% butylated hydroxytoluene - BHT - and 1% citric acid - CA) and novel extracts from potato (P) and apple (A) peels obtained by water (W) and 10%

ethanol (Et). After dipping, the apple cubes were drained on absorbent paper and packaged in plastic lidded containers (150 cc). Packaging were put in controlled chambers at 20±1°C to stimulate a short browning reaction or stored at 4±1°C (dark conditions up to 5 days) to simulate the average standard cool retail storage condition. Each dipping treatment consisted of 3 replicates represented by 3 different containers.

Procedure for potatoes and apple peel extracts

The extracts were prepared starting from i) organic yellow-paste potatoes cv. Bologna and ii) organic apples cv. Fuji by cryomaceration of peels which were maintained to direct contact with solid CO_2 (ratio peels/CO_2 1/1 w/w) over 24 hours, according to Venturi *et al*. (2019). The extraction was carried out using as solvents water and Et at 10% following the steps reported in figure 2 and the extracts were stored at -20°C in test tubes saturated with nitrogen, until dipping treatments.

Characterization of peel extracts

The total phenols content was determined calorimetrically at 700 nm, using the Folin-Ciocalteau reagent (Waterhouse, 2001). Phenols content was expressed as gallic acid equivalents. Antioxidant capacity was determined according to the TEAC antioxidant assay which was performed spectrophotometrically at 734 nm following Venturi *et al*. (2017). The radical cation ABTS (2,2'-azino- di-[3-ethylbenzthiazoline sulphonate]) was generated as described by Pellegrini *et al*. (1999). The activities of the extracts were expressed in terms of Trolox equivalent antioxidant capacity (TEAC).

Quality evaluation of fresh-cut apples

Fresh-cut apple characteristics were evaluated by a short test, 3h after cutting at 20°C, and by a cold storage test maintaining the cubes at 4°C until 5 days. The main quality parameters such as browning for the short test, and total solid sugars and firmness for the cold storage test were analyzed.

The level of browning was determined by color of apple cubes surface using a colorimeter (Eoptis, Mod. CLM-196 Benchtop, Tn., Italy) according to Buera *et al*. (1985). Results were expressed as Δ Browning Index (ΔBI) values by the follow equation:

$$\Delta BI = BIf - BIs$$

where
BIf = BI at the end of each observation time,
BIs = BI at the start of each experiment.

$$BI = 100^* (x-0.31)/0.172$$

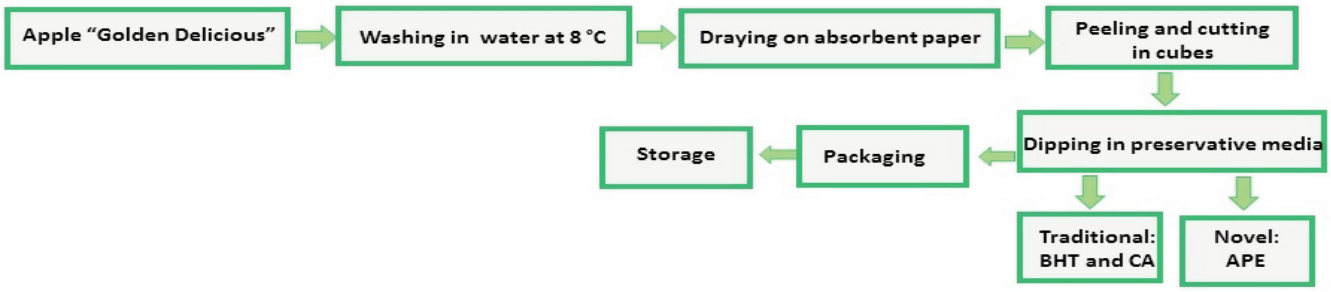

Fig. 1 - Experimental procedure to compare different preservative treatments on fresh-cut apple cubes with traditional (1% butylated hydroxytoluene - BHT - and citric acid - CA) and novel extracts from potato and apple (APE) peels.

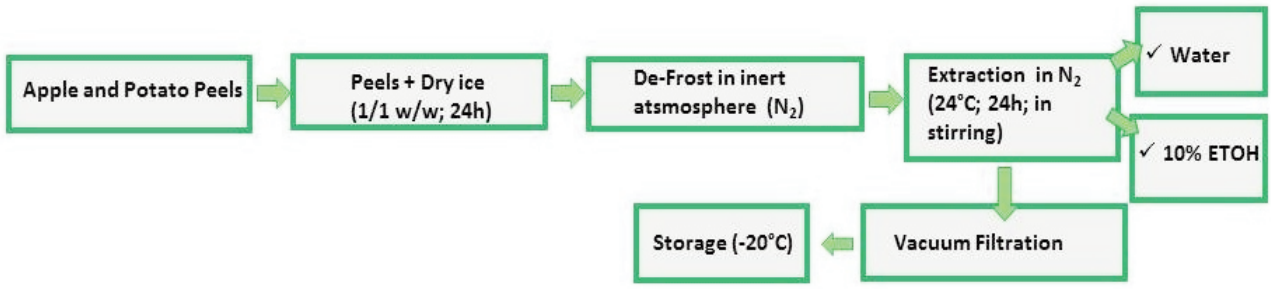

Fig. 2 - Procedure applied to obtain apple and potato extracts using as solvent water and 10% ethanol. Both extracts were stored at -20°C before use.

where
x = (a*+1.75L*)/(5.645L*+a*-3.012b*); L* defines the lightness, a* and b* define the red-greenness and blue-yellowness, respectively.

Total soluble solids (TSS), obtained from the fresh tissue sap of apple flesh cubes were measured using a hand refractometer (Mod. 2369-Bertuzzi, Milan, Italy) and expressed as °Brix.

Flesh firmness of apple flesh cubes was evaluated by a manual penetrometer (Mod. 53205, TR Turoni & C. Snc, Forlì, Italy), with a metal probe (8-mm diameter). The force needed to break parenchyma cells in the cortex was expressed in kilogram-force (kg/0.5 cm^2).

Statistical analysis

Statistical analysis of obtained data was conducted by PRISM 7 (GRAPHPAD, USA). Student *t* test and analysis of variance (ANOVA) with the test of mean comparisons according to Bonferroni were applied, with a level of significance at p≤0.05. All data are reported as mean values ± SE (standard error).

3. Results and Discussion

Peel antioxidant content

From the results reported in Table 1, a very remarkable total antioxidant activity was detected in both apple and potato peels extracts. However, on the basis of the high phenolic content, apple peels were characterized by the highest antioxidant capacity. It has been established that, both in red- and yellow-skinned apples, the highest nutraceutical power resides in the peel, representing the main source of antioxidants where values may reach 80% of total antioxidant capacity (Leccese *et al*., 2009), mainly determined by polyphenols (Wojdylo *et al.*, 2008).

Table 1 - Phenolic content and antioxidant capacity of potato and apple peel water and ethanol extracts

	Phenolic content (gallic acid mg/g dry weight)	Antioxidant capacity (µmol TEAC/mL)
Apple Peel		
Water extract	9.25 ± 0.26	0.33 ± 0.03
10% ethanol extract	13.14 ± 0.20 *	0.73± 0.02 *
Potato Peel		
Water extract	2.92 ± 0.41	0.17 ± 0.02
10% ethanol extract	3.95 ± 0.02 *	0.21 ± 0.01 *

Data are reported as mean ± SE (n= 3). In the columns, and within species, asterisks indicate significant differences between solvents according to Student t test (p≤0.05).

A great influence of solvent composition on antioxidant capacity of the extracts was stood out. The presence of a low ethanol percentage increased the extraction of antioxidant compounds like polyphenols in comparison with solely water in both species. In any case, cryomaceration of vegetal byproducts by means of solid carbon dioxide can be profitably applied to improve the green extraction of bioactive compounds, favoring the processes in solid/liquid extraction as demonstrated on other plant materials (Andrich *et al*., 2003; Zinnai *et al.*, 2015; Nari *et al*., 2018; Ascrizzi *et al*., 2019). According to the literature, potato and apple peels resulted a good source of total phenolics which can have health beneficial properties, mainly due to the presence of chlorogenic acid and caffeic acid (Wolfe and Wu, 2003; Friedman *et al*., 2017).

Short-time test: effect of dipping on browning of fresh-cut apples

In this study different preservative solutions have been used, in particular those commonly used by the IV gamma industry such as BHT and CA. The activity of these traditional solutions has been compared with that carried out by alternative solutions, deriving from agro-industry waste such as potato and apple peels. Nowadays, consumer awareness about green products is increasingly and in this contest the recovery of waste from the agro-industry can be considered as an evaluable source of chemical compounds of natural origin.

The main parameter negatively affecting the visual quality of fresh cut fruits, like apple, is the presence of oxidation on the surface and in the under layer flash tissue which occurs immediately after cutting. As reported in literature (Queiroz *et al*., 2008) and in previous studies (Venturi *et al*., 2019), it has been observed that the first hours after cutting are very critical due to the appearance of the initial oxidative process symptoms. Thus, the evaluation of browning in a short-time interval can be considered a useable and effective test to screen the efficiency of conservative treatments.

In figure 3 the Δ Browning Index (ΔBI) values determined at 20°C after 3 hours from dipping treatments with commercial and novel compounds are reported. The browning appearance was much more evident in water control dipping, while the tissue browning was significantly reduced when the preservative agents were added. Both apple and potato extracts obtained by 10% ethanol (EtA and EtP), showed the strongest anti-browning effects whilst

water extracts (WA and WP) had values comparable to those obtained with the use of commercial compounds BHT and CA. The beneficial effect exerted by apple and potato extracts could be linked to their phenolic content (Table 1). In the case of potato and its byproducts, the inhibition of the polyphenoloxidase (PPO) activity has been well recognized (Akyol *et al*., 2016). For this reason, potato extracts have been studied for their capacity in reducing lipid oxidation and improving nutritional properties of processed foods (Franco *et al*., 2016). As regard apple, phenolic rich peel extracts were found to be effective inhibitors of polyunsaturated fatty acid oxidation as demonstrated by a stronger inhibitory effect on fish oil oxidation (Sekhon-Loodu *et al*., 2013).

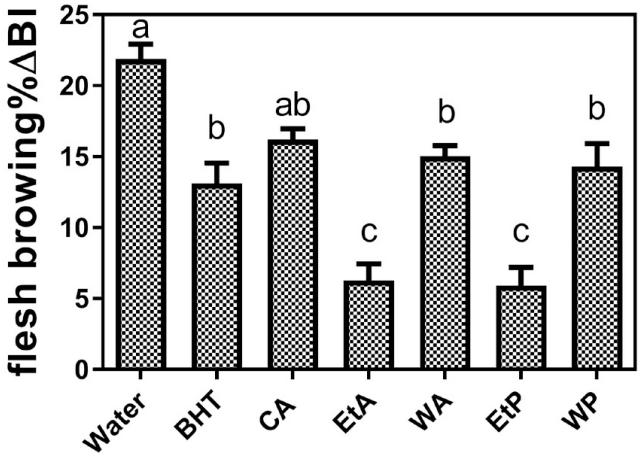

Fig. 3 - Short-time test: effect of dipping on flesh browning of fresh-cut apples. Δ Browning Index (ΔBI) percentage determined at 20°C, after 3 hours from dipping treatments: water; BHT (1% butylated hydroxytoluene); CA (1% citric acid); EtA (10% ethanol apple extract); WA (water apple extract); EtP (10% ethanol potato extract); WP (water potato extract). Data are means ± SE.

Cold storage test: effect of dipping on quality of fresh-cut apples

Temperature is considered the most important factor in the conservation of perishable products. The temperature strongly influences the respiration of the tissues, so much so that as the temperature increases, the respiration also increases (Fagundes *et al.*, 2013). An increase of 10°C has been observed to induce an increase in respiratory activity and an acceleration of the aging process of approximately 2-3 times. Therefore, the temperature must be as low as possible according to the tolerance thresholds of the different species. In general, most products can be stored at temperatures close to zero (0-1°C) with the exception of those are considered as a chilling sensitives (tomato, melon etc.) which must be stored at temperatures of 7-13°C. Storage at non optimal temperatures generates an alteration of cell membranes (Saltveit, 2002) which accelerate respiration and activate the enzymes involved in the detoxification of free radicals, such as copper/zinc superoxide dismutase, catalase and the enzymes of the ascorbate-glutathione cycle. In particular, minimally processed apples must be stored at a temperature not exceeding 4-6°C and need stabilizing treatments to maintain the quality level of fruits during cold storage (Senesi, 2008). Thus, the use of conservative compounds have to be appropriately tested also under cold conditions to address the requirement of keeping fruit quality during all the distribution chain.

In this work, during the storage at 4°C no significant differences were observed in the browning process in comparison with the phenomenon occurred after 3h at room temperature (data not shown). As a consequence, particular attention was focused on the maintenance of other quality parameters as firmness and TSS degree.

As concern apple tissue firmness, it is determined by cell size, biochemical and biophysical cell wall properties, cell-to-cell adhesion and tissue turgor (Toivonen and Brummell, 2008; Rux *et al*., 2017). The loss of texture and the degradation of tissues determine the softening of fruits not only during the ripening but also under particular storage conditions.

In the present study, when the processing procedures started, an average firmness of 2.70 kg/0.5 cm^2 was recorded. Under cold temperature at 4°C (Fig. 4), in water dipping treatment a texture decrease was observed with a tissue strength decline by about 25% and significant differences in comparison with all other treatments were observed. An interesting inhibition of tissue softening was recorded in the apple cubes treated with the peel extracts similarly to that observed using traditional media (BHT and CA). The positive effects of the natural novel compounds, confirmed also after 5 d of storage (Fig. 4), could be related to an attenuation of the physical and chemical changes affecting textural integrity as a consequence of enzymatic hydrolysis of cell wall pectic substances (Van Buggenhout *et al*., 2009). In particular, the softening increase observed in apple cubes after water treatment could be due to texture biochemical changes as reported in several minimally processed fruits and vegetables (Toivonen and Brummell, 2008).

As regard TSS content of Golden Delicious apple,

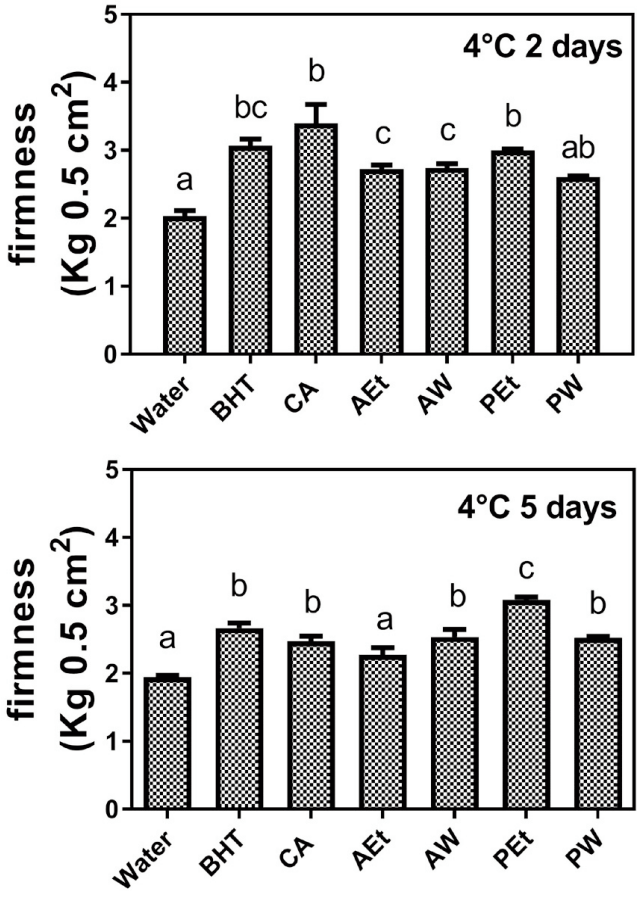

Fig. 4 - Cold storage test: effect of dipping on flesh firmness (kg/0.5 cm^2) of fresh-cut apples determined at 4°C after 2 and 5 days from dipping treatments: water; BHT (1% butylated hydroxytoluene); CA (1% citric acid); EtA (10% ethanol apple extract); WA (water apple extract); EtP (10% ethanol potato extract); WP (water potato extract). Data are means ± SE. Different letters indicate significant statistical difference at p≤0.05.

at commercial stage the changes associated with ripening were already accomplished, so that the maximum soluble level was settled on about 13°Brix. During storage this value should be maintained to preserve the sensory quality of fresh cut apple (Augusto *et al*., 2016; Musacchi and Serra, 2018). The low temperature was effective to maintain the TSS degree, indeed a weak decrement was measured in samples without any conservation treatments stored at 4°C for 2 days (Fig. 5). This was in accordance with several researches reporting no substantial changes in apple slices of cv. Gala coated with alginate and stored at 5°C with values ranging from 14. 6 to 12.8 °Brix (Olivas *et al*., 2007). Analogous results were obtained using apple extracts in contrast to the traditional additives BHT and CA that caused a more drastic TSS reduction. After 5 d storage a notable TSS decrease was recorded in apple cubes dipped in water whilst no significant variations in all other treatments were detected, suggesting a protective effect in minimizing carbohydrate breakdown. The possibility to use natural extracts from other different sources as postharvest treatment, has been also evaluated in several minimally processed apples with profitable results (Augusto *et al*., 2016).

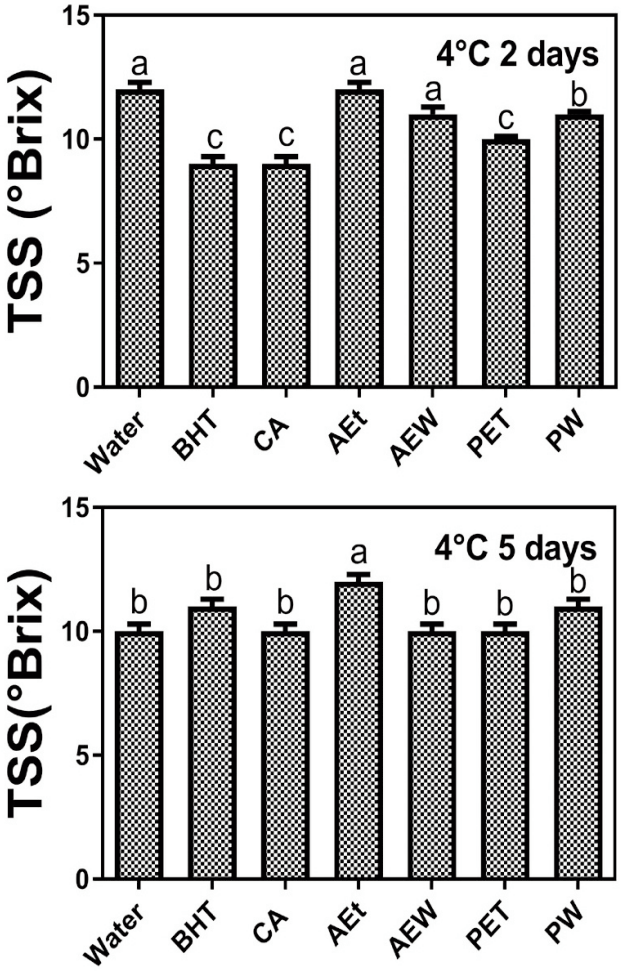

Fig. 5 - Cold storage test: effect of dipping on total solid content (TSS) expressed as °Brix of fresh-cut apples determined at 4°C after 2 and 5 days from dipping treatments: water; BHT (1% butylated hydroxytoluene); CA (1% citric acid); EtA (10% ethanol apple extract); WA (water apple extract); EtP (10% ethanol potato extract); WP (water potato extract). Data are means ± SE. Different letters indicate significant statistical difference at p≤0.05.

4. Conclusions

This study suggests that the residual waste of potato and apple can be considered as a exploitable source of valuable compounds for preservation of minimally processed apples. Potato and apple peel

extracts based treatments positively affected the visual quality of fresh-cut apples as anti-browning agent starting from the beginning of the preparation process. During cold storage of apple cubes, the application of these novel extracts delay the degradation mechanism of the flesh constituents maintaining a good level of firmness as well as improving the nutritional quality.

On the basis of the obtained results, the proposed cryomaceration procedure to recover compounds from potato and apple peels proved to be effective to formulate potential natural additives for postharvest processing. Thus, synthetic preservatives in freshly stored vegetables could be replaced or at least reduced.

Furthermore, the employment of potato and apple peels proposed in this study may contribute to manage the environmental and economic problems caused by the increasing amount of wastes and residues from food processing industries.

Metabolomic characterization of the plant materials tested in this work is under investigation and the next step of our research will be to establish the relationship between the observed effects on fresh-cut apples and the chemical composition of these novel extracts.

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Non-destructive detection of potato tubers internal defects: critical insight on the use of time-resolved spectroscopy

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Key words: absorption coefficient, bruise, internal brown spot, *Solanum tuberosum* cv. El Beida, TRS.

Abstract: Aiming at investigating the feasibility of time-resolved reflectance spectroscopy (TRS) for the non-destructive detection of internal brown spot (IBS) and other defects in 'El Beida' potatoes, 90 tubers were measured in 8 points by TRS for the absorption coefficient at 730 nm (μ_a730) and then transversally cut open for recording presence and position of internal defects and IBS severity. The μ_a730 was lower in healthy tissue than in defected ones and increased with increasing IBS severity with no difference between healthy and slightly IBS tissues. Tubers having at least one out of the eight μ_a730 measures ≥0.04262 cm^{-1} were considered "defected". Therefore, TRS tubers classification performance were: defected, 73.5%; healthy, 45.5%; slightly IBS, 57.1%; moderate IBS, 60%; and severe IBS, 100% of the cases. Misclassification could be due to the high variability in flesh color of 'El Beida' potatoes, as some healthy tubers showed *L, *b** and *C** color parameters very similar to that of defected ones, especially when IBS severity was slight or moderate, resulting in μ_a730 values not significantly different between healthy and IBS tissues. The feasibility of TRS in detecting internal disorders in potatoes must be investigated in other susceptible cultivar to see if flesh color can represent a real problem in the detection of defects linked to browning development.**

1. Introduction

Detecting internal defects (internal brown spot, hollow heart, heat

necrosis, black heart) in potatoes (*Solanum tuberosum* L.) is an important challenge for food engineering as potato is one of the main consumed products in the world: potato occupies the fifth position in terms of production after sugarcane, maize, wheat and rice (FAO, 2019).So, it is crucial to ensure tuber quality along the potato supply chain.

The presence of internal defects is not visible until tubers are cut or peeled and determines economic losses in potato industry as growers are not able to separate healthy from defected potatoes, causing waste during processing, and negatively influences consumer confidence. Usually only a representative sample is cut and, if internal defects are present, the whole lot is removed without verifying the real incidence of affected tubers, so increasing food waste. This problem can be tackled by using non-destructive techniques which potentially allow to segregate raw potato tubers according to the actual presence of internal defects, before the product reaches the fresh market or is processed.

Many noninvasive techniques (spectroscopic techniques, computer vision systems, ultrasound methods) have been investigated to assess internal defects in potato tubers with various performance results depending on the type of defect, as reviewed by Rady and Guyer (2015).

Spectroscopic techniques can detect potato defects as changes in absorbance have been found comparing sound and damaged tissues. However, the similarity of absorption characteristics between skin and damaged tissue represents a limiting factor for the segregation of defected tubers. Rady and Guyer (2015) reported that the classification rate of defected tubers by using spectroscopic techniques ranged from 50 to 98%. Recently, a transmission spectrum system in the visible/near infrared region was able to classify blackheart potatoes with an overall classification rate of 96.5% by using six selected wavelengths (711, 817, 741, 839, 678, and 698 nm) (Zhou *et al.*, 2015). Very good performances were reached by using imaging techniques. Infrared Hyperspectral imaging was able to detect hollow heart in 'Agria' potatoes achieving an accuracy of 89.1% of correct classification (Dacal-Nieto *et al*., 2011). Visible-Near Infrared and Short Wave Infrared hyperspectral imaging coupled with PLS-DA (partial least square discriminant analysis) were successfully used to detect black spot in raw tubers of three potato cultivars achieving an overall correct classification rate of 95.5% and 98.6%, respectively (López-Maestresalas *et al*., 2016).

Internal brown spot (IBS) is a physiological disorder of potato tubers which has an important economic impact in Italy. IBS incidence up to 50% has been observed under inductive environmental conditions and in susceptible cultivars (Parisi *et al*., 2014; Pentangelo *et al*., 2017). IBS is characterized by the presence of punctiform and/or enlarged rust-colored necrosis in the parenchymal tissues of the tubers. Irregular-shaped spots already appear in the vascular ring during the tuber bulking growth stage; IBS symptoms increase from the end of tuber filling to the complete tuber maturity (Raimo *et al*., 2018). IBS can affect different areas of the tuber depending on variety. In some cultivar, such as 'Luminella', necrosis areas are localized in the apical position, while in other varieties, such as 'Ricciona di Napoli', the symptoms can affect large portions of the parenchymal tissues, compromising potato tuber appearance and taste and altering the processing features (specific gravity and frying quality) of the tubers (Pentangelo *et al*., 2017; Raimo *et al*., 2018). Positive correlations have been found between IBS incidence and severity with tuber size and skin roughness (Parisi *et al.*, 2014; Raimo *et al.,* 2018). Environmental conditions, soil properties and irrigation rate also strongly affect IBS development, making difficult the prevention, the prediction and the cure of this disorder (Parisi *et al*., 2014; Pentangelo *et al*., 2015, 2017; Raimo *et al*., 2018). In addition, potatoes affected by IBS do not show external symptoms and at present it is not possible to segregate healthy from IBS tubers during mechanical grading and packaging (Parisi *et al*., 2014; Raimo *et al*., 2018). Recently, Vanoli *et al*. (2012) investigated the possibility of using time-resolved reflectance spectroscopy (TRS) to non-destructively detect IBS in 'Luminella' potato tubers, a well-known susceptible genotype.

TRS is a non-destructive optical technique which, in combination with proper models of photon migration, explores the fruit tissue at a depth of 1-2 cm with no or limited influence from the skin, allowing the measurement of the absorption (μ_a) and reduced scattering coefficients (μ_s) (Cubeddu *et al*., 2001; Torricelli *et al*., 2008). The absorption properties are related to the chemical composition (water, pigments), whereas the scattering properties are related to the structure (intercellular spaces, cell size and shape, starch granules). TRS has been mainly applied in postharvest studies for estimating the internal fruit

attributes related to maturity in apples, peaches, nectarines, plums, mangoes and pears, for discriminating fruit with different texture and sensory characteristics and for the detection of internal defects in fruits and vegetables (Rizzolo and Vanoli, 2016).

The development of internal disorders induces changes in the optical properties, as absorption increases with browning development (Vanoli *et al*., 2014) and scattering properties vary when a defect affects the fruit structure, as for mealiness, woolliness or watercore (Vanoli *et al*., 2010; Lurie *et al*., 2011; Vangdal *et al*., 2012; Rizzolo and Vanoli, 2016). Comparing healthy and browned fruit, the latter show higher μ_a values in the 670-940 nm range, as found in apples (internal browning), pears (brown heart), peaches (browning), plums (browning) and potatoes (IBS). The highest differences between the absorption spectra of browned and healthy tissues were found in the 670-780 nm range, and hence, these wavelengths were selected to distinguish healthy product from defected ones. High positive correlations were found among μ_a measured at 670 nm (μ_a670) and at 780 nm (μ_a780) and browning scores in plums (Vangdal *et al.,* 2012), nectarines (Lurie *et al.*, 2011) and in apples (Vanoli *et al*., 2014). It was possible to use μ_a750 to distinguish healthy 'Granny Smith' apples from those affected by internal browning with the former being characterized by μ_a750<0.030cm^{-1}; similarly, healthy 'Braeburn' apples had μ_a740<0.030 cm^{-1} while in 'Conference' pears, fruit with μ_a720≤0.034 cm^{-1} were not affected by brown hearth (Eccher Zerbini *et al*., 2002; Rizzolo and Vanoli, 2016). In potatoes, μ_a690 was used to segregate healthy from IBS tubers : tubers having μ_a690 values equal or higher than 0.039 cm^{-1} were considered as IBS and were correctly classified in the 81% of the cases. Healthy tubers showed μ_a690 values of 0.031±0.0032 cm^{-1} (mean±standard deviation) and were all correctly classified by TRS (Vanoli *et al*., 2012). These promising results were limited by the fact that the most part of the tubers had a small size and a round shape making quite easy the detection of IBS measuring each tuber by TRS in correspondence of four equidistant points around the equator.

The aim of this work was to detect IBS in 'El Beida', an oval shaped variety characterized by large size tubers with white flesh, in order to find the most suitable TRS set-up which allow to probe the whole bulk of each potato revealing also the presence of other defects such as necrosis, black spot and bruises.

2. Materials and Methods

Potatoes

Potato tubers cv. El Beida were supplied by a local grower in Bologna province (Italy) who found IBS in some potato samples. All potatoes were washed and dried with a paper towel. Defective samples showing bruises, rots, holes and greening were removed, and 120 tubers were selected for the experiment: 90 tubers were used for defect detection and 30 tubers for flesh color measurements. The tubers for defect determination were labeled, and morphological parameters including weight and diameters (x=longest axis, y= longest axis normal to x; z= longest axis normal to y) were measured. The geometric mean diameter (GMD) and the sphericity of each tuber were calculated according to Mohsenin (1986) as following:

$$GMD = (xyz)^{1/3}$$

$$Sphericity = GMD\ x^{-1}$$

Then, each tuber was measured by TRS and cut open for detecting IBS.

Time-resolved Reflectance Spectroscopy (TRS)

Each tuber was measured by TRS for the absorption coefficient at 730 nm (μ_a730), being this wavelength suitable for detecting IBS in potato (Vanoli *et al*., 2012). Considering both biological (large size of 'El Beida' tubers, IBS randomly distributed within the flesh), and the instrumenal characteristics (geometry of the TRS fibers), TRS measurements were performed in two regions of the tuber, at 15 mm distance from the sample center (on the right side-RING1 and on the left side-RING2), rotating the tuber 90° each time (0°, 90°, 180°, 270°) for a total of 8 measurement points.

A portable compact setup working at discrete wavelengths developed at Politecnico di Milano (Martinenghi *et al*., 2016) was used. The light source is a supercontinuum fiber laser (SC450-6W, Fianium, UK) providing white-light picosecond pulses, with the duration of a few tens of picoseconds. A custom-made filter wheel loaded with 14 band-pass interference filters (NT-65 series, Edmund Optics, New Jersey, USA) is used for spectral selection in the range 540-940 nm. Light is delivered to and collected from the sample by 1 mm fiber placed at 1.5 cm distance from the illumination point. A second filter wheel identical to the first one is used for cutting off the fluorescence signal originated from the sample when it is illuminated in the visible spectral region.

The light then is detected with a photomultiplier (HPM-100-50, Becker&Hickl, Germany) and the photon time-of-flight distribution is measured by a time-correlated single-photon counting board (SPC-130, Becker&Hickl, Germany). The instrumental response function has a full width at half maximum of about 260 ps and the typical acquisition time is 1 s per wavelength. A model for photon diffusion in turbid media was used to analyze TRS data to assess the bulk optical properties of the samples (Martelli*et al.*, 2009) to obtain the estimates of μ_a and μ_s at each wavelength.

IBS assessment

After TRS measurements, each tuber was transversally cut open in correspondence of the central part of the tuber, and at 15 mm from the center on the right and on the left side where the TRS fibers were positioned. Then, each equatorial section of each tuber was photographed, and the presence and position of internal defects and the IBS severity in correspondence of each TRS measurement point were recorded. Tuber without any visual IBS were considered healthy (H) while those affected by IBS at least in one section out the eight TRS measured sections were considered IBS (IBS).

IBS was also scored according to its severity as slight, moderate and severe considering the size of the tissue affected by IBS and the color intensity of the browned areas.

Flesh color

Thirty tubers were transversally cut open and color was measured on two opposite sides of the flesh with a spectrophotometer (CM-2600d, Minolta Co., Japan), using the primary illuminant D65 and 2° observer in the L^*, a^*, b^* color space. From a^* and b^* values, hue ($h°$) and chroma (C^*) were computed according to:

$h°$ = arctangent (b^*/a^*) × 360/(2×3.14) and $C^*=(a^{*2} + b^{*2})^{-2}$.

3. Results and Discussion

On average, 'El Beida' tubers had GMD = 67.2±0.6 mm (mean±SE) and sphericity=0.77±0.05 showing that potatoes studied in this experiment had medium-large size and cylindrical shape (Table 1), and were different from 'Luminella' potatoes used in the previous experiment (Vanoli *et al.*, 2012) which had GMD= 51.8±0.5 mm and sphericity= 0.91±0.01.

