

Quality evaluation of local apple varieties: physicochemical and antioxidant proprieties at harvest and after cold storage

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Abstract. A wide apple germplasm is present in Italy in which numerous local genotypes of specific cultivation environment have to be still evaluated for fruit quality attributes. This is the case of a long-established fruit area located in central Italy (Tuscany) where several ancient apple varieties survive with the perspective to be re-introduced for their excellent quality. The objective of this work was to determine over a 2-year period the physicochemical traits, total antioxidant capacity (TAC), and polyphenols (TP) content of three old local apple varieties: 'Paganina', 'Paradisa' and 'Rosa'. These characteristics were also assessed after 90 and 150 days at 4 °C cold storage, in normal atmosphere refrigerated cellars. For comparison purpose, the commercial apple cultivar 'Fuji' was considered. In general, the old varieties showed valuable quality properties, particularly due to a greater antioxidant power of fruits, although a variability between the two crop seasons, characterized by different weather conditions, was observed. After a dry summer, 'Rosa' showed very high TAC and TP values (2-fold higher than other varieties) that, after long-periods of cold storage, markedly decreased. These losses were not so noticeable in the others, suggesting a tendency to maintain a major stability during storage. These findings could meet the preference of demanding consumers for healthier foods who appreciate fresh fruits with protective properties by free radicals scavenging activity. Moreover, farmers who are focused on nearby and niche markets could have interesting in these local varieties to promote their valorization.

Key words: *Malus domestica* Borkh, antioxidant properties, pomological traits, post-harvest, storability.

INTRODUCTION

In a context characterized by a growing consumer's interest in locally produced foods, the safeguard of ancient fruit varieties appears relevant. These genotypes, characterized by high hardiness and suitable for niche markets, are considered by consumers more genuine and healthier (Darby et al., 2008; Denver & Jensen, 2014). The attractiveness is also due to increasing evidence about their high nutritional value. Recent researches showed that ancient varieties have higher nutraceutical traits than modern ones, particularly apple, one of the most commonly consumed fruits throughout the world (Donno et al., 2012; Jakobek et al., 2013). It has been proved that apple fruits are rich source of antioxidants, mainly phenolic compounds, able to neutralize free radicals and protecting towards degenerative disorders in humans (Vrhovsek et al., 2004; Scafuri

et al., 2016). In Italy, a wide apple germplasm is present but, while for the most popular varieties the antioxidant power is known, for varieties from specific cultivation areas the knowledge is often lacking or limited to few genotypes. This is the case of Lucca province in central Italy, a long-established fruit area in which ancient apple varieties survive with the perspective to be re-introduced for their excellent quality and sensory characteristics (Bartolini et al., 2015a).

Apples are generally stored over long periods using a wide range of technologies to extend the commercial life of fruits and keep them in the market longer (Farooq et al., 2012). For local farmers a regular cellar refrigerated storage is better than the sophisticated and expensive controlled-atmosphere technique. However, low temperatures can adversely affect the apple quality inducing some post-harvest changes on physicochemical attributes and antioxidant compounds content (Jan et al., 2012; Maragò et al., 2015).

Therefore, the main objective of this work was to determine the physicochemical traits, total antioxidant capacity, and polyphenols content in three old local apple varieties 'Paganina', 'Paradisa' and 'Rosa'. In addition, for the first time, the evaluation of cold storage effect on fruit quality characteristics was assessed.

MATERIALS AND METHODS

Plant material and cultivation site. The research was conducted over two consecutive harvesting seasons (2014–2015) on full bearing apple trees of three old local varieties: 'Paganina', 'Paradisa' and 'Rosa'. Trees, cultivated in a traditional area at high agricultural vocation (Tuscany, Lucca province, lat. 44.02 N, long. 10.27 E) were grown in three farms with similar pedo-climatic characteristics, using low input farming practices and a regular drip irrigation system. From fruit-set to harvest time the main climatic parameters were provided by the Hydrological Service of Tuscany (SIR). For comparison purpose, the popular apple cultivar 'Fuji' grown under the same cultivation conditions, was considered.

At physiological maturity, stage samples of fruits were analyzed to determine the main physicochemical parameters, total antioxidant capacity and total phenol content. In the second crop season, these analyses were also carried out on additional samples which were stored at 4 ± 0.5 °C, 90% relative humidity (RH) in a normal atmosphere for 90 and 150 days (d). The storage modality was selected as a representative conservation method used for apples by small farms.

Physicochemical analysis. Measurements of fruit weight, peel and flesh color, fruit firmness, pH, total soluble solids (TSS) and titratable acidity (TA) were obtained individually on 30 fruit/each variety. The size of fruits by weight was defined according to the following classification (Vitagliano et al., 2000): small (≤ 100 g), medium (100–190 g), large (> 191 g). Peel and flesh color (PC, FC) was evaluated using color charts according to UPOV Code (International Union for the Protection of New Varieties of Plants, Geneva) for apple. The relative area of red cover color of peel (CC) was visually estimated. Firmness was evaluated with a manual penetrometer on two peeled opposite areas at the equatorial region of apples, using an 11-mm-wide plunger. TSS was measured using a refractometer (°Brix) and TA was determined on fruit juice by titration a known volume of juice with 0.1 N NaOH to pH 8.1 endpoint. TA was expressed as milliequivalents of malic acid per 100 grams of fresh weight (meq malic

ac. 100 g FW⁻¹). Fruit weight loss was calculated as [(Initial weight-final weight)/ Initial weight]*100.

