Polyphenolic profiling in pulp and peel of Italian apple cultivars

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Summary
This study focuses on the polyphenolic content and antioxidant activity in various apple cultivars. Apples are widely recognized for their health benefits due to their high antioxidant content, particularly polyphenols, which help mitigate oxidative stress and prevent chronic diseases. The study explores the differences in polyphenol levels between the peel and pulp of ten apple cultivars grown in different regions of Italy. Results show that the peel consistently contains higher concentrations of polyphenols compared to the pulp in most of the apple cultivars examined. Among the polyphenols, catechin and epicatechin are the most abundant, particularly in the peel, contributing significantly to the antioxidant capacity of apples. Other compounds like chlorogenic acid, phlorizin, and cyanidin also show higher levels in the peel. The study underscores the nutritional value of consuming peel, which is often discarded. The findings suggest that promoting whole-apple consumption, including the peel, could enhance the health benefits associated with apple intake.

Keywords
apple; antioxidant capacity; polyphenol; fruit quality

Apples (*Malus domestica*) have a strong scientific interest due to their demonstrated beneficial effect on human health. Epidemiological and clinical studies have shown that a diet rich in fruit, including apples, can contribute to the prevention of chronic diseases such as heart disease, diabetes, and certain types of cancer [1]. These physical disorders are believed to be primarily caused by oxidative stress, particularly by free radicals and reactive oxygen species. Indeed, the high content of antioxidant components in apples, especially polyphenols, correlates this fruit with beneficial effects [2].

The polyphenol content of apples can be affected by genetic factors like apple variety (cultivar) and environmental factors like region, climatic conditions, weather, farming method, and soil composition. However, the influence of the variety is probably the most important, as judged by consistent effects found across several studies [3–6].

The evolution of the market in Italy over the years reflected the global trends, in which various cultivars of apples have been modified primarily under the influence of ease of cultivation in certain areas and the greater durability of the product. This selection has led to favouring the type of apple that is more commercially marketable without giving weight to its nutritional content. Recent studies have compared the antioxidant content of old and new cultivars, showing how the latter have lost their bioactive quality over time [7].

In contemporary times, consumers are increasingly inclined to adopt attitudes favouring their health, particularly by varying their diet and preferring nutritionally more complete foods [8, 9]. Moreover, there is a growing sensitivity towards food waste and environmental pollution associated with the food industry. This leads consumers to choose products of controlled and organic origin.

The combination of these consciousness-raising efforts will lead in the years to come to a new selection of apples, also thanks to marketing efforts that will make locally grown fruits with higher nutritional content more marketable.

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This study attempts to characterize the polyphenolic content of apple samples from different regions of Italy, distinguishing between pulp and peel. In fact, although the latter is usually discarded due to a mistaken dietary habit acquired over time, it has been repeatedly verified in the literature that it contains a higher antioxidant capacity.

Having a profile of the polyphenolic content in current cultivars of locally sourced apples not only contributes to characterizing their nutritional value but may also influence their future success in the economic market. Apples with a high polyphenolic content can offer advantageous commercial opportunities and contribute to meeting the new priorities of contemporary consumers.

MATERIALS AND METHODS

Samples

The ten apple cultivars studied in this study were: Renetta, Golden Brown (Ruggine dorata), Limoncella, Annurca, Rosata, Pinova, Golden delicious, Topaz, Kissabel-rouge and Gala. The samples were identified and purchased in local markets in Rome (Italy), cultivated in various Italian regions and all organically grown. A summary of the apples varieties characteristics and their tags are displayed in Tab. 1.

The samples were collected in late fall 2023 and 2 kg were collected for each of them. After being washed, pulp and peels were separated and stored at –20 °C until analysis for a maximum 10 days.

Standard and reagents

Gallic acid, (+)-catechin, (–)-epicatechin, chlorogenic acid, phlorizin, quercetin, phloretin, rutin, cyanidin, acetic acid, acetonitrile, sodium carbonate, methanol (HPLC grade), and hydrochloric acid (37%) were purchased from Merck (Darmstadt, Germany).

Preparation of extracts

Sample extraction

The polyphenols extraction procedure followed what was reported previously by Tarola et al. [10]. Briefly, the apple samples of peel and pulp were separated, and the pulp (5 g) and the peel (5 g) fractions were immediately homogenized with 10 ml of methanol containing 1% hydrochloric acid for 10 min using an sonicator Sonorex RH 100H (Bandelin, Berlin, Germany) at room temperature (25 °C). The extraction was repeated twice and the combined extracts were filtered through a membrane 0.45 μm pore size before being injected into high-performance liquid chromatograph (HPLC) to determine the individual phenolic compounds.