IBS was found in 26.7% of the tubers and the other defects were observed in 48.9% of the tubers, while 24.4% of tubers were healthy. Tubers affected by other defects showed small brown or grey spots under the skin and only in one case there was some internal necrosis area.

Table 1 - Morphological characteristics of potato tubers cv. El Beida

	Weight (g)	Diameter x (mm)	Diameter y (mm)	Diameter z (mm)	GMD (mm)	Sphericity
Mean	196.4	87.8	64.8	53.5	67.2	0.77
Min	96.7	65.4	52.1	44.2	54.0	0.67
Max	382.7	116.0	80.6	64.7	83.0	0.88
SD	55.5	11.0	6.3	4.9	6.1	0.04
SE	5.8	1.2	0.7	0.5	0.6	0.005

Table 2 - Absorption coefficient measured by TRS at 730 nm (μ_a730, cm-1) in healthy and in defected tubers and in relation to IBS severity

	Tissue type			IBS severity		
	Healthy	Other defects	IBS	Slight	Moderate	Severe
Mean	0.0375	0.0413	0.0420	0.0386	0.0404	0.0468
Min	0.0204	0.0228	0.0309	0.0309	0.0338	0.0338
Max	0.0542	0.0563	0.0651	0.0454	0.0517	0.0651
SD	0.0042	0.0053	0.0066	0.0033	0.0041	0.0082
SE	0.0002	0.0005	0.0007	0.0007	0.0007	0.0016

Considering the severity of IBS among IBS tubers, 29% showed slight severity, 42% moderate severity and 29% severe symptoms.

In a previous work on IBS detection in potatoes by TRS (Vanoli *et al.*, 2012), healthy and IBS affected tubers were measured in the 540-900 nm range and the highest relative percentage differences between the absorption coefficient values of these tissues were found in the 580-690 nm range, thus μ_a690 was chosen for IBS detection. However, also in correspondence of μ_a730 the relative percentage difference between healthy and IBS tissue was high. So, we choose to measure potato for the absorption coefficient at 730 nm. We select 730 nm also because in the current TRS set-up a higher power was available compared to 690 nm.

The μ_a730 was significantly lower in healthy tissue than in defected ones ($p \leq 0.05$), even if no difference was found between IBS affected tubers and those affected by other defects (Table 2).In IBS tubers, μ_a730 significantly increased with increasing IBS severity; however, no significant difference was found between healthy and slightly IBS tissues (Table 2).

Similarly, μ_a740 and μ_a750 measured in apples affected by internal browning, increased with the development of browning with healthy fruit showing the lowest μ_a740 and μ_a750 values and those affected by brown flesh the highest ones (Vanoli *et al.*, 2010, 2011). In addition, μ_a740 and μ_a750 values increased with increasing browning severity, even if this increase was significant only in fruit with moderate and severe browning while no difference was found between healthy flesh and that affected by slightly browning (Vanoli *et al*., 2010, 2011).

In order to fix the threshold value of μ_a730 above which a potato tuber can be classified as affected by IBS or by other defects, the mean and the 95% Confidence Intervals of μ_a730 value of defected tissues were computed (μ_a730= 0.04368 ± 0.00105 cm^{-1}). Hence, tubers having at least one out of the eight μ_a730 measures equal or higher than 0.04262 cm^{-1} were classified as affected by IBS or by other defects.

Figure1 shows the μ_a730 values for the eight measurement points for each of the 90 tubers under examination.

Only 73.5% of the tubers affected by defects was correctly classified by TRS measurements: 70.8% of tuber affected by IBS and 75.0% of tubers with other defects. Considering IBS potatoes, slightly affected tubers were correctly classified in 57.1% of the case, moderate IBS in 60% of the cases, while 100% of severe IBS affected tubers were identified. Probably when IBS was slight and moderate, the single brown spots within the potato flesh and/or brown area with slightly brown color make the IBS detection by TRS difficult.

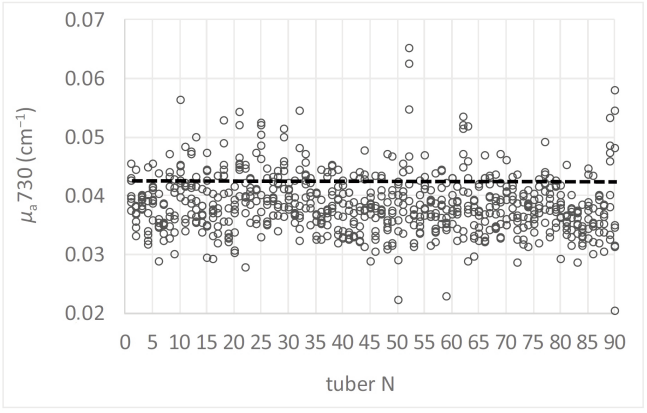

Fig. 1 - Values of the absorption coefficient measured by TRS at 730 nm in each tuber. The horizontal dashed line corresponds to the threshold value of μ_a730 for defect detection.

Figure 2 shows some examples of TRS measurements at 730 nm in correspondence of the 8 points (4 points for RING 1 and 4 points for RING 2) around the tuber in comparison with the actual localization of the defect within the flesh. Figures 2A and 2B show two IBS affected tubers correctly classified by TRS: when IBS spot are present, then the μ_a730 values were above the threshold values of 0.04262 cm^{-1} (see points 1, 3, 5 and 7 RING 1 and points 4 and 8 RING 2 for panel A; points 3, 5 and 7 RING 1 for panel B), while in healthy regions, the μ_a730 values were below the threshold values (see points 2 and 6 RING 2 for panel A; points 1 RING 1 and points 2, 4, 6 and 8 RING 2 for panel B). Figure 2C shows that TRS was able to reveal the presence of a bruise spot under the skin as only in point 4 RING 2 the value of μ_a730 was above the threshold of 0.04262 cm^{-1}. However, in panel D of Figure 2 is reported an example of an IBS affected tuber not correctly classified by TRS, as IBS spots are present in correspondence of the points 1 and 5 RING 1 and point 8 RING 2 but all the μ_a730 values are below the threshold of 0.04262 cm^{-1}.

It seems that the detection of defects in 'El Beida' potatoes did not depend on tuber size. Considering the distribution of potato tubers within 4 GMD classes (50-60 mm, 60-70 mm, 70-80 mm, 80-90 mm), 86.5% of the tubers belongs to 60-70 mm and 70-80

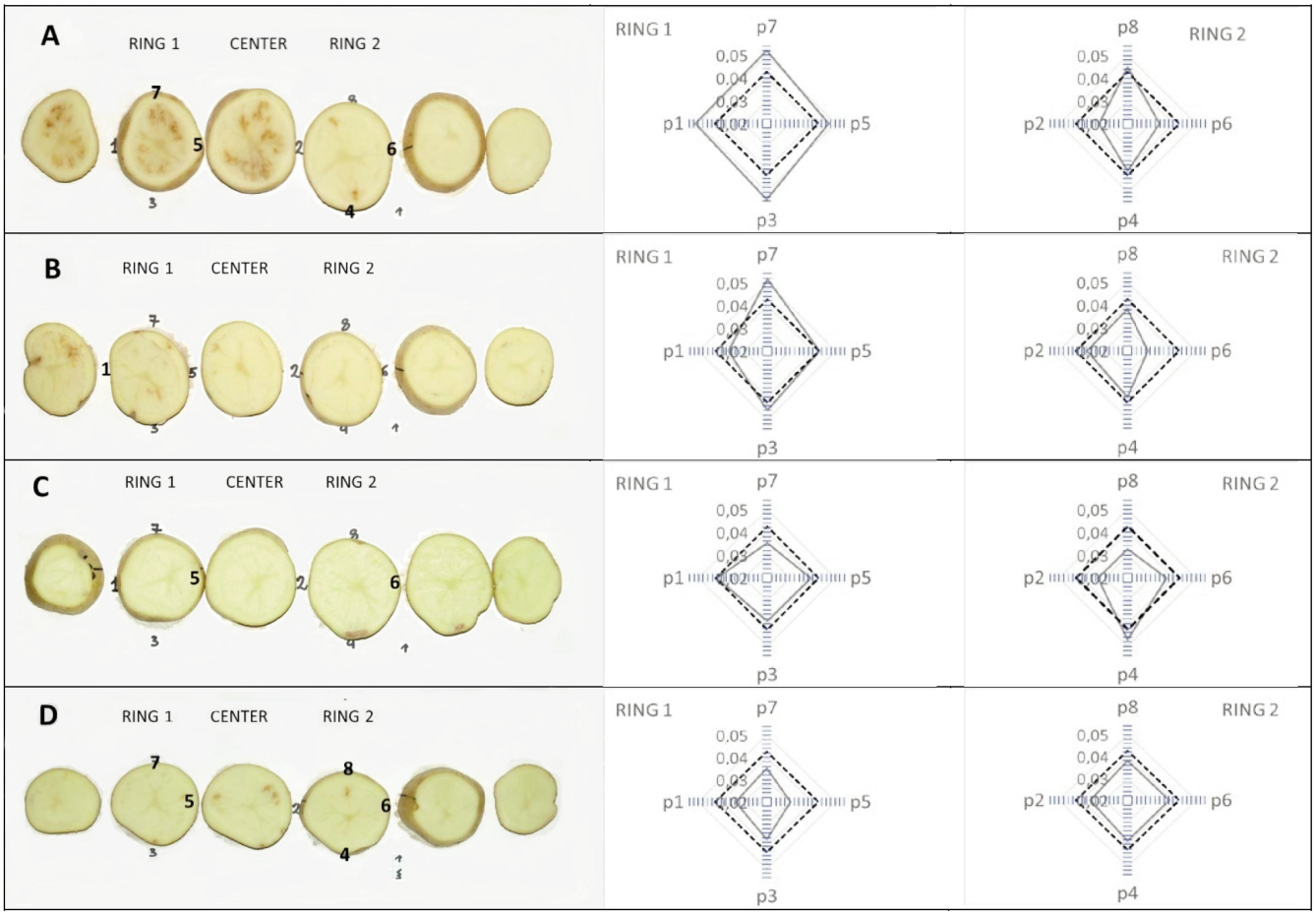

Fig. 2 - Comparison between localization of defects in potato tubers (left) and the corresponding TRS measurements at 730 nm (right). IBS tubers correctly classified by TRS (panels A and B); tuber with bruise correctly classified by TRS (panel C); IBS tuber not identified by TRS (panel D). The dashed lines correspond to the threshold value of μ_a730 for defect detection.

mm classes, where tubers correctly classified as defected by TRS are 70.0% and 75.0%, respectively (Fig. 3).

On the other hand, there were some problems in the identification of healthy tubers, as only 45.5% of healthy tubers was correctly classified by TRS. In this case it can be hypothesized a kind of relationship with tuber size, as the percentage of tuber correctly classified as healthy increased with tuber size, being correctly classified 0% of tuber for 50-60 mm class, 45.5% for 60-70 mm size and 71.1% for 70-80 mm size (Fig. 3). Healthy tubers considered by TRS as affected by defect were characterized by μ_a730 values ≥0.04262 cm^{-1} in at least 1 point out 8 measured ones by TRS (Fig. 4).

This misclassification could be due to differences in the flesh color of potato tubers. In fact, changes in the absorption coefficients measured by TRS in the

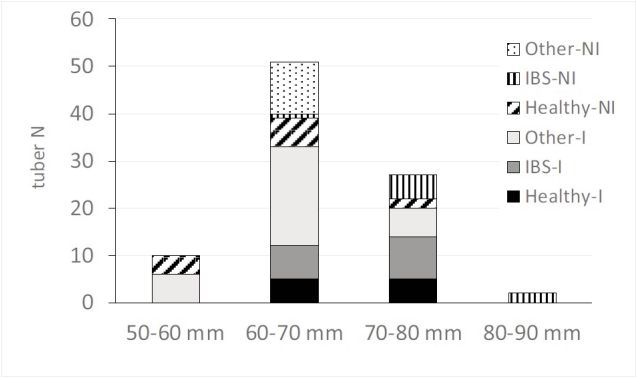

Fig. 3 - Healthy and defected tubers distribution according to 4 GMD classes correctly (I) or not correctly (NI) identified by TRS.

540-780 nm range reveal variations in the flesh color due to the presence of pigments (carotenoids, anthocyanins, chlorophylls) or to browning development.

Good correlations were obtained between TRS absorption spectra and total carotenoids content (R^2_{cv}=0.83 -0.93) and flesh color parameters (R^2_{cv}=0.78-0.96) in different mango cultivars (Vanoli *et al*., 2016).

In fruit affected by browning, high negative correlations were found between μ_a measured at 720 nm ('Conference' pears), 740 nm ('Braeburn' apples), 750 nm ('Granny Smith' apples) and *L** and *h°*, while positive correlations were observed for *a**, *b** and *L** (Eccher Zerbini *et al*., 2002; Vanoli *et al.,* 2010, 2011). Flesh color was significantly different between browned and healthy tissues, showing the former higher *a**, *b**, *C** and lower *L** and *h°* values than the healthy ones (Eccher Zerbini *et al*., 2002; Vanoli *et al.,* 2010, 2011). *L** and *h°* significantly decreased and *a** significantly increased with increasing browning severity, even if no clear distinction was found between healthy and slightly browned tissues.

In this experiment, 30 tubers not used for IBS detection were cut open and flesh color was measured. Six tubers showed IBS: in this case flesh color was measured on browned areas. 'El Beida' potatoes were characterized by a white flesh color (Table 3). As expected, tissue affected by IBS had lower values of *L** and *h°* and higher values of *a**, *b** and *C** than healthy tubers (Table 3), as previously observed in pears affected by brown heart (Eccher Zerbini *et al*., 2002) and in apples with internal browning (Vanoli *et al.*, 2010, 2011). Considering healthy potatoes, a high variability in flesh color is observed, with *L**, *b** and *C** values close to those of browned tissues. This scenario could explain why healthy tubers with darker flesh color have been classified by TRS as affected by defects. In the previous work on 'Luminella' potatoes (Vanoli *et al*., 2012), in which all healthy tubers were correctly classified, flesh color was not measured, so it can only be hypothesized that flesh color of this cultivar showed less variation than in 'El Beida' and with values of healthy tissue not so close to slightly affected tubers, making easier the correct tubers classification.

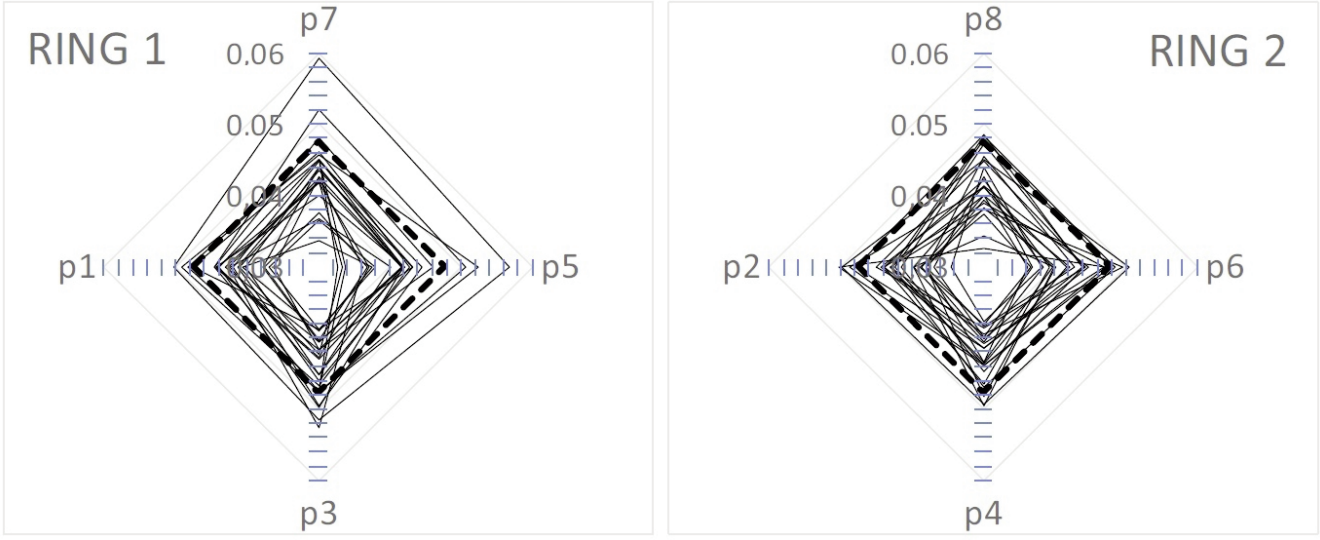

Fig. 4 - Values of the absorption coefficients measured at 730 nm in healthy tubers. The dashed lines correspond to the threshold value of μ_a730 for defect detection.

Table 3 - Color of healthy and IBS potato flesh

	Healthy flesh					IBS flesh				
	*L**	*a**	*b**	*C**	*h°*	*L**	*a**	*b**	*C**	*h°*
Mean	71.78	-2.21	15.80	15.95	97.93	62.96	0.21	19.23	19.27	89.74
Min	66.93	-2.85	14.38	14.49	96.90	56.30	-1.39	17.12	17.19	85.02
Max	74.80	-1.80	19.01	19.21	98.77	68.93	1.82	21.63	21.67	94.57
SD	1.91	0.24	1.04	1.05	0.63	4.28	1.19	1.99	1.98	3.53
SE	0.39	0.05	0.21	0.13	0.13	0.49	0.49	0.81	0.81	1.44

4. Conclusions

Time resolved reflectance spectroscopy has shown to be a feasible tool for detecting internal defects in potato tubers of medium-large size. However, there are some problems to be solved.
First at all, 'El Beida' potatoes showed high variability in flesh color, with some healthy tubers having color very similar to those affected by internal defects and so healthy tubers were misclassified by TRS measurements. On the other side, when IBS severity was slight or moderate, it was difficult to find significant differences between the absorption coefficients measured at 730 nm in healthy and IBS tissues, as flesh color was not so different. So, the feasibility of TRS in detecting internal disorders in potatoes must be investigated in other susceptible cultivar in order to see if flesh color can represent a real problem in the detection of internal defects linked to browning development.

In this study, eight measurement points were used to explore each tuber in a non-destructive way. This TRS set-up allowed to better explore the whole bulk of each potato: in fact, IBS detection did not depend on the tuber size. However, when IBS developed through some small and brown spots, the detection by TRS was very difficult. On the other hand, it's not possible to increase the regions explored by TRS, as when the fibers are positioned too close the tuber ends, the TRS signal is not reliable due to boundaries effect. At boundaries, photon can escape from the tissue and, if it not properly modelled, this might introduce overestimation of the absorption coefficient. The TRS set-up used in this study is based on the contact between the tubers and the optical fibers; the positions and the distance between the fibers determine the volume explored by TRS; perhaps, a non-contact system could allow to better localize the defect, if properly coupled to advanced modelling of the boundaries effect. It is worth noting that these effects can influence classical (not time resolved) NIR spectroscopy system since absorbance estimate are influenced not only by absorption and scattering properties of the tissue but also by the geometrical properties (size and shape).

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New active packaging for improving the shelf life and quality of tomato

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Abstract: Packaging materials play an important role in the quality preservation during postharvest storage and shelf life of fruits and vegetables. The properties of film can affect the gas composition and the physiology of the products. The Group Research Labs of SAES Getters S.p.A. have developed, in collaboration with its affiliate company SAES Coated Films S.p.A. a packaging system highly selective for ethylene, including a coating (1-2 µm), with good transparency and an additional anti-fog function, which can be deposited on various plastic films. The objective of the work was the evaluation of this new active packaging on 'cherry' type tomato berries during storage. The control consisted of a macro-perforated polypropylene film used for flow-pack packaging. Two experimental tests were conducted, the first during cold storage (10 ± 2°C) and the second at room temperature (22 ± 2°C). The analyses included visual appearance, weight loss, gas exchanges, relative humidity, volatile organic compounds (VOC), colour, titratable acidity, refractometric index (° Brix) and the concentration of lycopene and β-carotene. The use of the innovative packaging did not alter the main quality indicators of the tomato and the parameter that had the greatest impact on the product metabolism was the storage temperature. The VOC concentration was influenced by packaging, with a greater accumulation in the samples packed with active packaging compared to the control. Moreover, the same material determined a reduction in weight loss, a greater accumulation of CO_2 and a significant reduction in O_2, especially at 22°C. At the end of the storage, the berries stored in the active packaging showed higher levels of lycopene, compared to controls, and a reduction in β-carotene, indicating an active role of the material in modulating the content of bioactive compounds in the fruits. It can be concluded that the use of this innovative material can represent an effective tool for improving the postharvest management of tomato berries.

1. Introduction

The preservation of produce quality during postharvest depends on the packaging materials and storage conditions. The postharvest performance of produce is affected by pre-harvest factors and quality at harvest (Sharma *et al.*, 2014; Tyagi *et al.*, 2017). It is well known that quality is

obtained in the field and depends on growers choices and agronomic management. Nevertheless, postharvest stresses can induce different transcriptional and metabolic changes that may accelerate the senescence processes (Cavaiuolo *et al.*, 2017). During storage the products are still considered as living organs or tissues, thus their metabolism must be maintained active. Sugar content in the produce plays an important role for keeping the basal metabolism after harvest and higher sugar concentrations can allow longer shelf life, without compromise the quality. The main physiological processes that are associated with the postharvest performance are respiration rate and ethylene production (Colelli and Elia, 2009). Ethylene plays an important role in climacteric fruits and its effect depends on tissue sensitivity. In the most of climacteric products, lowering both processes, respiration and ethylene production greatly extend the storage and shelf life of the produce. Tomato (*Solanum lycopersicum* L.) is one of the most important products in the fresh vegetables market, particularly relevant in Italy, which is the first producer in Europe and among the ten most important producing countries in the world (Faostat, 2018). On a biological point of view, tomato is a climacteric fruit which has been widely studied as a model fruit for the production and sensitivity to ethylene accompanied by the characteristic rise in the respiratory rate during ripening. On a practical point of view, tomato berries are considered as a perishable product which can be easily subjected to quality loss during storage and shelf life, mainly because of the ethylene production and rise in respiration. For these reasons, innovative solutions and suitable packaging systems able to extend storability and shelf life of fresh produce with high transpiration rates such as tomato are needed (Agudelo-Rodriguez *et al.*, 2020). In fact, the packaging materials can play an important role in regulating gas exchanges between the environment and produce. The combination of gas modulation and optimal storage temperature can greatly extend the shelf life of produce (Fagundes *et al.*, 2015). Today, different solutions are available on the market for the preservation of fruit and vegetables using active packaging, with very different principles of action and effectiveness. The chemical and physical properties of the packaging materials can provide a barrier modifying the gas composition in the headspace or around the produce (Exama *et al.*, 1993). The gas composition of headspace in the boxes or in bags depends from the film gas permeability and produce respiration and ethylene production rates. Therefore, the film gas permeability and product physiology define the gas composition that both are affected by the storage temperature (Mangaraj *et al.*, 2009). In order to improve the film properties different materials can be added during the film extrusion or can be applied in one side of it. A good packaging film must keep its proprieties at different intervals of temperature, but especially at the low temperatures (Exama *et al.*, 1992). The packaging materials can help in reducing the water losses by reducing the transpiration and can also reduce the weight losses. These are key factors for the commercial quality of stored horticultural products. The barrier effect of the film can passively reduce the oxygen concentration and increase the carbon dioxide inside the package. The modification of the inner atmosphere by the action of the packaging material is also known as modified atmosphere packaging (MAP). MAP reduces respiration and ethylene production, thus is widely applied in the postharvest management of various fruits and vegetables. However, it is important to avoid hypoxic conditions that would derive from the oxygen depletion inside the package. This would result in the development of off-odours due to the triggering of a fermentative metabolism. Several additives can be included into the packaging film with various functions including the reduction of condensation (anti-fog additives) or the removal of specific gases such as ethylene. There are several ethylene absorbers that can be used for removing or reducing the concentration of this important plant hormone inside the packaging (Álvarez-Hernández *et al.*, 2018).

The Group Research Labs of SAES Getters S.p.A. have developed, in collaboration with SAES Coated Films S.p.A., and its affiliate company, a highly selective packaging system for ethylene, including an integrated absorber in the form of a coating (1-2 µm), with good transparency and an additional anti-fog function, which can be deposited on various plastic films.

The aim of this work was to evaluate the efficacy of this new film in improving the shelf life of tomato berries during shelf life.

2. Materials and Methods

Plant material and storage conditions

The experiment was conducted on tomato fruits (*Solanum lycopersicum* L. var. *cerasiforme*). Freshly

harvested berries were selected based on uniformity in size and lack of defects, which were estimated by a visual evaluation. Moreover, the colour uniformity was assured by selecting berries which showed similar hue and chroma values, which were measure by a colorimeter (for more details, see section "Fruit colour measurements"). Around 250 g of fruits were randomly divided into plastic punnets which were immediately flow packed with an automated flow packaging machine provided by SAES Getters S.p.A. Two distinct materials were used, a conventional macro perforated plastic film (control) and an innovative, highly selective active packaging system, characterized by a good transparency and an enriched with anti-fog additives and including an integrated ethylene absorber in the form of a coating (1-2 µm) (2DAF) (International Patent Application n° PCT/IB2016/050401, in the name of SAES Getters S.p.A.).

Punnets were then divided into two groups and placed in storage rooms (72 ± 2 RH%) maintained at 10 ± 2°C (optimal storage conditions) and 22 ± 2°C (sub-optimal storage conditions) for up to 13 days. Three punnets for each temperature/film were used. Sampling was performed after 1, 3, 6, 8, 10 and 13 days of storage.

Analysis of gas composition

At each timepoint gas composition within the punnets was performed by using the F-950 Three Gas Analyzer (Felix Instruments, Settle, USA). This instrument allowed measuring the concentration of oxygen (% O_2), carbon dioxide (% CO_2) as well as the pool of volatile organic compounds (ppm VOCs) including ethylene, esters and alcohols. Moreover, this instrument provides the value of relative humidity of the atmosphere analysed. Each punnet was transferred to 20°C for 30 minutes to equilibrate, before the analysis.

Weight loss estimation and sampling

At each time point, the fruit net weight within each punnet was measured with a laboratory scale (FZ500i digital milligram balance, A&D Company, Ltd., Japan).

Fruit colour measurement

At each time point, lightness (L*), hue angle (H=arctan(b*/a*) and chroma (C*= sqrt ($a^{*2}+b^{*2}$)) values were measured with a Minolta Chroma meter (CR-300 with an 8-mm aperture) calibrated against a standard white tile. Each record was reported as average of six measurements taken from six fruit.

Titratable acidity and total soluble solids content

Titratable acidity (TA) and total soluble solids (TSS) were measured from the juice squeezed from four fruits. TA was determined by titration of 5 g of juice with 0.1 N sodium hydroxide to a pH end point of 8.1, results are expressed as % citric acid. Titration was performed using a Titrator Compact G10S (Mettler, Toledo, USA). TSS were estimated using a portable digital (model 53011, Turoni, Italy) and results were expressed as °Brix.

β-carotene and lycopene determination

Tomato (four fruits) fruits were ground and juices were analysed for lycopene content using the spectrophotometric method adapted from Anthon and Barrett (2007). The lipophilic fraction of the juice was extracted and separated with a solution of hexane ethanol and acetone (2:1:1 v/v), the phases were separated, and the hexane phase was read at 503 nm. Pigments concentrations were expressed in mg Kg^{-1} on fresh weight (FW) basis.

Statistical analysis

All data were subjected to a one-way ANOVA followed by Sidak's multiple comparisons test. Statistics were performed using GraphPad Prism version 6 for Windows, GraphPad Software, La Jolla California USA, www.graphpad.com.

3. Results

Weight losses and gas composition inside the packages

In general, the tomato berries did not show much variations in weight during storage under the tested conditions. Only few changes were observed in 2DAF-storaged fruits, which showed higher weights compared to control fruits after 8 days at 10°C and after 13 days at 22°C (Fig. 1A, 1B).

Gas composition inside the packages was primarily affected by temperature and then by the material used. In fact, even if samples stored in the 2DAF film accumulated higher concentrations of CO_2 and consumed more O_2 compared to control samples, at 10°C these differences were not significant (Fig. 2A, 2C). At 22°C instead a strong decrement in O_2 and a progressive accumulation of CO_2, were observed. The minimum O_2 levels was reached after 8 days of storage, while the maximum peak of CO_2 was recorded after 10 days at 22°C (Fig. 2B, 2D).

As observed for respiratory gases, the relative

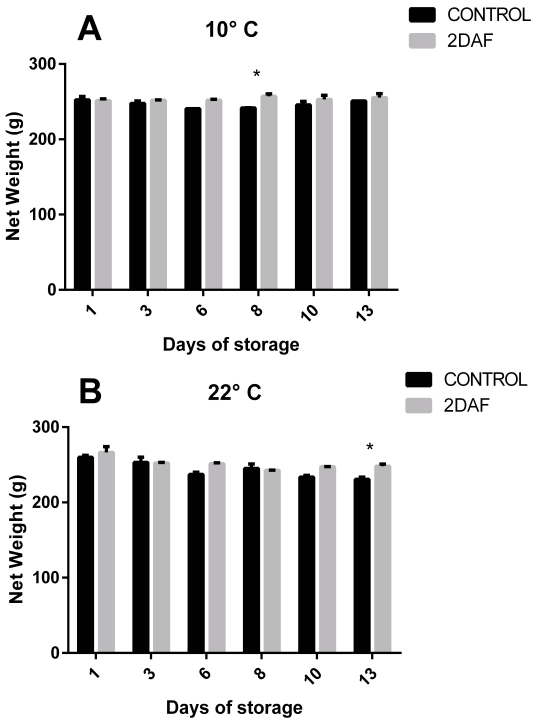

Fig. 1 - Tomato berries weight as affected by packaging material during storage at 10°C (A) and 22°C (B). Asterisks indicate significant differences between 2DAF and control *P<0.05; **P<0.01; ***P<0.001; ***P<0.0001; n=3.

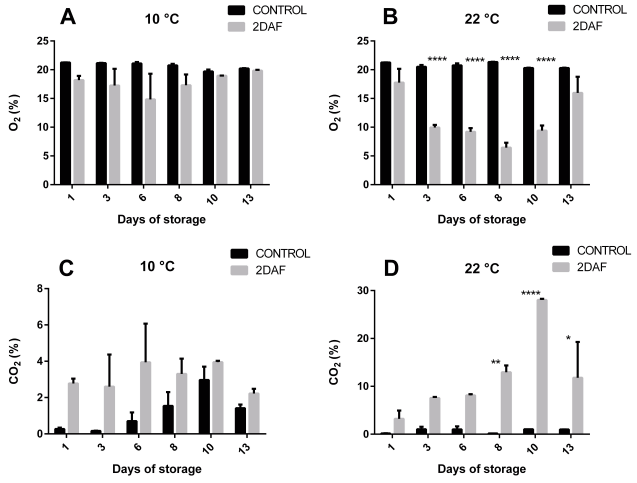

Fig. 2 - Changes in oxygen (A, B) and carbon dioxide (C, D) concentrations inside the packages, as affected by packaging material and storage temperature. Asterisks indicate significant differences between 2DAF and control *P<0.05; **P<0.01; ***P<0.001; ***P<0.0001; n=3.

humidity was affected by temperature. At 10°C no changes were observed between the two films (Fig. 3A), while at 22°C control packages accumulated a higher humidity in the inner atmosphere. These changes in comparison to 2DAF, were highly significant after 1 day of storage and significant (*P<0.05*) after 10 days (Fig. 3 B).

The accumulation of volatile organic compounds (VOCs) in control packages was limited and tent to fade during storage at both temperatures. On the other hand, the use of 2DAF allowed a more marked rise in VOCs concentration inside the headspace. VOCs maximum concentration was observed after 6 days at 10°C and after 8 days at 22°C. The highest concentration of VOCs was almost 7-fold higher at 22°C compared to the maximum concentration at 10°C (Fig. 3 C, 3D).

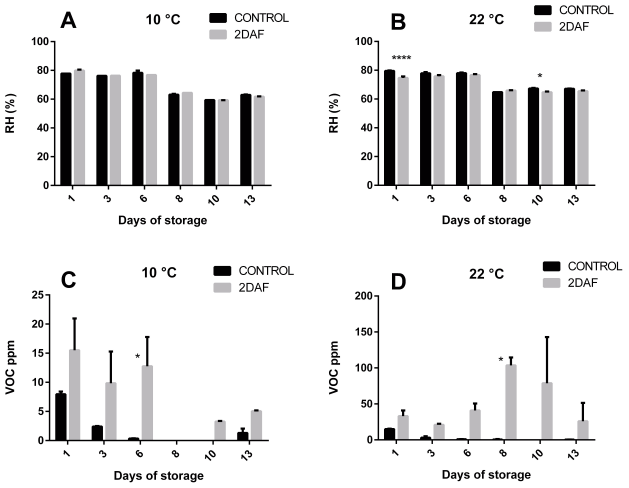

Fig. 3 - Changes in relative humidity (A, B) and volatile organic compounds (C, D) concentrations inside the packages, as affected by packaging material and storage temperature. Asterisks indicate significant differences between 2DAF and control *P<0.05; **P<0.01; ***P<0.001; ***P<0.0001; n=3.