Total antioxidant capacity (TAC) and total phenol (TP) analysis. Analyses were made on the same fruits (N = 30) previously subjected to the physicochemical determinations. Samples of 3 g (in triplicate) of fruit slices, constituted by flesh with peel, were immediately frozen and stored at -20 °C until extraction. Samples were homogenized using an ultra-Turrax blender at 4 °C to avoid oxidation. The extraction was performed in 80% ethanol for 1 h in a shaker in the dark and subsequently centrifuged at 4 °C for 10 min at 2,600 g. The supernatant was used for TAC (Total antioxidant capacity) and TP (total phenol) analysis.

TAC was evaluated using the improved Trolox equivalent antioxidant capacity (TEAC) method (Arts et al., 2004). The TEAC value was calculated in relation to the reactivity of Trolox, a water-soluble vitamin E analogue that was used as an antioxidant standard. In the assay, 40 µl of the diluted samples, controls or blanks added to 1,9690 µl ABTS^{•+} solution, resulted in a 20–80% inhibition of the absorbance. The decrease in absorbance at 734 nm was recorded 6 min after an initial mixing, and plotted against a dose–response curve calculated for Trolox (0–30 µM). Antioxidant activity was expressed as micromoles of Trolox equivalents pergram of fresh fruit weight (µmolTE gFW⁻¹). Trolox was purchased from Sigma Chemical Co. (St. Louis, MO).

TP content was determined according to the improved Folin-Ciocalteu (F-C) method (Waterhouse 2001). The assay provides a rapid indication of the antioxidant status of the studied material and is valuable for different food samples. The standard compound for the calibration curve was gallic acid (GA, Sigma Chemical Co, – St. Louis, MO). Total phenol content was calculated as milligrams of GA equivalent (GAE) per gram of fresh fruit weight (mg GAE gFW⁻¹). The absorbance of the blue colored solutions was read at 765 nm after incubation for 2 h at room temperature.

Statistical analysis. Instrumental data are reported as mean ± standard error of the mean (SEM). Analysis of variance (ANOVA), Student's *t*-test procedure and correlation analysis were performed using the package GraphPad Prism 5 (GraphPad Software, Inc.).

RESULTS AND DISCUSSION

Weather conditions. Climatic data, from May to October over two consecutive crop seasons (2014 and 2015), differed in terms of temperatures and distribution of rainfall (Table 1).

The summer 2015 was characterized by warmer temperatures by mean values of about 2 °C than 2014 which, instead, was noticeable for the very rainy condition during the early summer season. Indeed, up to August more than 400 mm of rainfall were recorded, of which 80% in July. This occurrence was infrequent for the considered geographical area where, average precipitations of the last 10 years, for the same period, ranged from 8.6 to 60.2 mm (SIR Toscana). By contrast, as usually happens, in 2015 conspicuous rainfall (more than 300 mm) occurred in October, close to harvest time. The total rainfall recorded during the fruit growth-ripening period (May–October) was 646 and 513 mm in the first and second year, respectively. An average of about 500 mm may be considered conforming to the multiannual data about Lucca province (SIR Toscana).

Table 1. Monthly minimum and maximum temperatures (°C) and cumulative rainfall (mm) from May to October, over a 2-year period (2014–15)

Month	2014				2015			
	Min.	Max.	Average	Rainfall	Min.	Max.	Average	Rainfall
May	11.8	23.7	17.8	39.8	13	24.5	18.8	34.4
June	16.0	29.4	22.7	48.0	16.8	30.2	23.5	29.8
July	17.4	28.7	23.2	314.6	20.7	33.9	27.3	7.4
August	17.3	29.1	23.2	4.0	18.4	31.9	25.2	80.6
<i>Av. temp.</i>	<i>15.6</i>	<i>27.7</i>	<i>21.7</i>		<i>17.2</i>	<i>30.1</i>	<i>23.7</i>	
<i>Total rainf.</i>				<i>406.4</i>				<i>151.2</i>
September	14.9	26.2	20.6	142.8	15.4	27.1	21.3	36.2
October (1–15)	14.9	24.6	19.7	63.0	14.4	21.4	17.9	230.0
October (15–31)	10.2	21.1	15.7	34.2	8.7	18.7	13.7	95.2
<i>Av. temp. (Sept.-Oct.)</i>	<i>13.3</i>	<i>24.0</i>	<i>18.7</i>		<i>12.8</i>	<i>24.9</i>	<i>17.6</i>	
<i>Total rainf. (Sept.-Oct.)</i>				<i>240.0</i>				<i>361.4</i>
<i>Av. temp. (May-Oct.)</i>	<i>14.9</i>	<i>26.6</i>	<i>20.8</i>		<i>15.9</i>	<i>27.9</i>	<i>21.9</i>	
<i>Total rainf. (May-Oct.)</i>				<i>646.4</i>				<i>513.6</i>

Physicochemical and antioxidant parameters of fruits at harvest. The harvest time and the main pomological traits of fruits are reported in Table 2. The physiological maturity of apples occurred in the second and third ten days of September for ‘Fuji’ and ‘Paradisa’, and in the second and third ten days of October for ‘Paganina’ and ‘Rosa’, respectively. Fruits presented the following morphological characteristics: ‘Paganina’-medium size, red-green peel (40–50% red cover colour), cream flesh and flat shape; ‘Paradisa’ – medium size, grey-yellow peel (5–10% red cover colour), greenish flesh and truncated cone shape; ‘Rosa’ – medium-large size, green-yellow peel (70–80% red cover colour), cream flesh and truncated cone shape; ‘Fuji’ (reference variety) – medium-large size, green peel (30–40% cover colour), cream flesh and spherical shape.

