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Original article

Antioxidant properties, sensory characteristics and volatile compounds profile of apple juices from ancient Tuscany (Italy) apple varieties

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Summary

Ancient apple varieties can be an important source of fruit products rich in polyphenols with a good taste and aroma. In this study juices were produced from three varieties of apples ('Panaia-red', 'Cipolla', and 'Nesta') grown in Tuscany (Italy). The phenolic contents, the sensory characteristics and the volatile compounds profile (VOCs) were analysed. The phenolic content was measured by spectrophotometer assays, and antioxidant activity was assessed using the in vitro test for the DPPH˙ radical. The VOCs profile was evaluated by DHS-GC-MS analysis and the sensory characteristics by a qualified sensory panel test. It was found that 'Panaia-red' juice had the highest level of phenolic compounds, except for flavonols, which were higher in the 'Cipolla' juice. 'Nesta' and 'Panaia-red' apple juices had the highest antioxidant activity. A total of forty-four volatile compounds were detected in the apple juices and a good score for taste and aroma was given to the juices by expert assessors. In comparison with commercial apple juices, the apple juices studied had approximately a double content of polyphenols, and, furthermore, they had a good aromatic profile and pleasantness with respect to the minimum threshold required by the sensory analysis (minimum score = 5).

Keywords
food analysis, mass spectrometry, *Malus* × *domestica* Borkh., polyphenols, sensory analysis

Abbreviations used
CE: Catechin Equivalent; DPPH˙: 1,1-DiPhenyl-2-Picryl-Hydrazyl; GAE: Gallic Acid Equivalent; LDL: Low Density Lipoprotein; FVN: Flavonols; CT: Condensed Tannins; TF: Total Flavonoids; TP: Total Phenolics, SSC: Soluble Solids Content; TA: Titratable Acidity; QE: Quercetin Equivalent; CYE: Cyanidin Equivalent.

Significance of this study

What is already known on this subject?
- Underutilized (for fresh consumption) apple varieties can be an important source of polyphenols.

What are the new findings?
- Production of juices with high polyphenols and antioxidant properties deserving these juices have a good aromatic profile and pleasantness.

What is the expected impact on horticulture?
- New products and valorization of underutilized apple varieties.

Introduction

Epidemiological studies have shown that diet plays a crucial role in the prevention of chronic diseases. A diet rich in fruits and vegetables is strongly associated with risk-reduction for cancer, diabetes, cardiovascular disease and Alzheimer's disease (Donaldson, 2004). For this reason, the National Cancer Institute and the National Research Council recommend at least five servings of fruits and vegetables daily (National Academy of Sciences CoDaH, National Research Council, 1989).

The protective effects of vegetables and fruits have been attributed to biologically active secondary plant metabolites, such as carotenoids, vitamins and polyphenols. Polyphenols, in particular, are bioactive non-nutrient plant compounds that have been found to provide a very strong antioxidant and free radical scavenging activity (Tsao et al., 2005).

The apple (*Malus* × *domestica* Borkh.) is one of the most widely grown and commonly consumed temperate fruit crops in the world, and it contains a large amount of natural phenolic phytochemicals (Shoji et al., 2004). There are five major groups of polyphenols in apples: hydroxycinnamic acids, flavan-3-ols, anthocyanins, flavonols and dihydrochalcones. The phenolic profile of apple is further complicated due to the polymerization of flavan-3-ols into dimers and polymers (procyanidins) as well as the glycosylation of other compounds, such as flavonols that are often associated with sugar moieties (Tsao et al., 2005).

Iacopini et al. (2010) have shown that some ancient and locally cultivated Italian apple varieties, when compared to international commercial varieties ('Golden' and 'Stark Delicious'), have a high level of antioxidant activity and this applies, in particular, to the ancient varieties rich in chlorogenic acid and epicatechin. Despite such potentially beneficial properties, these varieties have a limited diffusion as fresh products due to some of their commercially unwanted non-aesthetic properties. However, apples can be eaten either fresh and/or processed such as juice, cider, preserves and vinegar.

The composition of apple juice depends on variety, origin, growing conditions, quality of the fruit at harvest, ripening stage, processing procedures and postharvest storage (Alvarez et al., 2000). The major components of a raw apple juice, obtained after pressing apples, are water, sugars (such as fructose, sucrose, and glucose), malic acid, pectin, starch, polyphenols, proteins, vitamins (mainly ascorbic acid), and ashes (Lee and Mattick, 1989; Lea, 1990). Apple juice is a good phenolic source, and the most important polyphenols are chlorogenic acid (5-caffeoylquinic acid), quercetin glycosides, procyanidins, catechins and dihydrochalcones, such as phloridzin (phloretin-2'-O-glucoside) and phloretin-2'-O-xyloglucoside (Kahle et al., 2005; Felice et al., 2015; Maragò et al., 2015). Apple juice polyphenols have a high antioxidant capacity in vitro and it has been shown that the consumption of apple juice increases the antioxidant status of blood (Lee and Mattick, 1989), inhibits the proliferation of cancer cells, and increases anti-arteriosclerotic activity by reducing oxidation of low-density lipoprotein (LDL). In addition, such apple juice polyphenols have also been shown to possess growth-inhibitory properties in colon tumor cells (Barth et al., 2005; Kern et al., 2005).

However, the phenolic composition of juice is not the main characteristic for attracting consumers. Consumer's choice is driven especially by colour, taste (especially in terms of bitterness and astringency) and aromatic characteristics.