Hydrolysis of extracts

For the cyanidin analysis, the extracts of the apple samples were hydrolyzed following the method reported by Tarola et al. [11]. The method was modified by adapting it to the matrix, hydrolysis 4 mol·l^{-1} HCl, 50 min at 90 °C, consisting of 4 ml of the extracted sample in acidified methanol plus 1 ml of 37% HCl for 50 min in a 90 °C oven. After hydrolysis, the extracts were allowed to cool and made into methanol to reach 10 ml total volume. The extracts were filtered through a 0.45 μm pore size membrane before injecting HPLC.

HPLC analysis

HPLC analyses were performed on an HPLC system, an LC-10AT liquid chromatograph

Tab. 1. Characteristics of the ten Italian apple varieties studied.

Apple tag	Variety	Region	Old vs new variety	Peel colour	Pulp colour
M1	Rosata	Lazio	Old	Rose	Yellow
M2	Annurca	Campania	Old	Red	White
M3	Limoncella	Abruzzo	Old	Yellow	Yellow
M4	Gala	Trentino	New	Red	White
M5	Renetta	Trentino	Old	Yellow	Yellow
M6	Pinova	Trentino	New	Red	Yellow
M7	Golden delicious	Trentino	Old	Yellow	Yellow
M8	Topaz	Friuli	New	Red	Yellow
M9	Kissabel- rouge	Trentino	New	Red	Red-white
M10	Golden brown	Lazio	Old	Yellow-brown	Yellow

equipped with quaternary pumps FCV-10AL, a degasser DGU-14A (Shimadzu, Kyoto, Japan), a Rheodyne 7725 injection valve with a 20 μl loop (Rheodyne, Berkeley, California, USA) and a photodiode array detector SPD-M20A (Shimadzu). The analysis was carried out in triplicate for each sample. Chromatographic separations were performed on a C18 Kinetex (150 mm × 4.6 mm, 5 μm particle size) and a guard column, all supplied by Phenomenex (Torrance, California, USA). The elution of the samples involved the use of a gradient mobile phase with a flow rate of 0.6 ml·min^{-1} in a 50-minute chromatographic run, using two solvents: 2% acetic acid in water (solvent A) and acetonitrile (solvent B). The method involves a starting mobile phase composed of 3 % solvent A and 97 % solvent B. Solvent A is raised to 33 % in 15 min and then increased to 100 % in 30 min. Finally, the initial parameter is restored in the last 5 min of the chromatographic run.

Quantitative determinations were made by considering the respective peak areas of standards at maximum absorbance wavelength (from 280 nm to 520 nm, depending on the compound analyzed) and at the relative retention time versus the concentration (Tab. 2). The results are expressed in milligrams per kilogram of apple fruit.

The linearity of the method was evaluated using standard solutions at five concentration levels (0.1 mg·l^{-1} to 300 mg·l^{-1}) for the 9 compounds concerned. A linear regression coefficients R^2 greater than 0.991 for all the compounds examined was obtained and the limit of detection (*LOD*) for each compound was calculated, as shown in Tab. 2.

Tab. 2. Parameters of the polyphenols HPLC determination method.

	t_r [min]	λ [nm]	R^2	*LOD* [mg·l^{-1}]
Gallic acid	7.70	280	0.993	0.59
Catechin	12.95	280	0.997	0.31
Chlorogenic acid	12.40	320	0.992	0.24
Epicatechin	14.20	280	0.999	0.19
Rutin	16.10	350	0.991	0.06
Phlorizin	19.35	280	0.995	0.07
Quercetin	22.05	350	0.998	0.03
Phloretin	23.10	280	0.992	0.02
Cyanidin	13.55	520	0.991	0.02

t_r – retention time, λ – maximum absorption wavelength, R^2 – correlation coefficient, *LOD* – limit of detection.

Total phenolic content

Total phenolic content (*TPC*) was performed by spectrophotometric analysis using the Folin-Ciocalteu method [11]. The absorbance was measured at 725 nm in 1 cm path length cuvettes against the blank solution. The total content of phenols was expressed as milligrams of gallic acid equivalents (GAE) per kilogram of apple.

