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Title: Ancient apple cultivars from Garfagnana (Tuscany, Italy): a potential source for 'nutrafruit' production

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Keywords: ancient cultivars; antioxidant capacity; apple; cluster analysis; organoleptic properties; phenolic profile

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Abstract: Ancient apple cultivars are known for their organoleptic properties over a small geographic area, but little is known of their nutraceutical properties which might be useful in large-scale breeding programs. Nine ancient apple cultivars from Tuscany (Italy) were characterized for their organoleptic properties, phenolic profile and antioxidant activity. These cultivars had a high polyphenol concentration (principally flavanols and phenolic acids) and high total antioxidant capacity compared to most commercial apple cultivars. Fruit from the cultivars 'San Michele' and 'Del Debbio' showed a good compromise between fruit size and solid soluble content and might be suitable for fresh consumption, while fruit from 'Benito', 'Della Piastra', 'Lugliese Grisanti', 'Del Sangue' and 'Ruggine' had a high polyphenol content and excellent antioxidant capacity and may be suitable for breeding programs. 'Ruggine' fruit could also be used for sweet juices with high nutraceutical properties due to a high soluble solid content and high flavanol concentration.

Cover Letter

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Pisa, 15th January 2019

The Editor of *Food Chemistry*

Dear Editor,

We have revised our manuscript: "Ancient apple cultivars from Garfagnana (Tuscany, Italy): a potential source for '*nutrafruit*' production" following the suggestions of the referee 1 and receiving editor. We are grateful to both for their useful comments which have been very helpful in improving the manuscript. Please see in the attached file "responses to referees' comments" details of how we have accommodated the referees' suggestions and comments.

Best Regards,

Dr. Marco LANDI, PhD

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*Response to Reviewers

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Dear Editor,

We have revised our manuscript: "Ancient apple cultivars from Garfagnana (Tuscany, Italy): a potential source for '*nutrafrui*t' production" following the suggestions of the referee 1 and receiving editor. We are grateful to both for their useful comments which have been very helpful in improving the manuscript. Please see below details of how we have accommodated their suggestions and comments.

COMMENTS

Reviewer 1: It is appropriate to give brief information about the general characteristics, consumption patterns and production quantities of these local apple varieties in the introduction section of the article.
Our response: we already considered these aspects of crucial importance when writing the manuscript; however, our local varieties belong to a local germplasm collection and the data required by the referee are not available.

In the conclusion section, the findings are written as a result. Overall contribution to the science of this study should be written in more detail to the conclusion section.
Our response: thank you for that suggestion. We completely agreed with the referee's comment and therefore the conclusion section was completely rewritten with the goal to highlight the contribute to the science of our study rather than simply summarizing the main findings of our research.

The highlights are not remarkable. They should be reviewed.
Our response: We have completely refresh the highlights with the same attempt (of the previous point) to figure out the main contributes to the science of our study as well as its core of novelty.

This research is important for the introduction and dissemination of local apple varieties in Italy. However, the research seems to be more important on a local scale. So, more detailed information about the overall contribution of this research should be given.
Our response: Thank you for this critic point of view about this point of weakness. In accordance, we have mentioned in key sections (abstract, discussion, conclusions) that the use different old varieties, as those screened in our study, can be relevant not only at a local scale. We highlighted that some cultivars could be useful for different purposes on the base of their different characteristic; in particular, some of them could be useful for large-scale breeding programs, other cultivars for the production of juice with a high nutraceutical values, which is not only restricted to local uses.

Line 108-109 and Line 270-273 are written red underlined.
Our response: we apologize for this. The sentences were corrected.

Andre, C. M., Greenwood, J. M., Walker, E. G., Rassam, M., Sullivan, M., Evers, D., Perry, N.B., & Laing, W.A. (2012). Anti-inflammatory procyanidins and triterpenes in 109 apple varieties. Journal of Agricultural and Food Chemistry, 60(42), 10546-10554. https://doi.org/10.1021/jf302809k. Reference was given above not cited in the text but added to the references section. It should be corrected.
Our response: the reference was deleted from the references' list.

RECEIVING EDITOR'S COMMENTS:

The highlights are intended to showcase your most important results. Currently, they are mundane and lack context, requiring that the manuscript is read first. REVISE.
Our response: We have completely refresh the highlight with the attempt to figure out the main contributes to the science of our study as well as its core of novelty.

20 In many countries where apples have been growing for centuries, ancient apple cultivars are known locally, but few data are available describing the potential benefits of consumption or features that could be useful for apple breeding programs. Nine ancient cultivars from Garfagnana (Tuscany, Italy) were characterized for their phenolic profile and antioxidant activity. Besides cultivar-specific features, generally, a high polyphenol content (principally flavanols and phenolic acids) and total antioxidant activity were found with respect to these ancient apple cultivars. 'Rossa di Corfino' and 'Del Giappone' did not present relevant quality characteristics.
Our response: we have considered the changes proposed by the referee when revising the manuscript. However, the final outcome can be slightly different given that the final version was revised by a native speaker who also simplify and merged some sentences together.

27 Conversely, 'San Michele' and 'Del Debbio' were suitable for fresh consumption. - presumably most eating apples are "suitable for fresh consumption", be more specific.

Our response: Thank you for this suggestion. We have refreshed as follow: "'San Michele' and 'Del Debbio' fruits hadshowed a good compromise between fruit size and solid soluble content and might bewere suitable for the fresh consumption."

27 'Benito', 'Della 27 Piastra', 'Lugliese Grisanti', 'Del Sangue' and 'Ruggine' had a high polyphenols content and excellent antioxidant activity, making them also suitable for apple breeding programs. Finally, 'Ruggine', due to high soluble solid content and flavanol content, could be used for production of juice with putative health benefits.
Our response: we have considered the changes proposed by the referee when revising the manuscript. However, the final outcome can be slightly different given that the final version was revised by a native speaker who also simplify and merged some sentences together.

35 Apples cultivation in Western countries offers a high return on investment, largely because of mechanization, although highly skilled labour is need. Although hundreds of apple cultivars exist, globally, production is linked to just a few (...), such as Red Delicious, Golden Delicious, Gala and Fuji. The selection of these popular and extensively utilised cultivars has results in a uniformity of commercial apple orchards (...) and a dramatic loss in genetic biodiversity. However, interest in the preservation of autochthonous genetic heritage of fruit species is growing (...): ancient autochthonous varieties could represent an important source of innovative characteristics to satisfy modern fruit culture, such as shape and colour. However, the size, taste and texture of ancient-cultivars is not always commercially attractive, e.g. leathery peel, astringent taste, and a small size.
Our response: we have thoroughly considered the changes proposed by the referee when revising the manuscript. However, the final outcome can be slightly different given that the final version was revised by a native speaker who also simplify and merged some sentences together.

76 Standards for polyphenol profile and 2,2-diphenyl-1-picrydazil (DPPH) were purchased from Sigma-Aldrich (Milan, Italy).
Our response: done

85 About 3 kg of fruits from each cultivar were picked randomly on the commercial ripening date from specific positions on the trees, so that the micro-meteorological and edaphic impact was comparable. - then they weren't picked randomly; REVISE.
Our response: Thank you for this comment. We have revised as follow: **"**About 3 kg of fruit (from three different trees per cultivar) were randomly picked from each cultivar at a consistent position from the tree canopy at the commercial maturity date, so that the micro-meteorological and environmental impacts were comparable."

149 Organoleptic properties of the nine ancient apple cultivars are summarized in Table 1. It is a very basic requirement in scientific writing that actions are reported in the past tense (work was done) and results in the present tense (results are shown)
Our response: Thank you for this comment. We corrected the sentences in which the verb tens were wrong.

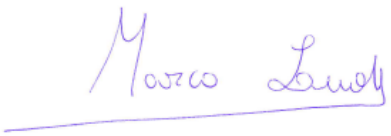
There are significant issues with the English including syntax, grammar, tense and choice of words. These must be resolved with the help of a native speaker or English language editing service, such as that provided by Elsevier https://webshop.elsevier.com/languageservices/languageediting. The authors are advised strongly again trying to make linguistic corrections independently, as this will further delay the paper and may lead to it being rejected.

Our response: the manuscript was carefully revised by a native speaker who is Dr. Annette Richardson from The New Zealand Institute for Plant & Food Research Limited (NZ). Meanwhile editing the manuscript, Dr. Richardson also raised some comments and proposed some suggestions to be undertaken. Therefore, all the co-authors are tankful to Dr. Richardson who was mentioned in "Acknowledgements".