Titratable acidity, total soluble solids, and fruit colour

Quality attributes of tomato berries were slightly affected by storage and packaging material. Titratable acidity was higher in control berries after 3 days of storage at 10°C, while at the other time points as well as at 22°C, no significant changes were observed between control and 2DAF-stored fruits (Fig. 4 A , 4 B).

Sugar concentration of tomatoes showed only few changes in fruits stored at 10°C, which, after 3 and 8 days showed higher °Brix values when packed in the 2DAF film (Fig. 4C, 4D).

No marked changes in colour were observed, with only two exceptions regarding 2DAF-stored berries, which showed significantly higher lightness and hue angle values after 10 days at 22°C (Fig. 5 A-F).

Fruit carotenoid concentrations

Both β-carotene and lycopene levels were quite stable during storage, however, it seems that the dif-

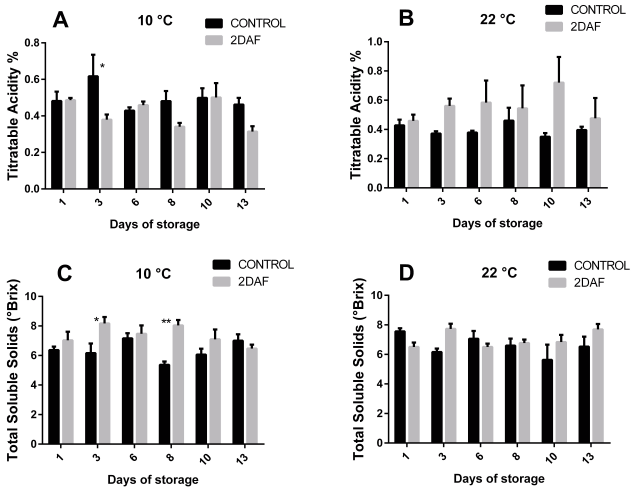

Fig. 4 - Changes in titratable acidity (A, B) and total soluble solids content (C, D) in tomato berries, as affected by packaging material and storage temperature. Asterisks indicate significant differences between 2DAF and control *P<0.05; **P<0.01; ***P<0.001; ***P<0.0001; n=4.

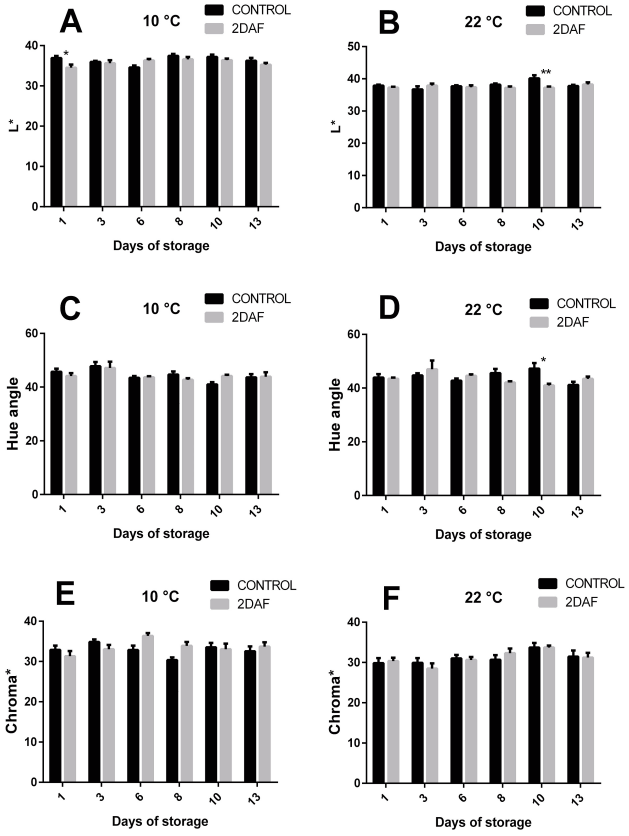

Fig. 5 - Changes in colour coordinate: lightness (A, B), hue angle (C, D) and chroma (E, F) measured on tomato berry skin, as affected by packaging material and storage temperature. Asterisks indicate significant differences between 2DAF and control *P<0.05; **P<0.01; ***P<0.001; ***P<0.0001; n=6.

ferent packaging used affected the accumulation these pigments, which were generally higher in 2DAF-stored samples. β-carotene concentration was significantly higher in 2DAF-stored samples after 8 days at both 10°C and 22°C (Fig. 6 A, 6B), while lycopene was significantly higher in the same packaging conditions, after 3 and 8 days at 10 °C and at the end of storage at 22°C (Fig. 6 C, 6D).

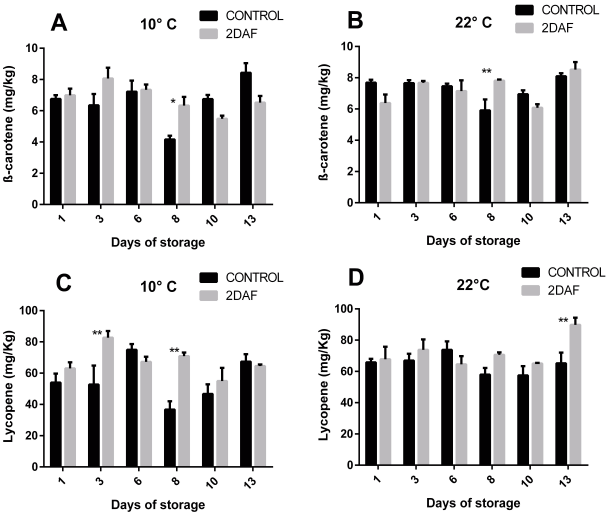

Fig. 6 - Changes β-carotene (A, B) and lycopene (C, D) concentration in tomato berries, as affected by packaging material and storage temperature. Asterisks indicate significant differences between 2DAF and control *P<0.05; **P<0.01; ***P<0.001; ***P<0.0001; n=4.

4. Discussion and Conclusions

The use of MAP has led to a significant improvement of storage management of various horticultural products in the last years (Arah *et al.*, 2016). Tomato is highly appreciated worldwide for its characteristic sensorial properties and for its nutraceutical value, mainly due to the presence of carotenoid pigments including β-carotene and lycopene as the most representative (Frusciante *et al.*, 2007). The experiment described in this paper helped in elucidating the effect of an innovative packaging solution in comparison to a traditionally adopted microperforated plastic film during storage at two temperatures. It is also important to consider that tomato is chilling sensitive and low temperature can induce injury and reduce the quality. The identification of adequate film gas permeability can extend the storability of tomato and preserving the fruit quality (Fagundes *et al.*, 2015).

As first consideration it is important to notice that none of the considered storage conditions determined a marked weight loss, thus it is possible to assume that there were no marked changes in the water content of berries. This last aspect has been considered when evaluating all the parameters which

have been assayed on fresh weight bases. Different storage conditions, together with genetic variability can affect the respiration of the produce during storage and shelf life (Colelli and Elia, 2009). Among the possible factors affecting the accumulation of O_2 and CO_2 inside the punnets, temperature was the most effective. It is possible that the lowest storage temperature (10°C) determined a decrease in the fruit metabolism and thus no significant changes were observed. On the other hand, the higher storage temperature (22°C) can be considered as optimal for several metabolic activities (Maul *et al.*, 2000). In this context, the effect of the different packaging was evident, with a more pronounced O_2 decrement accompanied to a progressive accumulation of CO_2 into the headspace, due to the respiratory activity of fruits. It is also to be considered that the control reference film was macro-perforated, thus it was expected to have a very/no low barrier effect toward gases. Nevertheless, it is interesting to notice that punnets wrapped in the control film accumulated higher humidity compared to those packed with the 2DAF film. High relative humidity is not recommendable because even though it can help in preventing the water loss, it could facilitate fungal development (Paull, 1999).

As for gases and relative humidity, the production and accumulation of VOCs was first influenced by storage temperature and then by the packaging film. Tomato aroma and flavour are characterized by a complex pool of volatile organic compounds (Krumbein and Auerswald, 1998). The approach adopted in this trial allowed us estimating the total concentration of volatiles in a simple, non-invasive and rapid way. As a drawback, it did not permit the identification of the single classes of molecules which could include, ethylene, esters and alcohols, among others. However, it is interesting to notice that punnets packed in the 2DAF film accumulated significantly higher amounts of VOCs, especially at the highest temperature of storage. It is well known that at low temperature the volatiles release from tomato fruits is limited due to the reduced metabolic activity and volatility of the molecules (Spadafora *et al.*, 2019). Also, the alteration of the gas composition in the atmosphere surrounding the product can induce the production of specific volatile metabolites (Rowan, 2011). This finding suggests the possibility to modulate the sensory properties of tomato in the postharvest phase, by selecting proper packaging strategies which allow accumulating/maintaining higher concentrations of quality-related volatiles. The sensory properties of tomato fruits are generally evaluated in terms of soluble solids content and acidity (Beckles, 2012). These two parameters (and the balance between them) are important determinants of tomato quality and consumers acceptance. Apparently, the conditions adopted in this trial were not effective in altering the acidity in a significant manner, although it was higher in cold stored control sample after 3 days. Majidi and colleagues (2014) reported that tomato fruit stored in MAP had significantly higher total soluble solids than those stored at the same temperature (13°C) without MAP. Similarly, in the present study, the combination of low temperature (10°C) and passive MAP, determined an increment in total soluble solids in the central phase of the storage period.

Even if few differences were found, the colour-related indexes did not change markedly in response to the packaging conditions. This was expected, because the tomatoes used in this trial were fully ripe, so the skin colouration was completely developed and uniform at the beginning of the storage. Also, the storage lasted up to 13 days, and in this time frame it is not common to observe marked colour alterations of tomato skin (Rosati *et al.*, 2000; Heredia *et al.*, 2009). On the other hand, significant changes involved the pigments composition of berries. It has been shown that berries lycopene and β-carotene content is not always well correlated with colour indexes, especially during storage (Pék *et al.*, 2010). Some connection can be hypothesized instead, between carotenoids and VOCs. In fact, some low molecular weight organic compounds derive from carotenoids catabolism (Perveen *et al.*, 2015), thus the use of the active packaging could have determined a change in the carotenoid metabolism leading to the coordinate increment of carotenoids and VOCs between 6 and 8 days of storage, as suggested by the results obtained. It has been previously observed that during postharvest, tomato fruits showed an increment in the levels of some important volatile compounds such as heptane and hexanal, and this increment was in line with highest β-carotene and lycopene concentrations in berries. The connection between carotenoids and VOCs was also observed since 6-methyl 5-hepten-2-one, a carotenoid-derived volatile compound, increased in fruits during storage (Franzoni *et al.*, 2018). This aspect worth to be further investigated as involves both the sensory and nutraceutical properties of fruits.

Considering the results obtained, it can be con-

cluded that the use of this innovative material can represent an effective tool for improving the postharvest management of tomato berries.

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Different growing conditions can modulate metabolites content during post-harvest of *Viola cornuta* L. edible flowers

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Abstract: Edible flowers are inflorescences traditionally used in various part of the world to enrich sweet and savoury recipes. The flowers of *Viola* spp. were appreciated since the Romans, and today the fresh products are now incorporated as ingredients in different culinary preparations. In this work, cultivation of potted *Viola cornuta* L. cv. Penny Lane was performed in greenhouse with different environmental conditions (basal heating, additional LED lighting and moisture management) and therefore the biomass production (number of flowers per square meter and plant dimension per pot) was assessed. The plants are characterised by flowers with dark purple and orange petals in the same corolla. The shelf-life of detached flowers was studied in post-harvest conditions at 0 and 4 days of cold storage at 4°C (polyethylene boxes, 12/12 h light/dark condition) to simulate the condition of I gamma products. Sugars and secondary metabolites were analysed. Basal heating seems not to increase flower number but could contribute to reach a well-balanced simultaneous presence of different antioxidant molecules (polyphenols, anthocyanins, carotenoids). Our data highlight that the short cold storage under light condition lead to an increase in the content of total polyphenols and antioxidant activity, although a general reduction in pigments and sugars is observed.

1. Introduction

Edible flowers are currently part of a niche market and perceived as a culinary novelty, even if their consumption is known for thousands of years. In fact, there are several historical evidences that highlight the use of the inflorescences to prepare and garnish dishes, from some ancient

civilisation as Greeks and Romans, to more recent times, e.g. the Victorian period in England (Mlcek and Rop, 2011; Cunningham 2015). Most appreciated species were roses (*Rosa* spp.), calendula (*Calendula officinalis* L.), saffron (*Crocus sativus* L.), dandelion (*Taraxacum officinale* L.), and elder inflorescences (*Sambucus nigra* L.) (Mlcek and Rop, 2011). In Indian and Chinese cultures, edible flowers are used as components of medicines based on herbs, in addition to culinary purposes (Wongwattanasathien *et al.*, 2010). Several edible flowers are beneficial to human health showing anti-infiammatory effects and antioxidant and ROS scavenging activities (Mlcek and Rop, 2011).

Today, around 180 specie are known to produce edible flowers (Lu *et al*., 2016), and *Viola* spp. are among the most common and currently consumed. These flowers are characterized by a sweet and refreshing taste, in addition to a pleasant velvety texture (Neumann and O'Connor, 2009; Koike *et al*., 2015). Edible Violas belong to 3 different species, namely *Viola cornuta* L. (horned pansy), *Viola tricolor* L. (Johnny Jumpup), and *Viola* × *wittrockiana* Gams (garden pansy) (Neumann and O'Connor, 2009). The plants are similar to each other except for flower size, which its diameter is in garden pansies (up to around 11.5 cm) > horned pansies (up to around 2.5 cm) > Johnny Jump ups (less than 2.5 cm in diameter) (Bailey, 1998; Kessler *et al.*, 1998). Over the years, intensive breeding programs selected new varieties with unique flower colours (pure-colour or multi-coloured flowers), greater flowers number, and plant temperature tolerance (Bailey, 1998). The cultivation of *V. cornuta* is similar to the one of *V.* × *wittrockiana.* These speciesare grown as autumn and spring bedding plants, although they are also raised for the summer and winter markets (Pearson *et al.*, 1995). In order to produce edible flowers safe for human consumption, chemical products, such as synthetic fertilizers and pesticides, has to be avoided during plants production; for this reason, only organic cultivation is allowed (Fernandes *et al.*, 2017). No special needs for cultivation are required, indeed well drained commercial potting soil can be used. Viola flowers are often cultivated in greenhouse, and properly defined environmental factors, such as temperature, photoperiod and irradiance are fundamental for the quality of the flowers (Gandolfo *et al.*, 2016). Pansies should be grown between 4 and 13°C, in order to reduce plant growth rate, internode elongation and to ensure high quality flowers (Cavins *et al.*, 2000). In fact, flower size (mm^2) decreased linearly with increasing temperature between 9 and 31°C (Pearson *et al.*, 1995). The ideal temperature for growth and flowering ranges from about 14°C to 21°C (Kessler *et al.*, 1998). Moreover, pansies are obligate FR (far red)-dependent long-day plants and, for this reason, FR radiation are required to promote the flowering process, in addition to red (R) radiation (Kozai *et al.*, 2016). Blue light is able to reduce the time required to produce flower buds in *V.* × *wittrockiana* (Rashidi *et al.*, 2018).

Full-bloomed, edible flowers can be sold in pots or, mainly, in small and medium rigid plastic packages to avoid their rapid drying and to preserve their fragile texture (Whitman, 1991; Kelley *et al*., 2001). However, flowers are high perishable so that different approaches were performed to prolong their shelf-life. Cold storage is documented for *V. tricolor* and *V. x wittrockiana*, using sealed low-density polyethylene film bags. These two species were able to preserve their commercial attractiveness up to 2 weeks of storage, when kept between 0 and 2.5 °C (Kelley *et al.*, 2003). More recently, different new post-harvest technologies were applied on *Viola* spp. Edible coatings (e.g. alginate), crystallization and osmotic dehydration improved violas shelf-life, as shown by a good visual quality for prolonged period (Fernandes *et al*., 2018 a, b, 2019 a, b). Coated pansies contained higher level of polyphenols and antioxidant activity than uncoated ones, on all assayed storage times (up to 14 days). Gamma irradiation are also tested, and this methodology increased polyphenols content and antioxidant activity in *V. tricolor* flowers, compared to no irradiated controls (Koike *et al.*, 2015). Edible flowers are selected and perceived by their fragrance, appearance, size and colour. Consumers prefer yellow and orange flowers rather than blue (Kelley *et al*., 2001, 2002). Within this regard*, V. cornuta* cv. 'Penny Lane' with orange-violet flowers have been selected for this work.

The aim of this work was to cultivate *V. cornuta* L. 'Penny Lane' and test the effect of different cultivation strategies for a higher production of flowers with good quantities of nutritional compounds. Moreover, the post-harvest treatment has been performed to analyze the change in bioactive compounds. Storage temperature was maintained around 4-6°C (cold storage) and flowers were also exposed to artificial light to simulate the refrigerated sector of the grocery market. Metabolites (polyphenols, anthocyanins, carotenoids, sugars) were analysed to determine the shelf-life of packaged flowers as I gamma products. To the best of our knowledge, any investigation of metabolites during post-harvest cold storage studies

were performed on *V. cornuta.*

2. Materials and Methods

Plant cultivation, greenhouse condition and flower blooming

Plants of *Viola cornuta* L. 'Penny Lane' with orange-violet flowers (Fig. 1) were purchased by Gruppo Padana - Ortifloricoltura dei Fratelli Gazzola S.S. Società Agricola (Paese, TV, Italy) and planted in 420 pots with a diameter of 14 cm (1 L volume). They were placed on 4 benches of an iron-glass greenhouse (called SAM-LAB) equipped with a climatic control system at the "Centro di Sperimentazione e Assistenza Agricola" (CeRSAA) in Albenga (SV) 43° 3' 14'' North and 8° 13' 1'' East). At the beginning of the experiment, plants were 3 cm height with a diameter of 2.5 cm. The substrate used was "TS4" soil from "Turco Silvestro" company (Albenga, SV, Italy), characterized by pH 6.5, electrical conductivity 0.56 dS/m, dry bulk density 250 kg/m^3 total porosity 90% v/v. The experimental design foresees 7 treatments (60 plants each) in the SAMLAB and reported in Table 1. The presence or absence of basal heating was guaranteed by either electric mat WARMSET at 50°C or water at 35°C (by hydraulic coil) and the addition of 1 or 2 hours of light after the astronomical sunset to extend the photoperiod, as reported in Table 1. In consideration of the number and the layout of the benches of the greenhouse and the possibility to subdivide the lighting of led lamps used for the experimentation, only the selected treatments were tested (Table 1). Each bench was equipped with 4 LED lamps placed at a distance of 1.50 meters from the surface of the pallet depending on the type of lamp and culture. For the tests, specifically, VALOYA B200 LED lamps with AP673L spectrum were used (blue 12% - green 19% - red 61% - far red 8% - PAR 92%). Each lamp has a total power consumption of 192 W, a photon flux in the range 400-700 nm of 284 μmol s^{-1} and a photon flux in the range 300-900 nm of 311 μmol s^{-1}). During the trial in the greenhouse the registered average temperature was 18°C and the average humidity was 64%. From 01/14/2019 to 02/28/2019 evaluations were carried out to observe whether different kind of basal heating and supplementary light could affect plant growth. At the end of the trials, the investigations included the measurement of plant diameter (cm) per each pot and the number of flowers per meter square. Thus, flowers were picked by hand in the morning for further analyses and cold treated.

Fig. 1 - Flowers of Viola cornuta cv. Penny lane grown in pot in SAMLAB greenhouse.

Flowers storage conditions

Fresh picked flowers were stored in polyethylene boxes at 4 °C with a 12 hours photoperiod in order to simulate better the condition of supermarket fridge

Table 1 - Greenhouse treatments (basal heating of benches and/or additional light) of Viola cornuta cv. Penny lane. Treatments are carried out for 3 months, from transplantation through flowering period until the end of trials

Treatment	Basal heating			Additional light		
	Electric mat 50°C	Hot water 35°C	Absent	1 hour	2 hours	Absent
1	X			X		
2	X					X
3			X		X	
4		X		X		
5		X				X
6		X			X	
7			X			X

counter. The refrigerated cells were equipped with LED lamps (Valoya, Finland) having the following spectrum: blue 21% - green 38% - red 35% - far red 6% - PAR 94%. Flower storage was evaluated after 4 days post-harvest (time 4) performing the following biochemical analyses: total phenolics content, antioxidant activity (DPPH assay), total anthocyanins content, total carotenoids content and total soluble sugars content. For each biochemical analysis three homogeneous biological replica were used. Each replica was stored at -20°C until further analyses. Fresh flowers (time 0) are used as control.

Polyphenol content

Polyphenols were extracted as reported by Bretzel *et al*. (2013). Fresh flowers (200 mg) were homogenized in 2 mL of methanol 70 %, and of total phenolic content was determined using the Folin-Ciocalteau assay (Singleton and Rossi, 1965). The absorbance was read at 765 nm in a UV-1800 spectrophotometer (Shimadzu Corp., Kyoto, Japan) and the total phenolic concentration was expressed as catechin equivalents per gram of fresh weight.

DPPH scavenging activity

The antioxidant activity of each sample was determined through the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) free radical scavenging assay, as described by Brand-Williams *et al.* (1995). The absorbance was read at 517 nm. Antioxidant activity was expressed in IC_{50}, which represent the concentration of the sample able to reduce the initial amount of radical DPPH by 50%. Consequently, lower IC_{50} value of sample corresponds to greater antioxidant activity.

Anthocyanin content

Total anthocyanins were extracted as reported by Bretzel *et al*. (2013). 200 mg of sample were homogenized in 750 µl of acidified methanol (MeOH/HCl 10:0.1). The absorbance was read at 535 nm and the total anthocyanin concentration was expressed as malvidin equivalents per gram of fresh weight.

Carotenoids content

Determination of total carotenoids content was determined using Lichtenthaler's formula (1987). Fresh flowers (100 mg) were added to 5 ml of methanol 99 % and it was incubated for 24 hours at 4°C. The absorbance was read at 665.2 nm, 652.4 nm and 470 nm.

Total soluble carbohydrates content

Total soluble carbohydrates content was estimated from dried flowers (20 mg) using anthrone protocol according to Yemm and Willis (1954). The absorbance was read at 630 nm, using glucose as external standard.

Statistical analysis

The normal distribution of the residuals and the homogeneity of variance was determined and then data were statistically analysed. The results of biomass (number of flowers and growth in pot) were expressed as mean values and analyzed using one-way analysis of variance (ANOVA) followed by Tukey's HSSD Test with p=0,05. The results of post-harvest treatments have been performed using ANOVA Student's t-test to determine the significant difference of each treatment between fresh samples (time 0) and samples after 4 days (time 4), with $p < 0.05$. Biochemical results were analysed by one-way ANOVA followed by Fisher's probable least-squares difference test with cut-off significance at $p \leq 0.05$ (StatView®, Version 5.0, SAS® Institute Corporation). The dependent variables were analysed using two-way ANOVA, with the factors ''Treatment" and ''Post harvest days'' (PHD).

3. Results and Discussion

Horned pansy (*Viola cornuta*) is a biannual plant with long flowering period through different seasons. It is also considered as cold-tolerant plant, since the minimum temperature for flowering is around 4°C, while the optimal temperature is around 26°C (Blanchard and Runkle, 2011). Winter temperature and light are very important factors to determine a good production of flowers (Boldt and Altland, 2019), and heating and supplemental lighting are often provided in greenhouse cultivation to improve the quantity and quality of flowers (Dieleman and Meinen, 2007; Oh *et al*., 2010). For this reason, horned pansy plants were subjected to different treatments (Table 1) to evaluate their effect on the growth and flowers number (Table 2) and thus the yield of edible flowers.

The results indicated that the treatment n. 7 (control, no basal heating, no additional light hours) and treatment n. 3 (no basal heating, 2 h of supplementary light) determined the larger diameter of plants (Table 2). However, there is no statistically significant difference (T student analysis) between treatment n. 3 and the control n. 7 (18.0 and 17.1 cm/plant respectively), probably due to the effect of light towards vegetative growth. The addition of basal temperature of the benches by hot water (treatment

5) corresponded to a decrease of the growth (15,6 cm/plant), while when also additional lighting was performed the decrease was not significant (treatments n. 1, 4, 6). The growth of the plants seems to be affected when the additional light is added, in the absence or with higher temperature of the benches (Table 2). The effect of supplemental LED lighting is known to affect positively many plant growth parameters of several plants, including pansy (Koksal *et al.,* 2015), so these results are in agreement with previous reports. The treatment 2 (electric mat 50°C) highlighted the lowest value of number of flowers (604.44 flowers/m^2) followed by treatment n.6 (636.94 flowers/m^2), while the other trials showed similar higher amounts (Table 2). Taken together, the biometric parameters suggest that the single elongation of photoperiod (2h) plays a positive role to increase the biomass and to produce more flowers, and the temperature is a secondary effect. The quality of plants in relation to light and temperature is debated since long time (Liu and Heins, 1997; Adams *et al.*, 1998), and the lower temperatures and higher irradiance seems to produce higher quality of flowers, including pansy (Pearson *et al.*, 1995; Boldt and Altland, 2019). The results presented here are in agreement of the observed influence of the exposure duration, intensity and combinations of light to the growth and flowering of *V.* × *wittrockiana* (Oh *et al.*, 2010). The lengthening of the photoperiod has been confirmed as important factor in *V.* × *wittrockiana* 'Rose', during experiments aimed to the determine the influence of photoperiod and phytochrome (Rashidi *et al.,* 2018). In that research, the night interruption decreased the plant dimension. The quality of flowers, especially the edible ones, are related to the visible characteristics and nutraceutical components (Benvenuti *et al.*, 2016). Thus, the influence of light and temperature for the production of different metabolites was also determined.

Edible flowers are considered a good source of antioxidant molecules (Rop *et al.*, 2012; Loizzo *et al.*, 2015) and polyphenols (including phenolic acids and anthocyanins) are considered the main antioxidant compounds. A first detail of phenolic composition and properties of *V. cornuta* edible flowers highlighted that their polyphenols content is lower than *V.* × *wittrockiana* (Moliner *et al*., 2019). The metabolites were analyzed at the time of harvest (time 0), and after short period of post-harvest in a chamber at lower temperature and in the presence of light (12h). The post-harvest treatment was chosen to mimic the condition of the benches of grocery stores. Pigments, as carotenoids and anthocyanins are the important compounds for evaluating the visual quality of flowers. At time of harvest (time 0), the worst treatment resulted the n. 3 (addition of 2h light), since the recorded amount of both pigments were the lowest, 0.14 and 5.63 mg/g FW for carotenoids and anthocyanins, respectively (Table 3). Instead, the highest values were determined with the treatment n.1 (temperature 50°C by electric mat and light 1h (0.32 and 10.42 mg/g FW for carotenoids and anthocyanins). The increased temperature, either by electric mat (n. 2) or by hot water (n. 5), did not support any increase in flower pigmentation, both for carotenoid and anthocyanins. Control flowers showed good carotenoid values (0.30 mg/g FW), while anthocyanins suffered without addition of light or temperature (6.93 mg/g FW). Of our knowledge only few papers have been published so far on the

Table 2 - Effect of different cultivation (basal heating and/or supplementary light) on biomass production of Viola cornuta cv. Penny lane

Treatment	Diameter (cm)	Number of flower per m^2
1 Electric mat 50°C + light 1 h	15.8±0.40 ab	838.42±42.9 a
2 Electric mat 50°C	16.2±0.46 ab	604.44±48.75 b
3 Light 2 h	18.0±0.40 a	864.42±40 a
4 Hot water 35°C + light 1 h	16.6±0.33 ab	851.42±33.8 a
5 Hot water 35°C	15.6±0.38 b	812.43±44.85 a
6 Hot water 35°C + light 2 h	16.1±0.24 ab	636.94±43.55 ab
7 Control	17.1±0.50 a	793.93±38.35 a

Plant diameter (cm) and the number of flowers (per meter square) were detected at the end of flowering period.
Data are expressed as mean value (n=60) and analyzed using one-way analysis of variance (ANOVA) followed by Fisher's probable least-square difference test with p=0.05.

Table 3 - Determination of carotenoids, anthocyanins, polyphenols, radical scavenging activity (DPPH assay), and soluble sugars of Viola cornuta flowers grown under different greenhouse conditions (AV1-7, see Table 1) and cold stored for 0 (time 0) o 4 (Time 4) days postharvest

Treatment	Carotenoids (mg/g FW)	Anthocyanins (mg/g FW)	Polyphenols (mg/g FW)	DPPH assay (IC50 mg/ml)	Soluble sugars (mg/g FW)
Time 0					
1 Electric mat 50°C+ light 1 h	0.32 ± 0.00 a A	10.42 ± 0.32 a A	12.42 ± 0.11 a A	0.82 ± 0.00 a A	209.69 ± 3.91 a A
2 Electric mat 50°C	0.27 ± 0.00 c B	8.05 ± 0.20 c A	9.93 ± 0.68 b B	0.80 ± 0.01 a A	183.84 ± 6.97 b A
3 Light 2 h	0.14 ± 0.01e B	5.63 ± 0.14 e B	11.00 ± 0.09 ab A	0.84 ± 0.02 abA	179.57 ± 3.98 b A
4 (Hot water 35°C + light 1 h)	0.29 ± 0.01 bc A	9.28 ± 0.49 bc A	11.85 ± 0.40 ab A	0.86 ± 0.02 ab A	207.45 ± 4.85 a A
5 Hot water 35°C)	0.28 ± 0.01 c A	10.29 ± 0.25 ab A	9.35 ± 1.06 b A	0.89 ± 0.01 b B	181.29 ± 3.84 b A
6 Hot water 35°C + light 2 h	0.26 ± 0.00 d A	6.93 ± 0.42 dA	7.36 ± 0.16 c B	1.19 ± 0.05 d B	183.90 ± 10.96 b A
7 Control	0.30 ± 0.00 b A	8.71 ± 0.57 cd A	7.66 ± 0.25 c B	1.09 ± 0.01 c B	179.90 ± 1.44 b A
Time 4					
1 Electric mat 50°C+ light 1 h	0.33 ± 0.01 b A	4.81 ± 0.19 d B	12.46 ± 0.25 a A	0.78 ± 0.01 a A	203.61 ± 2.03 a A
2 Electric mat 50°C	0.36 ± 0.01 a A	7.63 ± 0.18 c A	11.62 ± 0.16 b A	0.82 ± 0.02 aA	158.91 ± 2.90 d A
3 Light 2 h	0.21 ± 0.00 e A	9.22 ± 0.19 b A	10.88 ± 0.21 bc A	0.84 ± 0.01 b A	161.09 ± 0.36 d B
4 Hot water 35°C + light 1 h	0.28 ± 0.01 c A	10.73 ± 0.56 a A	11.65 ± 0.32 ab A	0.90 ± 0.02 c A	175.50 ± 1.30 c B
5 Hot water 35°C	0.23 ± 0.00 d A	6.65 ± 0.44 c B	10.43 ± 0.45c A	0.77 ± 0.02 a A	164.42 ± 3.41 d A
6 Hot water 35°C + light 2 h	0.25 ± 0.01 d A	4.75 ± 0.49 d B	9.32 ± 0.04 d A	0.94 ± 0.02 c A	159.48 ± 2.99 d B
7 Control	0.27 ± 0.01 c B	7.49 ± 0.79 c A	10.37 ± 0.27 c A	0.78 ± 0.01 a A	192.31 ± 2.56 b A
ANOVA p-value					
Treatment	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
PHD	0.0014	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Treatment × PHD	< 0.0001	< 0.0001	0.0041	< 0.0001	0.0060

Data are expressed as means (n=3, ± SE.) ANOVA followed by Fisher's probable least-square difference test was used, with a cut-off significance at p=0.05. Smalls letter indicate comparisons between treatments at the same postharvest day (PHD); capital letters indicate comparisons between the two PHD for the same treatment. Interaction between treatments and PHD were analysed by two-way ANOVA.

influence of light and temperature on the content of pigments in *Viola* spp. (Rashidi *et al.,* 2018), so these results will contribute to define the effect of these factors and their contribution to the pigmentation. Other metabolites of horned pansy were determined at time of harvest, such as polyphenols andsugars, that are expected as fundamental nutraceutical components, as well as the scavenger reducing power (by DPPH assay). With regards to total soluble carbohydrates (TSS) statistically significant differences were observed among the various treatments: higher sugars amounts were observed in the treatments n. 1 and 4, characterized by the addition of light (1 h) and higher temperature. Although the other treatments showed the similar less quantities of sugars, the lowest amount is observed in treatment n. 3 (TSS 179.57 mg/g FW). The highest amount of total polyphenols (12.42 mg/g FW) was measured in treatment n. 1 (basal electric heating at 50 °C with 1 hour of additional light), and the lowest value in the control and n. 6 (7.66 and 7.36 mg/g FW, respectively). The antioxidant activity is higher in the treatments of addition of temperature by electric mat (n. 1 and 2) with IC_{50} (DPPH assay) values of 0.80 and 0.82 mg/ml respectively, followed by the trials n.3 and 4. Flowers of the control and treatment n.6 showed the lower scavenger reducing activity. In the present work the concentration of total polyphenols ranged 7.36 and 12.42 mg GAE/g fresh weight. These values agree with those found in *V.* × *wittrockiana,* reported by other authors (Rop *et al.,* 2012). However, the polyphenol values could be underestimated by the method of extraction, as already shown in Gonzàles-Barrio *et al.* (2018). In fact, they reported different polyphenol amounts in *V.* × *wittrockiana* by using either acidic hydrolysis or maceration instead of the method adopted in this work (Bretzel *et al.,* 2014).