Fruit aroma is specific to each species and cultivar (Sanz et al., 1997). It is a complex phenomenon, arising from a mixture of different volatile compounds, each contributing to the overall sensory quality of fruit and therefore its derived products. In different studies, aroma compounds have been investigated with the aim of enhancing the quality of apples and apple products. In these studies, over 300 compounds have been identified in the aroma profile (Dixon and Hewett, 2000), but their individual contribution to the characteristic apple juice aroma has yet to be completed. The principal volatile fraction of aroma apple juice is constituted by esters, aldehydes and alcohols, but also ethers, fatty acids, lactones, terpenes and ketones. Each group of aroma compounds gives a typical odour characteristic to the apple juice flavour. Esters and aldehydes are present in lower concentrations in respect to alcohols, but they are responsible for the major part of the total aroma intensity of the juice, due to their low olfactory detection threshold values. Alcohols, however, have higher aroma threshold values, but, quantitatively, they are the largest group of aroma components in apple juice and they make an important contribution to the fruity flavour of the juice (Lea, 1990). According to Corollaro and co-workers (2013), descriptive sensory analysis is maybe the best approach to describe sensory perceptions using a comprehensive language that is close to the consumers.

The aim of this study was to analyse the phenolic composition, the antioxidant activity, and the VOCs profile of apple juices made from three ancient Italian varieties with a view to increasing understanding of the nutraceutical properties of these juices and their consumer acceptance, in terms of sensory quality.

Materials and methods

Fruit sampling for ripening characterisation and chemicals

Malus × domestica (Borkh.) genotypes of ancient Italian varieties ('Nesta', 'Panaia-red' and 'Cipolla') were grown in the Casentino area (Tuscany, Italy) (VV.AA., 2013) and fruits were collected at the end of October. In order to provide a characterisation of the ripening stage, three biological replicates ($n = 3$) of each variety were collected and each replicate was made up of four apple fruits randomly selected from different plants.

Gallic acid, Catechin, Quercetin, Cyanidin, Folin-Ciocalteau reagent, 1,1-DiPhenyl-2-PicrylHydrazyl (DPPH$^{\cdot}$ 90%) were purchased from Sigma Aldrich (Milan, Italy). All chemicals were of analytical or HPLC grade and all aqueous solutions were prepared by using ultra-pure water purified by Milli-Q System (Millipore, Milan, Italy).

Preparation of apple juice

Thirty kilograms of apples were collected for each of the three varieties. They were collected from the same plants used for the initial characterisation of the fruits and used for the preparation of the juices. All fruits were carefully washed, cut into pieces and squeezed at room temperature using a hand press machine. The juices obtained were divided into aliquots of 200 mL each and stored in sealed green glass bottles, which were pasteurized at 80°C for 20 minutes, cooled at room temperature and stored in a cold-room at 4°C until subsequent analysis.

Morphological and physiochemical parameters of fruit and juice

Fresh weight (g), height (cm), width (cm) and firmness (N cm^{-2}) of the apples were measured. Firmness was measured with a Fruit Pressure Tester Model FT327, using a 11 mm tip diameter. Soluble solids content (SSC), titratable acidity (TA) and pH were measured for all juices. SSC was determined with a hand-held refractometer (Sper Scientific, AZ, USA), and results expressed as °Brix. TA was determined by titrating 10 mL of juice with 0.1N NaOH to pH 8.1 using 1% phenolphthalein solution in ethanol, and results are given as malic acid equivalents (MAE) L^{-1}. pH was measured by a pH-meter and results expressed as Units.

Juice phenolic composition

The pasteurized juice samples used in these analytical determinations were filtered at 0.45 µm before analyses. Each analysis was performed on three independent ($n = 3$) juice samples per each variety and each sample was analysed in three technical replicates. An UV-VIS Agilent HP 8453 diode array spectrophotometer was used for the spectrophotometric determinations.

Total phenolic content (TP) of apple juices was measured by a colorimetric assay (Singleton and Rossi, 1965) with some modifications to reduce volumes. TP content was expressed as mg gallic acid equivalents (GAE) L^{-1} of juice.

Total flavonoid content (TF) of apple juices was determined using a colorimetric assay (Kim et al., 2003) with some modifications. TF content was expressed as mg catechin equivalents (CE) L^{-1} of juice.

Flavonols content (FVN) was determined using a colorimetric assay (Romani et al., 1996) with some modifications. FVN content was expressed as mg quercetin equivalents (QE) L^{-1} of juice.

Condensed tannins (CT) were determined using a colorimetric assay (Waterman and Mole, 1994) with some modifications. The total amount of CT content was expressed as mg cyanidin equivalents (CYE) L^{-1} of juice.

Juice antioxidant activity

Antioxidant activity of pasteurized and 0.45 µm filtered juice was measured by DPPH$^•$ assay method (Brand-Williams et al., 1995). An aliquot of 0.1 mL of an appropriate dilution (1:5) of each apple juice was added to 3 mL of DPPH$^•$ methanolic solution (12×10^{-5} M), and vortexed. Absorbance at 515 nm (A_{515}) was measured at different time intervals on a UV-VIS Agilent HP 8453 diode array spectrophotometer until the reaction reached a plateau, in order to find the time required to achieve the steady state. The initial concentration of DPPH$^•$ was controlled for every experiment by using a calibration curve made by measuring the absorbance at 515 nm of standard samples of DPPH$^•$ at different concentrations. The linear regression was $Abs_{515} = 0.0085 \times [DPPH^•]$ ($R^2 = 0.998$). The DPPH$^•$ radical scavenging effect was expressed as percentage reduction (%R) of the initial DPPH$^•$ absorption operated by test samples:

$$\%R \text{ of the initial DPPH}^\bullet \text{ absorption} = [(A_{DPPH}(t_0) - A_{sample}(t))/A_{DPPH}(t_0)] \times 100$$

where

$A_{DPPH}(t_0)$ is the absorbance of DPPH$^•$ at zero time;
$A_{sample}(t)$ is the absorbance of the solution added of the sample after time t.