Antioxidant activity

Antioxidant activity (*AOA*) was determined by two methods involving the measurement of the scavenging activity of two different radicals by spectrophotometric assays.

For the 2,2'-azinobis-(3-ethylbenzthiazolin-6-sulfonic) acid (ABTS) assay, the procedure followed the method of Thaipong et al. [12] with some modifications. The working solution was prepared by mixing the two stock solutions (7.4 mmol·l^{-1} ABTS solution and 2.6 mmol·l^{-1} potassium persulfate solution) in equal quantities and allowing them to react for 12 h at room temperature (± 25 °C) in the dark to produce $ABTS^{\bullet+}$ radical cation. The solution was then diluted by mixing 1 ml $ABTS^+$ solution with 60 ml methanol to obtain an absorbance of 1.1 ± 0.02 units at 734 nm using the spectrophotometer UV 1800 (Shimadzu). Fresh $ABTS^+$ solution was prepared for each assay. Fruit extracts (150 μl) were allowed to react with 2 850 μl of the $ABTS^+$ solution for 2 h in a dark condition. Afterwards, the absorbance was measured at 734 nm. The standard curve was linear between 0.2 mg·l^{-1} and 40 mg·l^{-1} for gallic acid, chlorogenic acid and cyanidin; 1 mg·l^{-1} and 300 mg·l^{-1} for catechin; 1 mg·l^{-1} and 100 mg·l^{-1} for epicatechin; 0.2 mg·l^{-1} and 20 mg·l^{-1} for quercetin and phloretin; 0.2–50 mg·l^{-1} for phlorizin and rutin, all of them covering the range from 10.0 % to 200.0 % of the expected concentration of each phenolic compound in apple samples.

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay was performed following the method of Thaipong et al. [12] by measuring the decrease in the absorbance of the working solution at 517 nm.

The results of both assays were expressed as milligrams GAE per kilogram of apple. As reported by Olszowy-Tomczyk [13], gallic acid has the advantage over 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) of a better antioxidative response.

Statistical analysis

Each antioxidant activity assay was done three times for the same extract to determine their reproducibility. Analysis of variance was used to test any difference in the obtained values. Correlations

among data obtained were calculated using Pearson's correlation coefficient.

Principal component analysis (PCA) was applied to highlight a natural grouping of samples depending on their different antioxidant capacity and antioxidant content in relation to the different cultivars. All the computations were performed using V-PARVUS [14].

RESULTS AND DISCUSSION

The apple chosen for this study included old varieties such as Annurca, Renetta, Golden delicious, Golden Brown, Limoncella and Rosata while the rest are news varieties as Topaz, Gala, Pinova, and Kissabel-rouge [15]. In Tab. 1 all apples are reported with a summary of their characteristics.

HPLC analysis

The characterization of the polyphenolic content was obtained using HPLC. Tab. 2 lists the polyphenolic compounds determined in the apple samples, along with their corresponding retention times and *LOD* values at the respective maximum absorption wavelengths. Among the various polyphenols identified, catechin emerged as the most abundant compound, particularly in the apple peel (Tab. 3). For instance, the M4 peel exhibited an impressive content of catechin, reaching up to 904.5 mg·kg^{-1}. Epicatechin, another notable flavanol, followed a similar trend, reaching, in the same apple, the highest value of both 250.8 mg·kg^{-1} in the peel and 91.3 mg·kg^{-1} in the pulp. These compounds are well-known for their antioxidant properties, which contribute to the overall health benefits of consuming apples. This high content underscores epicatechin and catechin's predominant role in the antioxidant capacity of the apple peel.

Chlorogenic acid, phlorizin, and cyanidin were also present in notable quantities, albeit generally lower than catechin and epicatechin. Chlorogenic acid showed the highest amounts, with the M1 peel containing 103.4 mg·kg^{-1} and highlighting its contribution to the apple's polyphenolic profile. Phlorizin, a dihydrochalcone known for its anti-inflammatory and anti-diabetic properties, was most concentrated in the M9 peel, reaching 211.8 mg·kg^{-1}. Cyanidin, an anthocyanin pigment, was more abundant in the peel, especially in the M6 variety, reflecting its role in the colouration and antioxidant activity of the fruit.