We hope that in this revised version the manuscript might be appropriate for the final acceptance.

Best Regards,

Dr. Marco LANDI, PhD



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Highlights

- ~~Nine ancient Tuscan apple cultivars were analysed for their nutraceutical properties~~
- ~~Organoleptic characteristics and phenol profile varied among cultivars~~
- ~~UPLC–MS coupled with PCA is a useful tool for apple cultivar screening~~
- ~~Different possible uses are proposed for each cultivar~~

- Polyphenolic profile was determined by UPLC-MS in ancient Tuscany apple cultivars
- Flavanols and phenolic acids are the most abundant polyphenols in ancient apples
- Procyanidin B1 has the highest correlation with the fruit's antioxidant capacity
- Phenolic content and antioxidant ability are higher than most cultivated apples
- Ancient apples should be re-evaluated for a "*nutrafruit*" production

Ancient apple cultivars from Garfagnana (Tuscany, Italy): a potential source for '*nutrafruit*' production

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Abstract

~~In many countries where apple has been growing since centuries,~~ A~~a~~ncient apple cultivars are known ~~at~~ for their organoleptic properties over a small geographic area ~~the local level~~, but ~~few data are available~~little is known o~~f~~n their nutraceutical properties~~,~~ which ~~features that~~ might~~could~~ be useful ~~for~~ in large-scale~~genetic~~ breeding programs. Nine ancient apple cultivars from ~~from Garfagnana (~~Tuscany~~,~~ (Italy) were characterized for their organoleptic ~~qualities~~properties, phenolic profile and antioxidant activity. ~~Besides cultivar-specific features,~~These cultivars had ~~generally~~ a high polyphenol concentration~~ntent~~ (principally flavanols and phenolic acids) and high total

antioxidant capacity ~~were found with respect~~compared to most commercial apple ~~apples~~ cultivars. ~~Cultivars 'Rossa di Corfino' and 'Del Giappone' did not show relevant quality characteristics.~~ Fruit from the cultivars ~~Conversely,~~ 'San Michele' and 'Del Debbio' ~~fruits had~~showed a good compromise between fruit size and solid soluble content and might be~~were~~ suitable for ~~the~~ fresh consumption, w~~W~~hile fruit from 'Benito', 'Della Piastra', 'Lugliese Grisanti', 'Del Sangue' and 'Ruggine' ~~, showed~~ had a high polyphenol~~s~~ content and excellent antioxidant capacity ~~making them~~and may be ~~also~~ suitable for breeding programs. ~~Finally,~~ 'Ruggine' fruit~~s~~ ~~,~~ could~~an~~ also be used for ~~the~~ sweet juices with high nutraceutical properties ~~because of~~due to a ~~due to~~ high soluble solid content and high flavanol ~~amount~~concentration. ~~can be used for production of juice with high nutraceutical properties.~~

Keywords: ancient cultivars, antioxidant capacity, apple, cluster analysis, organoleptic properties, phenolic profile

1. Introduction

Apples cultivation in Western countries offers a high return on investment, largely because represents one of the high-income crops, with a high of high mechaniszation, level andalthough highly skilled labour needsis also required. Although hundreds of apple cultivars exists , globally, most apple due to a large number of breeding programs, in the world the production is linked to just a few groups ofis from a few cultivars (Hokanson, Lamboy, Szewc-McFadden & McFerson, 2001) such as 'Red Delicious', 'Golden Delicious', 'Gala' and 'Fuji' (Hokanson, Lamboy, Szewc-McFadden & McFerson, 2001). In addition, the clonalThe selection ofextensive use of these popular and extensively utilised cultivars has resulteds in determined a uniformity of commercial apple orchards (Donno et al., 2012; Cerrutti, Bruun, Donno, Beccaro & Bounous, 2013), and which has led a dramatic loss inof genetic biodiversity.

NowadaysCurrently, the interest for in the preservation of autochthonous genetic heritages of fruit species is growing (Wojdylo, Oszmianski & Laskowski, 2008; Cerrutti et al., 2013; Ferreira et al., 2016). The ancient autochthonous varieties could represent an important source of innovative characteristics to satisfy theneeded for modern fruit-culture, such as particular fruit shapes, new tastes and , different peel and pulp colours in peel and pulp. However, often fruit of these 'ancient cultivars' also display unappreciated unacceptable organoleptic qualities such as leathery peel, astringent taste compounds and , but also a small size making them commercially unattractive.

In the last years,Recently the concept of food in Western countries has changed in Western countries whereso that, in addition to excellent organoleptic characteristics, consumers also require food with nutraceutical properties useful forthat benefit human health (Lobo, Patil, Phatak & Chandra, 2010; Roche et al., 2015). SurelyUndoubtedly, oOne of the most important features that make apple fruits interesting for researchers is their high polyphenolic content in polyphenols (Jakobek & Barron, 2016; Jakobek, García-Villalba & Tomás-Barberán, 2013; Maragò, Michelozzi, Calamai, Camangi & Sebastiani; 2016). These metabolites have been intensively studied in epidemiological research because they are particularly important crucial for the human diet as they

play a positive role in reducing the risk of many cardiovascular diseases and showed anticancer~~ogenic~~ properties (Rasouli, Farzaei & Khodarahmi, 2017). The main polyphenols ~~contained~~ found in apple fruit~~s~~ are anthocyanins, dihydrochalcones, flavanols, flavonols, hydroxybenzoic acid and hydroxycinnamic acid~~s~~ (Neveu et al., 2010). However ~~even though their~~ the concentration of polyphenols in fruit ~~is~~ can be strongly influenced by many factors ~~such as~~including geographic~~al~~ region, agronomic techniques, ~~conservation~~ fruit storage technique,~~method, stage of~~ fruit maturity ~~and~~ as well as fruit ~~variety~~ cultivar (Awad, Wagenmakers & De Jager, 2001; Treutter, 2001).

~~Besides of constitutive high level of phenols in apple cultivars, it~~It has been ~~displayed in many cases~~suggested that ancient apple cultivars could have a higher content of ~~these~~ nutraceutical compounds ~~compared to~~than commercial apple cultivars (Maragò et al., 2016; Iacopini, Camangi, Stefani & Sebastiani, 2010). Therefore, the ~~aim~~ goal of new research~~es~~ should be the selection of ~~some~~ ancient cultivars with appreciable organoleptic characteristics and nutraceutical content for the fresh fruit market or~~, alternatively,~~ the selection of ancient cultivars ~~more~~ with suitable properties for juice production.

In this work, we characterized the fruit of nine ancient Italian apple cultivars from Italy (Garfagnana, Tuscany) for their organoleptic qualities, polyphenol~~s~~ profile and antioxidant activity, and ~~on the basis of~~ used these ~~ir~~ characteristics ~~we~~ to suggest ~~different~~ possible ~~utilization~~uses for cultivars. The cultivars are enrolled in the bank of germplasm of Tuscany region (Regional Law N. 64, 16th November 2004).

2. Materials and methods

2.1. Reagents, solvents and standards

Methanol, formic acid and acetronile were purchased ~~by~~ from Carlo Erba Reagents (Cornaredo, Milan, Italy). Standards for polyphenol profile and 2,2-diphenyl-1-picrydazil (DPPH) were purchased ~~by~~ from Sigma-Aldrich (Milan, Italy).

2.2. Apple sampling and organoleptic characteristics

~~Fruits~~Fruit from ancient apple cultivars (*Malus domestica* Borkh.; 3-5 plants per cultivars) were harvested from an orchard situated at the experimental field "Centro vivaistico La Piana" in Camporgiano (44°08'17'' N, 10°19'05'' E; Lucca, Italy) ~~,~~which is located in the historical and geographical region of Garfagnana.~~Lucca (Italy)~~. The nine cultivars have been identified by ~~a code~~the abbreviations: B ('Benito'), RC ('Rossa di Corfino'), DP ('Della Piastra'), DG ('Del Giappone'), LG ('Lugliese Grisanti'), SM ('San Michele'), DD ('Del Debbio'), DS ('Del Sangue'), R ('Ruggine').