Other reports showed the influence of storage at different temperature in different flowers (Kelley *et al.*, 2003). Moreover, different packages used for the storage conditions can affect the quality of flowers (Landi *et al.*, 2018). Changes of appearance, and aesthetic value were performed on *V.* × *wittrockiana* (Kelley *et al.*, 2003). The results obtained at the time

of cold storage (time 4) were compared to those at the time of harvest (time 0). The data reported here indicated that the purple-pink flowers maintained the carotenoids content after 4 days of cold storage in the treatments n. 1, 4 and 6, whereas in the treatments n. 2 and 3 values of carotenoids increased. Meanwhile, in flowers of treatment n. 5 and 7 (control) the amount of carotenoids decreased (Table 3). After 4 days of postharvest treatment, anthocyanins are the most affected metabolites by cold storage. In fact, the amount of anthocyanins decreased in the treatments n. 1, 5 and 6, whereas in the treatment n. 3 values increased (9.22 mg/g FW). The treatment n. 1 showed the largest decrease, 10.42 mg/g FW at time 0 and 4.81 mg/g FW at time 4. However, the loss of pigmentation is not always documented, but it is peculiar of each species and variety, as already demonstrated in other species as *Acmella oleracea*, *Salvia discolor, Begonia semperflorens, Tropaeolum majus* (Landi *et al.*, 2018). The total polyphenols content after cold storage maintained the same values of that detected at Time 0, with the exception of treatments n. 2, 6 and control, where polyphenols increased, 11.62, 9.32 and 10.37 mg/g FW at time 4, respectively (Table 3). The antioxidant activity increased in the treatments with hot water (n.4, 5, 6). Different susceptibility to the storage process was observed in other edible flowers, with different changes (increase or decrease) on nutraceutical values up to 8 days of postharvest (Landi *et al.*, 2018). The soluble sugars dropped significantly in the treatment n. 4, since the values was the highest at time 0 but reduced at 80% at time 4 (207.45 and 175.5 mg/g FW). Other decrease in the content of sugar is observed for the treatments n.3 and 6. Soluble sugars are important nutritional components of the flowers and represent a good characteristic for the choice of edible flowers (Mlcek and Rop, 2011). However, there are few works on the sugar profile of edible flowers, e.g. *Rosa micrantha* (Guimarães *et al.*, 2010). In experiment done with cut lily flowers was discussed the role of reducing sugars, as a typical reaction of plants that defend themselves against injury due to chilling or frost (Van doorn and Han, 2011).

4. Conclusions

The different cultivation treatments used in this work are differently correlated with the analyzed metabolites. In order to obtain flowers with high quality of brilliant color, one hour of supplementary lighting and a basal heating of 50°C seem to be the right combination of factors. The cold storage imposed to the flowers as the post-harvest treatment indicated that the flowers treated with additional 2 h of light (treatment n. 3) retained values of the metabolites during the post-harvest, with the exception of sugars. However, even if the additional lighting seems to preserve the flowers from depigmentation and to maintain the nutraceutical compounds, the decreased content of the observed sugars could be a consequence of the phenomenon of senescence. Further studies on the influence of illumination on plastic bags and the evaluation of ethylene production can be useful for the definition of the post-harvest process in *V. cornuta.* In addition, the investigation of the other minor nutritional components can be crucial to define a more detailed condition of storage.

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Anthocyanin and carotenoid contents assessed by time-resolved reflectance spectroscopy in potato tubers (*Solanum tuberosum* L.) with different flesh colors

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Abstract: **This work aimed at studying the relationships between the absorption spectra acquired by time-resolved reflectance spectroscopy (TRS) and the carotenoid (CAR) and/or the anthocyanin (ANT) contents in 9 potato genotypes with different flesh color (white, yellow, red, purple). Fifty whole and intact tubers/genotype were non-destructively measured by TRS in the 540-980 nm range; white- and yellow-fleshed were ranked according to increasing μ_a540, the red ones according to μ_a670 and the purple ones according to μ_a780. Then, 5 tubers/genotype, corresponding to the highest, the lowest and 3 intermediate values of each μ_a range, were analyzed for flesh color and CAR and ANT contents. In white- and yellow-fleshed genotypes, μ_a540 ranged from 0.078 to 0.207 cm^{-1}, showing the highest value in 'Melrose' and in 'ISCI 133/12-1' and the lowest ones in 'Romantica' and in 'CN 07.16.3'. In red-fleshed tubers, μ_a670 ranged from 0.049 to 0.146 with no significant differences between genotypes; in purple-fleshed genotypes, μ_a780 ranged from 0.147 to 0.473, showing the highest values in 'Bleuet'. CAR content ranged between 0.071 to 5.937 mg kg^{-1} FW, displaying the highest amounts in the deep yellow genotypes 'Melrose' and 'ISCI 133/12-1' and the lowest ones in the white 'CN 07.16.3' and in the dark purple 'Bleuet' tubers. ANT content ranged from 31.63 to 798.44 mg kg^{-1} FW in red-purple genotypes, having the highest values in 'Bleuet'. By using TRS spectra and PLS analysis, it was possible to predict CAR (R^2_{CV}=0.79, RMSECV=0.89) and ANT (R^2_{CV} =0.81, RMSECV=95.53) contents and flesh color ($h°$) in yellow-fleshed genotypes (R^2_{CV} =0.93, RMSECV=0.67) and purple genotypes (R^2_{CV} =0.82, RMSECV=1.63).**

1. Introduction

Potatoes are grown throughout the world and are consumed in large quantities. Potatoes present wide biodiversity, with approximately 5000 known varieties, most of them developed through man selection (Fernandez-Orozco *et al*., 2013). Potatoes account for only about 2% of the food energy supply; however, they are the predominant staple for many countries.

Potato is mainly composed of water (80%) and carbohydrate, with starch being the most abundant; contributes up to 3.3% of dietary fiber, shows low amount of proteins and aminoacids with excellent nutritional value and is also rich in vitamins (ascorbic acid, folic acid, niacin, riboflavin, thiamine, pyridoxine) and in minerals such as potassium, phosphorous and calcium (Burlingame *et al.*, 2009; Fernandez-Orozco *et al.*, 2013; Zaheer and Akhtar, 2016). In addition to ascorbic acid, potatoes contain several phytochemicals such as polyphenols, anthocyanins, flavonoids, carotenoids, tocopherols, and alpha-linoleic acid, which have beneficial effects on human health due to their antioxidant activity (Ezekiel *et al.*, 2013; Zaheer and Akhtar, 2016). Although other fruits and vegetables have antioxidant content higher than that of potatoes, considering the large quantities in which potatoes are consumed throughout the world, their contribution to the human diet is very significant. Phytochemical content in potatoes is affected by various factors such as genotype, cultivation conditions and methods (organic *vs* conventional), developmental stage, postharvest storage, cooking and processing conditions (Lachman *et al.*, 2012; Ezekiel *et al*., 2013; Murniece *et al.*, 2013). Generally, the skin and/or the fleshes of potatoes varieties are white, yellow, or deep yellow. However, the introduction and availability of pigmented potatoes in which skin and/or fleshes are red, purple, blue, or orange have attracted consumers over the last two decades due to their high antioxidant content in terms of anthocyanins, carotenoids and total phenolics (Tierno *et al.*, 2016). The coloration pattern of the skin and fleshes of colored potatoes is variable, i.e., the skin alone may be pigmented, or the flesh may be partially or entirely pigmented.

Potato cultivars with white flesh contained fewer carotenoids as compared to cultivars with yellow or orange flesh (Ezekiel *et al.*, 2013; Fernandez-Orozco *et al.,* 2013; Kaspar *et al.*, 2013; Murniece *et al*., 2013). Carotenoid concentrations in white- and purple-fleshed potatoes were similar, while yellow potatoes having a 45-fold greater carotenoids concentration compared to white and purple potatoes (Kaspar *et al*., 2013; Hejtmankova *et al*., 2013). Lutein, zeaxanthin, violaxanthin and neoxanthin are the major carotenoids present in potatoes and β-carotene is present in trace amounts (Lu *et al.*, 2001; Ezekiel *et al*., 2013; Hejtmankova *et al*., 2013; Kaspar *et al*., 2013). Both total and individual carotenoid contents were positively correlated with tuber yellow intensity (Lu *et al*., 2001; Murniece *et al*., 2013).

Anthocyanins are present in considerable amounts in purple-red pigmented potatoes and their concentrations are considerably higher in the skin than in the flesh (Ezekiel *et al*., 2013). Purple-fleshed potatoes had higher anthocyanins compared to red-fleshed potatoes, while low or non-detectable amounts were found in yellow and white-fleshed cultivars (Nayak *et al.*, 2011, Lachman *et al*., 2012; Ezekiel *et al*., 2013; Kaspar *et al*., 2013; Kita *et al*., 2013; Lachman *et al*., 2013; Tierno *et al*., 2015, 2016; Akyol *et al*., 2016). The most common anthocyanins present in potatoes are pelargonidin, malvidin, petunidin, cyanidin, peonidin and delphinidin (Lachman *et al.,* 2012; Hejtmankova *et al*., 2013; Akjol *et al.,* 2016). Red-fleshed genotypes contain predominantly acylated glycosides of pelargonidin, while purple-fleshed clones contain predominantly acylated glycosides of petunidin, malvidin and peonidin (Lachman *et al.*, 2012; Hejtmankova *et al*., 2013; Kita *et al.,* 2013; Akyol *et al*., 2016; Tierno *et al*., 2016). Especially due to anthocyanins, pigmented potatoes also exhibit higher antioxidant activity in comparison to common yellow-fleshed potatoes (Lachman *et al*., 2009; Nayak *et al*., 2011; Lachman *et al*., 2012).

Anthocyanin and carotenoid contents are generally determined by analytical methods, such as gas-liquid chromatography (GLC), HPLC and UV-VIS spectrophotometry. These techniques, however, are costly and time-consuming and are not suitable for on-line applications in the food industry. Consequently, rapid, accurate, and non-destructive techniques have been studied to monitor antioxidant amounts in potato tubers. However, most of the published papers concern the estimation of dry matter, starch, proteins and sugars in potatoes and only a few articles deal with the prediction of anthocyanin and carotenoid contents in raw and processed potatoes (López *et al*., 2013). NIR spectroscopy applied on whole tubers was able to accurately identify samples containing different levels of soluble phenolics, anthocyanins and hydrophilic antioxidant capacity

belonging to a collection of 18 purple- and red-fleshed potatoes and to predict the total phenolic content in 98 potato varieties (López *et al.,* 2014; Tierno *et al*., 2016). Total and individual carotenoids, anthocyanins as well as total phenolics and antioxidant activity have been estimated with good/high accuracy by NIR, hyperspectral imaging, infrared and Raman spectroscopy during drying process, in homogenized potato chips and in lyophilized potatoes (Shiroma-Kian *et al*., 2008; Bonierbale *et al*., 2009; Liu *et al*., 2017; Mazurek *et al*., 2017; Escuredo *et al*., 2018; Sebben *et al.,* 2018). NIR was also used to differentiate accessions with low, medium and high concentrations of violaxanthin, antheraxanthin, lutein and β-carotene (Bonierbale *et al.,* 2009).

Among non-destructive optical techniques, Time-resolved Reflectance Spectroscopy (TRS) is gaining increasing interest (Nicolai *et al*., 2014). TRS has been mainly applied in postharvest studies for estimating fruit maturity, for discriminating fruit having different texture and sensory characteristics and for the detection of internal defects in fruits and vegetables (Rizzolo and Vanoli, 2016). TRS, in combination with proper models of photon migration, allows the complete optical characterization of a diffusive medium through the measurements of the absorption (μ_a) and of the scattering (μ_s) coefficients by probing flesh at a depth of 1-2 cm with no or limited influence from the skin (Cubeddu *et al*., 2001; Rizzolo *et al*., 2016). While scattering is related to the structure, absorption depends on the chemical composition of the tissue, mainly on the presence of pigments such as chlorophylls, anthocyanins and carotenoids. TRS absorption spectra measured in the 540-780 nm range were successfully used to predict total carotenoids content in mangoes in combination with partial least squares regression achieving a R^2_{cv}=0.83 and 0.93 depending on the cultivars (Vanoli *et al.,* 2016). In 'Haden' and 'Palmer' mangoes, the absorption coefficient measured by TRS at 540 nm (μ_a540), in correspondence of the tail of carotenoid absorption, significantly correlated (*r*=0.78-0.94) with total carotenoids, *all-trans*-β-carotene, *all-trans*-violaxanthin no.3, *all-trans*-violaxanthin no.1, no.2, no.6 ('Haden'), and 9-*cis*-violaxanthin no.2, no.3 ('Palmer') (Vanoli *et al.*, 2018). Furthermore, high positive correlations were also found among μ_a540 and *a** and yellowness index (*r*=0.83-0.98), as well as high but negative correlation between μ_a540 and H° (*r*=-0.83-0.98) (Rizzolo *et al*., 2016; Vanoli *et al*., 2016, 2018). The absorption coefficient measured in the 500-580 nm range was also related to the presence of anthocyanins, as found in plums and in red-fleshed peaches (Rizzolo and Vanoli, 2016).

The aim of this work was to investigate the relationships between TRS absorption spectra and carotenoids and/or anthocyanin contents in nine potato genotypes with white, yellow, red and purple flesh color.

2. Materials and Methods

Potato tubers

The experiment was carried out on 9 potato genotypes: 7 commercial varieties and 2 belonging to CREA-CI breeding programme. The 9 genotypes showed different flesh color: 2 had purple flesh ('Bleuet', 'Salad Blue'); 2 red flesh ('Magenta Love', 'ISCI 218/3'), 4 yellow flesh ('ISCI 133/12-1', 'Doribel', 'Melrose', 'Romantica') and 1 white flesh ('CN 07.16.3'), whose traits are reported in Table 1.

All the potato genotypes were grown in the experimental field in Budrio (Bologna Province, Northern Italy), 44°32'14" N - 11°32'03" E - 28 m a.s.l. in accordance to the Emilia-Romagna Region's IPM

Table 1 - Potato genotype characteristics

Genotype	Dealer	Ploidy	Skin colour	Flesh colour	Weight (g) mean ± SD	GMD (mm) mean ± SD
CN 07.16.3	Bernard SAS, France	2n=4x=48	yellow	white	203.5 ± 61.9	68.4 ± 4.7
Romantica	Danespo A/S, Denmark	2n=4x=48	dark red	cream	191.7 ± 42.7	68.4 ± 4.7
Doribel	Pizzoli spa, Italy	2n=4x=48	yellow	cream	203.8 ± 42.9	68.1 ± 4.8
Melrose	Romagnoli F.lli spa, Italy	2n=4x=48	reddish brown	deep yellow	187.0 ± 36.5	66.5 ± 4.1
ISCI 133/12-7	Not on the market yet	2n=4x=48	yellow	deep yellow	196.0 ± 60.4	67.7 ± 6.5
ISCI 218/3	Not on the market yet	2n=4x=48	red	red with yellow pigmentation	104.6 ± 24.5	55.1 ± 4.4
Magenta Love	GM Sottotetti srl, Italy	2n=4x=48	red	red	132.0 ± 36.0	58.4 ± 5.2
Salad Blue	D.T. Brown Seeds, United Kingdom	2n=4x=48	blue	parti-coloured purple	88.0 ± 18.8	51.5 ± 3.3
Bleuet	NewStyle Potatoes BV, The Netherlands	2n=4x=48	blue	deep purple	149.3 ± 37,7	62.5 ± 5.1

Guidelines. Potatoes were harvested at full maturity on August 13, 2018 by a mechanical potato digger and stored at 4°C, 90% relative humidity, up to February 14, 2019. At storage removal, 50 potatoes/genotype without external defects were selected, and the diameters (x=longest axis, y= longest axis normal to x; z= longest axis normal to y) were measured. Geometrical Mean Diameter (GMD) of each tuber was calculated according to Mohsenin (1986) as following:

$$GMD = (xyz)^{1/3}$$

Then each tuber was measured by TRS on two opposite sides in the central region in the 540-980 nm range for white- and yellow-fleshed ones, in the 670-980 nm range for red-fleshed and in the 780-980 nm range for purple-fleshed ones. Within each genotype, white- and yellow tubers were ranked according to increasing μ_a540, the red ones according to μ_a670 and the purple ones according to μ_a780. Then, 5 tubers/genotype, corresponding to the highest, the lowest and 3 intermediate values of μ_a540 (yellow), μ_a670 (red) and μ_a780 (purple) were selected for physical-chemical analyses. Each tuber was cut in half and the flesh was measured for color in correspondence of the two TRS measurement points; then samples were immediately deep frozen at -20°C until carotenoids (CAR) and anthocyanin (ANT) analysis.

Time-resolved Reflectance Spectroscopy (TRS)

A portable compact setup working at discrete wavelengths developed at Politecnico di Milano (Torricelli *et al*., 2015) was used. The light source is a supercontinuum fiber laser (SC450-6W, Fianium, UK) providing white-light picosecond pulses, with the duration of a few tens of picoseconds. A custom-made filter wheel loaded with 14 band-pass interference filters (NT-65 series, Edmund Optics, New Jersey, USA) is used for spectral selection in the range 540-940 nm. Light is delivered to and collected from the sample by 1 mm fiber placed at 1.5 cm distance from the illumination point. A second filter wheel identical to the first one is used for cutting off the fluorescence signal originating from the sample when it is illuminated in the visible spectral region. The light then is detected with a photomultiplier (HPM-100-50, Becker&Hickl, Germany) and the photon time-of-flight distribution is measured by a time-correlated single-photon counting board (SPC-130, Becker&Hickl, Germany). The instrumental response function has a full width at half maximum of about 260 ps and the typical acquisition time is 1 s per wavelength. A model for photon diffusion in a spherical turbid medium was used to analyze TRS data to assess the bulk optical properties of the samples (Martelli *et al*., 2009) to obtain the estimates of μ_a and μ_s at each wavelength.

Flesh color

Flesh color was measured with a spectrophotometer (CM-2600d, Minolta Co., Japan), using the primary illuminant D65 and 2° observer in the *L**, *a**, *b** color space. From *a** and *b** values, hue (*h*°) was computed according to:

$$h° = \text{arctangent}\,(b^*/a^*) \times 360/(2\times 3.14).$$

Carotenoids and anthocyanin analysis

CAR and ANT analyses were carried out on individually frozen samples by slicing flesh portion after skin removal.

For CAR analysis, 1 g of flesh was extracted with 2 mL of NaCl 20% in water and 4 mL of a solution of hexane/acetone/ethyl acetate 2:1:1 v/v/v (Picchi *et al.,* 2012). For ANT analysis, 1 g of flesh was extracted with 4 mL of ethanol/water 50:50 acidified with HCl, final concentration 0.2 M, pH=1.2 (Giusti and Wrolstad, 2001). Then the mixtures were accurately stirred, mixed, centrifuged at 4890 *g* for 5 minutes at 4°C, and the supernatants were used for the spectrophotometric analysis. The extracts were stored at -20°C until spectrophotometric analysis (Jasco, model V-630, Deutschland GmbH, Pfungstadt, Germany).

Total carotenoid content (CAR) was determined by measuring the absorbance at 441 nm and quantified considering the Epsilon value of 2540 g 100 g^{-1} for zeaxanthin (Baurernfeind *et al.,* 1971). CAR data were expressed as mg of zeaxanthin equivalent (ZE) per kilogram of fresh weight (mg ZE kg^{-1} FW).

Total anthocyanin content (ANT) was determined by measuring the absorbance at 503 nm (Giusti and Wrolstad, 2001) and quantified, considering the Epsilon values of 18420 Moles cm^{-1} for pelargonidin (Giusti and Wrolstad, 2001). ANT data were expressed as mg of pelargonidin equivalent (PE) per kilogram fresh weight (mg PE kg^{-1} FW).

Statistical analysis

Data of μ_a540, μ_a670 and μ_a780, CAR, ANT and flesh color (*h*°) were submitted to ANOVA considering genotype as factor (means compared by Tukey's test at P≤0.05%) by using the Statgraphics v. 5.2 (Manugistic Inc., Rockville, MD, USA) software package. TRS absorption spectra were processed by Unscrambler X 10.0.1 (Camo, Norway) in order to build Partial Least Square (PLS) Regression models for

CAR, ANT and *h*° prediction, without pretreatments of spectral data.

3. Results and Discussion

TRS absorption spectra

The TRS absorption spectra of the 5 selected white, yellow, red and purple tubers are illustrated in figure 1. The absorption spectra of white and yellow potatoes showed a maximum at 980 nm, corresponding to water, and high values at 540 nm, in correspondence to the tail of carotenoid absorption, as previously found by Rizzolo *et al.* (2016) and Vanoli *et al.* (2016, 2018) in mangoes. The absorption spectra of red potatoes showed a peak at 980 nm and high absorption at 670 nm, while in purple potatoes maxima were observed at 670 nm for 'Salad Blue' tubers and at 780 nm for 'Bleuet' ones, with a lower water peak. The absorption at 670 and at 780 nm could be linked to the presence of anthocyanins, as the prominent absorbance peaks of anthocyanin were around 500-550 nm (Giusti and Wolstrad, 2001) but some absorbance was also noticed above 650 nm (Laksmiani *et al*., 2016; Noda *et al.,* 2017). Contrary to what found in fruit such as apples, pears, peaches, mangoes and plums (Rizzolo and Vanoli, 2016), μ_a670 in potatoes was not linked to chlorophyll content, as no greening development was detected in tubers studied in this experiment.

In white and yellow genotypes, μ_a540 ranged from 0.078 to 0.207 cm^{-1} and showed the highest value in 'Melrose' and 'ISCI 133/12-1' and the lowest ones in 'Romantica' and 'CN 07.16.3' (Table 2). As for red-fleshed tubers, μ_a670 ranged from 0.049 to 0.146 cm^{-1}, with no significant differences between genotypes (Table 2). In purple genotypes, μ_a780 ranged from 0.147 to 0.473 cm^{-1} assuming the highest values in 'Bleuet' (Table 2).

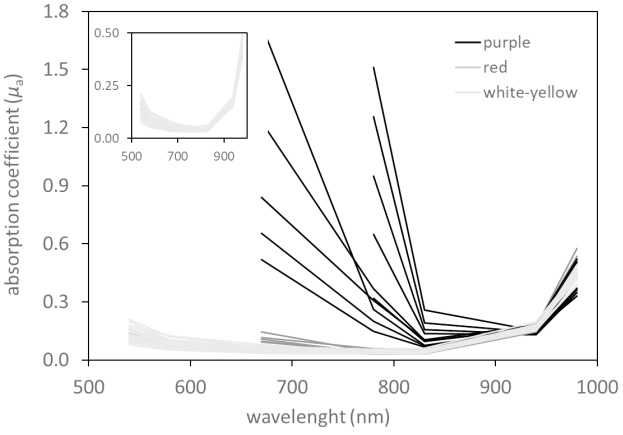

Fig. 1 - Absorption spectra of the five selected purple-red-yellow-white fleshed tubers. The inset figure shows the variability of the absorption spectra for white- and yellow-fleshed genotypes.

Table 2 - Values of the absorption coefficients measured by TRS at 540 nm (μ_a540), 670 nm (μ_a670) and 780 nm (μ_a780) used to rank white-yellow, red and purple potatoes, respectively

	Mean	Min	Max	SD
μ_a540 (cm^{-1})				
CN 07.16.3	0.104	0.078	0.136	0.023
Romantica	0.108	0.089	0.132	0.016
Doribel	0.141	0.101	0.176	0.029
Melrose	0.167	0.139	0.197	0.022
ISCI 133/12-1	0.161	0.122	0.207	0.033
μ_a670 (cm^{-1})				
ISCI 218/3	0.096	0.049	0.146	0.038
Magenta Love	0.080	0.055	0.107	0.021
μ_a780 (cm^{-1})				
Salad Blue	0.256	0.147	0.367	0.086
Bleuet	0.937	0.318	1.510	0.473

Carotenoid and anthocyanin contents

Carotenoid content (CAR) ranged from 0.071 to 5.937 mg ZE kg^{-1} FW, *i.e*. values comparable with the data found by Ezekiel *et al.* (2013), Hejtmankova *et al.* (2013) and Tierno *et al.* (2015), on other genotypes. CAR was present in white, yellow and also in red and purple (except in 'Salad Blue') genotypes, and showed the highest amounts in the deep yellow genotypes 'Melrose' and 'ISCI 133/12-1', intermediate contents in the red-fleshed 'ISCI 218/3' and in 'Magenta Love' and the lowest ones in the white genotype 'CN 07.16.3' and in the dark purple 'Bleuet' tubers (Table 3). These data confirmed that deep yellow-fleshed genotypes are usually characterized by much higher carotenoid contents than white- and red-fleshed ones (Ezekiel *et al*., 2013; Kaspar *et al.,* 2013; Tierno *et al.,* 2015; Kotíková *et al.,* 2016; Tierno *et al.,* 2016). In contrast, purple-fleshed tubers had either no carotenoids or a carotenoid content similar to that of white genotypes (Hejtmankova *et al.,* 2013; Kaspar *et al*., 2013; Kotíková *et al*., 2016).

Anthocyanins (ANT) were present in red and purple genotypes, with values ranging from 31.63 to 798.44 mg PE kg^{-1} FW (Table 3) in agreement with the findings of Ezekiel *et al.* (2013), Hejtmankova *et al.* (2013), Lachman *et al.* (2012, 2013), Kita *et al.* (2013) and Tierno *et al.* (2015) on other potato cultivars. ANT showed the highest amount in 'Bleuet' tubers,

Table 3 - Carotenoid and anthocyanin contents and pulp color (h°) of white, yellow, red and purple-fleshed potatoes

	CAR (mg ZE kg^{-1} FW)				ANT (mg PE kg^{-1} FW)				*h*° pulp			
	Mean	Min	Max	SD	Mean	Min	Max	SD	Mean	Min	Max	SD
CN 07.16.3	0.241	0.071	0.496	0.181	nd	nd	nd	nd	97.8	97.5	98.3	0.4
Romantica	0.997	0.323	1.498	0.563	nd	nd	nd	nd	97.4	96.7	98.2	0.6
Doribel	1347	0.278	2.244	0.716	nd	nd	nd	nd	96.1	95.9	96.3	0.2
Melrose	4540	3.457	5.937	0.955	nd	nd	nd	nd	92.2	91.1	93.8	1.1
ISCI 133/12-1	4063	3.323	5.291	0.751	nd	nd	nd	nd	94.0	93.4	95.1	0.7
ISCI 218/3	2.655	2.000	3.882	0.784	88.42	47.37	130.42	29.65	55.8	44.7	65.8	7.9
Magenta Love	2.285	1.276	3.244	0.812	144.49	80.62	245.84	65.96	38.4	21.3	58.1	15.4
Salad Blue	nd	nd	nd	nd	49.05	31.63	73.05	19.74	337.0	333.6	339.7	2.3
Bleuet	0.526	0.331	0.843	0.212	529.38	328.08	798.44	176.58	331.8	328.2	335.4	2.7

nd= not detected.

while did not significantly differ among the other 3 genotypes (Table 3). The highest ANT content was usually found in dark purple genotypes (Ezekiel *et al*., 2013; Hejtmankova *et al*., 2013; Lachman *et al*., 2012, 2013; Nayak *et al.,* 2011; Tierno *et al*., 2015, 2016). 'Salad Blue', characterized by a parti-coloured purple flesh, showed lower ANT content than the deep purple 'Bleuet' genotype, but similar ANT values to the red-fleshed genotypes, as previously observed by Hejtmankova *et al.* (2013), Kita *et al.* (2013), Lachman *et al.* (2012, 2013). ANT was not detected in white and yellow genotypes (Table 3) as found by Tierno *et al.* (2015); on the other hand, Kaspar *et al.* (2013) observed that white potatoes had no ANT, whereas yellow-fleshed ones showed 20-fold lower ANT concentrations than the purple ones.

Flesh color

Considering the white and yellow genotypes, 'CN 07.16.3' and 'Romantica' exhibited the highest *h*° values, 'Melrose' and 'ISCI 133/12-1' the lowest ones and 'Doribel' intermediate values (Table 3). 'CN 07.16.3' and 'Romantica' had a pale yellow flesh, even if classified white and cream, respectively (Table 1); 'Doribel' showed a slightly yellower flesh than 'CN 07.16.3' and 'Romantica', even if classified creamy as 'Romantica' (Table 1); 'Melrose' and 'ISCI 133/12-1' tubers had the yellowest flesh color, even if the yellow intensity was higher in 'Melrose' tubers: both these genotypes were classified as deep yellow-fleshed (Table 1). The flesh color of these 5 genotypes agreed with the respective carotenoid contents: more intense was the yellow color of the flesh, the higher the CAR content. Considering all the 5 genotypes, a high negative linear (r=-0.83, p<0.001) relationship was found between *h*° and CAR content of the flesh, in agreement with Lu *et al.* (2001), reporting a strong relationship between total and individual carotenoids and tuber yellow intensity, and Murniece *et al.* (2013), finding a positive correlation between carotenoid content and the *b**coordinate of the flesh in organically and in conventionally cultivated potatoes.

As for purple genotypes, a slight but significant difference in the flesh color existed between 'Bleuet' and 'Salad Blue', as the former showed a lower *h*°, indicating a darker purple color (Table 3). In addition, 'Bleuet' also had an 11-fold greater ANT content compared to 'Blue Salad'; this higher ANT content was responsible for the deeper purple color as confirmed by the negative and significant correlation between ANT content and *h*° (r=-0.89, p<0.001). Considering red-fleshed potatoes, 'Magenta Love' showed lower *h*° than 'ISCI 218/3', confirming that the former had a red color and the latter a deep orange color due to the presence of a slightly higher CAR and a slightly lower ANT contents in the flesh (Table 3). A negative and significant correlation was found between ANT content and *h*° (r=−0.87, p<0.001) also for red genotypes. Dependence of the flesh coloration of the tubers measured by the CIELab scale with phenol flavonoid contents was also observed by Escuredo *et al.* (2018) in 35 potato varieties with different flesh color.

Partial Least Square (PLS) Regression models

TRS absorption coefficients measured at the different wavelengths were used to develop Partial least squares (PLS) regression models for predicting CAR and ANT contents and *h*° color of the potato flesh. For each parameter, the best model was selected considering the lowest root-mean-square error of cross-validation (RMSECV), combined with the lowest number of latent variables (LV) and the highest coefficient of determination in cross-validation (R^2_{CV}). The

results of PLS regressions are reported in Table 4 and in figures 2 and 3.

A good result was obtained for the prediction of CAR contents, as the PLS model had a R^2_{CV} of 0.79 and an RMSECV of 0.89, being μ_a540 and μ_a580 the important variables (Fig. 2, top). A slightly better result was achieved for ANT prediction, as the performance of the PLS model showed R^2_{CV} of 0.81 and RMSECV of 95.53 (Fig. 2, bottom). The μ_a780 and μ_a830 were the important variables. However, figure 2 (bottom) showed that samples are not equally distributed according to ANT content. There are two groups: the larger one with ANT content up to 250 mg PE kg^{-1} FW, including the red genotypes and the purple genotype 'Salad Blue', and a second group with ANT content ranging from ~300 to 800 mg PE kg^{-1} FW corresponding to the deep purple-fleshed 'Bleuet' genotype. Probably, the highest ANT content, together with highest variability of 'Bleuet' tubers, strongly affected the performance of the PLS model for ANT content prediction.