Table 2. ‘Paganina’, ‘Paradisa’, ‘Rosa’ and ‘Fuji’ apple varieties: harvest time, peel and flesh colour, red cover colour (CC, %), longitudinal shape and size of fruits

	Paganina	Paradisa	Rosa	Fuji
Harvest time	10–20 October	20–30 September	20–30 October	10–20 September
Peel colour	red-green, 40–50CC	grey-yell., 5–10CC	green-yell., 70–80CC	green, 30–40CC
Flesh colour	cream	greenish	cream	cream
Shape	flat	truncated cone	truncated cone	spherical
Size	medium	medium	medium-large	medium-large

Apple fruits showed different physicochemical traits according to the variety and the harvest year (Table 3). As regards the mean values of the two years, the flesh firmness ranged between 4.6 and 8.2 kg 0.5 cm⁻², above the minimum threshold of 4.4 kg 0.5 cm⁻² considered an important qualitative index for apples (Kupferman, 1992). The highest TSS content (about 14 °Brix) was detected in ‘Paganina’ and ‘Rosa’ which were significantly different from ‘Fuji’ (about 11 °Brix). TA values ranged from 4.4 to 7.3 meq malic ac. 100 g FW⁻¹ in ‘Fuji’ and ‘Paganina’, respectively. As a consequence, variations in TSS/TA ratio from 1.8 to 2.7 were found.

Within each variety, apple pomological attributes in response to different summer weather conditions were observed. In 2015, year characterized by a warm and dry summer season, values of FF, TSS, TA and TSS/TA ratio were generally higher than the previous year. It has been observed that, among the environmental factors determining the apple quality, temperature was the most important correlating with fruit hardness, soluble sugar content, and sugar-acid ratio (Wei et al., 1998). In addition, conditions of moderate water deficit stress caused a TSS and TA increase (Navarro et al., 2010). Among the local varieties, 'Paradisa' had less variability of fruit parameters in response to summer stresses due to high temperatures and drought conditions.

In order to establish the influence of environmental conditions on physicochemical characteristics of fruits, analysis of variance, considering two main effects (years and varieties), was performed (Table 4). Significant interactions 'year x variety' for FF, TA and TSS/TA ratio were observed, confirming the role of weather conditions which can impact with the physiological responses of genotypes during the grown-ripening season (Bartolini et al., 2015b).

Concerning the antioxidant properties of apples, expressed by total antioxidant capacity (TAC) and total phenol (TP) as average of the two crop seasons (Table 3), local varieties proved values higher than the commercial variety 'Fuji' showing about 36 $\mu\text{mol TE g FW}^{-1}$ for TAC and 1 mg GAE g FW^{-1} for TP. Indeed, in the other three varieties the concentrations were from about 44 to 63 $\mu\text{mol TE g FW}^{-1}$ for TAC, and from 2 to 5 mg GAE g FW^{-1} for TP, confirming that the ancient cultivars may have higher levels of bioactive compounds than the modern ones (Jakobek & Barron, 2016). Changes in TAC and TP values in relation to the crop season were observed. In general, the lowest concentrations were found in 2015 by a mean decrease ranging from 30 to 70% respectively, in comparison to the previous year. On the contrary, 'Rosa' showed an increase of 2-fold higher, reaching values of about 90 $\mu\text{mol TE g FW}^{-1}$ for TAC, and around 7 mg GAE g FW^{-1} for TP. The raise in antioxidants recorded for 'Rosa' in 2015 was in agreement with findings of authors who found an antioxidant enhancement in apples and other fruits such as grape, acerola and apricot, under warm and dry summer conditions (Lima et al., 2005; Leccese et al., 2012). It has been suggested that water stress, caused by the deficit irrigation technique, can imply an activation of phenolic biosynthesis in the plants (Roby et al., 2004). The behavior of the other varieties was in accordance to Lata (2007) who has reported higher significant concentrations of total phenolics in wetter and cooler growing seasons. The influence of different climatic conditions on antioxidant power of fruits has been debating, confirming the importance of genotype who can differently react to the environment and, consequently, responses are cultivar-dependent (McGhie et al., 2005).

The 2-way ANOVA results (Table 4) showed that TAC and TP variations among varieties were greater than between harvest years, as found in other fruit species (Howard et al., 2003; Leccese et al., 2012). Nevertheless, the significant interaction 'variety x year' revealed that environmental growing conditions may impact the capacity of genotype to synthesise antioxidants which are influenced by biotic and abiotic factors (Besjada & Tomczak, 2012).