About 3 kg of fruit (from three different trees per cultivar) were randomly picked ~~of fruits~~ from each cultivar ~~were picked randomly (but~~ at a consistent ~~distance~~position from the tree canopy~~)~~ ~~on~~at~~at~~ the commercial ~~ripening~~ maturity date ~~randomly from specific positions on the trees~~, so that the micro-meteorological and ~~edaphic~~ environmental impacts ~~was~~ were comparable. Morphometric analyses of fruit were carried out using standard descriptors for apples (Bellini, Giordani, Picardi & Giannelli, 2008), using ten ~~fruits~~fruit of each cultivar. Physical and chemical harvest indexes were estimated on three randomly selected ~~fruits~~fruit per cultivar~~s~~ as follows: (i) fresh weight (FW) (g), height (mm), width (mm) and height/width ratio of ~~fruits~~fruit; (ii) flesh firmness (expressed as Kg cm^{-2}) was measured on a sample of ~~fruits~~fruit after removing the skin, on two opposite sides of each fruit, with a digital penetrometer equipped with an 11 mm tip (TR Snc, Forlì, Italy); (iii) soluble solid content (SSC, °Brix) was determined on a flesh juice sample using a digital refractometer (~~refractometer~~ Mod. 53 011, Turoni, Forli, Italy). Harvested ~~fruits~~fruit were then stored ~~in a cold chamber~~ at 2 °C and 95% relative humidity for one~~1~~ month before bio~~sampling~~chemical analysis. ~~At sampling, fruits~~Fruit were carefully washed twice with tap water and ~~finally~~ once with distilled water~~. Fruits were then~~ before they were peeled and ~~sliced with a sharp knife before removing~~ the core ~~portion~~ and seeds removed. ~~After that the s~~Slices were then

cut into small ~~portions~~ pieces (approximately 10 x 10 x 5~~0.5~~ m~~c~~m), randomly allocated to~~ized~~ ~~in~~ falcon tubes, frozen in liquid nitrogen~~N$_2$~~ and stored at -80 °C ~~until~~ for biochemical analys~~e~~is.

2.3. Phenol extraction

~~Flesh~~ A~~a~~pple flesh samples (about 1 g FW) were homogenized with 10 mL of 70% (v/v) 99.5% HPLC grade methanol by sonication for 30 min, keeping the temperature from 0 to 4 °C. After centrifug~~at~~ing samples at~~on (~~ 6000 *g* for 10 min at 4 °C~~)~~, the supernatant was collected and filtered with PTFE filters (0.20 µm pore size; Sarstedt, Verona, Italy). Each sample replicate consisted of FW material belonging to three different fruits of the same tree. Extracts were stored at −80 °C before analysis.

2.4. UPLC–MS analysis~~s method~~

The ~~P~~phenolic profile was determined as described in Assumpção et al. (2018) with ~~few~~ some modifications. The UPLC–MS analysis was performed using an Agilent 1290 Infinity II LC system (Agilent Technologies Italia S.p.A., Cernusco Sul Naviglio, Italy) consisting of a degasser, a binary pump, an autosampler, a column oven and ~~equipped with~~ an Agilent 6495A triple quadrupole MS with a~~. A~~ C18 column, 2.1 × 50 mm, 1.8 µm (Agilent Zorbax Eclipse Plus, Santa Clara, CA, USA) ~~was used~~ for separation of phenolic compounds. Solvent A consisted of 0.2% formic acid in water whereas solvent B was 0.2% formic acid in acetonitrile. The elution gradient was: 6% B (3 min), from 6 to 30% B ~~(in~~ 11 min), from 30 to 100% B ~~(in~~ 2 min), 100% B (2 min). The column temperature was 35 °C, the flow rate was 0.3 mL min^{-1}, and the injection volume was 2x10^{-6} L. MS parameters employed were as follows in ESI(+): gas temp: 150 °C; gas flow: 13 L min^{-1}; nebulizer: 50 psi; sheath gas heater: 350 °C; sheath gas flow: 12 L min^{-1}; capillary: 3500 V, HPRF funnel: 120; LPRF funnel: 40; in ESI(−): gas temp: 150 °C; gas flow: 13 L min^{-1}; nebulizer: 50 psi; sheath gas heater: 350 °C; sheath gas flow: 12 L min^{-1}; capillary: 1500 V; HPRF funnel: 120; LPRF funnel: 80. For quantification, an external standard method was used. A calibration curve was

constructed for each compound analysed ~~in~~ with at least five different concentrations from 1 to 500 μg L^{-1} ~~was constructed for each compound analysed~~ and ~~utilized to~~this was used to quantify the concentration of each compound in the samples. Data are expressed as μg g^{-1} FW.

2.5. Antioxidant capacity~~activity~~

A~~To assay the a~~ntioxidant capacity~~,~~ was measured ~~following~~using the method reported by Brand-Williams, Cuvelier and Berset (1995) ~~was followed~~. Briefly~~,~~ 15 μL of phenolic extract were added to 990 μL of a solution containing $3.12x10^{-5}$ M DPPH in methanol. The decrease in absorbance at 515 nm was measured against a solution blank (without extract) after a reaction time of 30 min at room temperature (~~that was preliminary~~ optimised ~~to observe~~for the highest antioxidant ~~effect of~~concentrations in the extract) using a spectrophotometer (Ultrospec 2100 pro, GE Healthcare Ltd., Chalfont St. Giles, Buckinghamshire, UK). Results were expressed as percentage of reduction of the initial DPPH absorption by the extracts and expressed as μmol Trolox g^{-1} FW.

2.6. Statistical analysis

Data ~~are expressed as mean ± standard deviation and are~~were subjected to a one-way ANOVA test and statistical differences ~~among~~ between the nine apple cultivars were calculated by least significant difference (LSD) test at 95% of confidence with GraphPad Software (GraphPad, La Jolla, USA). Data are expressed as mean ± standard deviation. Pearson's correlation coefficient between phenolic compounds and total antioxidant activity was carried out with GraphPad software. Hierarchical clusters, to group cultivars with similar phenolic profiles, were determined~~were carried out~~ using Ward's method~~, in order to see similarities between cultivars under phenolic aspect~~. The polyphenolic~~s~~ profile of each replicate (n=3) of the nine ancient cultivars were ~~processed~~ analysed using the Principal Component Analysis (PCA) ~~performing on the covariance matrix~~, to determine which phenols ~~most~~ contributed to ~~creating~~

distinctionsdifferences in the dataset. The Cluster and PCAs analysis were conducted using IBM SPSS Statistics 24 (IBM, New York, USA).

3. Results and Discussion

3.1. Organoleptic characteristics

Organoleptic properties of the nine ancient apple cultivars wereare summarized in Table 1. Significant differences were revealedappear amongwere found between the apples cultivars for fruit weight, height, width, firmness and soluble solids content (SSC). Fruit fresh weight varieddsd from 88.8 g for LG to 256.0 g forwith SM having the highest weight and LG the lowest one (Table 1). The cultivar SM together withand the cultivar DD also had also the highest fruit height and width and. In addition, DD fruitsfruit also had the highest height/width ratio (1.02 ± 0.02) as compared towhile fruit from the average all the other cultivar fruitsfruit (values ranged from 0.83 to 0.86.; mean average 0.84±0.04).

Lower values of fruit flesh firmness were recorded in the cultivars LG, DS and DD cultivars (4.38, 6.86 and 6.94 kg cm^{-2}, respectively) and the highest vales were in fruit from B and DP cultivarstrees (9.97 and 9.43 kg cm^{-2}, respectively). In most cases the values of fruit flesh firmness from this study were similar to those found in commercial cultivars harvested at commercial maturity , as in this study (Iacopini et al., 2010). Lower values were recorded in LG, DS and DD cultivars (4.38, 6.86 and 6.94 kg cm^{-2}, respectively) and the highest in B and DP cultivars (9.97 and 9.43 kg cm^{-2}, respectively).

The soluble solid content of fruit is an important quality parameter to test mainlyindicator particularly of the sugar content in apples. For values offruit SSC content, the ancient cultivars are were subdivided into three groups: DD, DS, LG and R cultivars fruit had with a significantly higher value of SSC (mean value of 16.5 ° Brix) than fruit from other cultivars, RC, DP and SM fruit were grouped in the middle with a mean SSC value of 13.9 ° Brix and, finally, fruit from B and DG cultivars with lowerhad the lowest values (12.1 ° Brix). Values reached byThe SSC values of fruit

from B and DG trees wereare lower thano those of commercial apples cultivars that usually range between 13 and 20° Brix (Drogoudi, Michailidis & Pantelidis, 2008; Iacopini et al., 2010).