To the best of our knowledge, there are a few papers in literature dealing with the non-destructive determination of antioxidant compounds in whole tubers. Tierno *et al.* (2016) found that NIR measurements on unpeeled intact potatoes combined with PLS-DA allowed to accurately identify samples containing different levels of total phenols, total

Table 4 - Performance of PLS regression models on original TRS absorption spectral data for prediction of total carotenoids (CAR) and total anthocyanin (ANT) contents and of flesh color (*h*°)

Dependent variables	TRS parameters	Variable number	Calibration		Validation	
			R^2_C	RMSEC	R^2_{CV}	RMSECV
CAR	μ_a540-980	5	0.84	0.73	0.79	0.89
ANT	μ_a780-980	1	0.81	90.61	0.81	95.53
h° white-yellow genotypes	μ_a540-980	4	0.94	0.54	0.93	0.67
h° purple genotypes	μ_a780-980	2	0.87	1.24	0.82	1.62

R^2C= coefficient of determination between predicted and measured values in calibration;
R^2CV= coefficient of determination between predicted and measured values in cross-validation;
RMSEC= root mean square error of calibration;
RMSECV= root mean square error of cross-validation.

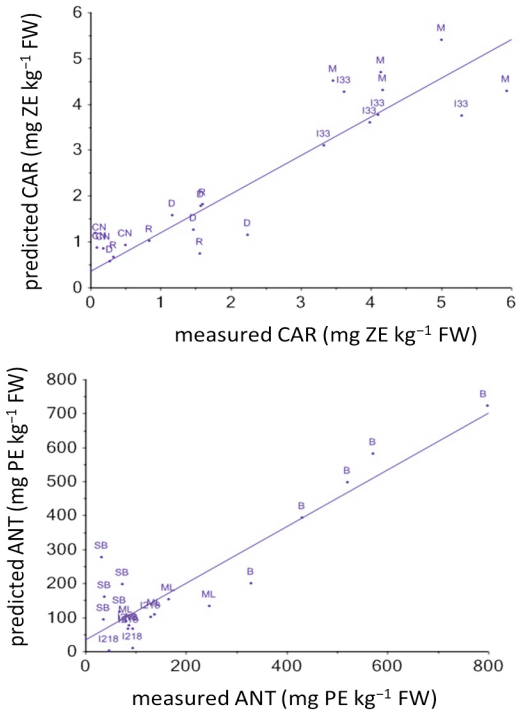

Fig. 2 - Measured and predicted CAR (top) and ANT (bottom) contents by PLS regression analysis.

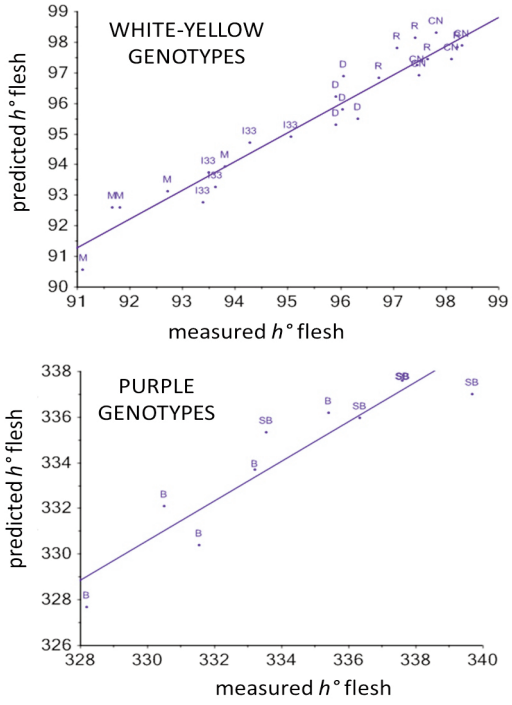

Fig. 3 - Measured and predicted flesh color of white-yellow (top) and purple (bottom) potato genotypes by PLS regression analysis.

monomeric anthocyanins and hydrophilic antioxidant capacity belonging to a collection of 18 purple- and red-fleshed potatoes. Regarding total carotenoids content, Tierno *et al.* (2016) found that NIRS was only capable of identifying samples with a high content of these compounds. Good models for predicting total phenol content have also been built by López *et al.* (2014) measuring 1157 whole potato tubers with NIR, and obtaining coefficients of determination of 0.88, 0.77 and 0.74 for calibration, cross-validation and external validation, respectively. However, when NIR technology was applied on freeze-dried and milled material (Bonierbale *et al.,* 2009; Escuredo *et al*., 2018; Liu *et al*., 2017) it was possible to successfully estimate CAR and/or ANT contents. Bonierbale *et al.* (2009), measuring 152 *Solanum phureja* germplasm accessions by NIR, estimated total carotenoids and zeaxanthin concentrations with R^2 values ranging from 0.63 to 0.92, and they were able to differentiate accessions with low, medium and high concentrations of violaxanthin, antheraxanthin, lutein or β-carotene. Total flavonoid content was predicted by NIR with R^2=0.82 (Escuredo *et al*., 2018) and total anthocyanin amount by hyperspectral imaging in purple-fleshed sweet potato during drying process achieving a coefficient of determination for calibration of 0.868 and a coefficient of determination for prediction of 0.866 by using ten key wavelengths (637, 660, 666, 700, 729, 761, 801, 837, 892, and 957 nm) (Liu *et al*., 2017).

The flesh color prediction model for yellow genotypes (Fig. 3, top) showed the best performance, as R^2_{CV} was 0.93 and RMSECV was 0.67 and, as found for CAR content, the important variables were μ_a540 and μ_a580. PLS models were separately developed for flesh color prediction of red and purple genotypes considering the very high differences in the *h*° values, being on average, 47 for red-fleshed tubers and 335 for purple-fleshed potatoes (Table 3). A good model was obtained for the prediction of flesh color of purple genotype with R^2_{CV} = 0.82 and RMSECV = 1.62 (Fig. 3, bottom), while no significant model could be developed for red genotypes. By using NIR spectra, Escuredo *et al.* (2018) were able to estimate the *b** coordinate of the flesh with R^2=0.75 by using NIR spectra, while poor results were obtained for the *a** and *L** coordinates in lyophilized creamy, yellow and purple-fleshed potatoes; on the other hand, Mazurek *et al.* (2017), successfully modeled *L** parameter (R^2=0.992) in potato chips.

4. Conclusions

TRS was able to quantify with a reasonable accuracy carotenoid and anthocyanin contents in yellow and in red/purple fleshed-genotypes, respectively. TRS also allowed the estimation of flesh color in yellow-fleshed-genotypes, without being influenced by the different color of the skin, and in purple-fleshed ones. However, TRS was not able to predict flesh color in red-fleshed genotypes. The highly significant correlations between *h*° color coordinate and CAR and ANT contents can be used for discriminating potato tubers with different concentrations of pigments. The encouraging results of this study indicated the potential application of TRS for the non-destructive determination of carotenoid and anthocyanin contents and for the flesh color estimation in whole and intact potato tubers. However, further studies with a larger set of samples will be advisable in order to obtain better and more reliable models.

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Effects of kaolin-based particle film on physiological, nutritional, nutraceuticals parameters and *Ceratitis capitata* infestations in peach fruit at harvest and after storage

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Abstract: The Mediterranean fruit fly (*Ceratitis capitata* Wiedemann) is a worldwide pest of economic importance because attacks a large number of agricultural crops and for the extent of the damage it causes. Among the alternative control strategies to the use of sprays with synthetic insecticides, a very important role can be played by powders obtained from rocks whose activity arise from the ability to form a film of white powder, which acts as a repellent and irritant to insects. This film can also interfere with plants' physiology and affect quality of fruit. In this study the efficacy of a commercial kaolin-based formulation to control medfly infestations was compared to synthetic insecticides commonly used against this pest (phosmet, alfa-cypermethrin, deltamethrin). The results showed a significant reduction of medfly attacks in fruits treated with insecticides (1.5% damaged fruit) or with kaolin (0.5% damaged fruits) compared to the untreated sample (10% damaged fruits), while physiological and quality parameters did not show relevant differences between treatments and control fruit. Overall results highlight how the use of kaolin represents a valid alternative to treatments with synthetic insecticides to control *C. capitata* attacks on peaches, while not affecting fruits' quality.

1. Introduction

Ceratitis capitata (Diptera Tephritidae), also known as the Mediterranean fruit fly is one of the most harmful insect pests to several fruit crops of the Mediterranean countries. It is a polyphagous~~ic~~ phytophagous that is considered highly invasive for the wide of host species and the high tolerance to low temperatures, compared to other fruit flies (Malacrida *et al.*, 2007). The fruits can be attacked early or close to harvesting: in the first case, the fly may sting the fruit several times for the oviposition and the developing larvae cause rottenness, early ripeness

and fruit drop. In case of late stings, the eggs laid shortly before harvesting, hatch during storage. Yet, even in case eggs would not hatch, wounds caused by stings could favor growth of decay causing fungi (D'Aquino *et al*., 2011). Given the extent of the damage caused by this fly, early monitoring is required, when fruits are not yet ripe. In Italy the control strategy against *C. capitata* is mainly carried out with traps for adult specimens or with chemical sprays, whose use is increasingly discouraged after the European Directive 2009/128/EC. Moreover, the development of resistant strains to conventional pesticides (Sparks and Nauen, 2015) has stimulated studies for alternative and biological methods based on low toxicity and environmentally friendly plant extracts and mineral products. One of these mineral products is the kaolin clay, a fine powder, mainly composed of kaolinite, which, sprayed onto trees as a water suspension, forms a white and thin particle film on leaves and fruit surface (Glenn *et al*., 1999; Mazor and Erez, 2004). Different mechanisms seem to be involved in contrasting medfly attacks on fruit. The particle film beside masking the colour of leaves, stems and fruits, making long-distance host recognition difficult (Saour and Makee, 2004), renders the host less attractive for the white color of the film, which is the least attractive colour for ovipositing females of *C. capitata* (Katsoyannos, 1987). Moreover, the hard and irritating surface of kaolin film exerts at some extent a repellent effect on several insects, included medfly (Saour and Makee, 2004; Salerno *et al*., 2019).

However, if the positive effect of kaolin to control several pests is well documented and consistent, its impact on physiological response of plants and fruit quality is contradictory, depending on several factors such as the commercial formulations of the powder, the climate conditions, the intensity and quality of solar radiation, and environmental temperature. Several studies show that kaolin particle films do not reduce photosynthesis and plant growth but mitigate water stress and photorespiration caused by intense solar radiation (Kerns and Wright, 2000; Glenn *et al*., 2002; Jifon and Syvertsen, 2003). Kaolin treatments were also reported to reduce leaf temperature and increase water use efficiency in artichoke (Basnizki and Evenari, 1975) and to decrease the rate of CO_2 absorption (presumably due to a partial block of stomata opening) in sorghum and cotton (Stanhill *et al*., 1976; Moreshet *et al*., 1979).

The aim of this study was to evaluate the effectiveness of kaolin versus synthetic insecticides to control medfly infestation and quality in stone fruit at harvest and during a simulated marketing conditions (SMC) of 7 days.

2. Materials and Methods

Plant material and treatment

The experiment was carried out in a stone fruit orchard located in north-western Sardinia (Lat 39° 50' N, Long 09° 38' E). Seven years old peach trees [*Prunus persica* (L.) Batsch.] cv. O'Henry, highly susceptible to medfly attacks, were chosen for the experiment. Trees were trained to a palmetta system, spaced 3 m along the rows and 4 m between the rows. To evaluate the efficacy of the different treatments, a randomized block design with 3 replicates of 3 trees per treatment was used. Each replicate was separated by the next one by four untreated trees. The following treatments were compared: i) Kaolin (Surround® WP, Geovita, Turin, Italy; dissolved in water at 30 g/L); ii) a sequence of synthetic insecticides representing a local protocol to control medfly, applied in the following order: one treatment with phosmet (Spada® 50 WG, Gowan, Ravenna, Italy, at 1.5 g/L); two treatments with alpha-cypermethrin (Fastac® 10 SC, BASF, Monza e Brianza, Italia, at 0.3 g/L) and two treatments with deltamethrin (Decis® EVO, Bayer Crop Science, Ravenna, Italy, at 0.12 g/L); iii) Untreated control.

Phosmet, an organophosphate insecticide, can penetrate through the plant surface with a limited transport in plant tissues (Agrochemicals Handbook, 1983), while Alpha-cypermethrin and Deltamethrin are synthetic insecticides belonging to the pyrethroid group, that kill insects for contact and are more stable than pyrethrins when exposed to air and sunlight (Worthing and Hance, 1991). All treatments, carried out by spraying the products on the plants to obtain a homogeneous coverage, started 42 d before harvest, when fruits were not susceptible to medfly attacks and repeated at week intervals until 7 d before harvest. This local protocol followed by growers relies on frequent treatments in order to maintain residue levels of insecticides sufficient to kill medfly adults on fruit surface. On the other hand, Kaolin was also sprayed at week intervals to maintain a continuous and even film on fruit surface, whose homogeneity would be reduced and made discontinuous by fruit growth in case less treatments had

been done.

Evaluation of medfly damage and storage condition

At harvest, the total number of fruits for each treatment showing visible damage by medfly, confirmed by the presence of larvae after dissecting the fruit, were counted and the percentages of damaged fruit on the total yield of each plant were calculated. The total number of fruit produced by each plant ranged between 278 and 321.

One hundred and twenty sound fruits (divided in replicated of 40 fruits each) for each treatment, free of any visible defect, were selected for storage. The fruits were placed in plastic trays and stored in a ventilated storage room kept at 20°C and 55-60% RH for 7 d. At the end of storage, fruits were inspected for damage by medfly (presence of larvae within the flesh after dissection) or for the presence of molds but with no evident sign of medfly's attack. The percentage of fruit damaged by medfly and that of fruit with the presence of molds were calculated.

Physiological and chemical determinations

Respiratory activity and ethylene production rates were determined after 1, 2, 3, 5 and 7 d of storage at 20°C and 55-60% RH using 10 sound fruit for treatment. Fruit were individually placed in 1 L jars, whose lids were fitted with two silicon septa and closed for 1 h prior CO_2 determination. At sampling time, the headspace air was mixed for 1 min by an electrical fan fixed inside the jar. CO_2 concentrations were determined by a combined CO_2/O_2 analyzer (Combi Check 9800-1, PBI-Dansensor A/S, Rinsted, Denmarck). The analyzer was connected to each jar by two tubes, each one ending with a needle inserted in one of the two septa to form a closed system. Respiration activity, as CO_2 release, was expressed as mL Kg^{-1} h^{-1}.

To determine ethylene concentration a 1-mL sample headspace air from each jar was withdrawn with a gas-tight syringe from the same septa used for CO_2 determination. Ethylene was assessed by a Varian 3300 GC (Australia Ltd., Victoria, Australia) equipped with a flame ionization detector (FID), Carbowax 20M 80/120 mesh Carbograph 1 AW 30 column (Alltech, Italy, Milan), and the column, injector and detector temperatures set at 60°C, 110°C and 180°C, respectively.

Chemical analyses were performed in triplicate at harvest and after 7 d of storage at 20 °C from puree obtained by homogenizing the fruit with a domestic homogenizer. The results of all chemical analyses are the mean values of three replications. According to the type of analysis, detailed procedures of sample preparation are described below.

Titratable acidity (TA), total soluble solid (SST), total phenolic compounds, glucose, fructose, sucrose, antioxidant activity and organic acid were determined on supernatant obtained by centrifugation of the puree at 13,000 x *g* for 20 min and filtered through a 0.45 µm acetate cellulose filter.

TA was measured using an automatic titrator (Metrom 720 SM Tritino, Switzerland) by titrating aliquots (10 g) of samples to an endpoint of pH 8.2 with 0.1N NaOH and expressing the result as g L^{-1} citric acid, while TSS were measured by a digital refractometer (Mod. PR-101, Atago, Tokyo, Japan) and expressed as percentage.

Total phenolic content was determined according to the Folin-Ciocalteu colorimetric method (Singleton and Rossi, 1965) and expressed as mg 100^{-1} g^{-1} gallic acid equivalents. Folin-Ciocalteu phenol reagent was from Fluka (Buchs, Switzerland).

Analyses of glucose, fructose and sucrose were performed according to Palma *et al*. (2018). Stock standard solutions of each carbohydrate were prepared in ultrapure water and quantified according to the linear calibration curves of standard compounds. Glucose, fructose, and sucrose were purchased from Sigma-Aldrich Co. (Milan, Italy).

Antioxidant activity was assessed using the free radical DPPH, according to Bondet *et al.* (1997). The mixture containing 3mL of a methanol solution of 6×10^{-5} mol L^{-1} of DPPH and 100 µL samples was allowed to react for 15 min in a cuvette. The decrease of absorbance at 515 nm of DPPH solution added with the sample was measured and the results expressed as Trolox equivalent antioxidant capacity (mmol L^{-1} TEAC). 2,2-diphenyl-1-picrydazyl (DPPH), and Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), a water-soluble analog of vitamin E reagent were from Fluka (Buchs, Switzerland).

Malic and citric acid measurement was performed according to Palma *et al*. (2013).

Total carotenoids were extracted from puree using a mixture of hexane/acetone/ethanol (2:1:1). The homogenized sample (10 g) was weighed into 100 mL glass vials with 50 mL of extranet solution. Samples were kept in constant agitation for 60 min. The solutions were left to separation into a distinct polar layer (35 mL) and non-polar layer (25 mL) containing carotenoids. Total carotenoids were determined by a spectrophotometric method using a UV-Vis spectrophotometer (Cary 50, Varian Australia Ltd., Victoria, Australia). Total carotenoids content

was calculated by comparing the absorbance of the carotenoids hexane solution with a calibration curve obtained using different concentrations of standard carotene at 451 nm (Kopec *et al.*, 2012). carotene was from Sigma-Aldrich Co. (Milan, Italy).

Firmness measurements were carried out by a testing machine (Mod. DO-FB 0.5 TS, Zwick Roell, Ulm, Germany) recording the highest resistance (F Max) opposed to the penetration of an 8-mm-diameter flat faced cylindrical plunger to a depth of 10 mm and moving at a speed of 3.3 mm s^{-1} and the deformation of the fruit surface at the highest resistance opposed before penetration (L at F max). The two parameters, F Max and L at F Max, were expressed as newton (N) and mm respectively. Ten fruits were used for each treatment.

Mass loss, expressed as percentage, was determined on 30 fruits for treatment, individually weighed at harvest and at the end of the storage period.

Statistical analysis

Statistical analysis was performed using Statgraphics Centurion software (Herndon, VA, USA), version XV Professional statistical program. Analysis of variance (ANOVA) was carried out according to a single-factor design, after testing (Skewness and Kurtosis) data normality assumptions. Appropriate data transformations were carried out when violations of normality assumptions were met. The number of replications differed depending on the type of analysis performed. Mean comparisons were performed using Duncan's multiple range test at $P \leq 0.05$.

3. Results

Evaluation of medfly damage at harvest and after storage

At harvest, kaolin and insecticides reduced the percentage of peaches with visible damages by medfly to 0.5 and 1.5%, respectively compared to 10% of untreated fruit (data not shown).

At the end of storage, fruit with visible damage and the presence of larvae within the flesh, observed after cutting the fruit, were 0.5±1% and 3±1.6% in kaolin and insecticide treatments, respectively, while in control fruit the infested fruits were approximately 16±2.1% (Fig. 1). Decay incidence due to the presence of molds was 7.5±2 % in kaolin treated fruit, 13±3% in those treated with insecticides and 19±5.2% in untreated ones. Consequently, the total loss was 8±3 % in kaolin treated fruit, 16±4.1 % in those treated with the insecticides and 35±7.3% in control ones (Fig. 1).

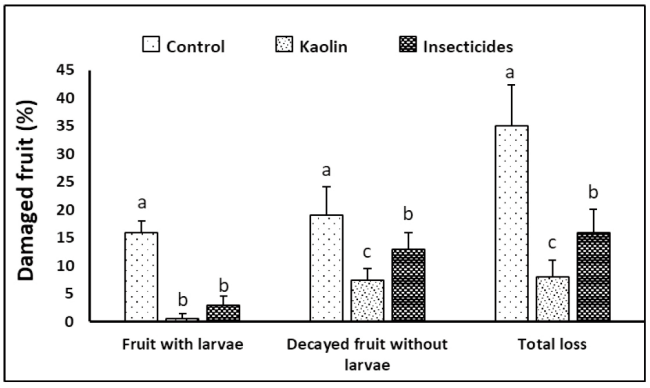

Fig. 1 - Incidence of fruit damaged by *Ceratitis capitata* in 'O'Henry' peach after 7 d of storage. Columns with different letters are significantly different at P≤0.05 according to Duncan's multiple range test. Vertical bars represent standard deviation (n=4).

Effect of treatments on physiological and chemical properties

Compared to harvest time, respiration rate almost doubled at the end of storage, but significant differences could not be detected among treatments (Fig. 2).

In contrast, overall ethylene production showed a decreasing trend during the first 2 d and then gradually increased with final values significantly higher than those recorded at harvest time (Fig. 3). Although significant differences were not detected among treatments, presumably due to the relatively high variability occurring among the fruit of the same treatment, fruit treated with insecticides showed constantly higher rates than the other two treatments (Fig. 3).

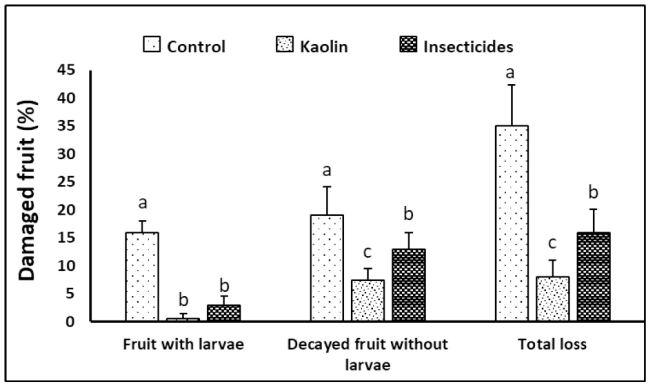

Fig. 2 - Respiratory activity as carbon dioxide release in 'O'Henry' peach as affected by pre-harvest treatments with kaolin or insecticides stored at 20°C. Columns with different letters are significantly different at P≤0.05 according to Duncan's multiple range test. Vertical bars represent standard deviation (n=10).

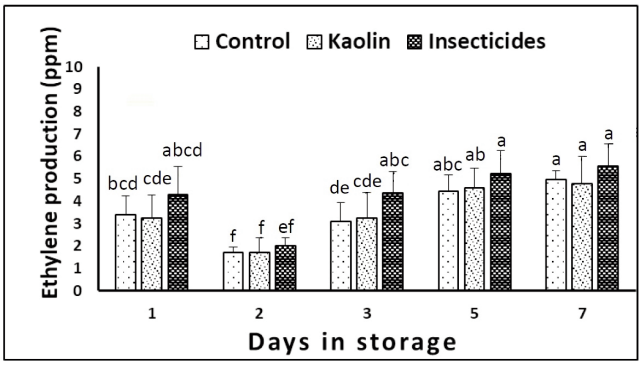

Fig. 3 - Ethylene production rates in 'O'Henry' peach as affected by pre-harvest treatments with kaolin or insecticides stored at 20°C. Columns with different letters are significantly different at P≤0.05 according to Duncan's multiple range test. Vertical bars represent the standard deviation (n=10).

In Table 1 are reported data concerning the chemical composition of peaches at harvest and after 7 d storage. TA showed an overall significant decline of about 9% (p= 0.024) after 7 d of storage, but differences among treatments were not significant neither at harvest time (p=0.720) nor at the end of storage (p=0.982). SST, differently than TA, showed an overall increase of about 6 % over storage (p=0.012). Values of individual treatments were slightly but significantly lower in kaolin treated fruit at harvest (p=0.042), while no significant difference could be detected among treatments after 7 d of storage (p=0.532).

Glucose, fructose and sucrose content did not show any difference among treatments neither at harvest time (p=0.952, p=0.914, p=0.241, respectively) nor at the end of storage (p=0.412, p=0.119, p=0.264, respectively), but while overall fructose levels increased with storage (p=0.018), sucrose decreased (p=0.041).

Regarding the other compounds, negligible variations occurred over storage in citric acid (p=0.048), total carotenoids (p=0.084) and malic acid (p=0.253) levels, while total phenols and antioxidant activity increased with final values of about 129 mg 100^{-1} g^{-1} gallic acid equivalents (p=0.001) and 1.8 mmol L^{-1} TEAC (p=0.001), respectively. However, both total phenols (p=0.240) and antioxidant activity did not show any significant difference neither at harvest time (p=0.291, p=0.183, respectively) nor after 7 d of storage (p=0.571, p=0.982, respectively).

Fruit firmness was affected by storage time but not by treatments (Table 2). In particular, F max and Lat F max decreased during storage with final values about 90 % lower than harvest time.

Mass loss, which on average was around 3 and 6%

Table 2 - Evolution of firmness as F Max (maximum force to penetration) and L at F Max (deformation of the fruit surface at F Max) and changes in weight loss (% reduction of the initial weight) in 'O'Henry' peaches as affected by pre-harvest treatments with kaolin or insecticides at harvest or after 3 or 7 d of storage at 20°C

Treatment	F Max (N)	L at F max (mm)	Weight loss (%)
	Harvest		*3 days*
Control	46.97 a	4.79 a	4.23 a
Kaolin	53.83 a	5.49 a	4.04 a
Chemical	49.71 a	5.07 a	4.13 a
	7 Days		*7 days*
Control	4.60 b	0.47 b	6.36 b
Kaolin	5.88 b	0.60 b	5.97 b
Chemical	4.80 b	0.49 b	6.04 b

Values in column for each storage time not followed by the same letter are significantly different at P≤0.05 according to Duncan's multiple range test.

Table 1 - Changes in chemical parameters in 'O'Henry' peaches as affected by pre-harvest treatments with kaolin or insecticides at harvest or after 7 d storage at 20°C

Chemical parameter or compound	Treatments					
	Harvest			7 days at 20°C		
	Control	Kaolin	Insecticides	Control	Kaolin	Insecticides
TA (%)	1.02 ± 0.05 a	1.02 ± 0.03 a	1.01 ± 0.01 a	0.92 ± 0.06 b	0.92 ± 0.05 b	0.92 ± 0.04 b
SST (°Brix)	14.7 ± 0.17 b	14.2 ± 0.25 c	14.7 ± 0.17 b	15.3 ± 0.30 a	15.1 ± 0.05 a	15.4 ± 0.30 a
Glucose (g 100 mL^{-1})	1.16 ± 0.10 a	1.16 ± 0.10 a	1.18 ± 0.04 a	1.20 ± 0.03 a	1.23 ± 0.01 a	1.24 ± 0.05 a
Fructose (g 100 mL^{-1})	1.61 ± 0.14 b	1.61 ± 0.14 b	1.57 ± 0.03 b	1.91 ± 0.01 a	1.95 ± 0.07 a	1.82 ± 0.07 a
Sucrose (g 100 mL^{-1})	9.51 ± 0.27 a	9.53 ± 0.56 a	9.03 ± 0.16 a	8.12 ± 0.07 b	8.12 ± 0.07 b	7.97 ± 0.17 b
Total phenols (g 100 mL^{-1})	121.7 ± 31.8 b	125.1 ± 49.5 ab	119.7 ± 31.0 b	128.8 ± 27.8 a	130.9 ± 29.4 a	129.5 ± 11.0 a
Tot carotenoids (g 100 mL^{-1})	7.60 ± 0.58 a	7.25 ± 0.50 a	6.81 ± 0.29 a	7.19 ± 0.44 a	7.36 ± 0.21 a	7.08 ± 0.76 a
Antioxidant (mmol L^{-1} TEAC)	1.76 ± 0.01 b	1.77 ± 0.01 b	1.77 ± 0.01 b	1.82 ± 0.01 a	1.86 ± 0.03 a	1.82 ± 0.01 a
Malic acid (g 100 mL^{-1})	1.23 ± 0.05 a	1.24 ± 0.05 a	1.24 ± 0.03 a	1.16 ± 0.13 a	1.14 ± 0.04 a	1.20 ± 0.05 a
Citric acid (g 100 mL^{-1})	0.23 ± 0.01 c	0.26 ± 0.02 ab	0.23 ± 0.03 bc	0.27 ± 0.03 ab	0.29 ± 0.01 a	0.27 ± 0.02 ab

Values within rows for each parameter not followed by the same letters are significantly different at P ≤0.05 according to Duncan's multiple range test. Each mean is followed by the standard deviation (n=3).

after 3 and 6 d, respectively, was not affected by treatments (Table 2).

4. Discussion and Conclusions

Different field as well as laboratory studies demonstrated the efficacy of kaolin treatments in reducing medfly punctures on fruit of different species (Mazor and Erez, 2004; D'Aquino *et al*., 2011; Lo Verde *et al*., 2011) and its higher efficacy when compared to traditional (organophosphates, pyrethroids) or novel insecticides, despite the excellent laboratory results of the last ones. For example, the high activity of spinosad (Adan *et al*., 1996; Vargas *et al*., 2002; Mangan *et al*., 2006), was not confirmed in field experiments to control medfly in citrus fruit, while kaolin tested in the same experiment, resulted very effective (Braham *et al*., 2007). Indeed, in contrast to traditional insecticides, whose efficiency may be affected by medfly developmental stage, population density, mode of action and environmental factors (ligth, temperature, rain) that can shorten their persistance (Braham *et al*., 2007), kaolin efficiency seems stable over time, provided a uniform coverage of fruit surface and absence of abundant rains (Mazor and Erez, 2004; Lo Verde *et al*., 2011). D'Aquino *et al*. (2011) found a significant lower percentage of damaged fruit at harvest in peaches and nectarines treated with kaolin compared to those subjected to a conventional treatment with organophosphates trichlorfon and fenthion, although the kaolin protective activity was higher in peaches rather than in nectarines owing to the lower adherence of the particles on nectarines surface and the difficulty to form a uniform film.

Our results showed a marked effect of both kaolin and insecticides in reducing the number of fruit with visible damage~~s~~ at harvest, but after one week of storage at 20°C, decay incidence caused by pathogenic fungi was markedly higher in fruit treated with insecticides than in those treated with kaolin. The lower performance of fruit treated with insecticides compared to those treated with kaolin may depend on the short persistence of pyrethroids: due to their rapid degradation rate, the level of residues on fruit surface after one week might be not sufficient to completely prevent medfly oviposition. As a result, in fruit damaged just before harvest, decay incidence at the end of storage caused by the activity of developing larvae or pathogenic fungi penetrated through stings was higher in insecticides treated fruit than in kaolin ones.

Kaolin sprayed on leaves and fruit surface, depending on particles size and film uniformity may affect the transmission of photosynthetically active, ultraviolet and infrared radiations, resulting in changes in surface temperature, photosynthetic activity, selective production of pigments, chemical composition, susceptibility to decay and physiological disorders (Jifon and Syvertsen, 2003; Lombardini *et al.*, 2005; Russo and Díaz-Pérez, 2005; Cantore *et al*., 2009).

Our results showed no difference in respiration and ethylene production rates of kaolin treated fruit compared to control or insecticides treated ones. These results can be explained considering that the size and porosity of the particles deposited on fruit surface would not affect gas exchange and the fruit ripening process.

Kaolin did not affect firmness and mass loss. These results are in contrast with those reported by Ergun (2012) with 'Galaxy' apples, who attributed the reduction of mass loss to the small size of the kaolin particles which by partially blocking stomata and lenticels would have led to a reduction of gases and water vapor exchange with the environment. A reduced transpiration rate of kaolin was also detected in bean leaves (Tworkoski *et al*., 2002), groundnut (Khan and Morey, 1980), and tomatoes (Cantore *et al*., 2009).

Differently, either no or an inconsistent effect of kaolin film on transpiration and stomatal conductance were reported by others (Kerns and Wright, 2000; Glenn *et al*., 2001; Jifon and Syvertsen, 2003; Russo and Díaz-Pérez, 2005; Glenn, 2012; Lobos *et al*., 2015). Genetic variability among species, differences in growth environment, possible kaolin-induced physical skin modifications, kaolin formulations, may be only some among the numerous factors leading to contrasting results (Wand *et al*., 2006; Conde *et al*., 2016).