Table 3. ‘Paganina’, ‘Paradisa’, ‘Rosa’ and ‘Fuji’ apple varieties over a 2-year period (2014–15): main physicochemical and antioxidant parameters: flesh firmness (FF, kg 0.5 cm⁻²), total soluble sugars (TSS, °Brix), titratable acidity (TA, meq malic ac. 100 g FW⁻¹), sugars/acids ratio (TSS/TA), total antioxidant capacity (TAC, μmol TE g FW⁻¹), total phenols (TP, mg GAE g FW⁻¹). Mean ± SEM. Within each variety, asterisk indicates significant differences between years by Student *t*-test ($P \leq 0.05$). Among varieties, different letters indicate significant differences at $P \leq 0.05$

	Paganina			Paradisa			Rosa			Fuji		
	2014	2015	mean	2014	2015	mean	2014	2015	mean	2014	2015	mean
FF	3.9 ± 0.3	5.3 ± 0.5*	4.6 ± 0.4c	5.4 ± 0.3	5.9 ± 0.5	5.6 ± 0.3b	6.2 ± 0.1	10.2 ± 0.7*	8.2 ± 0.4a	5.9 ± 0.2	6.8 ± 0.3*	6.3 ± 0.2b
TSS	12.6 ± 0.2	15.1 ± 0.6*	13.8 ± 0.4a	12.2 ± 0.4	13.1 ± 0.2	12.6 ± 0.3ab	13.8 ± 0.3	14.9 ± 0.2*	14.3 ± 0.3a	11.0 ± 0.3	11.6 ± 0.3	11.3 ± 0.3b
TA	6.8 ± 0.2	7.9 ± 0.1*	7.3 ± 0.2a	8.4 ± 0.2	5.9 ± 0.1*	7.1 ± 0.2a	3.7 ± 0.1	8.3 ± 0.2*	6.0 ± 0.1ab	3.6 ± 0.1	5.2 ± 0.1*	4.4 ± 0.1b
TSS/TA	1.8	1.9	1.8b	1.4	2.2*	1.8b	3.7	1.8*	2.7a	3.1	2.2*	2.6a
TAC	64.9 ± 0.8	43.2 ± 0.6*	54.0 ± 0.7a	51.5 ± 8.5	37.6 ± 0.1	44.5 ± 4.5a	35.4 ± 3.3	89.9 ± 1.1*	62.6 ± 4.4a	42.3 ± 1.5	30.4 ± 0.4*	36.3 ± 1.0a
TP	3.4 ± 0.5	0.8 ± 0.1*	2.1 ± 0.3ab	3.9 ± 0.8	0.5 ± 0.1*	2.2 ± 0.4ab	2.7 ± 0.2	7.4 ± 0.3*	5.0 ± 0.2a	1.9 ± 0.3	0.9 ± 0.5*	1.4 ± 0.4b

Table 4. Two-way ANOVA results. Variables: FF (FF, kg 0.5 cm⁻²), TSS (°Brix), TA (meq malic ac. 100 g FW⁻¹), TSS/TA, TAC (μmol TE g FW⁻¹) and TP (mg GAE g FW⁻¹)

Main effects	FF <i>P</i>	TSS <i>P</i>	TA <i>P</i>	TSS/TA <i>P</i>	TAC <i>P</i>	TP <i>P</i>
Year (Y)	< 0.0001***	< 0.0001***	< 0.0001***	< 0.0001***	0.0109*	0.8940 ns
Variety (V)	< 0.0001***	< 0.0001***	< 0.0001***	< 0.0001***	< 0.0001***	< 0.0001***
Interac. YxV	< 0.0001***	0.0519 ns	< 0.0001***	< 0.0001***	< 0.0001***	< 0.0001***

Physicochemical and antioxidant parameters of fruits after cold storage. To assess the storability of apples, in the second crop season (2015) physicochemical and antioxidant performances were tested after 90 and 150 d at 4 °C cold storage (Table 5).

Table 5. ‘Paganina’, ‘Paradisa’, ‘Rosa’ and ‘Fuji’ apple varieties (year 2015). Physicochemical parameters of fruits at harvest time (0) and after 90 and 150 days at +4 °C cold storage: weight loss (%), flesh firmness (FF, kg 0.5 cm⁻²), total soluble sugars (TSS, °Brix), titratable acidity (TA, meq malic ac. 100 g FW⁻¹), and sugars/acids ratio (TSS/TA). Mean ± SEM. Within each variety, different letters indicate significant differences at $P \leq 0.05$; ns not significant. For the weight loss, asterisks indicate significant differences between storage days by Student *t*-test ($P \leq 0.05$)