Minor polyphenols such as gallic acid, rutin, quercetin, and phloretin were detected. Gallic acid, although present in relatively low contents, was consistently higher in the peel than in the pulp for all apples except for M5. Rutin and quercetin, while important for their antioxidant and anti-inflammatory properties, were often below the detection limits in the pulp, emphasizing the peel's richer polyphenolic content. Phloretin was determined in a few samples and only in M5 pulp.

In conclusion, a stark contrast in polyphenolic content between the peel and pulp is a recurring theme across most apple cultivars examined. With few exceptions, the peel consistently exhibited higher contents of polyphenols compared to the pulp. This difference can be attributed to the peel's role in protecting the fruit against environmental stressors, pathogens, and UV radiation, necessitating a higher content of bioactive compounds and highlights the nutritional value of the peel, which is often discarded during apple consumption.

Polyphenol content and antioxidant activity

Tab. 4 shows the *TPC* and antioxidant activity, assessed using the DPPH and ABTS methods, for the apple cultivars in both the peel and pulp tissues. The results reveal significant differences between the peel and pulp. Specifically, *TPC* in the peel ranged from 840 mg·kg^{-1} to 2 031 mg·kg^{-1} (expressed as GAE), while the pulp displayed *TPC* values ranging from 237 mg·kg^{-1} to 1 365 mg·kg^{-1}. When comparing the mean values obtained for the two apple parts, the peel showed, in most cases, three times higher *TPC* than the pulp, which is consistent with findings reported by Piagentini and Pirovani [15] and Khanizadeh et al. [16] who reported similar differences in phenolic contents between the peel and pulp of various fruits. Notably, the M4 sample exhibited a very low difference between pulp and peel (a ratio of 1.3), as it showed a very high *TPC* value in the pulp and M1 having the highest *TPC* value in peel (2 031 mg·kg^{-1}) and a very low value in pulp (388 mg·kg^{-1}) showed a high ratio of 5.2. The apple varieties with the lowest amount in the pulp were the new apples, Topaz (M8) and Kissabel-rouge (M9).

Regarding *AOA* measured by the DPPH assay, the trend of results mirrors that of the *TPC*, with higher values in the peel compared to the pulp. Peel values range from 93 mg·kg^{-1} for M1 to 70 mg·kg^{-1} (expressed as GAE) for M8. There are also notable differences among the pulps, with M1 having the lowest value (15 mg·kg^{-1}) and M4 the highest (79 mg·kg^{-1}). This finding is consistent with the results reported by Chinnici et al. [17]. However, the peel-to-pulp *AOA* mean ratio was

Tab. 3. Polyphenol content in the apple variety examined.