DHS-GC-MS analysis

The volatile compound profile was obtained by DHS–GC-MS technique. An Agilent 7820 GC-Cromatograph equipped with a 5977 MSD with EI ionization was employed. A 5 mL aliquot of each apple juice sample was added in 20 mL screw cap head space vials, supplemented with 2 g NaCl and immediately sealed. A Gerstel MPS2 XL autosampler equipped with automated dynamic headspace DHS, Thermal Desorption Unit (TDU) and liquid CO_2-cooled Programmable temperature vaporizer (GERSEL CIS 4) was used for ensuring consistent VOCs extraction and injection conditions. The chromatographic settings were as follows: injector in solvent vent mode set at -20°C during thermal desorption, then 12°C sec^{-1} up to 300°C, hold time 10 min, J&W innovax column (50 m, 0,20 mm i.d., 0.4 um df); oven temperature program: initial temperature 40°C for 1 min, then 10°C min^{-1} until 130°C, then 5°C min^{-1} until 210°C, then 20°C min^{-1} until 260°C, hold time 3 min. MS detector was operated in scan mode in the m/z range 29–330, at three scans sec^{-1}. The deconvoluted peak spectra, obtained by Agilent MassHunter software, were matched against NIST 08 spectral library for tentative identification. The peak area of each compound (measured on specific ions), were normalized as % of the total area of identified compounds in each chromatogram. Since the selected ions for quantitation had different relative intensities in the mass spectra of their respective compounds, and no information on the ionization yields are available, no quantitation inferences can be made on relative compound concentration within the same analysis. However, the comparison among relative response for individual analites in different samples is valid, being the analytical conditions consistent through the experiment.

Sensory analysis

The sensory analysis of the fruit juices was carried out by the ASTRA Innovation and Development Agency (Faenza, Italy). The agency used a panel of sixteen expert assessors (n=16) with different technical knowledge (sommeliers, enologists and agronomists) and trained according to ISO (International Standards Organization) standards UNI ISO 8589.

Using the quantitative descriptive analysis method (QDA), six parameters were evaluated (1. colour intensity; 2. olfactory intensity; 3. sweetness; 4. acidity; 5. ratio of sweetness to acidity; 6. aroma intensity) for the sensory fingerprinting with a score from 1 (absent) to 9 (very intense). The analysis was completed by adding hedonic profiles according to visual, olfactory, and taste pleasantness, and an overall score using a scale from 1 up to 9.

Each expert was given transparent plastic cups labelled with three digit codes (corresponding to the juice samples) containing about 20 ml of juice. Each taster was offered a tray with samples served at 20°C and arranged randomly.

Statistical analysis

The results presented in tables and graphs were reported as means ± standard deviation (SD). Statistical differences in polyphenols content and antiradical activity among varieties were determined by one way-ANOVA after verification of normality and homoscedasticity assumptions and means separated by post-hoc LSD-test at $p = 0.05$. The volatile compounds and the panel test data were analysed by non-parametric Kruskal-Wallis ANOVA (since these data were not normally distributed) and if significant, pairwise sample mean comparison was done using Mann-Whitney test.

Three independent inhibition percentages curve of DPPH$^•$ radical for 'Nesta', 'Panaia-red' and 'Cipolla' apple juices were obtained from the three biological replicates. At each measuring time (5, 20, 40, 60, 90 and 120 minutes) two aliquots for each biological replicate were used and their values pooled. Data were interpolated for each biological replicate by a Logarithmic curve (percentage of inhibition of DPPH$^•$= (a x ln (t) + b)). In Figure 2 the lines represent the curve obtained by the mean of three biological replicates for the different apple varieties. The estimates of the coefficients a and b in each variety were done for each of the three biological replicates. Data were analysed by ANOVA followed by LSD-test at $p = 0.05$.

Results

Fruit and juice physicochemical parameters

The quality of apples at ripening can be measured by fruit size, colour, soluble solid content, titratable acidity and firmness. Regarding morphological characteristics, 'Nesta' had the smallest fruit size (60 ± 3.3 g) and part of the skin red. 'Panaia-red' fruits were the largest (111 ± 5.3 g) and part of the skin was also red as in 'Nesta' variety. 'Cipolla' fruits were of medium size (83 ± 4.1 g) and both their skin and flesh had red streaks (Figure 1). Regarding fruit firmness, 'Cipolla' had the highest value (36.3 ± 2.55 N cm^{-2}), while 'Panaia-red' had the lowest (24.5 ± 3.53 N cm^{-2}). 'Nesta' fruits had an intermediate value (32.4 ± 2.10 N cm^{-2}) (Table 1).

Juice yield from fresh fruits is strongly affected by cultivar, fruit phenological stage, and juice production method (Van der Sluis, 2005). In our case juice yields were: 37% (v/w) for 'Nesta', 34% (v/w) for 'Panaia-red' and 40% (v/w) for 'Cipolla'.

SSC, pH and TA are quality parameters related to the stability of bioactive compounds in fruits and their derived products, and were determined in juices immediately after their production.