3.2. Polyphenolic component

Twenty-seven phenolic compounds from five main groups (flavonols, flavanols, anthocyanins, dihydrochalcones and phenolic acids) were found fruit of inthe ancient apple cultivars and subdivided into five groups (flavonols, flavanols, anthocyanins, dihydrochalcones and phenolic acids) (Table 2). The most common compounds were from Among these groups, the most represented were the flavanol (catechin, epicatechin, procyanidin B1, procyanidin B2, procyanidin B3 and procyanidin B4) and the phenolic acid group (chlorogenic acid, neochlorogenic acid, cryptochlorogenic acid, *p*-coumaroyl glucose, *p*-coumaroylquinic acid, gallic acid, caffeoyl glucoside, protocatechuic acid, feruroyl glucose and *t*-cinnamnic acid) with differences among cultivars. Finally, amongThe only anthocyanin, cyanidin-3-O-galactoside, was only found in the fruit flesh from one cultivar (DS)s, only cyanidin-3-O-galactoside was detected in the cultivar DS. In agreement with the literature, tThe main compounds found in fruit from this study were chlorogenic acid and epicatechin and this has been previously found in commercial apple cultivars (Chen, Zhang, Li & Ma, 2012; Bi, Zhang, Chen, Zhang, Li & Ma, 2014) (Chinnici, Bendini, Gaiani & Riponi, 2004; Iacopini et al., 2010) and other studies of ancient cultivars as found in commercial (Chen, Zhang, Li & Ma, 2012; Bi, Zhang, Chen, Zhang, Li & Ma, 2014) and ancient cultivars (Iacopini et al., 2010; Panzella, Petriccione, Rega, Scortichini & Napolitano, 2013, Kschonsek, Wolfram, Stöckl and Böhm, 2018). It has been observed that ancient cultivars in many cases account for higher level of total phenolic and in particular chlorogenic acid (Kschonsek, Wolfram, Stöckl and Böhm, 2018).

The total polyphenolic concentration (TPC) in fruit ofIn eight (B, DP, DG, LG, SM, DD, DS, and R) out of the nine cultivars studied ranged from 703.0 to 1201 µg g^{-1} FW (Table 2), the total polyphenols content (TPC) ranged from 703.0 to 1201 µg g^{-1} FW which is higher than the TPC

values found in fruit from ~~other~~ commercial cultivars such as Golden Delicious, Granny Smith and Red Delicious (Tsao, Yang, Young & Zhu, 2003; Kalinowska, Bielawska, Lewandowska-Siwkiewicz, Priebe, Lewandowski, 2014; Remorini et al., 2015; Masi et al., 2017) (Table 2). However ~~W~~we are aware that t~~T~~he comparison between the ~~of~~ results of the present experiment and those of ~~obtained in this work to those of~~ other studies ~~might~~ may be misleading given that~~,~~ ~~as~~ phenolic composition of apple fruit can also be influenced by agronomic and environmental~~al,~~ ~~edaphic and more in general and pedo-climatic conditions~~ factors (Awad et al., 2001; Treutter, 2001). To overcome the confounding effects of environmental factors over the genetic background of cultivars ~~Only~~ the s~~S~~creening of commercial and ancient cultivars would need to be carried out under ~~grown in~~ the same experimental conditions ~~would, avoids the possible confounding effects of environmental factors over the genetic background~~.

To ~~highlight~~ determine the similarities and ~~the~~ differences in the polyphenolic profile among cultivars, ~~in terms of~~regarding ~~polyphenol profile, in Figure 1~~ a heatmap, ~~which represents with different colours~~of the four major phenolic groups found in the ~~apple~~ flesh of fruit from each cultivar and ~~a~~ a cluster analysis are ~~reported~~shown in Fig. 1. The cluster analysis divided~~s~~~~ed~~ the nine cultivars into three clusters ~~by choosing~~using a relatively large and safe cutting value at the linkage distance of 10. The first cluster wa~~i~~s~~was~~ composed ~~by a large part~~ of the cultivars ~~(~~R, DP, B, DD and SM~~)~~, the second ~~one~~ cluster ~~consist~~consisted~~s~~ of~~was composed by~~ cultivars LG and DS ~~cultivars~~, while ~~the last group was composed by~~ RC and DG ~~cultivars~~ belonged ~~s~~ to the third ~~group~~cluster. ~~In terms of~~ ~~Regarding~~In terms of polyphenol~~s~~ composition, the first cluster~~group~~ was generally characterized by fruit with a high flavanol content ~~in flavanols~~ and a low ~~level~~ ~~amount~~concentration of phenolic acids ~~with the exception~~except for ~~of~~ fruit from~~the~~~~for exception to~~ cultivar B, which ~~also associated~~had a high level of dihydrochalcones and phenolic acids ~~to~~ as well as a high flavanol content ~~even high level of dihydrochalcones and phenolic acids~~. Cultivars from t~~To t~~he second ~~group is~~cluster were characterized by ~~belong cultivars with~~ a high content of phenolic acids and a low content in flavanols in fruit, whereas the last cluster of cultivars ~~was~~

~~characterized composed by cultivars~~produced fruit with both a ~~a~~ low ~~content in~~ flavanol~~s~~ and a low phenolic acid content~~s~~.

To ~~investigate unveil~~examine ~~deeper~~ the differences in fruit polyphenolic compositions~~s~~ which ~~allow~~ underpinned the separation of ~~the~~ cultivars ~~to be separated~~ into the cluster~~s~~ shown in Fig. 1, a principal component analysis (PCA) ~~on the polyphenol profile~~ was performed (Fig. 2). The first two principal components (PC1 and PC2) accounted for 57.80 and 33.72% of the total variance in~~of~~ the original data. In the loading plot (Fig. 2a), the first dimension was determined ~~principally consists was mainly constituted by~~~~of~~by epicatechin, procyanidin B3 and B2 concentrations, whilst ~~primarily~~ chlorogenic acid concentration determined the~~originates~~~~formed the~~ second component with positive scores indicating high concentrations of compounds. ~~As shown in the score plot (Fig. 2b), t~~There ~~has been~~~~is a~~was considerable differentiation ~~among~~ between apple cultivars in the four groups a~~As~~ shown in the score plot (Fig. 2b),~~.~~ Cultivars R, DP, SM and DD showe~~d~~~~d~~ positive scores for PC1 and negative scores for PC2, except ~~for~~ ~~B~~ cultivar B ~~that~~ showed~~s~~~~d~~ a positive score for both PC ~~the~~ components~~. A negative score in PC2 indicates a low chlorogenic acid content~~. The cultivars DS and LG ~~had reached~~~~reach~~had positive scores for PC2 and negative scores for PC1, indicating that these two cultivars were characterized by a low flavanol~~s~~ content. The remaining cultivars, DG and RC, were characterized by the lowest ~~content in~~ phenol concentration~~s~~ ~~according to the~~with negative scores ~~reached~~ for both principal components. These results generally ~~are in line with~~support the ~~previous~~ cluster analysis showed in Fig. 1 although ~~. However, a discrepancy for the~~ cultivar B ~~has emerged,~~was group~~ed~~~~ing it~~ separately but still close to cultivars ~~(but closely related) from~~ R, DP, DD and SM in the PCA analysis, due ~~for~~ to ~~a~~ higher ~~content in~~ chlorogenic acid concentrations in fruit~~tent~~.

Antioxidant ~~analysis~~capacity

The highest values ~~of~~ for antioxidant capacity ~~determined~~ measured by the DPPH assay were found in fruit from the cultivars LG and R (8.56 and 8.31 µmol TE g^{-1} FW, respectively), whereas the

lowest value was detected in cultivar RC (2.44 µmol TE g^{-1} FW) (Fig. 3). The cultivars with the greatest antioxidant capacity (LG and R), ~~are~~were~~were~~ localized in different position in PCA score plot (Fig. 2b~~, score plot~~), with negative scores for one of the two components (LG for PC1 and R for PC2), reflecting ~~an opposite~~differences in ~~content~~ concentrations ~~in~~ of chlorogenic acid (high in LG and low in R) and flavanols (high in R and low in LG). It is ~~N~~noteworthy~~,~~ that fruit from ~~Interesting to note that B~~ cultivar B, ~~with~~ which had a high ~~value in~~ antioxidant capacity (8.13 µmol TE g^{-1}), had ~~an~~ intermediate ~~content~~ concentrations of both~~in~~~~of~~ chlorogenic acid and flavanols (Tab. 2 and Fig. 3).