Although no specific study at our knowledge has been set up to specifically evaluate the effect of kaolin treatments on fruit quality, generally results reported in the literature indicate a positive effect of kaolin on overall quality. These positive effects of kaolin, seems to rely on its ability to reduce organs' surface temperature and to enhance solar radiation reflection, which, while reducing the risk of sunburns improves skin color development, lowers the photorespiration process and increases photosynthetic efficiency (Glenn *et al*., 2002; Wand *et al.*, 2006; Glenn, 2009).

In grapevine, kaolin treatments stimulated the phenylpropanoid, flavonoid-flavonol and anthocyanin-pathways thus increasing total phenolics and anthocyanins content in ripe berries, but had no effect on pH, TA and SST (Conde *et al*., 2016). Our results, in agreement with previous findings (Glenn, 2012; Lobos *et al*., 2015; Conde *et al*., 2016), denoted no effect of kaolin on TA and SST, but also on juice antioxidant activity, glucose, fructose, sucrose and organic acids contents both at harvest time and during storage.

Despite kaolin treatment was not able to completely control medfly attacks, its performance was superior to synthetic insecticides in controlling direct damage by medfly due to the presence of larvae but also indirect damage even when larvae did not develop, for the lower incidence of decay caused by wounds pathogens. Therefore, kaolin seems to be a promising alternative to conventional insecticides to manage medfly infestation in peaches. One potential disadvantage of kaolin at commercial level is the fact that the white film persists on fruit surface after harvest and that to be removed fruit should be washed, an operation that normally is not done on peaches intended for fresh consumption. However, the presence of kaolin on fruit surface could be exploited positively commercially by reporting in the label that the white film covering the fruit is a proof that no synthetic insecticides were used in the growing process. On the other hand, in case fruit are destined to the processing industry rinsing the fruit would not be a problem.

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Effects of 1-MCP and ethylene on preservation of quality and vase life of Alstroemeria (cvs. Hercules and Mayfair) cut flowers

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Key words: aging, catalase, chlorophyll, climacteric, leaf yellowing, vase life.

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Abstract: In order to improve the vase life characteristics and postharvest quality of Alstroemeria cut flowers cv. Hercules and Mayfair, the effects of 1-methylcyclopropene (1-MCP) and ethylene have been investigated in a completely randomized design with three replications. First, cut flowers were fumigated at 0, 0.5, 1 and 1.5 µL L^{-1} of 1-MCP concentrations for 24 h and then exposed to 1 µL L^{-1} of ethylene for 18 h. This experiment was conducted at 20 ± 2°C, 60-65% RH, and a 12/12 h light/dark photoperiod. The results showed that 1-MCP treatment significantly increased postharvest durability in both cultivars, compared to the control. Also, results of 1-MCP treatment on leaf chlorophyll index in both cultivars, confirmed the role of 1-MCP in preventing external ethylene action as the main factor of Alstroemeria leaf yellowness. Based on data, Mayfair cultivar was more sensitive to ethylene in comparing to Hercules cultivar, although 1-MCP treatment reduced the active oxygen species in both cultivars by reducing biosynthesis and ethylene action or by direct increase in antioxidant enzyme activity.

1. Introduction

Alstroemeria cultivars are consumed as cut flower due to their variation in the flower pattern and color which spotted with dark colors, and this caused an increase in their global trade during last decades (Ferrante *et al*., 2002). Alstroemeria cut flower quality is often decreased by prematurity yellowing of leaves which reduce viability of leaves before appearing the secondary florets (Ferrante *et al*., 2002).

From commercially point of view, the leaf green color preservation is one of the most important qualitative characteristics playing a role in aesthetic valuation of this ornamental plant (Mutui *et al*., 2006). The leaf yellowing is affected by various factors such as poor storage conditions, deficiency of internal cytokinin, exposure to internal and/or external ethylene, darkness, accumulation of abscisic acid, leaf aging and damage (Ferrante *et al*., 2009).

Ethylene is one of the most important limiting factors in maintaining

the quality and life of many cut flowers due to accelerating aging in the post-harvest stage. Alstroemeria cultivars showed varied range of sensitivity to ethylene. Although they produce very little ethylene, they are sensitive to external ethylene (Chanasut *et al*., 2003) so that low concentration of this gaseous hormone causes leaf yellowing, abscission of petals and accelerating flower aging (Reid, 1989). For these reasons, many efforts are being made to gain more information about compounds inhibiting and blocking ethylene biosynthesis and action. 1-MCP patented by Edward Sisler, acts as a blocking agent of ethylene action by binding to ethylene receptors. In other words, 1-MCP binds competitively to ethylene binding site with affinity higher than that of ethylene and blocks downstream signal transduction, thereby prevents the expression of genes induced by ethylene, determining an extension of the shelf life of many climacteric cut flowers (Serek *et al*., 1994; Ahmadi *et al.*, 2008, 2009; Daneshi Nergi and Ahmadi, 2014). Although some silver-containing compounds could inhibit ethylene action, they have toxicity effects on human health and the environment, limiting their application on crops. Moreover, to reduce the people concerns on these harmful and hazardous compounds, the demand of using safe compound to extend post-harvest life in crops especially edible products is also increasing.

The purpose of this study was to evaluate the efficacy of 1-MCP on extending the longevity of cut flowers and on reducing leaf yellowing in Alstroemeria cvs. Mayfair and Hercules. In most Alstroemeria cultivars the first aging symptom of cut flowers is the onset of yellowness in the leaves which occurs earlier than petals aging or abscission. The activity of antioxidant enzymes in petals and ethylene production of both cultivars were also evaluated.

2. Materials and Methods

In this experiment, cut flowers of Alstroemeria cvs. Mayfair and Hercules were harvested based on commercial indices from a greenhouse located in Pakdasht, Tehran, Iran. After harvesting, cut flowers were immediately transported dry to the Postharvest Laboratory at Tarbiat Modarres University. Stems were re-cut under water to a length of 45 cm and held in 500 ml vase solution containing 200 mg/L 8-hydroxyquinoline sulfate (8-HQS), and 3% sucrose. (Rasouli *et al*., 2015). Then cut flower stems were placed in 200 L glass chambers and treated with 1-MCP at concentrations of 0, 0.5, 1, and 1.5 µL L^{-1} for 24 hours (Daneshi Nergi and Ahmadi, 2014).

Ethyl Bloc powder prepared from US AgroFresh was used for applying 1-MCP treatment. Considering the given concentrations, certain amounts of Ethyl Bloc were weighed and placed in Petri dishes, then warm water was added to the Petri dishes inside glass chambers. Immediately, the lids of glass chambers were hermetically sealed by adhesive tape (Daneshi Nergi and Ahmadi, 2014). After 24 hours of treatment with 1-MCP, the lids of the chambers were removed and cut flowers were ventilated with fresh air for one hour. Then, by closing and sealing the lids again, ethylene was injected into each chamber, to expose all flowers to 1 µLL^{-1} exogenous ethylene for 18 h. When the ethylene treatment was completed, the lid of glass containers was opened and the vases containing flowers were placed on the laboratory table. The experiment was conducted at 20± 2°C, 60-65% RH and a 12/12 h light/dark photoperiod at an illumination of 15 µmol m^{-2} s^{-1}.

Vase life

One of the most important criteria for evaluation of postharvest quality is the viability of cut flowers. In this study, the flower life is the time interval between the beginning of treatment until the cut flowers lose their ornamental value. When 50% of petals falling down in cut Alstroemeria, it was considered as the end of vase life quality (Mutui *et al*., 2006).

Relative fresh weight and chlorophyll content

In order to measure this trait, the weight of flower was measured using digital scale (KERN model) with an accuracy of ± 0.001 g. This trait was calculated during the evaluation period using equation: relative fresh weight (percent) equal to (wt/wt = 0) in this regard wt: flower weight (g) on day 0, 3, 6, 9 and 12, wt = 0: flower weight g per day (He *et al*., 2006). To measure the chlorophyll content, 0.2 g of leaf sample was used to extract its chlorophyll in 80% acetone. Then, in a volumetric flask, 25 ml of the extract was filtered, acetone reached 25 ml volume and chlorophyll was completely extracted (Arnon, 1949).

Evaluation of catalase activity

About 200 mg of frozen petal cell mass, sampled on day 6 of the experiment, was extracted in 3 ml of 25 mM sodium phosphate buffer (pH = 6.8) and centrifuged for 4 min at 15°C. The enzyme solution was used to measure enzyme activity. Then, the reaction mixture was prepared including 25 mM of sodium phosphate buffer (pH = 6.1), 10 mM of hydrogen peroxide and enzymatic extract. Catalase activity was

measured at the wavelength of 240 nm and calculated as a delta of absorbance at 240 nm per mg protein. All enzymatic extraction stages were performed on ice (Cakmak and Horst, 1991).

CAT activity = (final Abs-Initial Abs)/protein

Evaluation of superoxide dismutase activity

About 200 mg of petal tissue, sampled on day 6 of experiment, was extracted in 3 ml of HEPES-KOH buffer containing 0.1 mM of sodium EDTA (pH = 7.8). The resulting homogenate was centrifuged for 30 minutes at 12000 rpm at 4°C. The resulting supernatant was used to measure superoxide dismutase activity. The reaction composition in the final volume of three ml is as follows:
HEPES-KOH buffer (50 mM) containing 0.1 mM of sodium EDTA at pH = 7.8, sodium carbonate (50 mM) at pH = 10.2, L-methionine (12 mM), Nitro Blue Tetrazolium (NBT) (75 mM), Riboflavin (1 mM), and the enzyme extract were appropriately considered as one unit of superoxide dismutase activity as an enzyme that results in 50% inhibition of nitrobutetrazolium at 560 nm (Swanson, 1955). Adsorption of the reaction mixture was measured using BIO-RAD spectrophotometer.

Measurement of ethylene

After finishing ethylene treatment three flowers from each treatment were placed in a 1.8 L sealed glass bottle and kept at 20 ± 2°C for 48 h, same as experimental condition. By a gas-tight syringe, gas samples were taken through head spaces and were injected into a GC manufactured by Agilent America Co. Model GC-6890N fitted with a capillary column and a flame ionization (FID) detector. The carrier gas was helium at 6.5 mL min^{-1}, injection temperature was 180°C and column temperature was 60°C (Daneshi Nergi and Ahmadi, 2014).

Statistical analysis of the data

The experiment was conducted in a completely randomized design (CRD) with four treatments and three replications. The data were analyzed using statistical software SAS version 9 and the means were compared by the least significant difference (LSD) test (P=0.05). The figures were drawn using Excel software.

3. Results

Vase life

This experiment showed that the vase life of cut flowers cv. Hercules was longer than cv. Mayfair, although vase life of both cultivars was affected by 1-MCP application. Maximum plant longevity was 12 days for cv. Hercules under 1.5 µl 1-MCP treatment and the longevity of cv. Mayfair was extended to 10.8 days under 1 µL L^{-1} of 1-MCP, which were significantly different at level as indicated by Least significant difference (LSD), comparing to the control. No significant differences were observed between 1-MCP treatment levels in any of cultivars at 1% level by an LSD (Fig. 1).

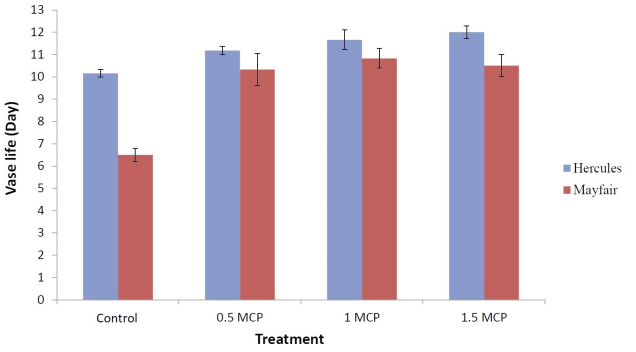

Fig. 1 - Vase life of Alstroemeria treated with concentrations of 1-MCP at 0, 0.5, 1 and 1.5 µL L^{-1}. Vertical bars represent standard deviation.

Relative fresh weight

The results of analysis of variance and mean comparison of relative fresh weight of cut flower of Alstroemeria cv. Hercules showed that the highest relative fresh weight (119.5%) was obtained on the sixth day after applying treatment, which was statistically significant at 1% probability level. The lowest relative fresh weight (90.2%) was obtained on the twelfth day after applying treatment, which was not significantly different from the relative fresh weight on day 0, but it was significantly different with relative fresh weight at other times, at probability level 1% (Fig. 2). In cv. Mayfair, the highest relative fresh weight (106.32%) was showed at 1 µL L^{-1} of 1-MCP treatment, which was statistically significant at 1% probability level with 0.5 and 1.5 µL L^{-1} of 1-MCP treatments. Control samples revealed the lowest relative fresh weight (62.9%) (Fig. 3).

Chlorophyll content

Data showed that 1-MCP treatments affected significantly the chlorophyll content in cv. Hercules. The highest content of chlorophyll (1.13 mg) was revealed at 1 µL L^{-1} of 1-MCP concentration which

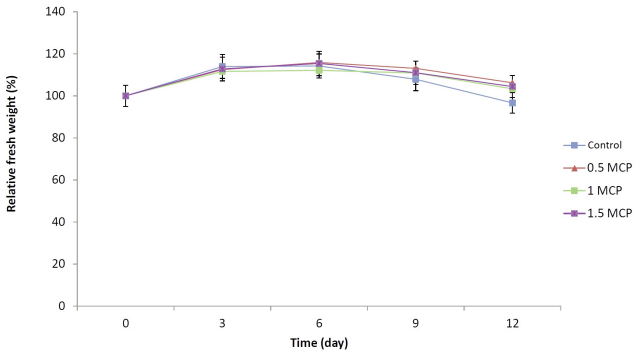

Fig. 2 - Relative fresh weight of cut flower Alstroemeria cv. Hercules treated with concentrations of 1-MCP at 0, 0.5, 1 and 1.5 µL L^{-1}. Vertical bars represent standard deviation.

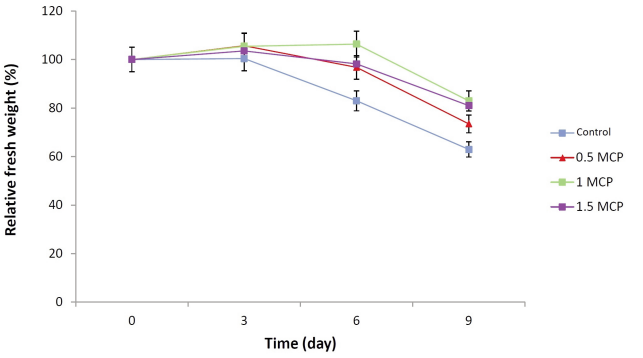

Fig. 3 - Relative fresh weight of cut flower Alstroemeria cv. Mayfair treated with concentrations of 1-MCP at 0, 0.5, 1 and 1.5 µL L^{-1}. Vertical bars represent standard deviation.

was not significantly different from 1.5 µL L^{-1} of 1-MCP. The lowest chlorophyll content (0.94 mg) was measured in leaves treated without any 1MPC (Fig. 4).

In Mayfair cultivar, the effect of treatment on chlorophyll content was significant at 5% probability level so that the maximum chlorophyll content (1.63 mg) was revealed at 1.5 µL L^{-1} of 1-MCP treatment which was statistically significant at 5% probability level, comparing to control (Fig. 5).

Endogenous ethylene production

Ethylene production was measured 48 hours after ethylene treatments. Cut flowers cv. Hercules showed the lowest endogenous ethylene production at 1 µL L^{-1} of 1-MCP, which was not significantly different to ethylene biosynthesized under 1.5 µL L^{-1} of 1-MCP (Fig. 6). Cut flower cv. Mayfair revealed lowest ethylene production under 1.5 µL L^{-1} of 1-MCP

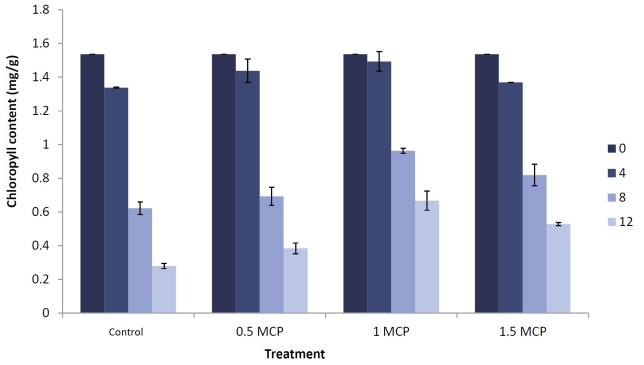

Fig. 4 - Relative chlorophyll content on days 0, 4, 8 and 12 of cut flower of Alstroemeria cv. Hercules treated with concentrations of 1-MCP at 0, 0.5, 1 and 1.5 µL L^{-1}. Vertical bars represent standard deviation.

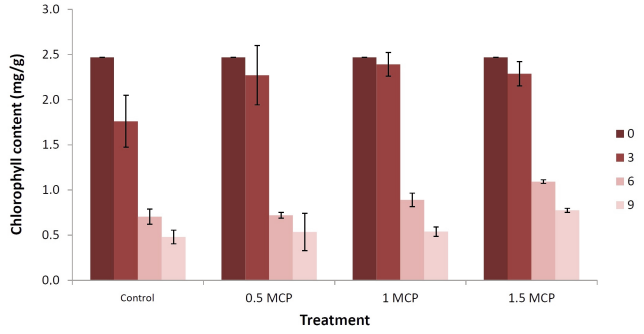

Fig. 5 - Relative chlorophyll content on days 0, 3, 6 and 9 of cut flower of Alstroemeria cv. Mayfair treated with concentrations of 1-MCP at 0, 0.5, 1 and 1.5 µL L^{-1}. Vertical bars represent standard deviation.

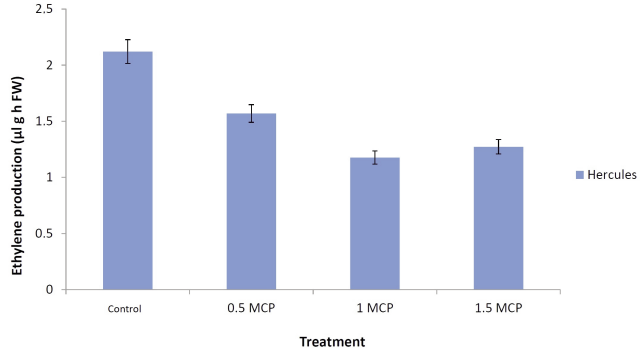

Fig. 6 - Ethylene production of cut flower of Alstroemeria cv. Hercules 48 h after finishing ethylene treatment. Vertical bars represent standard deviation.

application, showing significant differences in respect to other treatments (Fig. 7). Data showed that cv. Hercules produced higher content of ethylene in comparison to cv. Mayfair.

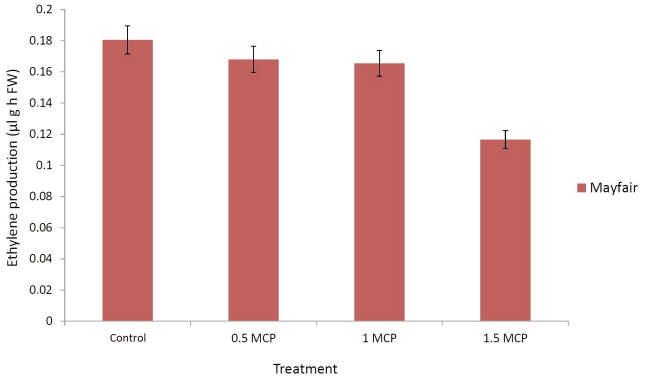

Fig. 7 - Ethylene production of cut flower of Alstroemeria cv. Mayfair 48 h after finishing ethylene treatment. Vertical bars represent standard deviation.

Catalase activity

Data on cut flowers Alstroemeria cv. Hercules showed that 1-MCP had a positive effect on catalase activity, so that the highest activity was evaluated under 1 µL L^{-1} (55/98 absorption delta/mg protein) concentration, which was significantly different at 1% probability level, comparing to other treatment based on LSD test (Fig. 8).

Flower petals of cv. Mayfair showed the highest level of catalase activity under 1 and 1.5 µL L^{-1} of 1-MCP concentration, without any significant difference. The lowest catalase activity was revealed in control sample which did not receive any 1-MCP (27.79 absorption delta / mg protein) (Fig. 8).

Superoxide dismutase activity

The analysis of data in cut flowers Alstroemeria cv. Hercules showed that the maximum activity of superoxide dismutase was appeared in 1-MCP at concentration of 1.5 µL L^{-1} (82.34 enzyme units/mg protein) according to LSD test, without any significant difference with 1 µL L^{-1} concentration (Fig. 9).

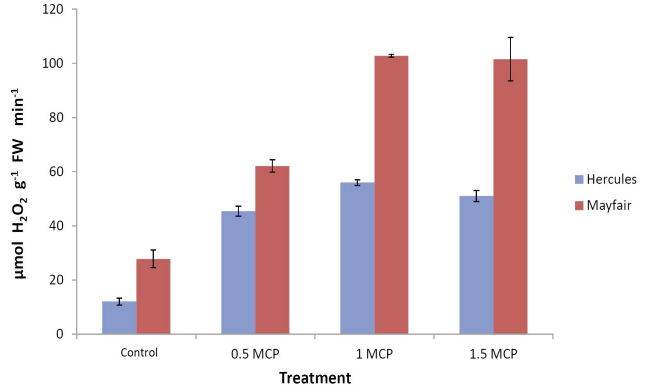

Fig. 8 - Catalase activity in Alstroemeria flowers treated with concentrations of 1-MCP at 0, 0.5, 1 and 1.5 µL L^{-1}). Vertical bars represent standard deviation.

In the cut flower of Alstroemeria cv. Mayfair, the maximum activity of superoxide dismutase was obtained from the treatment of 1-MCP at a concentration of 1 µL L^{-1} (90.97 units of enzyme/mg protein) which was no significantly different from other 1-MCP concentrations according to LSD test (Fig. 9). The lowest level of superoxide dismutase activity was related to control samples, which had a significant difference at 1% probability level with other treatments.

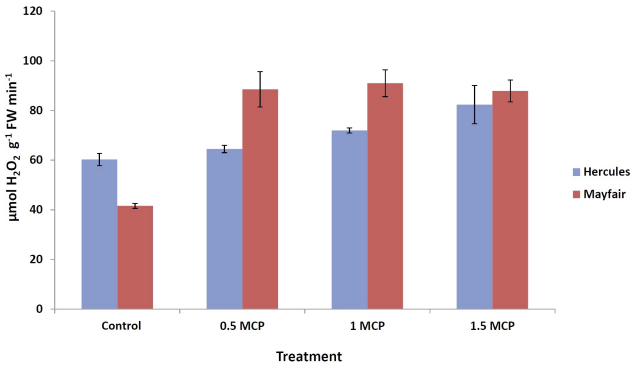

Fig. 9 - Superoxide dismutase activity in Alstroemeria flowers treated with concentrations of 1-MCP at 0, 0.5, 1 and 1.5 µL L^{-1}. Vertical bars represent standard deviation.

4. Discussion and Conclusions

Vase life is one of the most important factors for evaluating the quality of cut flowers. The role of ethylene as the most important accelerating factor in aging of plant organs has been entirely investigated, especially in climacteric crops. In this experiment, 1-MCP was used to reduce the deleterious effects of ethylene (Fig. 1). Based on the results, ethylene accelerated quality deterioration and leaf senescence in the flowers of two Alstroemeria cultivars, while 1-MCP increased vase life of both Alstroemeria cvs. by alleviation of the harmful effect of ethylene. 1-MCP treatment preserves quality and prolongs post-harvest life by preventing ethylene action and subsequently preventing endogenous ethylene production.

1-MCP compound, like other ethylene action inhibitors such as silver thiosulfate, delayed flower aging when the flowers were exposed to 1 µLL^{-1} of ethylene (Serek *et al.*, 1995). This experiment showed that 1-MCP treatment decreased the indigenous ethylene biosynthesis in cut flowers of Alstroemeria (Fig. 6 and 7). Accordingly, it seems that the prolonged vase life of cut flowers of Alstroemeria

by application of 1-MCP could be related to the role of 1-MCP in inhibition of internal ethylene production. Similar to our results, Chutichudet *et al.* (2011) reported that 1-MCP can prevent ethylene production and increase the life of cut flowers. In carnation cut flower cv. Fortune, it was shown that vase life increased with increasing concentration of 1-MCP (Ranjbar and Ahmadi, 2015).

The results of the present study showed that the relative fresh weight of cut flowers increased and then reduced over time, which was higher in control samples, comparing to 1-MCP-treated cut flowers (Fig. 2 and 3). The reduction in flowering stem weight of flowers treated with 1-MCP during storage may be affected by the interference of 1-MCP in ethylene self-production system (Porat *et al*., 1995). The report of Daneshi Nergi and Ahmadi (2014) on rose cut flowers cv. Sparkle treated with 1-MCP and ethylene showed that the effects of treatment on the relative fresh weight were significant over time (Daneshi Nergi and Ahmadi, 2014).

According to the present results, 1-MCP at 1 and 1.5 µL L^{-1} concentration had the better yield on chlorophyll content in cv. Hercules (Fig. 4). Also, in cv. Mayfair, 1.5 µL L^{-1} of 1-MCP treatment performed better than control samples (Fig. 5). Chlorophyll, as the main pigment involved in light harvesting, plays essential role in terms of absorption and utilization of light energy in photosynthesis. Basically, the leaf aging is associated with a reduction in chlorophyll content. It was reported that reduction of chlorophyll content of Alstroemeria leaves coincided with leaves aging (Ferrante *et al*., 2002). Ethylene accelerates chlorophyll degradation in the leaves of many cut flowers. The results of both cultivars showed that 0.5 µL L^{-1} of 1-MCP could not prevent chlorophyll degradation, which was consistent with some reports (Çelikel *et al*., 2002; Seglie *et al*., 2010). It seems that protection of chlorophyll in leaves of cut flowers Alstroemeria under 1-MCP treatment could be related to preventing the action of external ethylene, known as the main cue of leaf yellowing in ornamental plants. In Asian pear, 1-MCP treatment delayed chlorophyll degradation, which was attributed to the effect of 1-MCP treatment on suppression of enzymes involved in on ethylene production pathway (Cheng *et al*., 2012). The process of leaves yellowing in cut Alstroemeria is under the control of genetic background and environmental conditions. Data of Hercules cultivar showed that 1 µL L^{-1} of 1-MCP resulted in lower ethylene synthesis than other treatments (Fig. 6). At the concentration of 1.5 µL L^{-1} in Mayfair cultivar, 1-MCP resulted in lower ethylene synthesis than other treatments (Fig. 7). In both cultivars, 1-MCP treatments prevented ethylene production. The pre-treatment with 1-MCP for preventing ethylene production is consistent with the study results of rose cv. Samantha (Xue *et al*., 2008) and clove (Seglie *et al*., 2010).

Catalase is considered an important biological factor and its major function is in the decomposition of hydrogen peroxide to water and oxygen and preventing creation of hydroxyl radicals (Spanou *et al.,* 2012). Superoxide dismutase like Cu-Zn superoxide dismutase, Mn superoxide dismutase and outside cell superoxide dismutase play a critical role in inhibition of superoxide (Miao and St Clair, 2009).

In fact, catalase and superoxide dismutase play roles in protecting the metabolism balance of oxygen in plant tissues (Xie *et al.,* 2003). Superoxide causes lipid peroxidation, cell membrane damage and finally senescence; 1-MCP can affect enzyme activities, which remove superoxide (Li *et al.,* 2007).

In the cut flower of Hercules, no significant difference was found between 1-MCP treatments, but the activity of catalase was significantly increased compared to control (Fig. 8). The results of Mayfair cultivar cut flower showed that no significant difference was found between 1 and 1.5 µl of 1-MCP but catalase activity increased significantly compared to control (Fig. 8). Ethylene-stimulated aging could change the cell structure and increased the concentration of reactive oxygen species such as superoxide radicals, hydroxyl radicals and hydrogen peroxide. Therefore, 1-MCP treatment reduced the reactive oxygen species and consequently delayed aging by reducing the biosynthesis and action of ethylene or by directly increasing the activity of antioxidant enzymes (Fig. 9). Consistent with the results of this experiment, an increase was observed in antioxidant enzymes activity of 1-MCP -treated Gladiolus flowers (Hassan and Ali, 2014). It seems that 1-MCP treatment reduced the oxidative stress in cut flowers. In other words, activity of these enzymes is a factor for the protection of cells against oxidative stresses (Zhou *et al*., 2014). In asparagus, 1-MCP hindered the ethylene signal transduction and resulted in a delay by affecting ethylene biosynthesis, and enhancing superoxide dismutase activity (Zhang *et al.,* 2012). In addition, it has been found that applying 1-MCP delayed aging, but its effect varied depending on the flower organ, cultivar, and development stage and treatment concentration. It seems that catalase activity depends on the plant species, plant type and experimental condi-

tions during aging. Finally, it can be concluded that increasing the activity of antioxidant enzymes reduces the aging of flowers.

In general, 1-MCP treatment had a positive effect on improving physiological and biochemical characteristics resulted in an extended postharvest longevity of Alstroemeria cultivars. The higher concentrations of 1-MCP revealed better effect in comparing to low concentrations, especially on ethylene production. According to the results, 1-MCP treatments, by increasing the protein content and the activities of catalase and superoxide dismutase, improved the quality and increased vase life of cut flowers of Alstroemeria.

In conclusion, based on this study, the application of 1-MCP acting as ethylene action inhibitor, could be recommended to increase postharvest life and extended the longevity in Alstroemeria cut flowers.

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Volatile compound and gene expression profiles associated with the storage of two peach fruit genotypes differently sensitive to chilling injuries

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Key words: cold storage, C-repeat-binding factors, lipoxygenase pathway, *Prunus persica*, volatile organic compounds.

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Abstract: The patterns of volatile organic compounds (VOCs) emission and the expression of genes associated with the lipoxygenase (LOX) pathway have been studied in harvested peach fruit of two cultivars ('Flaminia', FL, and 'Red Haven', RH) during and after cold storage. Two temperature storage conditions have been applied for two weeks: 0.5 and 5.5°C, the latter recognized to be an inducer factor of chilling injury (CI) of the flesh. Fruit were also monitored during 3 days of shelf-life (SL) at room temperature after removing from the cold storage. A different behaviour between cultivars has been observed in terms of internal browning (more evident in FL after 2 weeks) and extractable juice (already reduced in RH at the end of 1 week of storage at 5.5°C). Although some common responses have been observed (e.g. a general increase of 2-hexenal and 2-hexenal-E at the end of both cold storage conditions), LOX pathway-associated volatiles (aldehydes, alcohols, esters) showed different trends in relation to the genotype and the applied stress, with apparently no specific correlations with the incidence of CI. The expression level of five LOX pathway-associated genes (*PpLOX1, PpLOX4, PpHPL1, PpADH1, PpAAT1*) have been analysed and the results point out that a genotype-dependent behaviour is present, but specific responses (up-regulation of *PpLOX1* and *PpAAT1* during SL) appear to be present in both cultivars. In addition, the expression of two *C-repeat-binding factors* (*PpCBF1* and *PpCBF6*), recognized to be involved in the responses of plant tissues to low temperature stress, showed marked changes in relation to the applied temperature, suggesting that these genes might play a regulatory role in the overall metabolism of cold stored peaches.

1. Introduction

Peach fruit are highly perishable at ambient temperature and they are normally stored under refrigeration, making possible to maintain commercial quality up to 2-4 weeks, depending on the specific cultivars and

storage conditions (Ramina *et al*., 2008). However, low temperatures may lead to the development of chilling injury (CI), which negatively affect fruit quality shattering important organoleptic quality traits such as taste and flavor, as well as inducing the appearance of physiological disorders such as internal browning and mealiness/woolliness (Crisosto and Mitchell, 2002; Lurie and Crisosto, 2005). CI symptoms typically evolve during post-storage/shelf-life conditions and can also compromise the fruit capability to ripen after storage, resulting in quality levels not compatible with marketability. Different cvs possess variable levels of tolerance/susceptibility against CI (Brizzolara *et al*., 2018; Nilo-Poyanco *et al*., 2018). For peach fruit a specific range of temperatures, called "killing zone" (2.2-7.6°C), has been associated to CI symptoms which tend to appear more rapidly and to become more severe than at 0-1°C (Crisosto and Valero, 2008). Several works investigated the causes of CI onset arguing that a key aspect in peach fruit response to "killing zone" temperatures is the lower ethylene synthesis that, in turn, may only in part activate the ripening machinery (Fernández-Trujillo *et al*., 1998; Walsh *et al*., 2001; Zhou *et al*., 2001; Pons *et al*., 2015; Wang *et al*., 2017).