'Panaia-red' juice had a significantly higher amount of SSC (18.0 ± 0.91 °Brix), in comparison with 'Nesta' and 'Cipolla' (14.0 ± 0.21 and 14.0 ± 0.82 °Brix, respectively). Acidity in apple juice is an important sensory attribute, associat-

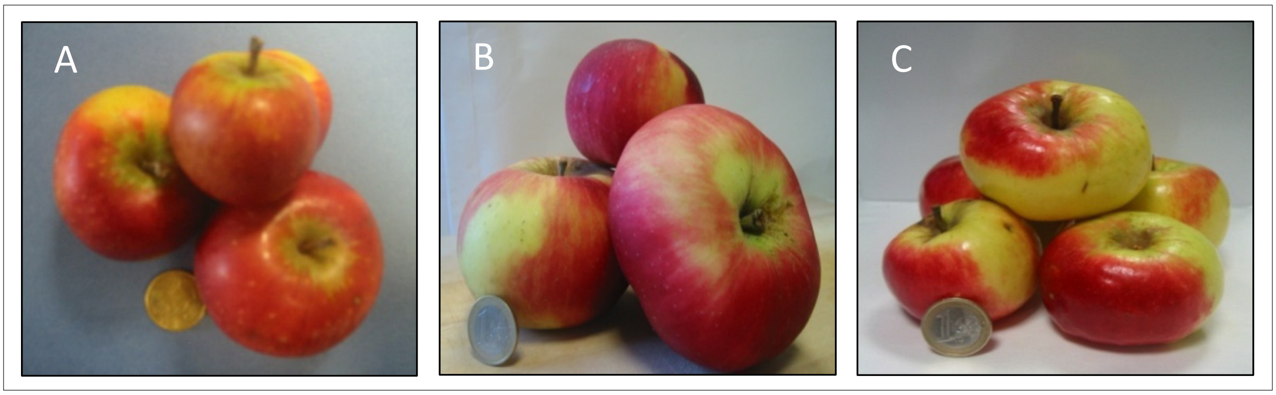


Figure 1. Apple fruits used to produce apple juices: A) 'Nesta' apples; B) 'Panaia-red' apples; C) 'Cipolla' apples.

Table 1. Fresh weight (g), height (cm), width (cm) and firmness (N cm^{-2}) of apple fruits of 'Nesta', 'Panaia-red' and 'Cipolla' varieties at harvest. Data ($n = 3$) are mean ± standard deviation. ANOVA *P*-values, *** = $p \leq 0.001$. In the same row, different letters indicate significant differences according to LSD-test at $p = 0.05$.

Parameter	'Nesta'	'Panaia-red'	'Cipolla'	*P*-value
Fresh weight (g)	60 ± 3.3 C	111 ± 5.3 A	83 ± 4.1 B	***
Height (cm)	4.1 ± 0.22 C	5 ± 0.2 A	4.5 ± 0.23 B	***
Width (cm)	5.2 ± 0.08 C	6.7 ± 0.09 A	5.8 ± 0.04 B	***
Firmness (N cm^{-2})	32.4 ± 2.10 B	24.5 ± 3.53 C	36.3 ± 2.55 A	***

Table 2. Soluble solids content (SSC), titratable acidity (TA) and pH in apple juices of 'Nesta', 'Panaia-red' and 'Cipolla' varieties. Data (n=3) are mean ± standard deviation. ANOVA *P*-values, $p \leq 0.001$ = ***; $p \leq 0.05$ = *; n.s. = not significant. In the same row, different letters indicate significant differences according to LSD-test at $p = 0.05$.

Parameter	'Nesta'	'Panaia-red'	'Cipolla'	*P*-value
SSC (°Brix)	14.0 ± 0.21 B	18.0 ± 0.91 A	14.0 ± 0.82 B	***
pH (Units)	3.5 ± 0.05	3.5 ± 0.20	3.8 ± 0.10	n.s.
TA (MAE L^{-1})	3.8 ± 1.35 A	2.3 ± 0.81 AB	1.6 ± 0.30 B	*

Table 3. Total phenolic content (TP), total flavonoid content (TF), flavonols content (FVN), condensed tannins (CT) in apple juices of 'Nesta', 'Panaia-red' and 'Cipolla' varieties. Data ($n = 3$) are mean ± standard deviation. ANOVA *P*-values: $p \leq 0.001$=***; $p \leq 0.05$=*. In the same row, different letters indicate significant differences according to LSD-test at $p = 0.05$.

Parameter	'Nesta'	'Panaia-red'	'Cipolla'	*P*-value
TP (mg L^{-1})	675 ± 24.6 B	778 ± 27.3 A	639 ± 30.1 C	***
TF (mg L^{-1})	113 ± 7.9 B	179 ± 12.8 A	164 ± 19.2 A	***
FVN (mg L^{-1})	96 ± 5.6 C	109 ± 12.0 B	130 ± 8.1 A	***
CT (mg L^{-1})	343 ± 53.2 B	384 ± 38.7 A	333 ± 17.2 B	*

ed with juice flavour and astringency (Aguilar-Rosas et al., 2007). With respect to TA, 'Nesta' juice had the highest value (3.8 ± 1.35 MAE L^{-1}), whereas Cipolla juice had the lowest (1.6 ± 0.30 MAE L^{-1}). The pH was similar among all three juices and was in the range of 3.5 ± 0.05 – 3.8 ± 0.10 units (Table 2).

Phenolic content and antioxidant activity

The phenolic profiles of the 'Nesta', 'Panaia-red' and 'Cipolla' juices were evaluated by the determination of the TP and of the three main apple phenolic classes: the TF, the CT and the FVN. The analyses of the phenolic content revealed that the 'Panaia-red' juice is the one with the highest TP and CT contents in comparison with those of 'Nesta' and 'Cipolla' (respectively 778 ± 27.3, 675 ± 24.6 and 639 ± 30.1 mg L^{-1} for TP and 384 ± 38.7, 343 ± 53.2 and 333 ± 17.2 mg L^{-1} for CT). On the other hand, 'Cipolla' had the highest amount of FVN (130 ± 8.1 mg L^{-1}). Moreover, 'Panaia-red' and 'Cipolla' juices showed the highest amount of TF (179 ± 12.8 and 164 ± 19 mg L^{-1}, respectively) (Table 3).