In Table 3 significant positive correlations ~~are summarized the correlation coefficients for~~between phenolic compound concentrations~~s~~ ~~for which , following the row, a positive and significant correlation with the~~and total antioxidant ~~ability~~capacity (DPPH) ~~was found; following the row~~are summarized~~:~~: ~~procyanidin B1, catechin, epicatechin, procyanidin B2, chlorogenic acid and procyanidin B3~~. ~~Clearly, the chemical structure of the each phenol compound plays a key role in determining its antioxidant activity (Abbas et al. 2017).~~ Our data~~D~~~~The data~~ are ~~in line with~~similar to ~~other~~ previous work~~s~~ ~~(Stanger, Steffens, Soethe, Moreira & Do Amarante, 2017; Wojdylo et al., 2008),~~ in which procyanidins, cat~~h~~echin, epicatechin and chlorogenic acid largely contribute ~~have a pivotal role for the contribution~~ to the~~in~~ antioxidant activity in apple ~~fruits~~fruit (Stanger, Steffens, Soethe, Moreira & Do Amarante, 2017; Wojdylo et al., 2008) ~~).~~ although some authors have reported that only procyanidins and epicatechin had a significant role in apple peel and juice (Iacopini et al. 2010; Oszmianski, Wolniak, Wojdylo & Wawer, 2007; Tsao, Yang, Xie, Sockovie, & Khanizadeh, 2005; Grzesik, Naparło, Bartosz & Sadowska-Bartosz, 2018). Clearly, the chemical structure of each phenolic compound plays a key role in determining its antioxidant activity (Abbas et al. 2017). The reactive centres are the aromatic –OH groups (particularly 3',4'-dihydroxy catechol group) and their~~its~~ activity is enhanced by other substituents (Abbas et al. 2017). ~~Concerning~~In the flavonoid molecule, t~~T~~he antioxidant activity ~~of flavonoid~~ is principally related to the O-dihydroxy groups at~~the~~ the B-ring because of~~, to~~ (i) the presence of a C 2-3 double bond, (ii)

to the 3- and 5-hydroxy group and, finally, (iii) to the 4-oxo function in the A- and C-rings (Abbas et al. 2017). ~~However, some authors reported that primarily procyanidins and epicatechin, have a significant role in apple peel and juice, being the most important individual antioxidants in apple (Iacopini et al. 2010; Oszmianski, Wolniak, Wojdylo & Wawer, 2007; Tsao, Yang, Xie, Sockovic, & Khanizadeh, 2005; Grzesik, Naparlo, Bartosz & Sadowska-Bartosz, 2018).~~

Conclusion

In this study, the phenolic profile and total antioxidant activity of fruit from nine ancient apple cultivars from Garfagnana (Tuscany, Italy) were ~~analyzed~~analysed. The cultivars belong to a germplasm collection and their local use and consumption has been progressively abandoned and replaced ~~in favour of the (few)~~by ~~most~~modern commercial~~ized~~ cultivars. Our dataset offers clear evidence~~s~~ that ancient apple cultivars should be re-evaluated not only ~~at the~~for local consumption~~level~~, but ~~they can represent~~as a peerless source of genetic variability ~~(e.g., in terms of~~for both organoleptic ~~features) as well as~~and nutraceutical properties ~~which can proficiently~~that can be exploited for large-scale breeding programs. ~~In particular, leveraging on different S~~cultivar-~~s~~pecific features of ancient cultivars could also be used for ~~,~~ different commercial uses ~~can be proposed for different ancient cultivars (~~e.g., juice production, fresh fruit consumption and ~~,~~ extraction of targeted metabolites~~)~~. We are aware that some work ~~has~~needs to be done ~~to allow~~for these cultivars to compete economically with the most cultivated ~~current~~ cultivars, ~~but that~~however the ancient cultivars studied ~~herein~~ showed a higher total flesh polyphenol ~~content~~concentration ~~in flesh respect~~compared to the main commercial cultivars such as 'Golden Delicious', 'Fuji~~i~~', 'Pink Lady' and 'Royal Gala' (Masi et al., 2017; Tsao et al., 2003; Veberic, Trobec, Herbinger, Hofer, Grill & Stampar, 2005), ~~and a~~as well as higher antioxidant activity (Maragò et al., 2015). This shows ~~can represent an~~the added values of these cultivars and~~-~~ promising starting point for future "*nutrafruit*" production.

~~Twenty-six phenolic compounds, regrouped in five principal groups (flavonols, anthocyanins, flavanols, dihydrochalcones and phenolic acids), were detected in the nine cultivars and, in a general way, the ancient cultivars showed a higher total polyphenol content in flesh respect to main commercial cultivar such 'Golden', 'Fujii', 'Pink Lady' and 'Royal Gala' (Masi et al., 2017; Tsao et al., 2003; Veberic, Trobec, Herbinger, Hofer, Grill & Stampar, 2005), and a higher antioxidant activity (Maragò et al., 2015). The analyses revealed that RC and DG cultivars had a low appeal in terms of organoleptic and nutritional characteristic. The cultivars SM and DD showed excellent organoleptic characteristics (good compromise between fruit size and SSC) and moderate antioxidant capacity that make them suitable for fresh consumption. As regards the remaining cultivars (B, DP, LG, DS and R), they showed a high polyphenol content and excellent antioxidant activity, but some organoleptic features should be improved, making them therefore interesting genetic sources for breeding programs. Furthermore, R cultivar, due to their high SSC and flavanol content, might be used for a juice production with high nutraceutical properties.~~

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~~Legends of the~~ Figures legends

Figure 1. Heatmap visualization of the four major phenolic groups detected on fruit from~~in~~ nine ancient apple cultivars from Garfagnana (Italy). The i~~I~~ntensity of different colours shown in the ~~bars~~ scale bar at the top of the figure represents the ~~content~~ concentration each of the phenol groups: phenolic acid, flavanols, flavonols and dihydrochalcones (~~value~~ expressed in μg g^{-1} FW of fruit) ~~(left side)~~. On the right side of the figure ~~is reported~~ the hierarchical clustering of cultivars determined from ~~according to~~ the phenolic profile of each cultivar is shown~~s~~. The cultivars are B: 'Benito'; RC: 'Rossa di Corfino'; DP: 'Della Piastra'; DG: 'Del Giappone'; LG: 'Lugliese Grisanti'; SM: 'San Michele'; DD: 'Del Debbio'; DS: 'Del Sangue'; R: 'Ruggine'.

Figure 2. Principal Component Analysis (PCA) describing the separation of phenolic compounds on the base of PC1 (57.8 % of variation) and PC2 (33.7% of variation) (a), and the separation of each cultivar replicate using a bidimensional plot ~~on their relative amount of each phenolic compounds~~based on PC1 and PC2 (b). ~~PCA is illustrated using a bidimensional plot in which components 1 and 2 explain 91.52% of data variability.~~ The cultivars are B: 'Benito'; RC: 'Rossa di Corfino'; DP: 'Della Piastra'; DG: 'Del Giappone'; LG: 'Lugliese Grisanti'; SM: 'San Michele'; DD: 'Del Debbio'; DS: 'Del Sangue'; R: 'Ruggine'~~B: Benito; RC: Rossa di Corfino; DP: Della Piastra; DG: Del Giappone; LG: Lugliese Grisanti; SM: San Michele; DD: Del Debbio; DS: Del Sangue; R: Ruggine~~.

Figure 3. Total a~~A~~ntioxidant capacity determined by DPPH assay on fruit from~~in~~ nine ancient apple cultivars from Garfagnana (Italy). Each value is the mean of three replicates ± standard deviation. For each phenol, means flanked by the same letter are not significantly different after one-way ANOVA test with cultivars as source of variability following LSD test (P=0.05). The

cultivars are B: ‘Benito’; RC: ‘Rossa di Corfino’; DP: ‘Della Piastra’; DG: ‘Del Giappone’; LG: ‘Lugliese Grisanti’; SM: ‘San Michele’; DD: ‘Del Debbio’; DS: ‘Del Sangue’; R: ‘Ruggine’.