Varieties	Storage (days)	Weight loss (%)	FF	TSS	TA	TSS/TA
Paganina	0	-	5.3 ± 0.5 a	15.1 ± 0.6 ns	7.9 ± 0.1 a	1.9 c
	90	4.5	3.6 ± 0.3 b	14.4 ± 0.1	4.8 ± 0.2 b	2.9 b
	150	11.9*	3.4 ± 0.2 b	13.8 ± 0.1	3.9 ± 0.1 c	3.5 a
Paradisa	0	-	5.9 ± 0.5 ns	13.1 ± 0.2 a	5.9 ± 0.1 a	2.2 ns
	90	5.0	5.4 ± 0.3	13.4 ± 0.3 a	6.1 ± 0.1 a	2.0
	150	10.3*	4.5 ± 0.2	11.6 ± 0.4 b	4.9 ± 0.2 b	2.4
Rosa	0	-	10.2 ± 0.7 a	14.9 ± 0.2 ns	8.3 ± 0.2 a	1.8 c
	90	1.9	5.0 ± 0.1 b	16.0 ± 0.3	6.2 ± 0.1 b	2.6 b
	150	3.8*	6.7 ± 0.6 b	14.9 ± 0.1	4.6 ± 0.4 c	3.2 a
Fuji	0	-	6.8 ± 0.3 ns	11.6 ± 0.4 ns	5.2 ± 0.1 a	2.2 c
	90	4.6	6.9 ± 0.5	10.0 ± 0.4	2.9 ± 0.1 b	3.5 b
	150	6.7*	6.6 ± 0.4	10.1 ± 0.1	2.6 ± 0.1 b	3.8 a

Concerning the physicochemical parameters, apple fruit-weight loss ranged from about 2% to 12%, similarly to whom reported for several apple cultivars (Golias et al., 2008; Guerra et al., 2010). This occurrence, as a consequence of the increase in transpiration and respiration rates (Blampired, 1981; Erturk et al., 2003), was particularly strong in ‘Paganina’ after the longest storage. On the other hand, the lowest weight loss was found in ‘Rosa’, probably due to its peel structure which appears thick with presence of wax (data not shown).

Flesh firmness, one of the most storage-susceptible parameter, did not change over time in ‘Paradisa’ and ‘Fuji’, while ‘Paganina’ and ‘Rosa’ had a significant firmness decrease after 90 d, without further declines. This finding supports several investigations in which a gradual FF reduction with an extending storage period was observed (Ghafir et al., 2009; Jan et al., 2012). Apple’s softening in post-harvest, due to respiration and evapo-transpiration phenomena (Kov et al., 2005; Fuller, 2008), could negatively influence the fruit quality, predisposing fruits to diseases (Hoehn et al., 2003).

With the exception of ‘Paradisa’, TSS content did not change in comparison to harvest, in agreement with results obtained by Golias et al. (2008). About the total soluble sugars, literature reports some contradictions (stability, increase or decrease) as a function of the variety rather than storage conditions (Ghafir et al. 2009; Guerra et al., 2010). Thus, uncertainty for TSS as a valid parameter assessing the apple tolerance to cold storage arises (Farooq et al., 2012). During storage, as a result of the respiration process consuming organic acids, a progressive and significant decrease of TA was recorded (Jan et al., 2012). Changes in TA had a significant effect on TSS/TA ratio, widely considered as an important feature for apple flavor (Abbott et al., 2004). The ratio

gradually increased, but ‘Paradisa’ maintained a mean value close to 2, denoting a good balance between sugars and acids, main factors for the eating apple quality (Sadar et al., 2016). This variety, object of a sensory evaluation carried out by people with visual disability able to assess the intrinsic quality attributes, was well appreciated for sweetness, juiciness and flavor (Bartolini et al., 2015a).

TAC and TP values, recorded at harvest time and at the end of the two cold storage periods, are showed in Fig. 1.