The analysis of the antioxidant activity, obtained by measuring the DPPH$^\bullet$ percentage reduction, proved that the three apple juices were significantly active as antioxidant agents. Moreover, the DPPH$^\bullet$ curves showed that the *a* coefficient for 'Nesta' juice was statistically higher (22.3) than those of 'Panaia-red' and 'Cipolla' juices (21.2 and 17.7, respectively). The *b* coefficient was lower in 'Panaia-red' juice (-20.7) than in 'Nesta' and 'Cipolla' juices (-14.43 and -13.7, respectively) (Figure 2).

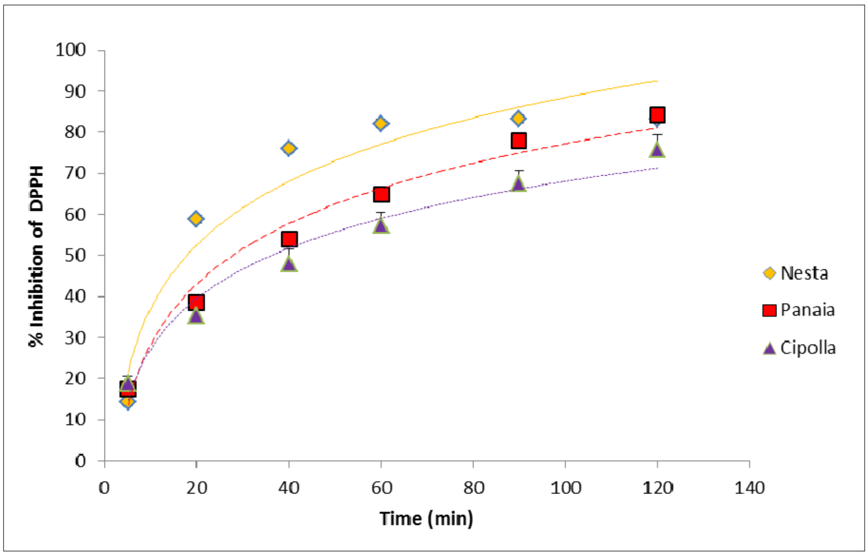
Apple juice VOCs profile

In this study, the VOCs were analysed by a DHS-GC-MS system, and 44 different volatile molecules present in apple juice aroma were detected. Seven of these are unknown since no reliable matching was found in literature for their mass spectra (Table 4). Significant differences among varieties were observed in the relative content of the volatile compounds and the main chemical groups were esters, followed by alcohols and aldehydes. 'Nesta' juice was characterized by a significantly higher relative content of ethyl isobutanoate, 1-propanol, 1-butanol-2-methyl + 1-butanol-3-methyl, "unknown 4" and β-damascenone in comparison with the other juices. The relative contents of propyl isovalerate and benzaldehyde were similar to 'Cipolla' juice. 'Nesta' and 'Panaia-red' juices had similar relative contents of 1-butanol, which was significantly higher than in 'Cipolla'. Relative contents of 1-butanol-3-methyl acetate, 3-hydroxybutanone, 1-hexanol and 2-hexen-1-ol were significantly higher in 'Panaia-red' juice with respect to the other juices. 'Cipolla' juice had the largest number of significantly different volatile compounds (i.e., ethyl acetate, "unknown 1", ethyl propionate, ethyl isobutanoate, methylbutanoate, 2-methylbutanoic acid, isobutylacetate, 1-propanol, ethyl butanoate, ethyl-2-methyl butanoate, butyl acetate, propyl isovalerate, 1-butanol, 1-butanol-3-methyl acetate, 1-butanol 2-mehyl, 3-hydroxybutanone, 1-hexanol, 2-hexen-1-ol, "unknown 4", ethyl 3-hydroxybutanoate, benzaldehyde and β-damascenone) with respect to 'Nesta' and 'Panaia-red' juices (Table 4).

Sensory analysis showed that 'Nesta' juice had a good olfactory intensity with a typical cooked apple fragrance and an amber/orange colour. The taste was found to be very sweet and slightly acid. The aroma was described as intense and good. In conclusion, 'Nesta' juice was very fragrant and tasty and got a score of 7.

'Panaia-red' juice had a distinct olfactory intensity with pear, wood, yeast and floral notes. It also had an orange/golden colour, and its taste was sweet, but acid. It was the most acid of the three juices. The aroma was intense and with a taste of cooked apple and cider. It had a preference score of 6.53.

'Cipolla' juice had a very good olfactory intensity with pear, fresh apple, grape must and floral notes and an amber colour. Its taste was very sweet and its acidity was the lowest of the three juices. The aroma was intense and pleasant with cooked apple, wood and caramel notes. It had a score of 6.81 (Figure 3).



Coefficient	'Nesta'	'Panaia-red'	'Cipolla'	*P*-value
a	22.3 A	21.2 B	17.7 C	***
b	-14.43 B	-20.7 A	-13.7 C	***
r	0.96	0.99	0.98	-

FIGURE 2. Inhibition percentages of DPPH radical in 'Nesta', 'Panaia-red' and 'Cipolla' apple juices. Logarithmic curves (percentage of inhibition of DPPH$^\bullet$ = (a × ln (t) + b)) in the graph are representative of the different apple varieties. Data ($n=3$) are mean ± standard deviation. ANOVA P-values: $p\leq0.001$=***.
In the same row, different letters indicate significant differences according to LSD-test at $p=0.05$. r is the correlation coefficient of the regression curves.

