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

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).

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 (cm³) 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 crosshead 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

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).

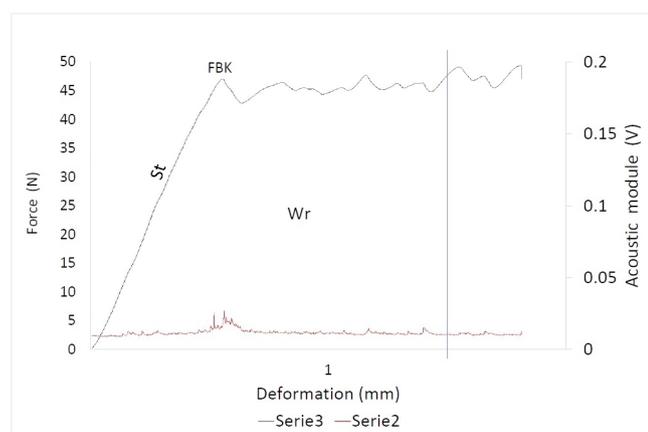Fig. 2 - Force/deformation curve and acoustic profile of 'Pomella Genovese' apples. FBK= Force at the breaking point; Wr = Work; St= stiffness.

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·) 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:

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

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

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.

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 possi-
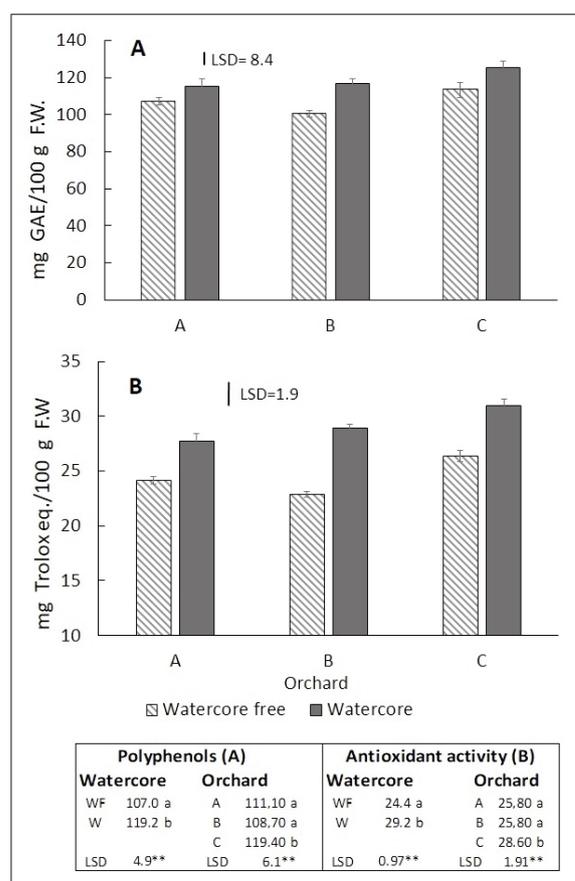

Fig. 4 - Total phenols content (A) and antioxidant activity (B) of watercore-free (WF) and watercore (W) fruit of 'Pomella Genovese'from three different orchards. Average ± standard error. LSD is reported in each figure (P>0.01). In the box at the bottom the averages and LSD values are indicated for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

ble 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.

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-
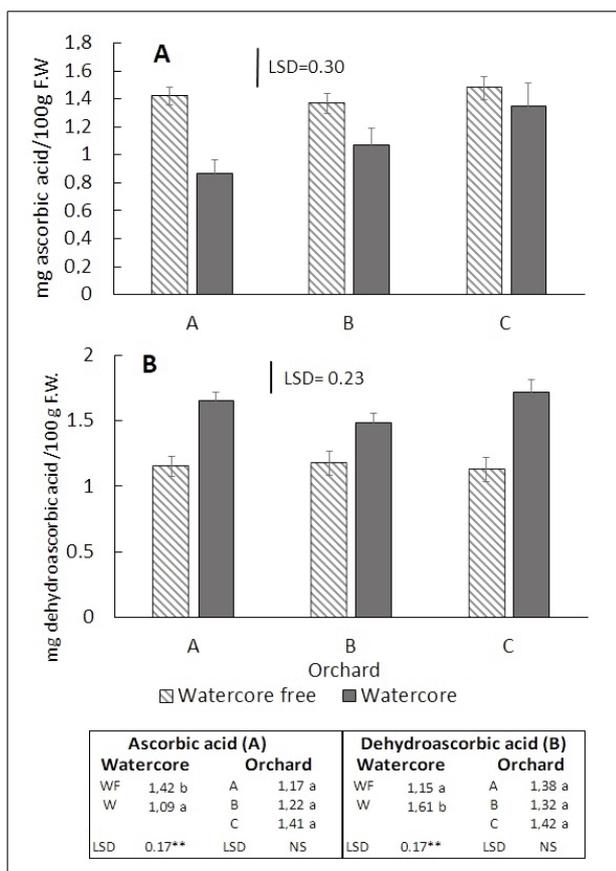

Fig. 5 - Ascorbic (A) and dehydroascorbic (B) acid content of watercore-free (WF) and watercore (W) fruit of 'Pomella Genovese' apple from three different orchards. Average ± standard error. LSD is reported in each figure (P>0.01). In the box at the bottom the average and LSD values are indicated for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

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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