Table 1. Organoleptic characteristics on fruit from~~of~~ nine ancient apple cultivars from Garfagnana (Italy). Each value is the mean of three replicates ± standard deviation. For each parameter, means flanked by the same letter are not significantly different after one-way ANOVA test with cultivars as source of variability ~~following~~ using an LSD test (P=0.05). The cultivars are B: 'Benito'; RC: 'Rossa di Corfino'; DP: 'Della Piastra'; DG: 'Del Giappone'; LG: 'Lugliese Grisanti'; SM: 'San Michele'; DD: 'Del Debbio'; DS: 'Del Sangue'; R: 'Ruggine'.

	Apple cultivars								
Parameters	B	RC	DP	DG	LG	SM	DD	DS	R
Weight (g)	102.60 ± 19.12^{cd}	108.40 ± 29.14^{cd}	110.30 ± 27.20^{cd}	107.10 ± 25.54^{cd}	88.80 ± 20.13^{d}	256.00 ± 7.54^{a}	198.00 ± 26.87^{b}	130.40 ± 10.86^{c}	119.40 ± 13.11^{cd}
Height (mm)	53.26 ± 2.50^{b}	53.23 ± 5.75^{b}	56.53 ± 2.74^{b}	53.53 ± 4.21^{b}	51.73 ± 4.17^{b}	74.96 ± 6.90^{a}	80.26 ± 4.36^{a}	58.60 ± 4.32^{b}	52.33 ± 4.15^{b}
Width (mm)	63.78 ± 3.36^{c}	62.82 ± 6.12^{c}	65.29 ± 6.49^{c}	65.12 ± 5.42^{c}	60.82 ± 6.62^{c}	90.51 ± 10.52^{a}	78.85 ± 6.71^{b}	67.90 ± 3.65^{bc}	62.56 ± 3.63^{c}
Firmness (kg cm^{-2})	9.97 ± 2.02^{a}	8.37 ± 1.49^{ab}	9.43 ± 1.74^{a}	8.16 ± 1.12^{ab}	4.38 ± 0.82^{c}	8.65 ± 0.98^{ab}	6.94 ± 1.22^{b}	6.86 ± 1.11^{b}	8.92 ± 1.58^{ab}
SSC (°Brix)	12.13 ± 0.55^{c}	14.46 ± 1.10^{b}	13.83 ± 0.70^{b}	12.13 ± 0.70^{c}	16.40 ± 0.85^{a}	13.60 ± 0.17^{b}	16.60 ± 0.65^{a}	15.90 ± 0.52^{a}	16.96 ± 0.40^{a}

Table 2. P~~rofile of p~~henolic compound concentrations~~s~~ (μg g^{-1} FW) ~~in~~ on fruit from ~~for~~ nine ancient apple cultivars from Garfagnana (Italy). Each value is the mean of three replicates ± standard deviation. For each phenol, means flanked by the same letter are not significantly different after one-way ANOVA test with cultivars as source of variability following LSD test (P=0.05). The cultivars are B: 'Benito'; RC: 'Rossa di Corfino'; DP: 'Della Piastra'; DG: 'Del Giappone'; LG: 'Lugliese Grisanti'; SM: 'San Michele'; DD: 'Del Debbio'; DS: 'Del Sangue'; R: 'Ruggine'.

Polyphenols	Ancient apple cultivars								
	B	RC	DP	DG	LG	SM	DD	DS	R
Flavonols									
Q-galactoside	0.26 ± 0.15^{a}	0.07 ± 0.005^{b}	0.09 ± 0.01^{b}	0.06 ± 0.01^{b}	0.05 ± 0.01^{b}	0.03 ± 0.004^{b}	0.04 ± 0.01^{b}	0.32 ± 0.06^{a}	0.07 ± 0.02^{b}
Q-glucoside	1.06 ± 0.97^{a}	0.93 ± 0.13^{ab}	0.26 ± 0.04^{b}	0.2 ± 0.04^{b}	0.38 ± 0.13^{b}	1.12 ± 0.12^{a}	0.38 ± 0.08^{b}	1.03 ± 0.33^{a}	0.2 ± 0.05^{b}
Q-arabinopyranoside	0.45 ± 0.07^{c}	0.39 ± 0.06^{c}	0.2 ± 0.03^{d}	0.22 ± 0.06^{d}	0.16 ± 0.06^{de}	0.77 ± 0.16^{a}	0.23 ± 0.04^{d}	0.64 ± 0.16^{b}	0.07 ± 0.02^{e}
Q-arabinofuranoside	0.72 ± 0.22^{bc}	0.52 ± 0.16^{c}	0.3 ± 0.04^{d}	0.22 ± 0.06^{d}	0.16 ± 0.06^{d}	1.13 ± 0.24^{a}	0.23 ± 0.05^{d}	0.9 ± 0.22^{b}	0.09 ± 0.03^{d}
Q-rhamnoside	1.56 ± 0.97^{ab}	1.79 ± 0.55^{ab}	0.5 ± 0.12^{c}	0.29 ± 0.05^{c}	0.19 ± 0.05^{c}	2.1 ± 0.16^{a}	1.23 ± 0.34^{b}	1.62 ± 0.42^{ab}	0.95 ± 0.28^{bc}
Quercetin	-	-	0.03 ± 0.02^{b}	0.04 ± 0.04^{b}	-	-	-	0.33 ± 0.06^{a}	-
Kaempferol	-	-	0.01 ± 0.002	0.02 ± 0.005	-	-	-	-	-
Total	4.05 ± 1.92^{ab}	3.70 ± 0.85^{b}	1.39 ± 0.17^{c}	1.05 ± 0.22^{c}	0.94 ± 0.31^{c}	5.15 ± 0.40^{a}	2.11 ± 0.51^{c}	4.83 ± 1.11^{ab}	1.38 ± 0.40^{c}
Anthocyanins									
Cyanidin 3-O-galactoside	-	-	-	-	-	-	-	0.42 ± 0.15	-
Total	-	-	-	-	-	-	-	0.42 ± 0.15	-
Flavanols									
Catechin	52.13 ± 8.19^{c}	1.16 ± 0.14^{e}	71.40 ± 19.16^{b}	71.74 ± 16.97^{b}	51.23 ± 8.59^{c}	32.19 ± 7.48^{d}	17.06 ± 1.69^{de}	48.74 ± 14.08^{cd}	93.96 ± 6.40^{a}
Epicatechin	329.84 ± 60.59^{bc}	9.03 ± 1.13^{e}	342.66 ± 22.55^{b}	163.73 ± 12.98^{d}	151.88 ± 31.62^{d}	283.77 ± 36.70^{c}	266.87 ± 16.52^{c}	123.96 ± 20.09^{d}	409.21 ± 8.08^{a}
Procyanidin B1	13.72 ± 1.96^{a}	-	7.67 ± 0.29^{cd}	8.51 ± 1.02^{c}	11.25 ± 1.15^{b}	6.46 ± 1.02^{d}	5.54 ± 0.38^{d}	9.77 ± 1.38^{bc}	13.83 ± 1.87^{a}
Procyanidin B2	15.38 ± 2.29^{a}	0.72 ± 0.07^{f}	8.33 ± 0.61^{c}	3.97 ± 0.32^{e}	5.09 ± 1.03^{de}	12.67 ± 1.22^{b}	15.53 ± 1.35^{a}	5.91 ± 0.55^{d}	14.7 ± 0.46^{a}
Procyanidin B3	134.24 ± 20.36^{ab}	3.71 ± 0.24^{f}	138.14 ± 6.27^{ab}	66.89 ± 6.48^{d}	42.70 ± 5.03^{e}	104.44 ± 13.92^{c}	141.04 ± 10.52^{a}	58.02 ± 13.19^{d}	122.30 ± 4.47^{b}
Procyanidin B4	3.55 ± 1.15^{a}	-	1.98 ± 0.26^{bc}	-	-	3.35 ± 0.75^{a}	2.47 ± 0.43^{b}	0.91 ± 0.06^{c}	1.41 ± 0.31^{c}
Total	548. 86 ± 59.35^{b}	14.62 ± 1.35^{e}	570.18 ± 40.15^{b}	314.84 ± 27.83^{d}	262.15 ± 47.16^{d}	442.88 ± 57.57^{c}	448.51 ± 29.30^{c}	247.31 ± 48.79^{d}	655.41 ± 14.40^{a}
Dihydrochalcones									
Phloridzin	32.72 ± 5.06^{b}	37.48 ± 17.6^{ab}	11.44 ± 2.8^{c}	9.97 ± 2.61^{c}	29.36 ± 3.11^{b}	47.93 ± 2.36^{a}	23.28 ± 14.05^{bc}	14.99 ± 5.14^{c}	10.14 ± 3.87^{c}
Phlor-xyl-glucose	118.96 ± 22.17^{a}	70.68 ± 20.7^{b}	20.79 ± 4.96^{d}	14.85 ± 4.3^{d}	71.35 ± 14.68^{b}	40.51 ± 2.52^{c}	37.8 ± 12.25^{cd}	17.69 ± 3.84^{d}	16.57 ± 3.35^{d}
Total	151.68 ± 25.20^{a}	108.16 ± 35.71^{b}	32.23 ± 7.65^{cd}	24.82 ± 6.90^{d}	100.71 ± 16.09^{b}	88.44 ± 2.45^{bc}	61.09 ± 26.80^{c}	32.68 ± 8.93^{cd}	26.71 ± 7.06^{d}
Phenolic acids									