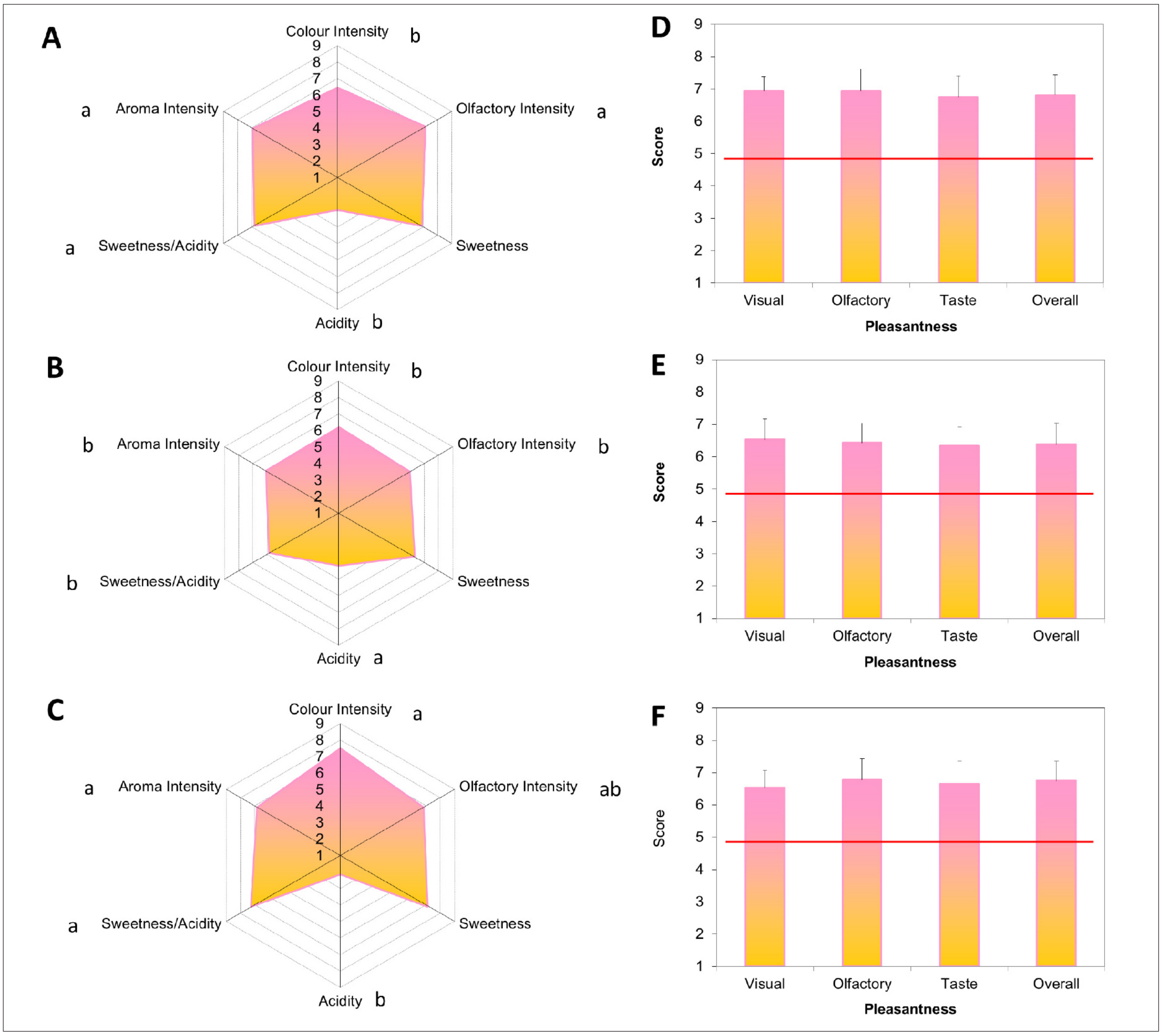


Figure 3. Juice panel test results: A) 'Nesta' sensory fingerprint; B) 'Panaia'-red sensory fingerprint; C) 'Cipolla' sensory fingerprint; D) 'Nesta' pleasantness score; E) 'Panaia-red' pleasantness score; F) 'Cipolla' pleasantness score. Sensory data are mean values and pleasantness data are mean ± standard deviation. Data of the sensory fingerprint were analysed by Kruskal-Wallis ANOVA test ($n=48$; df = 2) and if significant, pairwise sample mean comparison was done using Mann-Whitney test. Different letters for the same sensory trait in the different varieties indicate significant differences at $p=0.05$. In pleasantness graph the red line at 5 mark the minimum threshold required by the sensory analysis.

Discussion

In this study the phenolic content, the antioxidant capacity and the aromatic profile of the juice obtained from three ('Nesta', 'Panaia-red' and 'Cipolla') ancient Italian apple varieties were studied.