One of the most important ripening-related quality traits for fruit in general and, especially, for peaches is the profile of volatile organic compounds (VOCs). During peach fruit ripening a number of different VOCs (e.g. alcohols, terpenes, esters, lactones) are synthesized and play a role in making fruit attractive and for consumer acceptance (Aubert and Milhet, 2007; Yang *et al*., 2009). The lipoxygenase (LOX) pathway, by using fatty acids as precursors, is responsible for the production of specific VOCs that play important roles in fruit ripening, also acting as precursors for other metabolites. The LOX pathway has been also studied in relation to the physiological responses of peaches to cold storage and the changes in terms of overall organoleptic quality (Ortiz *et al*., 2009; Zhang *et al*., 2011). In addition to the description of VOC profiles and enzyme activity pattern, these works correlate specific LOX pathway-associated gene expression with the onset of CI. Indeed, Zhang *et al*. (2011) suggested that the decreased levels of fruity note volatiles in peaches with CI, were the consequence of modifications in expression of two lipoxygenase (*PpLOX1* and *PpLOX2*) and one alcohol acyltransferase (*PpAAT1*) genes.

The different metabolic profiles of peach fruit stored under CI-inducing or "safe" low temperatures have been also related to molecular mechanisms effective to control the expression of structural and regulatory genes. Considering the latter, the C-repeat (CRT) / dehydration-responsive element (DRE) binding factor 1 (CBF/DREB1) is recognized to be involved in plant responses to abiotic stresses (Thomashow, 2010). Plant CBF proteins are encoded by multigene families and specific members show distinct expression patterns in relation to low-temperature stress. The involvement of CBF in responses to cold storage has been reported in tomato fruit, in which an up-regulation of *LeCBF1* has been associated with a reduction of CI during storage (Zhao *et al*., 2011).

Differential expression of 6 isolated *CBF* genes has been observed in peaches stored under refrigeration (Liang *et al.*, 2013). These authors demonstrated that *PpCBF* 1/5/6 were up-regulated during cold storage at 0°C and this was accompanied by a decrease of CI symptoms.

In this work, we report the effects of 2 weeks of cold storage (0.5 and 5.5°C), followed by shelf-life at room temperature (20°C) on the profiles of LOX pathway-derived VOCs and the expression of LOX-pathway and *CBF* genes in peaches of two different genotypes ('Flaminia' and 'Red Haven', a typical Italian cultivar from Tuscany region and a worldwide commercially important cultivar, respectively).

2. Materials and Methods

Fruit material, sampling procedure and CI symptoms evaluation

Peach (*Prunus persica L. Batsch*) fruit belonging to 'Red Haven' (RH, yellow fleshed) and 'Flaminia' (FL, yellow-fleshed, +35 days from RH harvest) have been harvested at flesh firmness values of about 36 and 44 N for RH and FL, respectively, from a commercial orchard located at Casciana Terme (Pisa, Tuscany, Italy). The fruit selection, the refrigeration treatments and the sampling procedures are those described by Brizzolara *et al*. (2018). Briefly, after harvest fruit were selected for homogeneity in terms of fruit size and peel color and were analysed using NIR technology (NIR-Case, SACMI, Italy), which has been previously calibrated for firmness (manual penetrometer), total soluble solids (TSS) (optical refractometer) and peel color (arbitrary scale) for each cv. Peaches (thirty for each treatment) have been stored in cold chambers at two low temperature conditions (0.5 and 5.5±0.5°C) and, as non-refrigerated control, at 20±0.5°C. Relative humidity under cold storage was kept at 85% and monitored using a TGU-4500

(Tinytag Ultra 2, Gemini, United Kingdom) data logger. Peaches were sampled at harvest (T0) and after 1 and 2 weeks of cold storage. Nine fruit per treatment were additionally kept for 3 days under shelf life (SL, 20°C) conditions to investigate the post-storage phase. Firmness (N) was measured on nine fruit using a manual penetrometer (GY-3, Newtry) equipped with an 8 mm tip.

Chilling injury symptoms have been evaluated following the same protocol described by Brizzolara *et al*. (2018) using an arbitrary scale. Briefly, a total of nine peaches has been evaluated for each sampling time and treatment. For internal browning evaluation, peaches have been cut in two halves and, on each part, brown areas have been assessed with the open source software 'ImageJ', for a total of 18 measurements (2 for each sampled fruit) for every thesis and sampling time. Half fruit has been considered as representing 50% of the whole fruit, and browning incidence (%) has been calculated as the average of the nine measured fruit. Juiciness has been assessed by processing with a juice extractor (Moulinex Juice Extractor JU350B27) 50 g of pooled mesocarp tissue from three peaches (in triplicates, nine fruit in total), and it has been expressed as percentage of extracted juice normalized on control levels. At each sampling time, three fruit per replicate were processed and a random pool of mesocarp tissue was immediately frozen in liquid nitrogen and stored at -80°C. Powdered tissue has been used for molecular analyses, while for VOC analysis, mesocarp samples of the same three fruit were crushed in a 1M NaCl solution by means of a T25 Ultra-Turrax^{R} (IKA, Germany) and the obtained puree was immediately frozen in liquid nitrogen and stored at -80°C.

Volatile organic compounds (VOCs) analysis

For aroma volatile compound analysis, the same method previously adopted by Brizzolara *et al*. (2017, 2018) was used. Analyses have been performed using Agilent Technologies (6890N, United States) equipment: a gas chromatograph coupled with mass spectrometer (5973 Network Mass Selective Detector, Agilent Technologies) and equipped with an autosampler (MPS2, Gerstel Multipurpose sampler, Germany) has been used. Peach samples were incubated at 40°C for 2 h, volatile compounds were sampled for 45 min using solid phase micro extraction (SPME) fiber (Supelco Inc., Bellefonte, PA, United States) technology, with a polydimethylsiloxane / divinylbenzene (PDMS/DVB, 1 cm long, 65 µm thickness, 0.357 µL volume) sorptive coating. The fiber was desorbed into the split/splitless liner of the GC for 5 min in splitless mode, setting at 250°C the injector temperature. The GC was equipped with a 30 m × 0.25 mm i.d. capillary column (HP-5MS, 5% phenyl methyl siloxane) having a film thickness of 0.25 µm. Helium was employed as carrier gas with a flow rate of 1.2 mL min^{-1}. The GC oven heating program started at 40°C and increased to 250°C at a rate of 5°C min^{-1} with a total run time of 32.5 min. Each sample was analysed in triplicate. A mass spectrometer (5973 Network Mass Selective Detector, Agilent Technologies) coupled to the GC was used as detector for compounds quantification/identification.

Compound identification has been performed deconvoluting and analysing each chromatogram using the automated mass spectral deconvolution and identification system (AMDIS, National Institute of Standards and Technology, Gaithersburg, MD, United States). Each chromatographic peak spectra has been compared with those of the National Institute for Standards and Technology (NIST98, Version 2.0, United States) data bank considering only results with 90%, or more, of matching. Peak retention indices (RI) have been used to optimize data bank screening. Raw peak areas have been normalized on the sum of the areas of all the identified peaks for each specific chromatogram.

Total RNA extraction and qRT-PCR analysis

Frozen mesocarp samples were grounded to powders using ceramic mortar and pestle pre-cooled with liquid nitrogen. 100 mg of grounded tissue were weighted. Total RNA extraction was carried out following the protocol of Spectrum™ Plant Total RNA Kit (Sigma-Aldrich, Italy), including DNA digestion with the On-Column DNase I Digestion Set (Sigma-Aldrich, Italy). RNA concentration and purity were determined with Nanodrop 2000 spectrophotometer (Thermo Scientific, Italy), verifying an absorbance ratio 260/280 nm between 1.8-2 and 260/230 nm between 1.3-2. Integrity of the RNA extracted was demonstrated by the existence of intact ribosomal bands on a 1% (weight/volume) agarose gel. Next, reverse transcription (RT) of the RNA templates to cDNA was carried out in a final volume of 20 µL using iScript™ cDNA Synthesis (Bio-rad Inc, Italy) following the manufacturer instructions. The experimental design of the relative expression analysis included three biological replicates. The approach followed was sample maximization according which, in one run of the qPCR reaction, one gene should be tested on all the samples in the experimental set (Helleman

et al., 2007). Additionally, in each qPCR reaction a non-template control (NTC) was amplified as a negative control. Analyses were performed by ABI Prism 7300 sequence detection system (applied biosystem). The reaction system consisted of 5 µl of KiCqStart™ SYBR® Green qPCR ReadyMix™, with ROX™ (Sigma-Aldrich, Italy), 3 ng of cDNA template, 0.3 µl of revers and forward primers in working solution of 10 nM. DDW (Sigma-Aldrich, Italy) was added to reach a final volume of 10 µl. The RT-qPCR cycle was set to as follows: initial denaturation at 95°C for 2 min, followed by 40 cycles of amplification with denaturation at 95°C for 15s and annealing and elongation at specific temperature suited to the primer melting temperature (Tm) for 1 min. Following the 40 cycles a melt cycle was performed at 95°C for 15 s, 60°C for 1 min, 95°C for 15s and 60°C for 15s. Expression levels were normalized employing ubiquitin C (PpUBC) housekeeping gene as a reference. Genes and primers have been selected based on previously published work dealing with LOX pathway gene expression in peach fruit (Zhang *et al*., 2010; 2011).

Statistical analyses

Presented data produced with all the employed analytical techniques have been analysed using one-way analysis of variance (ANOVA) statistical tools ($p \leq 0.05$) and Tukey's Honest Significant Difference (HSD) *post hoc* test in order to identify compounds or genes significantly differing in terms of relative intensity or expression level between tested treatments and sampling times. Before performing the analysis, ANOVA assumptions have been tested for all the investigated genes and compounds.

3. Results and Discussion

Technological parameters and CI incidence after cold storage and during shelf life conditions

Both low temperatures were effective in slowing down the loss of firmness rate in both cultivars. At the end of 1 and 2 weeks of storage higher firmness values have been detected in FL fruit stored at 0.5°C than at 5.5°C (Table 1) and, although not statistically significant at the end of the first storage week, a similar trend has been observed in RH fruit. After 3 days of shelf-life (SL) at room temperature following cold storage, fruit were still firmer than control samples. These data confirm previously published researches on peach fruit firmness changes under and after cold storage (Zerbini *et al*., 2011; Cano-Salazar *et al*., 2012; Wang *et al*., 2013).

Considering the CI incidence, a marked difference has been observed between the two considered cultivars (Table 1). As far as internal browning is concerned, RH fruit did not show any symptoms at the end and after both cold storage conditions. Instead, FL peaches developed a marked incidence of mesocarp browning after 2 weeks storage + SL, with some limited symptoms under control conditions (20°C). The two cultivars behaved differently also in terms of extractable juice. In fact, FL peaches showed no difference of this parameter among samples after 1 week of storage, and a significant reduction of extractable juice was detected in the sample kept at 5.5°C for 2 weeks + SL when compared with both control (20°C) and 0.5°C + SL. On the other hand, RH fruit after 1 week at 0.5°C and 5.5°C + SL displayed significant lower values of extractable juice than control fruit. These samples were also those showing sig-

Table 1 - Flesh firmness, incidence of internal browning and extractable juice values in peaches during two weeks of cold storage and subsequent three days of shelf life

	0 week	1 week					2 weeks				
	T0	0.5	5.5	20	0.5 + SL	5.5 + SL	0.5	5.5	20	0.5 + SL	5.5 + SL
A - Flaminia											
Firmness (N)	44.4±7.9 a	45.1±5.3 a	26.8±1.5 bc	5.6±3.7 e	45.1±5.3 a	26.8±1.5 bc	39.8±3.6 a	22.0±2.2 cd	4.5±2.3 e	35.8±3.6 ab	12.8±2.2 de
Int. Browning (%)	0.0 d	0.0 d	0.0 d	0.0 d	0.0 d	0.0 d	0.0 d	0.0 d	11.0±1.0 c	60.0±4.5 a	51.0±3.6 b
Extractable juice (%)	78.1±4.1 c	77.7±4.2 c	80.5±5.0 bc	85.7±1.4 bc	87.1±4.3 bc	86.7±5.0 bc	84.3±7.2 bc	83.1±4.8 bc	92.4±1.6 ab	100.0±3.8 a	78.6±3.8 c
B - Red Haven											
Firmness (N)	36.3±4.1 ab	41.2±3.4 a	36.1±2.5 ab	2.8±1.0 d	41.1±3.3 a	36.0±2.6 ab	40.7±2.5 ab	29.3±7.3 bc	1.7±1.5 d	38.6±2.5 ab	20.8±7.3 c
Int. Browning (%)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Extractable juice (%)	88.1±0.7 c	91.9±1.4 bc	92.5±2.7 abc	100.0±4.3 a	98.4±2.6 ab	88.9±2.5 c	88.8±1.2 c	90.1±4.9 bc	95.5±0.7 ab	93.0±1.4 abc	78.2±3.8 d

'Flaminia' (A) and 'Red Haven' (B) were analysed at harvest (0), at the end of 1 and 2 weeks of cold storage at 0.5 and 5.5°C, and after three additional days of shelf life (0.5 and 5.5°C + SL). Samples kept at 20°C represent the room temperature control. The average values of nine replicates ± SD for each treatment and measured parameter are reported. Letters indicate the results of Tukey's Honest Significant Difference (HSD) post hoc test, performed independently for each cultivar.

nificantly reduced values after 2 weeks of storage +SL (Table 1).

These data concerning CI incidence under and after cold storage are in agreement with the published literature concerning the effects of different temperatures and the role played by the genetic background (Lurie and Crisosto, 2005; Zhang *et al*., 2011; Pons *et al*., 2015; Bustamante *et al*., 2016).

Cold storage effects on LOX pathway-related VOCs

As general trend, during peach fruit ripening volatile compounds associated with green-notes, such as C6 aldehydes and alcohols, tend to decrease while the levels of molecules associated with fruity-notes, such as esters and, in particular, lactones follow an opposite trend (Defilippi *et al*., 2009; Zhang *et al*., 2011; Brizzolara *et al*., 2018).

Considering aldehydes, synthesized through the activities of lipoxygenase (LOX) and hydroperoxide lyase (HPL), three compounds have been targeted: hexanal, 2-hexenal and 2-hexenal-E (Fig. 1). 2-hexenal and 2-hexenal-E significantly decreased from harvest to the second week in control fruit (kept at 20°C) (Fig. 1), confirming what has been previously demonstrated in other works (Defilippi *et al*., 2009; Ortiz *et al*., 2010). Differently from hexanal, 2-hexenal and 2-hexenal-E showed an increasing trend, compared to controls, in both cultivars after 1 week of storage either at 0.5 and 5.5°C. In addition, these two compounds showed, in general, a decreasing trend during SL at room temperature, reaching the control levels (Fig. 1).

Moving downstream in the LOX pathway, C6 aldehydes produced by LOX and HPL are used by other enzymes as precursor for the production of C6 alco-

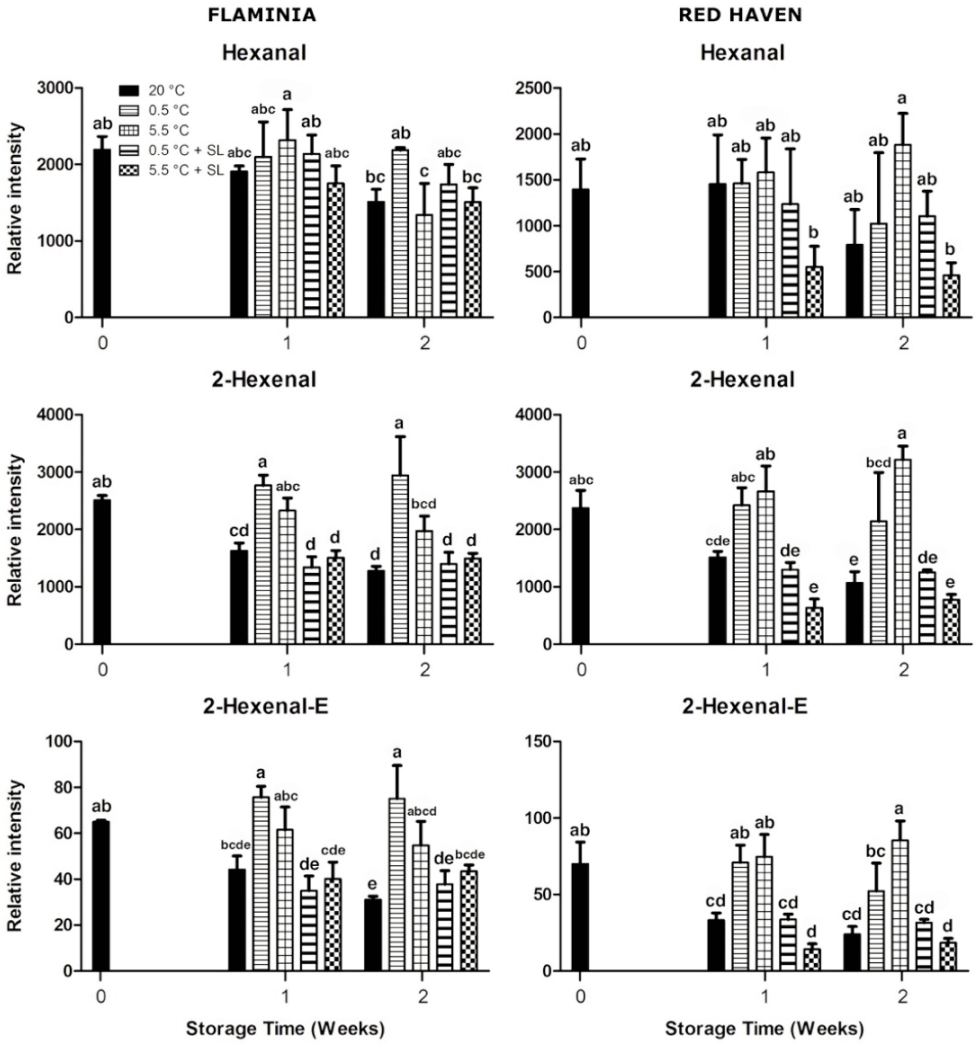

Fig. 1 - Analysis of targeted LOX pathway-derived aldehydes. Mesocarp samples (cv. Flaminia, left panel; cv. Red Haven, right panel) were analysed at harvest (0), at the end of 1 and 2 weeks of cold storage at 0.5 and 5.5°C, and after three additional days of shelf life (0.5 and 5.5°C + SL). Samples kept at 20°C represent the room temperature control. The average value of three biological replicates is reported with bars representing SD. Letters indicate the results of Tukey's Honest Significant Difference (HSD) post hoc test, performed independently for each cultivar.

hols. Specifically, aldehyde dehydrogenase (ADH) is the enzyme responsible for the production of alcohols from aldehydes (Lara *et al*., 2003; Defilippi *et al*., 2005). Considering the results on C6 alcohols level, hexanol, 2-hexen-1-ol and 3-hexen-1-ol accumulation trends are reported in figure 2. Interestingly, a temporary increase of these three compounds was detected after 1 week at 20°C only in FL. After 1 week of cold storage, hexanol and 3-hexen-1-ol resulted significantly reduced only in FL after both 0.5 and 5.5°C treatments. In both cultivars, 2-hexen-1-ol significantly increased in 0.5°C stored samples after 2 weeks when compared with control fruit (Fig. 2). While for hexanol similar levels have been detected in both cultivars, 2-hexen-1-ol and 3-hexen-1-ol were generally higher in RH and FL peaches, respectively.

Our result only in part confirm those reported by Zhang *et al*. (2011), who showed an increase of 2-hexenol and 3-hexenol, in peaches during shelf life following 2 weeks of cold storage at 0°C.

In the LOX pathway, the addition of an acyl moiety to alcohol, reaction catalysed by acyl transferase (AAT), results in the production of esters (Lara *et al*., 2003; Defilippi *et al*., 2005). This enzyme drives the production of these important compounds that impart fruity notes (Schwab *et al*., 2008). In addition to C6, also C5 compounds, including 2-pentenal, can be synthetized from an additional branch of the LOX

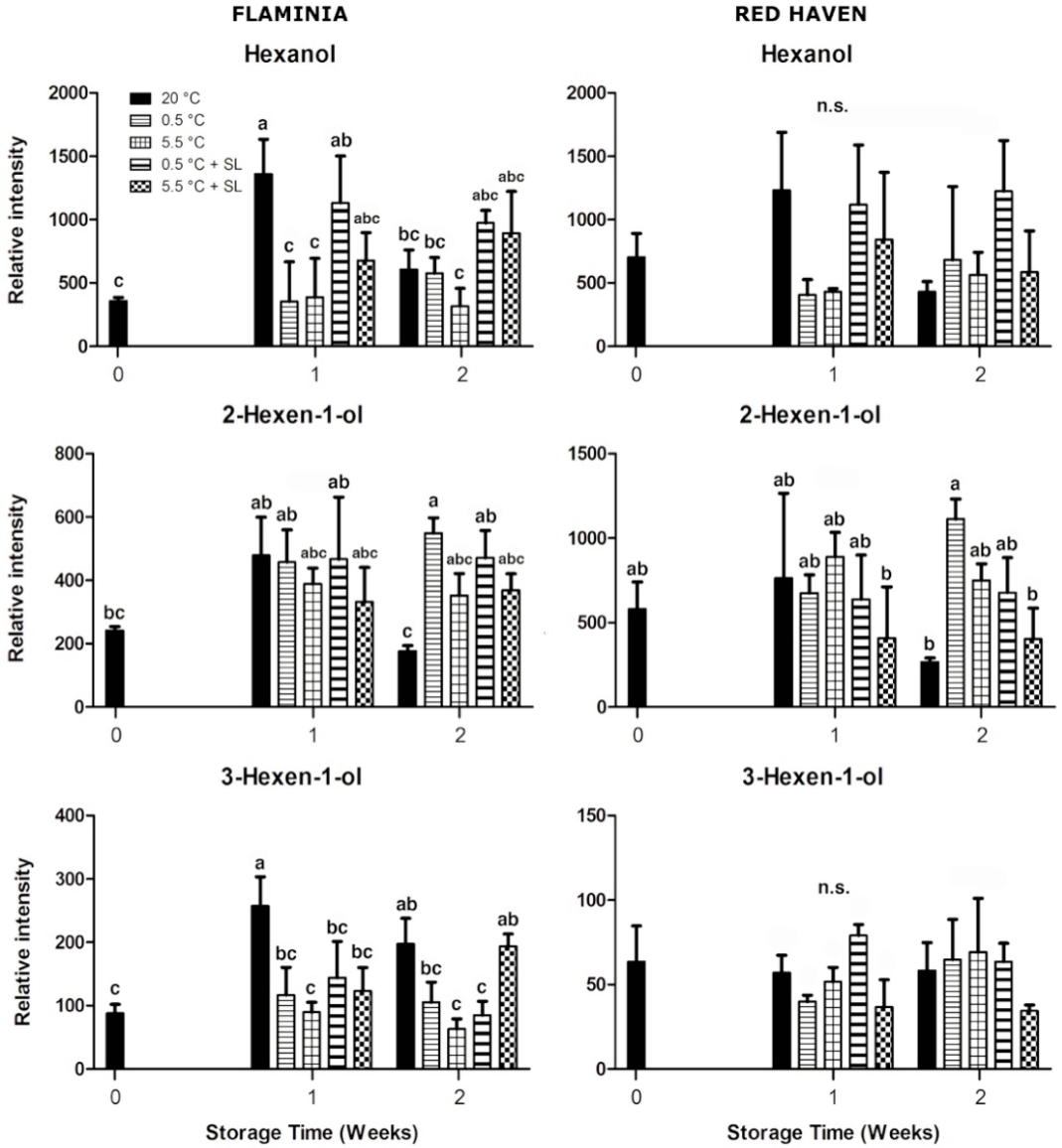

Fig. 2 - Analysis of targeted LOX pathway-derived alcohols. Mesocarp samples (cv. Flaminia, left panel; cv. Red Haven, right panel) were analysed at harvest (0), at the end of 1 and 2 weeks of cold storage at 0.5 and 5.5°C, and after three additional days of shelf life (0.5 and 5.5°C + SL). Samples kept at 20°C represent the room temperature control. The average value of three biological replicates is reported with bars representing SD. Letters indicate the results of Tukey's Honest Significant Difference (HSD) post hoc test, performed independently for each cultivar.

pathway (Gardner *et al.*, 1996; Nielsen *et al*., 2004; Veronico *et al*., 2006; Shen *et al*., 2014 a) and specific pentyl esters, derivatives of C5 compounds, have been considered. The targeted analysis that have been performed includes hexyl, 2-hexenyl, 3-hexenyl, 2-pentenyl and 4-pentenyl acetate compounds (Fig. 3). In general, FL fruit showed no significant difference among samples after 1 week of storage regarding hexyl, 2-hexenyl and 3-hexenyl acetate. The most significant change has been observed in RH cv concerning the levels of 2-hexenyl acetate that showed (as in FL samples) a decreasing trend during postharvest ripening at room temperature and increased at the end of 1 and 2 weeks of cold storage under both low temperature conditions. A similar increasing trend of 2-hexenyl acetate was also observed in cold stored FL samples, even though the differences are not statistically significant for both temperatures and

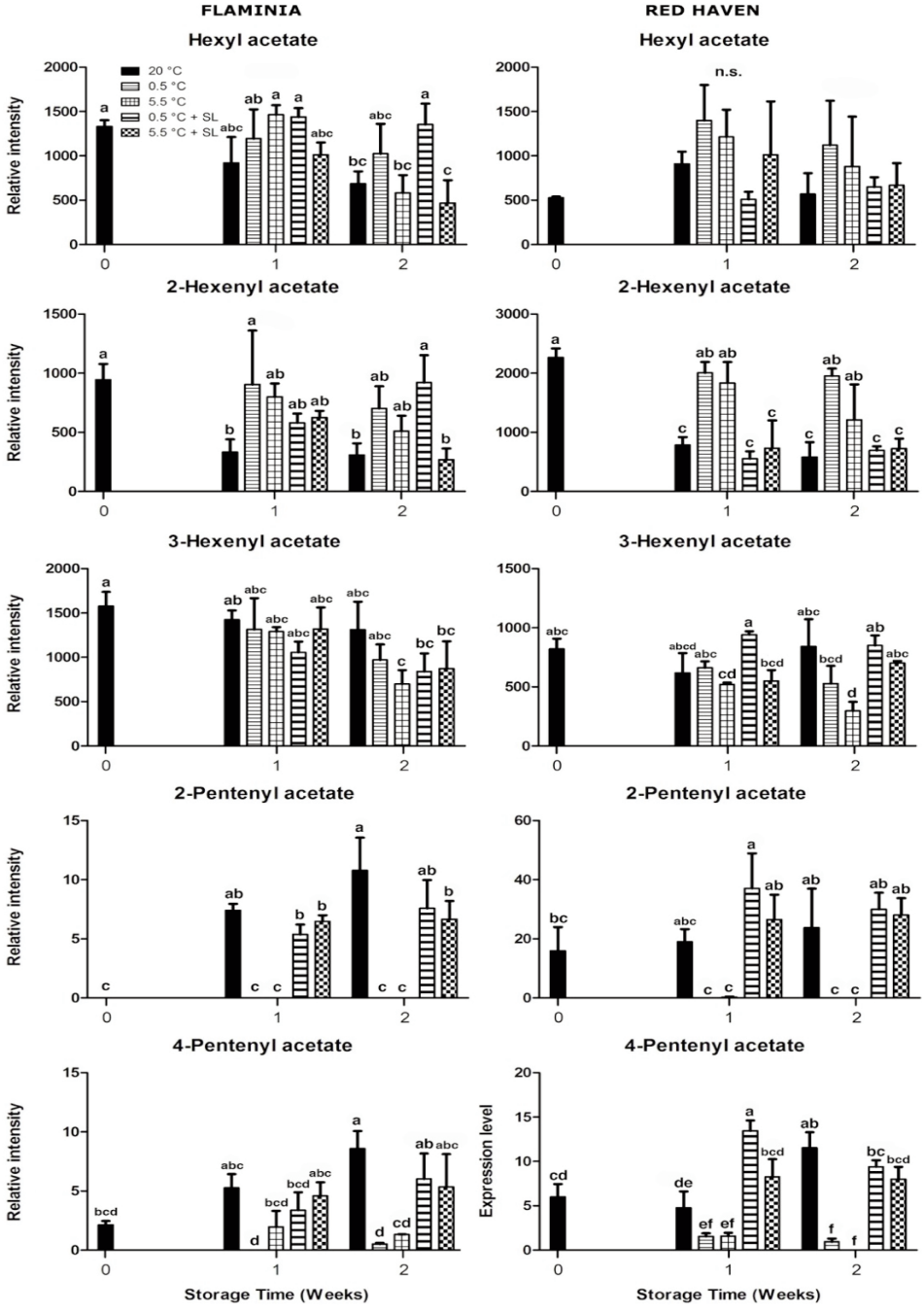

Fig. 3 - Analysis of targeted LOX pathway-derived esters. Mesocarp samples (cv. Flaminia, left panel; cv. Red Haven, right panel) were analysed at harvest (0), at the end of 1 and 2 weeks of cold storage at 0.5 and 5.5°C, and after three additional days of shelf life (0.5 and 5.5°C + SL). Samples kept at 20°C represent the room temperature control. The average value of three biological replicates is reported with bars representing SD. Letters indicate the results of Tukey's Honest Significant Difference (HSD) post hoc test, performed independently for each cultivar.

sampling times.

Interestingly, the two targeted pentyl esters (2-pentenyl and 4-pentenyl acetate), which show an increasing trend during postharvest ripening at 20°C, were markedly reduced by cold storage. 2-pentenyl acetate, in particular, showed almost undetectable levels in all samples at the end of cold storage at both temperatures. A general recover of both pentyl esters levels during SL was observed, with values reaching those of the controls (Fig. 3). Previous works demonstrate that chilling injured peaches show a lack of esters production and it has been argued that this could be the result of an inhibition of AAT1 activity (Ortiz *et al*., 2009). Also in other fruits, CI appearance has been linked to ester biosynthesis: this is the case of papaya in which CI symptoms appearance is accompanied by lower activities of LOX and AAT (Galli *et al*., 2008). Our data do not clearly confirm this behaviour although a general trend of lower hexyl and 2-hexenyl acetate in 5.5°C FL samples stored for 2 weeks was observed.

Expression analysis of LOX pathway genes

The expression of genes involved in the LOX pathway (*PpLOX1*, *PpLOX4*, *PpHPL1*, *PpADH1* and *PpAAT1*) is reported in figure 4.

Concerning *PpLOX1* and *PpLOX4* gene expression, specific pattern of accumulation of these transcripts has been identified in the two cultivars and different conditions. In FL control peaches (20°C) *PpLOX1* and *PpLOX4* revealed an identical trend showing a significant increase from harvest to the first week followed by a decrease during the second week. On the other hand, in RH control fruit the expression levels of these two genes showed a stable (*PpLOX4)* or decreasing (*PpLOX1)* trend in 2 weeks after harvest. *PpLOX1* appeared to be inhibited at the end of both low temperature storage conditions in FL, and a significant increase of its expression level has been recorded during SL in all samples. This increase during SL has been observed also in RH fruit. *PpLOX4* expression was significantly reduced and increased in FL and RH peaches, respectively, after 1 week of cold storage at both temperatures. An up-regulation of this gene was detected in both cultivars in 5.5°C + SL samples after 1 week of storage.

Considering *PpHPL1*, a decreasing trend of expression was detected in FL control fruit only. Genotype-related difference has been also observed when comparing the expression trends of cold stored peaches with significant increases in both SL samples of RH fruit after 1 week of storage.

PpADH1 and *PpAAT1* genes revealed a similar expression trend, almost mirroring the pattern detected for *PpLOX1* (Fig. 4). A peak of expression after 1 week has been detected in FL control samples for both genes, while in RH control peaches *PpADH1* expression showed a decreasing trend and *PpAAT1* expression remained unchanged. Besides the reduced expression of *PpADH1* detected in cold treated FL samples after 1 week, limited or non-significant changes of *PpADH1* expression levels were detected in cold stored and SL samples of RH (1 and 2 weeks) and FL (2 weeks). Interestingly, in both cultivars *PpAAT* gene expression showed a significant up-regulation in all SL fruit of either temperatures, if compared to samples collected at the end of cold storage. This behaviour is similar to that observed for *PpLOX1*. Thus, the expression of these two genes seems highly sensitive to temperature conditioning with a general decrease observed during cold storage and an expression recovery during SL at room temperature.