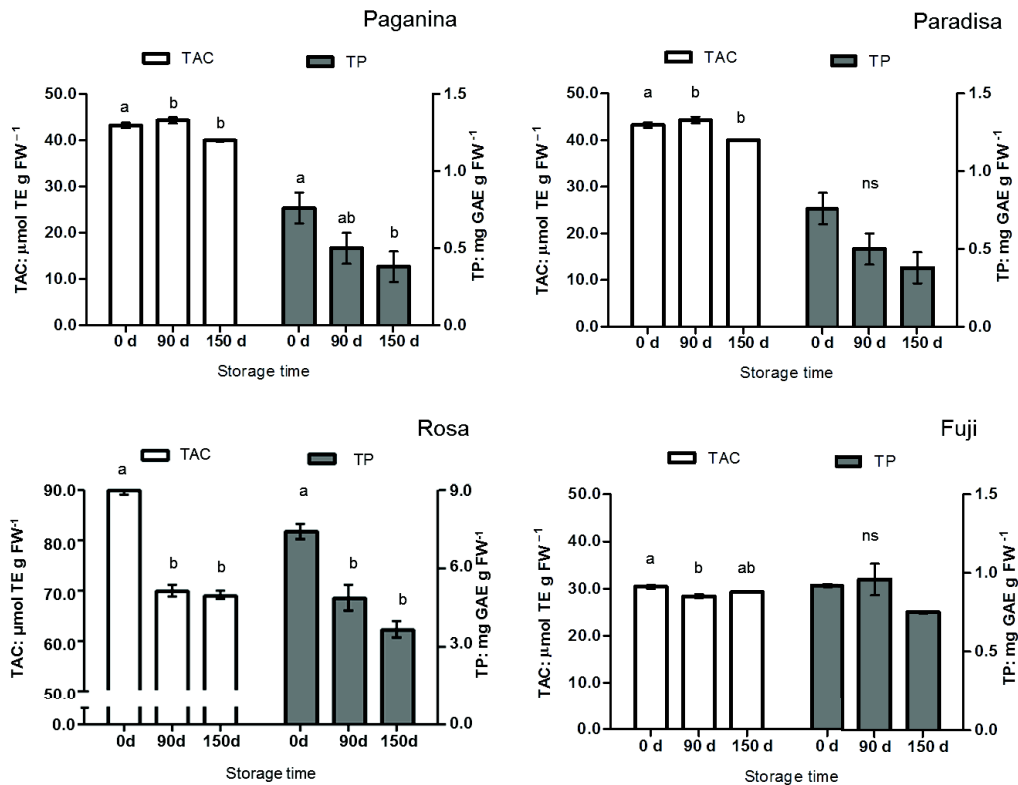


Figure 1. ‘Paganina’, ‘Paradisa’, ‘Rosa’ and ‘Fuji’ apple varieties (year 2015). Total antioxidant capacity (TAC) and total phenols (TP) at harvest time (0) and after 90 and 150 days at +4 °C cold storage. Mean ± SEM. Different letters indicate significant differences at $P \leq 0.05$.

Significant differences between fresh and stored samples were observed and varieties showed a similar trend in TAC and TP content decreases. In comparison to the initial average antioxidant values, ‘Fuji’ had the lowest declines while the local varieties, characterized by the highest contents at the beginning of storage, showed values which dropped over time from 7.5 to 22% for TAC and 26 to 50% for TP. The greatest losses were found in ‘Rosa’ that, however, preserved elevated contents because, in the year of storage trials, it was characterized by the highest antioxidant values at harvest (more than 2-fold higher than the other varieties). This general decline of antioxidant power was in agreement with Tarozzi et al. (2004) who have found that cold storage impoverishes the antioxidant properties of apples, particularly in skin tissue. In contrast, other authors have observed that cold storage did not affect the TP content of certain apple varieties

which may show stable or increased antioxidant concentrations (Napolitano et al., 2004; Matthes & Schmitz-Eiberger, 2009). The different evidence existing in literature suggest that the capacity to maintain over time the antioxidant content of fruits would be primarily related to variety, hence the importance to extend the knowledge within the wide apple Italian germplasm.

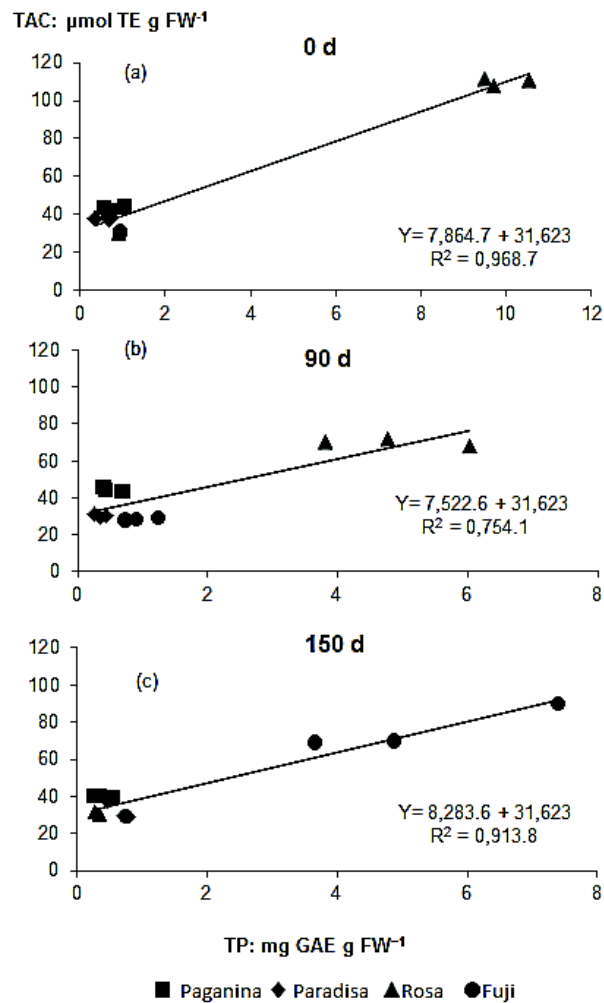


Figure 2 (a, b, c). Linear regression between total antioxidant capacity (TAC) and total phenols (TP) at harvest time (0) and after 90 and 150 days at +4 °C cold storage.

In order to verify a possible relation between TAC and TP of the studied varieties, regression analysis was carried out. From harvest to the end of both cold storage periods, a significant and positive relationship was shown (Fig. 2, a, b, c). As it has been observed for other genotypes, polyphenols are important bioactive compounds contributing to the antioxidant properties of apples (Leccese et al., 2009; Donno et al., 2012). Although the local varieties showed higher TAC values in comparison to ‘Fuji’, over time the correlation coefficients were influenced depending on variety. Indeed, after the longest

storage period, the standard variety 'Fuji' was able to maintain more constant level of polyphenols, important nutraceutical suppliers with health-promoting effects for humans (Scafuri et al., 2016).