The phenolic profiles of the 'Nesta', 'Panaia-red' and 'Cipolla' juices were evaluated by the determination of the TP, the TF, the CT and the FVN. Iacopini and collaborators (2010) studied the antioxidant properties of four ancient Italian apple varieties ('Mora', 'Nesta', 'Panaia-red' and 'Ruggine') in comparison with two, well known, commercial varieties ('Golden Delicious' and 'Stark Delicious'). In their research, TP and TF contents were analysed in whole apples and although the units of measurement are different (per fruit FW vs. per juice volume), the results, nonetheless, shows that 'Panaia-red' was the apple with the highest content of TP and TF with the 'Nesta' having the lowest amount of TP and TF (Iacopini et al., 2010). The amount of TP and TF recovered in the juices were higher when the variety had higher values of phenolic compounds in the fruits. Kahle and collaborators (2005) analysed the phenolic content of several commercial apple juices. The amount of total polyphenols was in the range of 152.2 ÷ 459.0 mg L^{-1}. In comparison with these commercial apple juices, 'Nesta', 'Panaia-red' and 'Cipolla' juices had approximately double the content of polyphenols. 'Panaia-red' was also one of the varieties with the highest fruit antioxidant capacity (Iacopini et al., 2010). These results are in accordance with previous works, where the phenolic content and the antioxidant activity of 'Nesta', 'Panaia-red', 'Cipolla' and 'Golden Delicious' apple juices were investigated (Felice et al., 2015; Maragò et al., 2015). In addition, 'Panaia-red' apple juice resulted to have significant antioxidant capacity on human umbilical vein endothelial cells (HUVECs) stressed by H_2O_2 (Felice et al., 2015).

Table 4. Normalized volatile compounds (% of peak area relative to specific target ions) in apple juices in apple juices of 'Nesta', 'Panaia-red' and 'Cipolla' varieties. Data were analysed by Kruskal-Wallis ANOVA test ($n=27$; df=2), (*=p<0.05, n.s.=not significant). In the same row, different letters indicate significant differences according to Mann-Whitney test. Aroma descriptors at the given concentrations are reported from http://www.thegoodscentscompany.com/.

Compound	'Nesta'	'Panaia'	'Cipolla'	P-values	RT (min)	Aroma descriptors	Conc %
Acetaldehyde	3.49	2.63	1.01	n.s.	3.8	Pungent, ethereal, aldehydic, fruity	0,1
Ethyl acetate	1.22 B	0.16 C	9.99 A	*	5.97	Ethereal, fruity, sweet, weedy green	10
Ethanol	4.09	2.06	4.07	n.s.	6.7	Strong alcoholic, ethereal medical	100
Unknown 1	0.31 B	0.02 C	0.88 A	*	6.9	NR	
Ethyl propionate	0.16 B	0.01 C	6.63 A	*	7.2	Sweet fruity rum juicy fruit grape pineapple	10
Ethyl isobutanoate	1.64 A	0.19 B	0.27 B	*	7.3	Sweet, etherial and fruity with pungent, alcoholic, fusel and rummy nuances	10
Propyl acetate	2.03	0.18	2.44	n.s.	7.5	Solvent, celery, fruity, fusel, raspberry, pear	10
Methyl butanoate	0.00 B	0.00 B	0.16 A	*	7.7	Fruity apple sweet banana pineapple	10
2-methylbutanoic acid	0.05 B	0.01 C	6.42 A	*	8.16	Pungent acid roquefort cheese	10
Isobutyl acetate	0.66 B	0.02 C	4.71 A	*	8.18	Sweet fruity ethereal banana tropical	10
1-propanol	7.93 A	2.49 B	1.12 C	*	8.52	Alcoholic fermented fusel musty	10
Ehtyl butanoate	0.07 B	0.003 C	14.22 A	*	8.6	Fruity juicy fruit pineapple cognac	1
Ethyl 2-methyl butanoate	0.09 B	0.01 C	4.34 A	*	8.87	Sharp sweet green apple fruity	10
Butyl acetate	0.95 B	0.50 C	2.48 A	*	9.20	Ethereal solvent fruity banana	10
1-propanol-2-methyl	1.46	1.36	0.77	n.s.	9.5	Ethereal winey	10
1-butanol-2-methyl acetate	1.31	5.49	2.33	n.s.	9.99	Over ripe fruit sweet banana juicy fruit	100
Ethyl pentanoate	0.17	0.07	0.05	n.s.	10.21	Sweet fruity apple pineapple green tropical	10
Propyl isovalerate	0.02 A	0.00 B	0.08 A	*	10.28	Bitter sweet apple fruity	100
1-butanol	24.03 A	26.63 A	8.21 B	*	10.5	Fusel oil, sweet, balsam	10
Ethyl butenoate	0.03	0.04	0.40	n.s.	10.85	Pungent chemical diffusive sweet allicious caramel rum	10
1-butanol-3-methyl acetate	11.49 B	13.07 A	2.65 C	*	10.88	Sweet, banana, fruity with a ripe estry nuance	1
1-butanol-2-methyl + 1-butanol-3-methyl	11.36 A	4.51 B	4.23 B	*	10.46	Roasted wine onion fruity	100
(E)-2-hexenal	3.75	1.01	1.17	n.s.	11.5	Green, banana, aldehydic fatty, cheesy	10
Unknown 2	0.10	0.03	0.10	n.s.	11.8	NR	
Ethyl tiglate	0.00	0.00	0.01	n.s.	12.07	Sweet fruity tutti frutti tropical berry floral caramel	10
1-pentanol	0.95	1.20	0.17	n.s.	12.17	Alcoholic, fusel, fermented, chocking and musty with sweet white wine top notes with over ripe banana and yellow apple nuances.	1
Hexyl acetate	0.07	0.01	0.15	n.s.	12.56	Fruity green apple banana sweet	10
Enknown 3	0.15	0.17	0.06	n.s.	13.4	NR	
3-hydroxybutanone	3.60 B	9.11 A	1.29 B	*	13.60	Sweet buttery creamy dairy milky fatty	1
1-hexanol	7.94 B	21.00 A	6.83 B	*	13.92	Ethereal, fusel oil, fruity, alcoholic, sweet, green	10
2-hexen-1-ol	1.41 A	3.29 A	0.45 B	*	14.91	Fresh, green leafy, fruity, unripe banana	10
Unknown 4	5.39 A	3.27 B	2.02 B	*	14.9	NR	
Acetic acid	0.37	0.25	0.46	n.s.	15.87	Sharp, pungent, sour, vinegar	10
Unknown 5	0.19	0.07	0.07	n.s.	15.9	NR	
Uurfural	1.01	0.31	0.40	n.s.	16.4	Sweet, woody, almond, fragrant baked bread	1
Unknown 6	1.42	0.28	0.36	n.s.	16.6	NR	
Unknown 7	0.26	0.13	0.11	n.s.	16.9	NR	
Ethyl 3-hydroxybutanoate	0.04 B	0.01 C	0.24 A	*	17.29	Fruity, green grape, tropical, apple skin	10
Benzaldehyde	0.29 A	0.12 B	0.21 A	*	17.9	Strong sharp sweet bitter almond cherry	10
Ethanol (2-ethoxy ethoxy)	0.19	0.13	0.85	n.s.	19.37	Slightly ethereal	100
Ethyl benzoate	0.03	0.01	0.03	n.s.	20.71	Fruity dry musty sweet wintergreen	100
B-damascenone	0.08 A	0.02 B	0.05 B	*	23.8	Natural sweet fruity rose plum grape raspberry sugar	10
Benzenethanol	0.14	0.16	0.26	n.s.	25.6	Sweet, floral, fresh and bready with a rosey honey nuance	10