Chlorogenic acid	420.40 ± 35.98^{c}	246.79 ± 31.86^{e}	241.43 ± 7.95^{e}	279.22 ± 6.93^{e}	487.86 ± 4.21^{b}	300.47 ± 18.80^{de}	330.45 ± 31.33^{d}	574.27 ± 56.07^{a}	283.91 ± 30.6^{de}
Neochlorogenic acid	6.36 ± 1.15^{c}	2.76 ± 0.30^{e}	2.26 ± 0.30^{e}	10.72 ± 0.77^{a}	11.04 ± 1.25^{a}	8.25 ± 0.66^{b}	3.98 ± 0.42^{d}	4.66 ± 0.36^{d}	7.45 ± 0.29^{b}
Cryptochlorogenic acid	0.54 ± 0.26^{bc}	0.76 ± 0.42^{b}	0.92 ± 0.02^{ab}	0.32 ± 0.07^{c}	1.2 ± 0.32^{a}	-	0.55 ± 0.01^{bc}	0.93 ± 0.17^{ab}	-
p -Coumaroyl glucose	0.61 ± 0.20^{d}	0.90 ± 0.42^{cd}	0.88 ± 0.33^{cd}	1.15 ± 0.13^{c}	3.10 ± 0.15^{a}	2.26 ± 0.15^{b}	0.47 ± 0.03^{d}	0.52 ± 0.15^{d}	0.56 ± 0.06^{d}
p -Coumaroylquinic acid	54.37 ± 8.86^{c}	22.17 ± 1.85^{ef}	21.18 ± 2.69^{f}	57.17 ± 3.90^{bc}	62.92 ± 4.06^{b}	72.65 ± 5.28^{a}	28.53 ± 2.75^{e}	20.79 ± 3.07^{f}	37.54 ± 4.15^{d}
Gallic acid	-	-	-	-	-	-	-	0.06 ± 0.01^{a}	0.03 ± 0.005^{b}
Caffeoyl glucoside	-	0.15 ± 0.04^{b}	-	-	0.39 ± 0.05^{a}	0.17 ± 0.03^{b}	-	0.09 ± 0.01^{c}	0.1 ± 0.01^{c}
Protocatechuic acid	0.11 ± 0.01^{b}	0.13 ± 0.04^{b}	-	-	0.14 ± 0.07^{b}	0.16 ± 0.04^{ab}	0.21 ± 0.02^{a}	0.14 ± 0.03^{b}	0.1 ± 0.01^{b}
Feruloyl glucose	14.85 ± 1.70^{d}	11.79 ± 1.85^{d}	18.88 ± 3.41^{c}	11.94 ± 2.18^{d}	35.03 ± 3.49^{a}	25.76 ± 2.23^{b}	3.38 ± 0.42^{e}	4.68 ± 0.42^{e}	5.65 ± 0.47^{e}
t-Cinnamnic acid	-	-	1.33 ± 0.95	1.77 ± 1.21	-	-	-	-	-
Total	497.24 ± 44.14^{b}	285.45 ± 33.94^{d}	286.88 ± 12.43^{d}	362.30 ± 6.72^{cd}	601.68 ± 7.98^{a}	409.72 ± 23.21^{c}	367.57 ± 33.69^{cd}	606.13 ± 56.85^{a}	335.34 ± 34.49^{d}
Total polyphenols	**1201.83 ± 37.42^{a}**	**411.93 ± 70.16^{e}**	**890.68 ± 52.26^{c}**	**703.01 ± 27.07^{d}**	**965.48 ± 45.08^{bc}**	**946.20 ± 82.53^{bc}**	**879.28 ± 66.12^{c}**	**891.37 ± 57.51^{c}**	**1018.84 ± 48.17^{b}**

Table 3. Pearson's correlation coefficient between ~~phenols~~ polyphenol concentration and ~~antioxidant~~ the antioxidant capacity (DPPH) on fruit from nine ancient apple cultivars form Garfagnana (Italy). The cultivars are B: 'Benito'; RC: 'Rossa di Corfino'; DP: 'Della Piastra'; DG: 'Del Giappone'; LG: 'Lugliese Grisanti'; SM: 'San Michele'; DD: 'Del Debbio'; DS: 'Del Sangue'; R: 'Ruggine'. **: P<0.01; ***: P<0.001

Phenolic compound~~Correlation~~	Correlation coefficient~~Polyphenols content~~
Chlorogenic acid	0.53**
Catechin	0.60***
Epicatechin	0.60***
Procyanidin B1	0.85***
Procyanidin B2	0.54**
Procyanidin B3	0.53**

Declaration of statement

DR, ELP, LG, ML, and RM designed the experiments. ELP and ELP executed the experiments. ELP, GC and ML analyzed results. DR, GC, LG, and RM discussed results and conclusions of the study. ELP wrote the manuscript. DR, ELP, LG, ML, and RM edited manuscript drafts.

Figure 1

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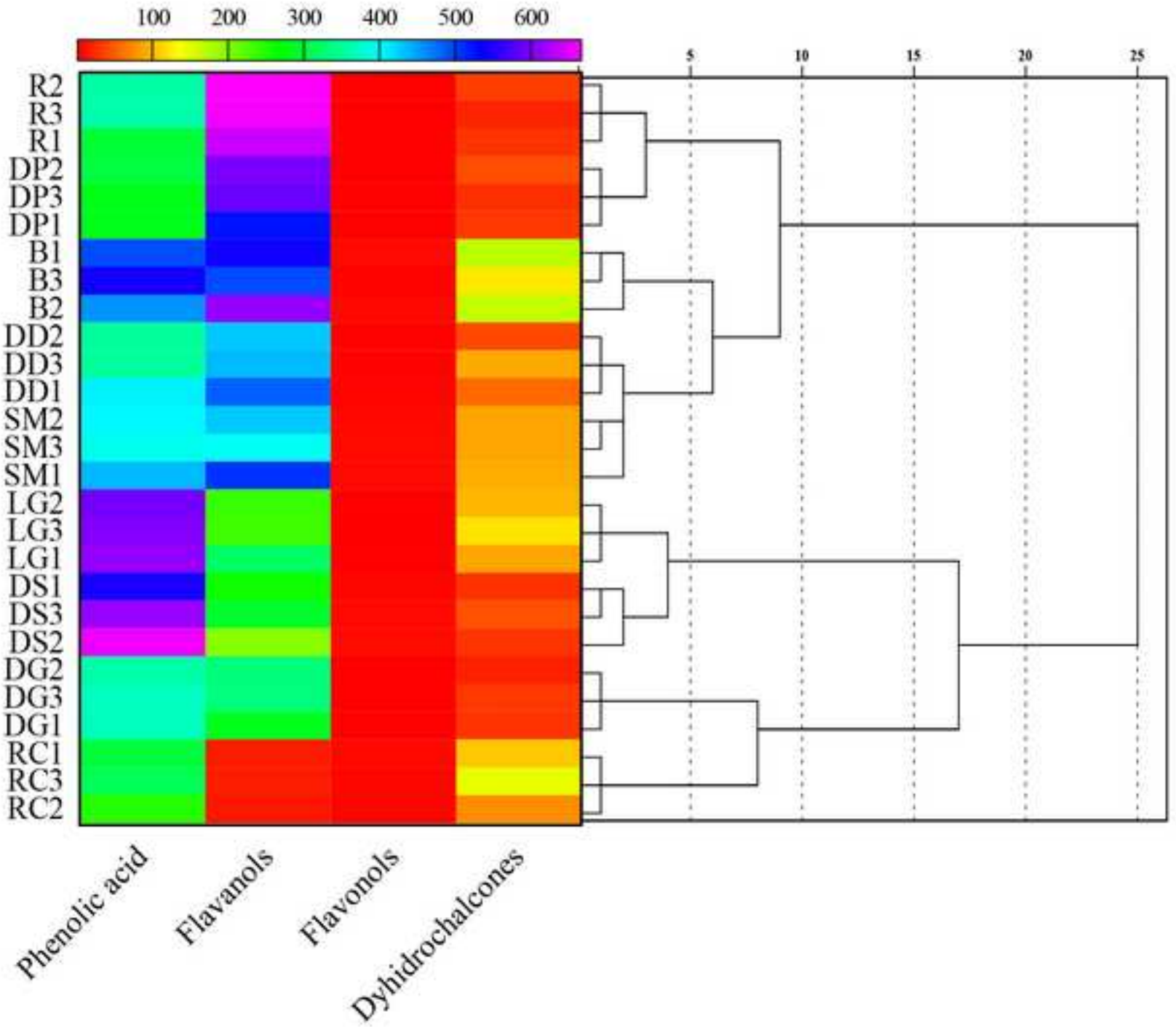