Apple variety		Gallic acid [mg·kg^{-1}]	Catechin [mg·kg^{-1}]	Chlorogenic acid [mg·kg^{-1}]	Epicatechin [mg·kg^{-1}]	Rutin [mg·kg^{-1}]	Phlorizin [mg·kg^{-1}]	Quercetin [mg·kg^{-1}]	Phloretin [mg·kg^{-1}]	Cyanidin [mg·kg^{-1}]
M1	Peel	15.1 ± 0.6	473.8 ± 18.4	103.4 ± 5.0	204.2 ± 4.1	3..3 ± 0.6	54.8 ± 2.3	24.1 ± 1.0	< *LOD*	9.3 ± 3.8
	Pulp	6 ± 0.2	11.1 ± 0.4	28.1 ± 1.0	20.0 ± 0.7	< *LOD*	< *LOD*	2.6 ± 0.1	< *LOD*	7.6 ± 0.3
M2	Peel	15.9 ± 0.6	990.9 ± 46.0	50.6 ± 1.6	174.8 ± 6.8	21.1 ± 0.7	144.2 ± 5.7	3.2 ± 0.2	3.5 ± 0.1	70.3 ± 1.6
	Pulp	5.9 ± 0.1	289.6 ± 7.3	< *LOD*	22.2 ± 0.6	< *LOD*	< *LOD*	< *LOD*	< *LOD*	8.8 ± 0.3
M3	Peel	11.1 ± 0.2	214.5 ± 9.7	36.0 ± 1.4	197.4 ± 8.9	48.2 ± 1.4	117.6 ± 4.8	8.7 ± 0.3	3.9 ± 0.1	43.9 ± 0.3
	Pulp	9.2 ± 0.3	153.5 ± 6.3	28.1 ± 1.4	64.4 ± 3.9	12.5 ± 0.3	12.9 ± 0.3	< *LOD*	< *LOD*	8.4 ± 0.4
M4	Peel	8.9 ± 0.2	904.5 ± 9.7	59.8 ± 2.4	250.8 ± 6.1	65.4 ± 0.4	152.1 ± 4.0	4.1 ± 0.2	< *LOD*	12.9 ± 0.5
	Pulp	5.6 ± 0.2	220.1 ± 7.3	38.1 ± 1.6	91.3 ± 4.1	8.9 ± 0.3	10.9 ± 0.3	3.4 ± 0.2	< *LOD*	10.9 ± 0.3
M5	Peel	7.4 ± 0.2	148.4 ± 6.3	68.8 ± 2.2	220.7 ± 2.6	14.2 ± 0.2	126.5 ± 0.5	< *LOD*	< *LOD*	14.4 ± 0.2
	Pulp	10.5 ± 0.2	161.8 ± 6.3	73.2 ± 2.2	64.7 ± 2.6	< *LOD*	12.3 ± 0.2	< *LOD*	2.4 ± 0.1	8.6 ± 0.3
M6	Peel	11.7 ± 0.2	427.9 ± 13.4	84.7 ± 2.2	247.2 ± 8.3	46.9 ± 0.6	52.6 ± 0.22	9.5 ± 0.2	2.6 ± 0.1	104.7 ± 1.8
	Pulp	6.1 ± 0.2	87.0 ± 3.5	37.3 ± 1.2	59.4 ± 0.7	6.8 ± 0.1	14.8 ± 0.5	2.3 ± 0.1	< *LOD*	7.6 ± 0.4
M7	Peel	12.4 ± 0.3	552.9 ± 22.8	48.1 ± 1.4	144 ± 6.3	52.8 ± 1.7	112.5 ± 4.0	9.3 ± 0.4	< *LOD*	10.2 ± 0.2
	Pulp	9.2 ± 0.3	14.9 ± 0.5	21.7 ± 0.7	49.8 ± 2.3	< *LOD*	19.2 ± 0.7	< *LOD*	< *LOD*	8.0 ± 0.3
M8	Peel	10.2 ± 0.3	169.6 ± 6.3	< *LOD*	78.8 ± 3.3	27.2 ± 0.8	15.2 ± 0.4	9.5 ± 0.3	< *LOD*	26.9 ± 0.3
	Pulp	8.4 ± 0.2	< *LOD*	3.3 ± 0.1	24.3 ± 1.5	< *LOD*	< *LOD*	< *LOD*	< *LOD*	8.0 ± 0.3
M9	Peel	11.2 ± 0.3	331.3 ± 13.8	12.6 ± 0.5	131.9 ± 4.5	47.2 ± 0.8	211.8 ± 6.9	10.9 ± 0.1	4.2 ± 0.1	65 ± 1.8
	Pulp	11 ± 0.3	126.8 ± 4.7	20.2 ± 0.5	53.4 ± 2.3	< *LOD*	10.2 ± 0.2	< *LOD*	< *LOD*	14.6 ± 0.3
M10	Peel	9.3 ± 0.2	210.9 ± 3.5	55.6 ± 1.2	123.6 ± 0.7	16 ± 0.1	68.9 ± 0.5	< *LOD*	< *LOD*	9.4 ± 0.4
	Pulp	5.8 ± 0.2	< *LOD*	< *LOD*	56.2 ± 2.3	4.9 ± 0.2	3.4 ± 0.2	< *LOD*	< *LOD*	9.3 ± 0.3

The data, expressed as milligrams of gallic acid equivalents per kilogram of sample, are represented in mean ± standard deviation. Identification of apple varieties is given in Tab. 1.
LOD – limit of detection.

Tab. 4. Total polyphenolic contents and antioxidant activity for the pulp and peel of all apple varieties.