[a] RT = retention time; NR = not reported; * = $p \leq 0.05$.
Aroma descriptors at the given concentrations are reported from http://www.thegoodscentscompany.com/.

The flavour of apple juice arises from many chemical compounds, and 8–23 of them seem to be responsible for the odour–flavour attribute (Konig and Schreier, 1999; Aprea et al., 2012). Aguilar-Rosas and collaborators (2007) properly identified eight volatile compounds in apple juices and analysed also the percentage losses in volatile concentration among fresh apple juice and two different kind of pasteurized juices (Aguilar-Rosas et al., 2007). Aprea and collaborators (2012), instead, identified seventy-two compounds in eighteen different apple cultivars, with the majority of esters. The volatile profiles reported in literature for other apple ('Jonagold', 'Golden Delicious', 'Braeburn', 'Granny Smith', 'Jazz' and 'Pink Lady') juices, indicate that 1-butanol was the most abundant alcohol and volatile compound (Komthong et al., 2007; Gan et al., 2014).

On the bases of the olfactory characteristics of the compounds retrieved in the aroma profile we found some correspondences between the sensory and aromatic data. 1-butanol, that was associated to sharp olfactory notes in cider (Mangas et al., 1996), was most represented in 'Panaia-red' followed by 'Nesta' and 'Cipolla' juices, in agreement with the acidity sensation perceived by the tasters (Figure 3). In 'Panaia-red' the woody and floral notes are probably provided by 1-hexanol, 2-hexenal and ethyl benzoate. On the other hand, ethyl butanoate, ethyl acetate, ethyl butenoate and butyl acetate, which correspond to the fresh apple, caramel, sweet, apple, pear, pineapple and fruity flavour, were higher in 'Cipolla' juice in agreement with the perceived sensory characteristics.

Recently, Gan and collaborators (2014) studied the aroma profile of apple juices produced by five different apple cultivars ('Golden Delicious', 'Braeburn', 'Granny Smith', 'Jazz' and 'Pink Lady') harvested in three different countries (New Zealand, South Africa and Chile). They identified 16 volatile flavour compounds, mainly esters, alcohols and carboxylic acids. In all the juices, acetates, specifically butyl- and hexyl- were the dominant esters acetate (in a range of 4.76×10^{-1} – 6.0×10^{-3} µl L^{-1} for butyl acetate and 6.59×10^{-1} – 3.50×10^{-3} µl L^{-1} for hexyl acetate) and they were associated with the fruity and sweet odour descriptors.

Food aroma is not delivered only by volatile compounds. In fact, flavour is the combination of volatile and non-volatile compounds (as taste and texture) released during eating. This combination of compounds is responsible for tastes, perceived by taste buds, by receptors present in the oral cavity and for olfactory sensations, perceived retro-nasally by the olfactory epithelium (Deibler et al., 2004). Modifications to a product's non-volatile composition may have a significant impact on the aroma profile. Two of the major components of polyphenols flavour are bitterness and astringency. Astringency perception is connected with a formation of a complex between polyphenols and proteins in the mouth. Bitterness, on the other hand, is caused by the interaction between polar molecules and the lipid portion of the taste papillae membrane (Spanos and Wrolstad, 1992). The balance between bitterness and astringency depends on the molecular weight of polyphenols. For that reason, condensed tannins are the major compounds linked with these flavours. Moreover, sweetness is inversely proportional to condensed tannin content (Spanos and Wrolstad, 1992). This is in accordance with our results. 'Panaia-red' juice had the highest value of condensed tannins and thus lowest value of sweetness. The taste of 'Panaia-red' juice was nonetheless good, with an overall panel score of 6.53. In conclusion, apple juices produced by ancient Tuscan varieties had double the content of polyphenols in comparison with commercial apple juices studied (Kahle et al., 2005). They also exhibited good antioxidant properties (with respect to inhibition of the DPPH radical) and a good organoleptic characteristics specific to each variety. Therefore, these findings demonstrate that it is possible to produce apple juices rich in polyphenols still maintaining a good taste and aromatic profile. However, the assessment of the method used for sensory evaluation to reduce assessors' differences is also very important (Bavay et al., 2013). For that reason, it is important to conduct this kind of experiment, according to the recommendations of ISO standards and guidelines.

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