Figure 2a

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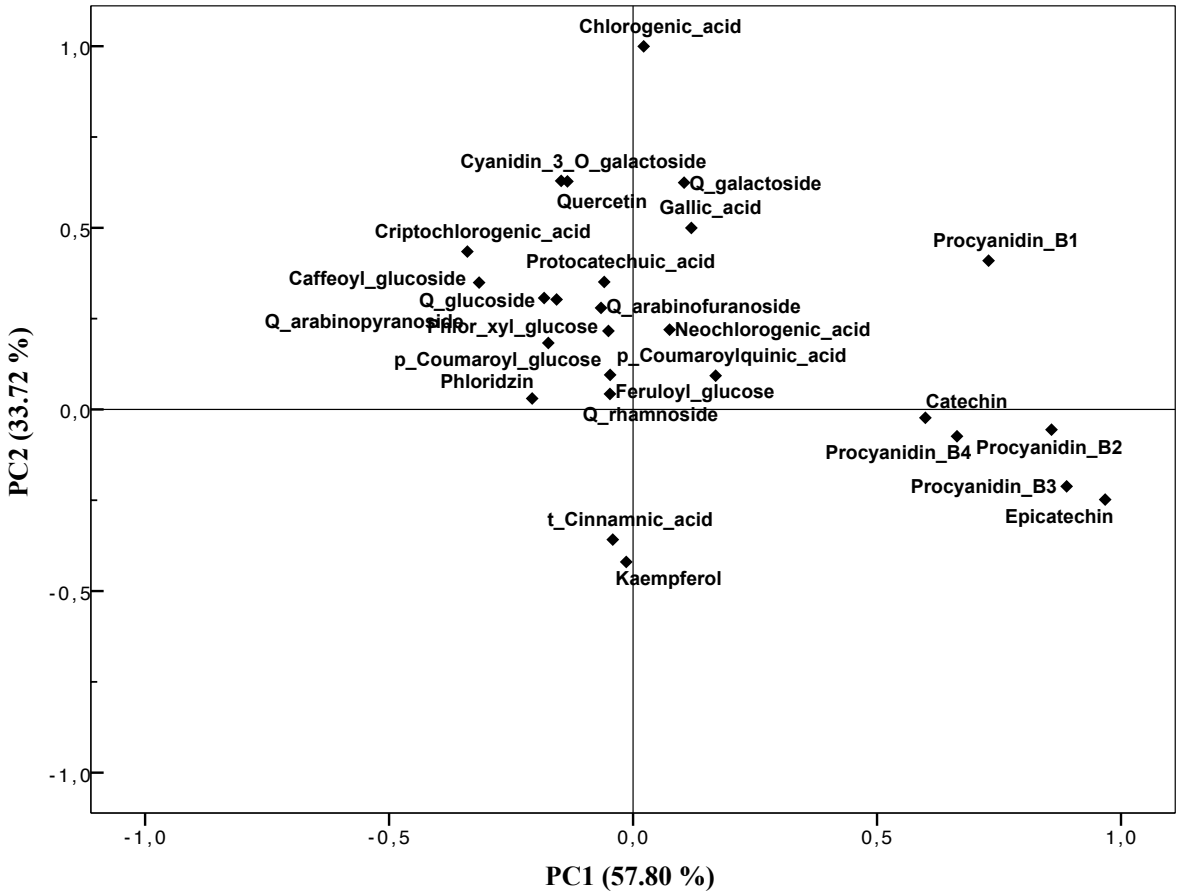

Figure 2b

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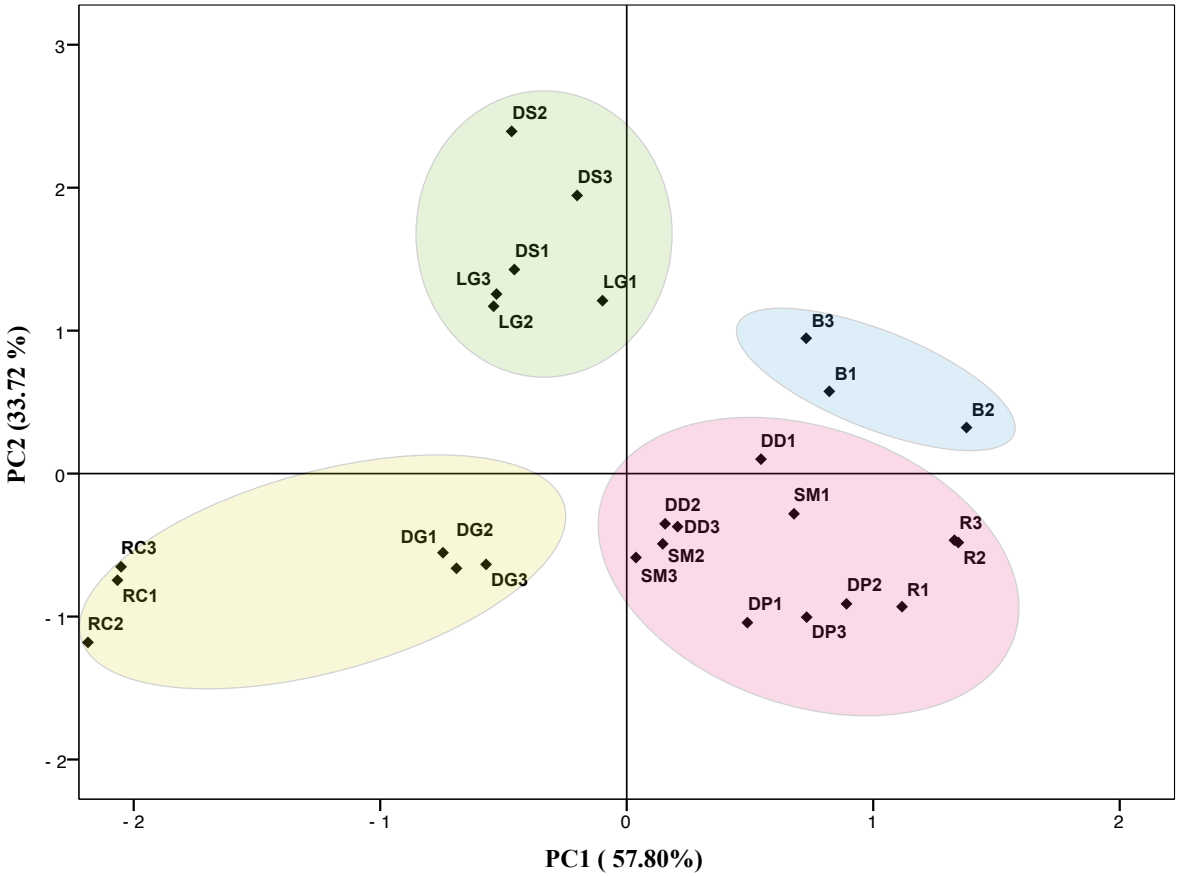

Figure 3

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