In general, our expression data confirm what reported in literature on peach fruit ripening in particular concerning *PpHPL1*, that shows a decreasing trend, paralleled by reduced amount of C6 aldehydes (Zhang *et al*., 2010; Shen *et al*., 2014 b). A decreasing *HPL* expression level during ripening has been observed also in other fruit species, such as tomato (Howe *et al*., 2000). The expression of *LOX1* and *4*, *ADH1* and *AAT1* during ripening appears to be different in relation to the genotype. In fact, the transient up-regulation of these genes observed in FL control fruit after 1 week of storage (followed by a down-regulation) was not observed in RH fruit.

Considering the effect of cold stress on *LOX* genes, an induction on *PpLOX1* and *PpLOX3* expression in peach fruit during shelf life after storage at 0.5 and 5.5°C, flanked by higher ethylene synthesis, has been reported by Zhang *et al*. (2011). In our trials, the effect of "rewarming" (shelf life at room temperature after cold storage) is clearly evident when considering the expression trend of *PpLOX1* (thus confirming the data by Zhang *et al*., 2011) but not of *PpLOX4*, suggesting that members of this multigene family have different regulatory mechanisms and may be selectively involved in the responses of peaches to postharvest temperature conditioning.

As observed for *PpLOX1, PpAAT* seems highly sensitive to temperature conditioning showing a marked expression recovery during post-cold storage SL. Similarly, Ortiz *et al*. (2009) reported an enhancement of *PpAAT* gene expression after cold storage,

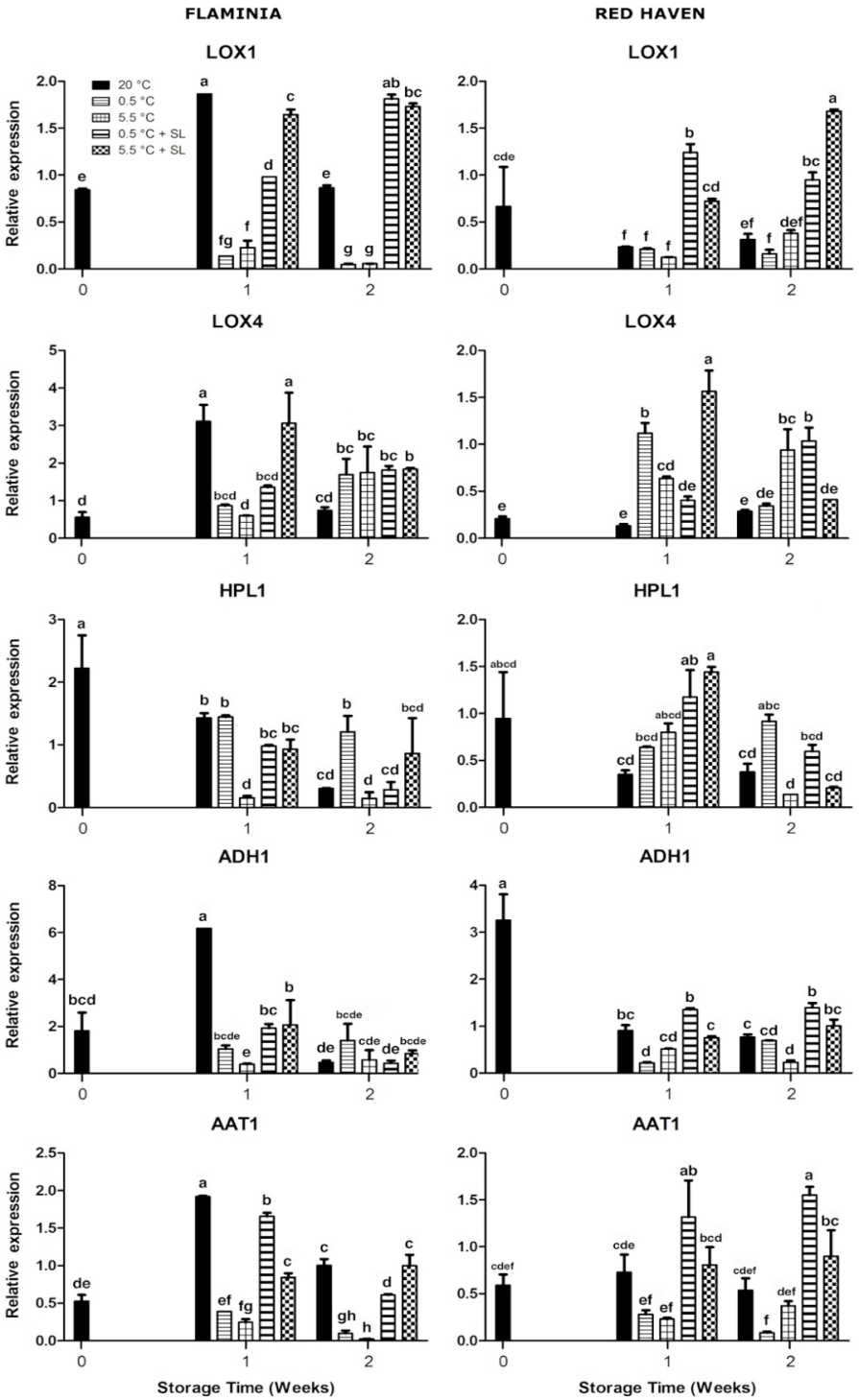

Fig. 4 - RT-qPCR analysis of selected LOX pathway-related genes. Mesocarp samples (cv. Flaminia, left panel; cv. Red Haven, right panel) were analysed at harvest (0), at the end of 1 and 2 weeks of cold storage at 0.5 and 5.5°C, and after three additional days of shelf life (0.5 and 5.5°C + SL). Samples kept at 20°C represent the room temperature control. The average value of three biological replicates is reported with bars representing SD. Letters indicate the results of Tukey's Honest Significant Difference (HSD) post hoc test, performed independently for each cultivar.

indicating a renewed ability of synthetizing esters after cold stress. Our results (Figs. 3, 4) confirm this observation.

Considering the possible relationships between CI and LOX pathway, Zhang *et al*. (2011) reported that in peach fruit LOX pathway genes showed lower transcript levels in fruit stored at a CI-inducing temperatures (e.g. 5°C) compared to fruit kept at 0°C, with transcript abundance that tends to decrease with extended storage periods and the onset of CI symp-

toms. Our gene expression data do not show any clear relationship with CI incidence, while, considering VOCs, only for hexyl acetate and 2-hexenyl acetate significantly lower concentrations have been detected in samples with CI symptoms.

Expression analysis of CBF genes

The expression pattern of two *C-repeat-binding factors* genes (*PpCBF1* and *PpCBF6*) has been analysed in FL and RH samples in relation to cold storage treatments (Fig. 5).

As a general trend the two genes appeared to be significantly up-regulated at the end of 1 and 2 week storage either at 0.5 and 5.5°C in both cultivars. In FL samples after 1 week and in RH fruit after 2 weeks, it appears that the lower the storage temperature the higher is the expression of these two genes. During 20°C SL after cold storage, either at 0.5 and 5.5°C, the expression of these genes markedly decreases reaching the level of the control, confirming that *CBF* gene expression is highly responsive to temperature conditions. It is interesting to note that RH peaches showed higher levels of expression, in particular considering *PpCBF1*.

Similar results in terms of expression pattern during refrigeration of *PpCBF* genes have been previously reported in peach fruit, confirming that these regulatory genes are generally sensitive to low temperatures (Liang *et al*., 2013). Specifically, *PpCBF1/6* appeared to be induced exclusively under cold storage, revealing very low or non-detectable levels during post-storage shelf life, and their level of expression was significantly higher under CI-delaying temperature (0°C), when compared to chilling inducing conditions (5°C). Indeed, this paper reports that the up-regulation of *CBF* genes correlated with a decreasing level of CI symptoms, such as flesh browning, as well as with a reduction of firmness loss. Our data clearly show that *CBF* expression is higher at 0.5°C than at 5.5°C in FL after 1 week and in RH after 2 weeks of storage, highlighting the different behaviour and regulatory mechanisms characterizing peach genotypes. It has been reported that *CBF* genes in peach fruit are activated over different time periods, with *PpCBF5* being activated at high levels in several hours, *PpCBF6* later within three days and *PpCBF1* requiring 1 week to be expressed at high levels (Liang *et al*., 2013). This temporal distribution of *CBF* genes activation during cold storage suggests that each gene plays a specific role, and it has been argued that while *PpCBF5* seemed to be associated with an early response to low temperatures, *PpCBF1/6* appeared to be more involved in protection to prolonged cold storage. However, the possible relationship between the expression trends and levels of these *CBF* genes and the sensitivity to postharvest cold stress of peach fruit remains to be elucidated. This is an important topic for future research in elucidating regulatory mechanisms involved in low temperature responses considering that the over-expression of peach *PpCBF1* allowed Wisniewski *et al.* (2011) to get to an increased freezing tolerance of apple trees of about 4-6°C. Moreover, the overexpression of several *CBF* genes in different plant species, including *Arabidopsis*, grape, potato and apple, appears to increase plant tolerance to cold stress (Novillo *et al*., 2007; Pino *et al*., 2008; Siddiqua and Nassuth, 2011;

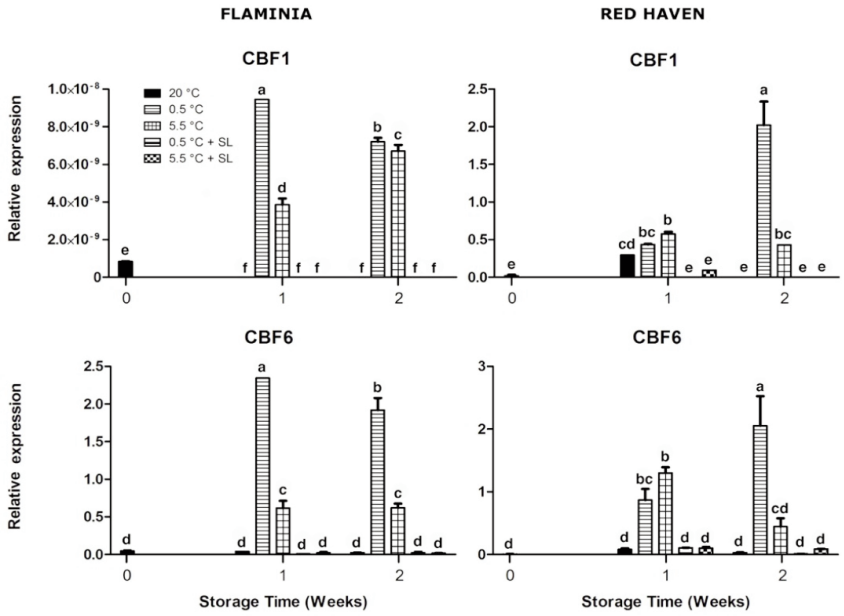

Fig. 5 - RT-qPCR analysis of two C-repeat-binding factors (CBF) genes. Mesocarp samples (cv. Flaminia, left panel; cv. Red Haven, right panel) were analysed at harvest (0), at the end of 1 and 2 weeks of cold storage at 0.5 and 5.5°C, and after three additional days of shelf life (0.5 and 5.5°C + SL). Samples kept at 20°C represent the room temperature control. The average value of three biological replicates is reported with bars representing SD. Letters indicate the results of Tukey's Honest Significant Difference (HSD) post hoc test, performed independently for each cultivar.

Takuhara *et al*., 2011).

4. Conclusions

Peach genotypes show marked difference in terms of responses to cold storage, with some cvs less susceptible to CI and others developing an array of symptoms during shelf life after a few days/weeks of refrigeration. This different behavior observed here in RH and FL peaches, as already reported in a previously published paper (Brizzolara *et al*., 2018), appears to be associated with different expression pattern of two *CBF* genes (*PpCBF1* and *PpCBF6*), recognized to be involved in the responses of peaches to cold storage.

If the general picture of the regulatory role played by *CBF* genes has been described in plants, the possible mechanisms involving CBF transcription factors in modulating molecular and physiological responses of fruit tissues to postharvest cold stress remain to be elucidated. Peaches represent an interesting model for such studies, based on the fact that the expression of the two *CBF* genes analyzed in the present paper appears to be differently affected by temperatures more (5.5°C) or less (0.5°C) inducing the appearance of CI symptoms.

In addition to the expected effect of genotype, specific genes of the LOX pathway also show different expression pattern depending on the storage temperature and the shelf-life, with some of them (e.g. *LOX1, ADH and AAT1*) representing good candidates for additional studies aimed at identifying metabolic and molecular markers of CI onset and incidence in cold stored peaches.

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Physio-chemical quality attributes of 'Italia' grapes from organic and conventional farming at harvest and during storage

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Key words: antioxidant activity, nutritional quality, postharvest, respiration rate, table grapes, *Vitis vinifera* L.

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Abstract: This study was aimed to investigate the quality at harvest and during storage of organically and conventionally grown 'Italia' grapes, collected from 2 different locations in Southern Italy. Four vineyards were chosen in order to have an organic and a conventional farm in each location. Before harvest, six plants per vineyard were randomly selected and considered as treatment replicate. Three bunches were harvested and labelled from each plant. In laboratory each bunch was weighed and thirty berries per bunch were detached and used for initial determination which included morphological (berry weight and dimension, peel thickness) and physical (berry color and firmness) attributes, maturity indices (respiration rate, soluble solids content and titratable acidity), and nutritional composition (phenol content, antioxidant activity, sugar and organic acid composition, ascorbic acid content). Then, the bunches from each replicate were kept in individual 15-L jars at 0°C and connected to a humidified air flow throughout the whole experiment. After 7 and 14 days of storage, respiration rate, weight loss, physical and nutritional attributes were also monitored on 20 berries per bunch. Location and agricultural practices affected to a different extent several grapes quality attributes, both at harvest and during storage. Maturity stage, sugar content and berry color were significantly affected by the location, while antioxidant-related compounds were significantly higher in organic grapes. Plant production and bunch weight were significantly higher for conventionally grown grapes, which also received the highest evaluation of external appearance, in terms of stalk dehydration and berry general aspect. Differences among conventional and organic grapes were maintained, for each location, during storage at 0°C. Conventional grapes maintained a higher visual quality during storage, resulting after 14 days below the limit of marketability (score 3) but above the edibility limit (score 2); whereas in one location organic grapes were judged not edible. Results showed a higher nutritional value in grapes obtained with the organic farming system although in terms of visual quality, storability and yield, conventional fruit had a better performance.

1. Introduction

More than 403'000 hectares of organic grapes are grown worldiwide,

constituting 5.7 percent of the world's grape-growing area (7.1 million hectares in 2016, according to FAO-STAT). In Europe, over 340'000 hectares (8.7 percent of the harvested grape area) are organic; Spain and Italy, cultivated more than 100'000 hectares of organic grapes, followed by France with over 78'000 hectares. The Italian National Information System on Organic Agriculture (SINAB) reported for the 2012 an increase of 6.4% of the organically cultivated land compared to the previous year, with a total of 49,709 organic operator. Regions in Southern Italy have the largest organic areas (Sicilia, Puglia and Calabria) and the largest number of organic farms with an increase of operators (20.3%) in Puglia Region, while most of the processors are located in northern Italy (especially Emilia Romagna and Lombardia). As known, grapes contain a wide range of nutritional and functional component such as vitamins, minerals, organic acids, enzymes as well as phytochemicals (Walzem, 2008), among which phenolics, particularly flavonoids, anthocyanins and resveratrol, are the most important because are held accountable for their health benefits (Yang *et al*., 2009). However, as known, both pre- and postharvest practices may affect the amount of these nutritional and functional compounds as well as many other elements of horticultural crops (Lee and Kader, 2000). Among the preharvest conditions, genotype, environmental conditions, cultural practices, and maturity at harvest influence quality attributes of grapes such as the concentration of phenolic compounds (Sellappan *et al.*, 2002). Particularly the application of organic and conventional agricultural techniques may affect the table grapes quality. Generally organic agriculture optimizes the health and productivity of interdependent communities of soil life, plants animals and humans. In fact, organic agriculture does not use synthetic pesticides and fertilizers (Briar *et al.,* 2007), but only ecological products. Several studies showed that the use of organic or conventional techniques significantly influenced the production, in terms of number of bunches on the vine stock and the average weight of the bunch (Detoni *et al.,* 2007). However, in the last years researchers focused their attention on the influence of organic cultivation on the content of secondary metabolites although no clear behaviors were observed. Higher concentration of bioactive compounds in plants grown with the organic system were reported by several authors, which were considered as the results of the plant exposure to situation that leads to an increase of natural defenses (Winter and Davis, 2006). Dani *et al.* (2007) reported that organic crop influenced the phenolic content and the antioxidant activity of white and purple grape juices, but for some study difference observed 1 month before harvest, were not observed at the harvest time (Mulero *et al*., 2010). Regarding differences observed during storage, *Thompson seedless* grapes from organic orchard showed more desirable color and lower browning index, and generally higher nutritional content than conventional grapes, with similar decay incidence (Zahedipoura *et al.,* 2019), but there is not much literature to this regard. Based on the above considerations, this paper had the principal aim of comparing the physio-chemical attributes at harvest and during storage of table grapes cultivated with organic and conventional techniques also taking into account the effect of two different production areas in Puglia Region (southern Italy).

2. Materials and Methods

In this experiment two organic farms were chosen in 2 different locations of the Puglia Region, one at Castellaneta (78 m above sea level) in province of Taranto (LOC1) and one in Adelfia (151 m on sea level) in province of Bari (LOC2). The climate for both locations is Mediterranean semi-arid, characterized by hot and dry summers and moderately cold and rainy winter seasons, with annual mean temperature of 14-15°C and mean annual rainfall within 450-500 mm. For each location a conventional farm, with similar characteristics, and in the same area (within 1 km) was chosen as control, resulting in a comparison among 2 different organic with 2 conventional farms. In each location, 6 plants were used as replicate, and 3 bunches for each replicate were collected in the same day, at the commercial maturity stage, with a total of 18 bunches per field. Soils were composed by 63.2% sand; 22.1% clay; and 14.7% silt for Bari location and 69.8% sand; 15.1% silt and 15.1% clay for Taranto. The conventional vineyard was managed according to common viticultural practices for the growing area, including winter mineral nutrition, spring-summer fertigation, and irrigation with seasonal volume of about 2000 m3/ha by drip irrigation. The organic farming was managed according (EC) Reg. 834/07 and Reg. 889/08.

At harvest bunches were weighed, closed in a sealed container to measure respiration rate, before to detach 30 berries per bunch. On these 30 berries per bunch biometrical attributes were evaluated, including berry weight and dimensions (major and

minor axes), and peel thickness. Following, bunches from each replicate were kept in individual 15-L jars at 0°C and connected to a humidified flow of air for the entire duration of the experiment. After 7 and 14 days of storage, respiration rate, weight loss, physical and nutritional attributes were also monitored on 20 berries per bunch. After berry detachment stalks were protected by excessive dehydration by using adhesive tape around the berry abscission zone.

Quality indexes

Initially and after 6 and 14 days, bunches were individually scored using a 5 to 1 subjective scale, with 5 = excellent, no defects, 4 = very good, minor defects, 3 = fair, moderate defects, 2 = poor, major defects, 1 = inedible. A score of 3 was considered as the limit of marketability and a score of 2 as the limit of edibility.

Then 20 berries for each bunch were used the following quality assessment:

- peel color using a spectrophotometer (Konica Minolta CM 2600d, Japan) in the CIE L*a*b* mode, and then calculating Hue Angle and Chroma values;
- flesh firmness with a manual firmness tester by measuring force required by a 2-mm probe to penetrate the tissue for 5 mm in two opposite locations;
- titratable acidity on 4g of grape juice for each replicate, using an automatic titrator (Crison, Titromatic 1S, Barcelona, Spain) with 0.1 N NaOH solution to pH 8.1 and reported as percent of tartaric acid;
- soluble solids content (SSC) using a refractometer (Atago, PR-32; Tokyo, Japan);

For respiration rate 3 bunches of each plant were closed in a sealed container to let CO_2 accumulate. Samples of gas (0.1 mL) were collected through a rubber septum and injected into a gas chromatograph (Shimadzu, model 17A, Kyoto, Japan) equipped with a thermal conductivity detector (230°C). Separation of CO_2 was achieved on a Carboxen 1006 plot (30 m X 0.53 mm, Supelco, Bellefonte, PA USA), with a column flow of 7 mL min^{-1}, and oven temperature of 180 °C; the difference in concentration was then referred to the sample weight, to the elapsed time, and to the head space volume. The sample weight at each storage time was also used to calculate weight loss.

Nutritional quality

After previous determinations, part of the berries were peeled, and the skin was frozen for the analysis of phenols and antioxidant activity whereas the juice was used for sugars composition and Vitamin C.

Ascorbic and dehydroascorbic acid were analysed on fresh samples by high performance liquid chromatography (HPLC Agilent 1200 Series, Waldbronn, Germany) equipped with a binary pump, an autosampler, and a photodiode array detector (DAD) (Zapata and Dufour, 1992).

The following determinations were performed on the frozen samples:

- organic acid and sugar composition by using the HPLC equipped with the (DAD) array and a refractometric detector (Pérez *et al.,* 1997).
- total phenolics of grape skins by the Folin-Ciocalteu reagent (Singleton and Rossi, 1965), dilutions were carried out in duplicate and calculated using a calibration curve obtained with gallic acid, reading the absorbance at 575 nm.
- antioxidant activity by spectrophotometer using the DPPH (2,2 diphenyl1-1-picrylhydrazil) method (Brand-Williams *et al*., 1995).
- Phenolic composition using the same extract for total phenolics analysed with HPLC procedure (Bonilla *et al.,* 1999).

Statistical analysis

On the data collected after harvest, a split plot design for the location and treatment was run, whereas on the whole data set a split plot with location as main plot, treatment as subplot, and time as third plot was assessed. Finally, due to the presence of significant 3th order interaction, a split plot for each location (for treatment and time of storage) was run.

3. Results and Discussion

The effect of location (LOC1 and LOC2), and cultivation system (organic and conventional) on quality attributes is shown in Tables 1 (physical and physiological attributes including productivity) and 2 (chemical composition) at harvest. Both area of cultivation and cultivation system showed a significant effect ($p<0.05$) on most of physical attributes (Table 1), with significant interaction only in the case of firmness, and chroma, but generally the effect of the cultivation system was the same in both locations. Particularly conventionally grown grapes showed higher visual quality, firmness, and production per plant, which in turn induced lower dimensions of the berries. At the same time LOC2 (Bari) also induced

higher productivity, higher visual quality and firmness compared to LOC1 (Taranto). Respiration rate was higher in organic grown grapes than in conventional grapes and in LOC2 compared to LOC1, indicating a higher metabolic activity for these fruit.

As shown in Table 2, total phenolics, flavonols and antioxidant activity were higher in organic grapes and in LOC1, compared to respectively conventional grapes and LOC2. For soluble solids content and titratable acidity there was a significant interaction, but looking to sugar and acid composition (data not shown), no significant differences due to the cultivation system were found for total organic acids, with significant highest amount of tartaric acid and glucose, among sugars, in organically grown grapes and in LOC1.

Differences among conventional and organic grapes were maintained, for each location, during storage at 0°C. Conventional grapes maintained a higher visual quality during storage, resulting below the limit of marketability (score 3) but above the edibility limit (score 2) at 14 days, whereas in LOC1 (Taranto) at that time organic grapes were judged not edible (data not shown). These results may be explained with the higher metabolic activity of organic table grapes, which reduce their storability. Firmness was higher in conventional grapes than in organic grapes for samples grown in Taranto area while no differences were observed for grapes grown in Bari. Also in another study the authors reported higher firmness for conventionally grown grapes than for organic, being related to the higher thickness of

Table 1 - Productivity, morphological indexes, physical and physiological quality attributes at harvest of organic and conventional grapes grown in the locations of Taranto (LOC1) and Bari (LOC2) in the Apulia region (Italy)

	Treatment		Location		Location x treatment
	Organic	Conventional	LOC1	LOC2	
Production per plant (kg)	23.5 b	31 a	25 b	29 a	NS
Visual Score	4.1 b	4.5 a	3.9 b	4.7 a	NS
Hue Angle	102.3 NS	103.7 NS	102.4 NS	103.7 NS	* NS
Chroma	7.5 NS	7.4 NS	8.1 a	6.9 b	*
Respiratory activity	3.8 a	2.6 b	1.1 b	5.3 a	NS
Morphological indexes					
Berry lenght (mm)	29.6 a	27.4 b	27.8 b	29.3 a	NS
Berry width (mm)	24 a	22.4 b	27.9 b	23.7 a	*
Berry weight (g)	10.9 a	9.5 b	27.10 b	10.8 a	NS
Peel thickness	0.99 NS	1.04 NS	27.11 NS	1.13 NS	NS

Different letters indicate significant differences among mean values according to Tukey's test (P value≤0.05).
NS= not significant.

Table 2 - Chemical composition at harvest of organic and conventional grapes grown in the locations of Taranto (LOC1) and Bari (LOC2) in the Puglia region (Italy)

	Treatment		Location		Location x treatment
	Organic	Conventional	LOC1	LOC2	
Titratable acidity (TA)	0.4 b	0.5 a	0.5 a	0.4 b	*
Soluble solids content (SSC)	1.70 NS	16.7 NS	16.9 NS	16.8 NS	*
SSC/TA ratio	41.4 a	37.6 b	38 NS	41 NS	*
Flavan-3-oli	47.4 NS	48.5 NS	42.5 b	53.5 a	*
Hydrocinammic deriv.	4.3 NS	4.6 NS	4.9 a	3.9 b	NS
Flavonols	0.015 a	0.005 b	0.014 a	0.006 b	NS
Total phenols	387.6 a	307.3 b	437.7 a	257.2 b	NS
Antioxidant activity	940 a	641 b	987 a	594 b	NS
Ascorbic acid	0.4 NS	0.4 NS	0.2 b	0.6 a	NS
Dehydroascorbic acid	2.8 NS	2.8 NS	2.8 NS	2.8 NS	NS

Different letters indicate significant differences among mean values according to Tukey's test (P value≤0.05).
NS= not significant.

the epicuticular layer (Zahedipoura *et al.,* 2019). Similar results were also observed for organic kiwifruits (Amodio *et al.*, 2007), where firmness was not related to the thickness of the skin, being conventional fruit firmer despite the thin skin and the more advanced maturity stage. In the present study, the slight differences in skin thickness resulted not significant, and difference observed for one location could be due to berry dimensions and water turgor. Moreover, firmness of fruit can be affected by several agricultural practices such as sunlight exposure and (Sams, 1999) fertilization. Mineral content of soil and plant was not assessed in this experiment, but Amodio *et al*. (2007) shown a possible effect of different mineral composition of organic and conventional fruit.

Figures 1 and 2 show the changes of phenolic content and antioxidant activity of grape samples as a function of storage time for both conventional and organic production obtained in Taranto and Bari.

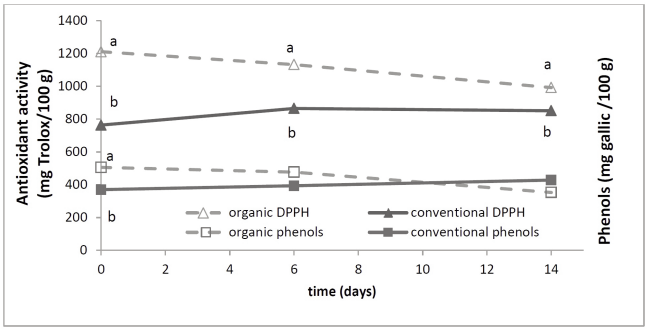

Fig. 1 - Evolution of the antioxidant activity and phenol content during storage of organic and conventional table grapes grown in LOC1 (Taranto). Different letters indicate significant differences among mean values according to Tukey's test (*P* value ≤0.05).

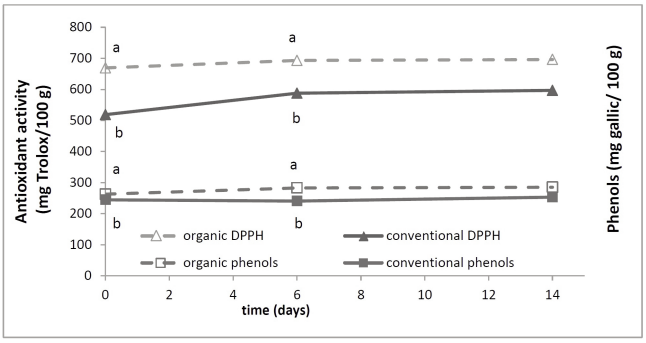

Fig. 2 - Evolution of the antioxidant activity and phenol content during storage in organic and conventional table grape grown in LOC2 (Bari). Different letters indicate significant differences among mean values according to Tukey's test (*P* value ≤0.05).

Phenolic content of organic table grapes was, in general, higher than that of conventional samples probably because the conventional growing practices utilize levels of pesticides that can result in a disruption of phenolic metabolites in the plant that have a protective role in plant defense mechanisms (Macheix *et al.,* 1990). Particularly, in the moment of harvest a phenolic content of 505.1±52.4 mg gallic acid 100 g^{-1} for organic sample and 369.8±57.8 mg gallic acid 100 g^{-1} for conventional table grapes grown in Taranto were observed ($p<0.05$) (Fig. 1); nevertheless, during storage this difference was progressively reduced becoming not significant after 6 days of storage. At harvest significantly higher values for antioxidant activity were observed for organic grapes (1210.7±134.3 mg Trolox 100 g^{-1} fw) than in conventional ones (763.4±97.6 mg Trolox 100 g^{-1} fw) (Fig. 1) as well as after 6 and 14 days of storage at which organic samples showed an antioxidant activity of 992.5 mg Trolox 100 g^{-1} fw and conventional samples of 850.9 mg Trolox 100 g^{-1} fw.

For samples grown in Bari area significant differences were maintained between the phenolic content and antioxidant activity of organic and conventional up to 6 days of storage (Fig. 2) although, in this case antioxidant activity was much lower than in LOC1.

Phenolic content of 'Italia' grapes was higher compared with those reported in literature studying the antioxidant and phenolic composition of different grape cultivars grown with conventional system ranged between 148.5 mg gallic acid 100 g^{-1} for 'Chasselas Dorè' and 123.1 mg gallic acid 100 g^{-1} for 'Nepoca' grapes (Mulero *et al*., 2010). Similar differences were observed in some diffferent harvests (Macheix *et al.,* 1990). On the other hand, other authors pointed out that the concentration of phenolic compounds of the skin changes greatly depending on the variety and also on the grape ripening stage (Riu-Aumatell *et al.*, 2002). Also in the case of antioxidant activity values were higher in comparison with the results of reported on literature. Values in the range of 55.7-274.2 µg g^{-1} extract in grape skins on four varieties are reported (Anastasiadi *et al.,* 2010), while the antioxidant activity was higher for organic grapes (5.70 mM Trolox g^{-1}) compared with conventional grapes (4.40 mM Trolox g^{-1}) (Mulero *et al*., 2010). As reported from several authors, high variability of phenols content in grapes may be caused by many factors including genotype, ripening stage, environmental and growing condition. Among these organic farming have shown in different studies on

grapes (Zahedipour *et al.*, 2019) and other fruit as kiwifruits (Amodio *et al*., 2007), and raspberry (Ponder and Hallmann, 2019) to positively affect antioxidant compounds, and particularly phenolics, representing a defense mechanism released from plant cells in response to biotic and abiotic environmental stress (Macheix *et al*., 1990; Zhang *et al*., 2011). In organic farming, synthetic pesticides are banned, therefore, plants need to create their own defence mechanisms against pests and diseases (Young *et al*., 2005). An increase of vitamin C and antioxidant enzymes activity was also observed in passion fruit (De Oliveira *et al*., 2017). In terms of vitamin C content at harvest and during storage organic and conventional table grapes presented almost the same values without any significant differences. Particularly, when the organic system was applied the vitamin C values were in the range of 1.46-3.12 mg 100 g^{-1} in Taranto and 2.18-3.36 mg 100 g^{-1} in Bari while for conventional samples vitamin C values were between 1.01-2.94 mg 100 g^{-1} in Taranto and 1.90-3.22 mg 100 g^{-1} in Bari (data not shown). Many factors are responsible for the wide variation in vitamin C content of fruits and vegetables at harvest. Maturity at harvest, harvesting method, and postharvest handling conditions also affect the vitamin C content of fruits and vegetable (Lee and Kader, 2000).

4. Conclusions

Results showed a higher phenolic content and antioxidant activity for grapes obtained with the organic farming system although in terms of visual quality, and yield performance conventional fruit had a better performance, resulting in a higher storability. These results confirmed previous finding on different antioxidant properties of organic fruit, but pointed out to the necessity of increasing their shelf-life in order to allow a better distribution, also considering international request of organic grapes.

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