CONCLUSIONS

Results of this study provide new knowledge about the physicochemical and antioxidant properties of apple fruits belonging to old varieties grown under a long-established fruit area in central Italy. Compared with the world-wide-cultivated 'Fuji', the three local varieties, 'Paganina' 'Paradisa' and 'Rosa', showed valuable quality properties. In particular, they stood out for a greater antioxidant power of fruits, although a variability, linked to the weather conditions of the two investigated crop seasons, was observed. After a dry summer, 'Rosa' showed very high TAC and TP values that markedly decreased after long-periods of cold storage. These losses were not so noticeable in the other varieties, suggesting a tendency to maintain a major stability during storage in normal atmosphere refrigerated cellars. Further investigations will be likely extended to better ascertain the effect of cold-storage time on antioxidant properties of local apples. Anyway, the quality traits established for the old analyzed varieties could meet the preference of demanding consumers for taster and healthier foods, who appreciate fresh fruits for high antioxidant power with protective properties by free radicals scavenging activity. Moreover, the adopted simple storage technique might be useful to farmers who are focused on nearby and niche markets.

In conclusion, consumers and farmers should have ready access to information on the valuable quality characteristics of the old but relevant apple varieties. This is a further and valid attribute to take into account for a re-introduction of local varieties to promote their valorization and also contributing to the preservation of biodiversity. Moreover, breeders could be interested in these genotypes to create apple parental lines with high quality profile.

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REFERENCES

- Abbott, J.A., Saftner, R.A., Gross, K.C., Vinyard, B.T. & Janick, J. 2004. Consumer evaluation and quality measurement of fresh-cut slices of 'Fuji,' 'Golden Delicious,' 'GoldRush,' and 'Granny Smith' apples. *Postharv. Biol. Technol.* **33**, 127–140.
- Arts, M.J.T.J., Dallinga, J.S., Voss, H.P., Haenen, G.R.M.M. & Bast, A. 2004. A new approach to assess the total antioxidant capacity using the TEAC assay. *Food Chem.* **88**, 567–570.
- Bartolini, S., Viti, R. & Ducci, E. 2015a. Local fruit varieties for sustainable cultivations: pomological, nutraceutical and sensory characterization. *Agrochimica* **59**(4), 282–294.
- Bartolini, S., Leccese, A. & Viti, R. 2015b. Quality and antioxidant properties of apricot fruits at ready-to-eat: influence of the weather conditions under Mediterranean coastal area. *J. Food Process Technol.* **7**:1 <http://dx.doi.org/10.4172/2157-7110.1000538>.
- Besiada, A. & Tomczak, A. 2012. Biotic and abiotic factors affecting the content of the chosen antioxidant compounds in vegetables. *Veg. Crops Res. Bul.* **76**, 55–78.
- Blampired, G.D. 1981. A relationship between water loss and storage breakdown of 'Mcintosh' apple. *Hort. Science* **16**(4), 525–526.

- Darby, K., Batte, M.T., Ernst, S. & Roe, B. 2008. Decomposing local: A conjoint analysis of locally produced foods. *Am. J. Agr. Econ.* **90**(2), 476–486.
- Denver, S. & Jensen, J.D. 2014. Consumer preferences for organically and locally produced apples. *Food Qual. Prefer.* **31**, 129–134.
- Donno, D., Beccaro, M.G., Mellano, D., Torello-Marinoni, D. & Cerutti, A.K. 2012. Application of sensory, nutraceutical and genetic techniques to create a quality profile of ancient apple cultivars. *J. Food Qual.* **35**, 169–181.
- Erturk, U., Akbuclak, B. & Ozer, M.H. 2003. Quality changes of some apple cultivars stored in normal atmosphere for long period. *Acta Hort.* **599**, 665–672.
- Farooq, Rab, A., Khan, N. & Iqbal, I. 2012. Physico-chemical quality of apple cv. Gala must fruit stored at low temperature. *Fuuast J. Biol.* **2**(1), 103–107.
- Fuller, M.M. 2008. The ultrastructure of the outer tissues of cold-stored apple fruits of high and low calcium content in relation to cell breakdown. *Ann. Appl. Biol.* **83**, 299–304.
- Ghafir, S.A.M., Gadalla, S.O., Murajei, B.N. & El-Nady, M.F. 2009. Physiological and anatomical comparison between four different apple cultivars under cold-storage conditions. *Afric. J. Pl. Sci.* **3**(6), 133–138.
- Golias, J., Mylova, P. & Nemcova, A. 2008. A comparison of apple cultivars regarding ethylene production and physico-chemical changes during cold storage. *Hort. Sci.* **35**(4), 137–144.
- Guerra, M., Valenciano, J.B., Marcelo, V. & Casquero, P.A. 2010. Storage behaviour of ‘Reinette du Canada’ apple cultivars. *Span. J. Agric. Res.* **8**(2), 440–447.
- Hoehn, E., Gasser, F., Guggenbuhl, B. & Kunsch, U. 2003. Efficacy of instrumental measurements for determination of minimum requirements of firmness, soluble solids, and acidity of several apple varieties in comparison to consumer expectations. *Postharvest Biol. & Technol.* **27**, 27–37.
- Howard, L.R., Clark, J.R. & Brownmiller, C. 2003. Antioxidant capacity and phenolic content in blueberries as affected by genotype and growing season. *J. Sci. Food Agric.* **83**, 1238–1247.
- Jakobek, L., Garcìa-Villalba, R. & Tomàs-Barberà, F.A. 2013. Polyphenolic characterization of old local apple varieties from South-Eastern European region. *J. Food Comp. Anal.* **31**, 199–211.
- Jakobek, L. & Barron, A.R. 2016. Ancient apple varieties from Croatia as a source of bioactive polyphenolic compounds. *J. Food Comp. Anal.* **45**, 9–15.
- Jan, I., Rab, A., Sajid, M., Ali, A. & Shah, S. T. 2012. Response of apple cultivars to different storage durations. *Sarhad J. Agric.* **28**(2), 220–225.
- Kov, E., Hertog, E. & Vanstreels, E. 2005. Relationship between physical and biochemical parameters in apple softening. *Acta Hort.* **68**, 573–578.
- Kupferman, E. 1992. Retaining firmness of apples in storage. *Tree Fruit Postharv. J.* **3**, 4–6.
- Łata, B. 2007. Relationship between Apple Peel and the Whole Fruit Antioxidant Content: Year and Cultivar Variation. *J. Agric. Food Chem.* **55**(3), 663–671.
- Leccese, A., Bartolini, S. & Viti, R. 2009. Antioxidant properties of peel and flesh in ‘GoldRush’ and ‘Florina’ scab-resistant apple (*Malus domestica*) cultivars. Antioxidants in scab-resistant apple cultivars. *New Zeal. J. Crop Hort. Sci.* **37**, 71–78.
- Leccese, A., Bartolini, S. & Viti, R. 2012. Genotype, harvest season, and cold storage influence on fruit quality and antioxidant properties of apricot. *Int. J. Food Prop.* **15**(4), 864–879.
- Lima, V.L.A.G., Mélo, E.A., Maciel, M.I.S., Prazeres, F.G., Musser, R.S. & Lima, D.E.S. 2005. Total phenolic and carotenoid contents in acerola genotypes harvested at three ripening stages. *Food Chem.* **90**, 565–568.
- Maragò, E., Iacopini, P., Camangi, F., Scattino, C., Ranieri, A., Stefani, A. & Sebastiani, L. 2015. Phenolic profile and antioxidant activity in apple juice and pomace. Effect of different storage condition. *Fruits* **70**(4), 213–223.
- Matthes, A. & Schmitz-Eiberger, M. 2009. Polyphenol content and antioxidant capacity of apple fruit: effect of cultivar and storage conditions. *J. Appl. Bot. Food Qual.* **82**, 152–157.

- McGhie, T.K., Hunt, M. & Barnett, L.E. 2005. Cultivar and growing region determine the antioxidant polyphenolic concentration and composition of apple grown in New Zealand. *J. Agr. Food Chem.* **53**, 3065–3070.
- Napolitano, A., Cascone, A., Graziani, G., Ferracane, R., Scalfi, L., Di Vaio, C., Ritieni, A. & Fogliano, V. 2004. Influence of variety and storage on the polyphenol composition of apple flesh. *J. Agric. Food Chem.* **52**, 6526–6531.
- Navarro, J.M.; Pérez-Pérez, J.G.; Romero, P. & Botia, P. 2010. Analysis of the changes in quality in mandarin fruit, produced by deficit irrigation treatments. *Food Chem.* **119**, 1591–1596.
- Roby, G., Harbertson, J.F., Adams, D.A., Matthews, M. & Matthews, M. 2004. Berry size and vine water deficits as factors in wine grape composition: Anthocyanins and tannins. *Aust. J. Grape Wine Res.* **10**, 100–107.
- Sadar, N., Urbanek Krajnc, A., Tojnko, S., Tijssens, L.M.M., Schouten, R.E. & Unuk, T. 2016. Development and distribution of quality related compounds in apples during growth. *Sci. Hort.* **213**, 222–231.
- Scafuri, B., Marabotti, A., Carbone, V., Minasi, P., Dotolo, S. & Facchiano, A. 2016. A theoretical study on predicted protein targets of apple polyphenols and possible mechanisms of chemoprevention in colorectal cancer. www.nature.com/scientificreports.
- SIR. Servizio Idrologico della Regione Toscana. Centro Funzionale della Regione Toscana (www.sir.toscana.it).
- Tarozzi, A., Marchesi, A., Cantelli-Forti G. & Hrelia, P. 2004. Cold storage affects antioxidant properties of apples in caco-2 cells. *J. Nutr.* **134**, 1105–1109.
- Vitagliano, C., Stefani, A. & Massai, R. 2000. Tuscan germplasm: apple. In: Valorization of Tuscan germplasm. ARSIA (ed.), pp. 173–178 (in Italian).
- Vrhovsek, U., Rigo, A., Tonon, D. & Mattivi, F. 2004. Quantitation of polyphenols in different apple varieties. *J. Agric. Food Chem.* **52**, 6532–6538.
- Waterhouse, A.L. 2001. Determination of total phenolic. *Current Protocols in Food Analytical Chemistry* Wroslad, R.E. ed. 1.1.1–1.1.8.
- Wei, Q.P., Li, J.R. & Su, H.R. 1998. Studies on the correlation between apple quality and ecological factors. *J. Shandong Agric. Univ.* **29**, 532–536.