Apple variety		Total polyphenolic content [mg·kg^{-1}]	Antioxidant activity [mg·kg^{-1}]	
			DPPH assay	ABTS assay
M1	Pulp	388.4 ± 52.4	14.5 ± 2.4	23.7 ± 3.0
	Peel	2 031.3 ± 286.5	92.9 ± 18.9	65.4 ± 8.3
M2	Pulp	677.2 ± 92.4	45.9 ± 8.2	39.3 ± 4.2
	Peel	1 720.1 ± 159.2	90.5 ± 9.3	61.8 ± 9.4
M3	Pulp	633.5 ± 45.1	50.1 ± 5.2	40.7 ± 4.2
	Peel	1 335.9 ± 238.0	85.7 ± 13.1	60.6 ± 6.2
M4	Pulp	1 365.4 ± 196.9	78.6 ± 5.6	56.6 ± 7.8
	Peel	1 855.7 ± 279.7	81.5 ± 11.5	57.2 ± 4.9
M5	Pulp	614.4 ± 47.3	71.5 ± 9.4	51.5 ± 4.7
	Peel	1 737.5 ± 198.1	91.4 ± 13.7	68.5 ± 7.2
M6	Pulp	518.8 ± 48.4	55.2 ± 6.1	43.8 ± 7.8
	Peel	1 866.1 ± 150.4	89.0 ± 8.8	62.1 ± 4.7
M7	Pulp	278.9 ± 38.1	23.6 ± 3.4	28.2 ± 3.5
	Peel	1 104.7 ± 188.0	82.5 ± 15.1	57.6 ± 6.7
M8	Pulp	237.1 ± 29.9	20.2 ± 3.3	26.6 ± 4.9
	Peel	845.6 ± 163.3	69.5 ± 6.9	51.1 ± 8.3
M9	Pulp	237.1 ± 33.0	26.3 ± 5.1	29.6 ± 2.5
	Peel	1 089.2 ± 141.5	77.5 ± 7.1	55.1 ± 4.1
M10	Pulp	486.5 ± 74.3	51.6 ± 5.8	44.1 ± 3.2
	Peel	1 345.6 ± 232.5	73.6 ± 6.5	53.1 ± 5.3

The data, expressed as milligrams of gallic acid equivalents per kilogram of sample, are represented in mean ± standard deviation. Identification of apple varieties is given in Tab. 1.
DPPH – 2,2-diphenyl-1-picrylhydrazyl, ABTS – 2,2'-azinobis-(3-ethylbenzthiazolin-6-sulfonic) acid.

2.53 ± 1.63 despite the high variability between the varieties. Also, in this case, as just reported for *TPC*, the ratio values of M1 and M4 differed significantly from the average.

In the case of the ABTS assay, the *AOA* ranged between 51 mg·kg^{-1} and 69 mg·kg^{-1} (expressed as GAE) for peels and between 24 mg·kg^{-1} and 57 mg·kg^{-1} for pulps. However, regardless of the assay employed, it was found that the M1, M2, M5, and M6 peels had the highest radical-scavenging activity, with mean values of 90 ± 1.6 mg·kg^{-1} and 65 ± 3 mg·kg^{-1} for the DPPH and ABTS assays, respectively. In contrast, the peels of M8, M9 and M10 varieties showed the lowest antioxidant capacity, in the range of 70–78 mg·kg^{-1} as measured by DPPH assay and 51–55 mg·kg^{-1} as measured by ABTS assay.

The present study did not show a perfect pattern of the advantages of old apple varieties over new ones in terms of *TPC* or *AOA*. For instance, in the peel samples, the old variety Rosata (M1) stood out as having the highest *TPC* as well as the highest DPPH-based antioxidant activity. The old variety Renetta (M5) had the highest ABTS-based antioxidant activity. However, the new variety Pinova (M6) had the second-highest *TPC* in the peel, as well as substantial *AOA* (DPPH- and ABTS-based). The new variety Gala (M4) exhibited the highest *TPC* and antioxidant activity (both DPPH and ABTS assay-based) in the pulp. The lowest parameters in peel samples were observed in the new variety Topaz (*TPC*, *AOA* DPPH- and ABTS-based). The old variety Rosata had the lowest *AOA* in the pulp (both DPPH and ABTS assay-based).

The results highlight a significant nutritional loss when the peel is discarded. Consuming whole apples, including the peel, maximizes the intake of these beneficial compounds, enhancing the fruit's health benefits. The peel's richness in polyphenols supports its use in food processing and nutraceutical applications, where extracting and utilizing these bioactive compounds can add value.

Correlation analysis

To establish the extent to which polyphenols contribute to the antioxidant properties of apples, Pearson's correlation coefficients between *AOA* and *TPC* contents were determined for both the pulp and peel (Tab. 5). A significant positive correlation ($p < 0.01$) between *TPC* and *AOA*, as determined by ABTS and DPPH assays, was found for both the peel ($r = 0.748$ and $r = 0.709$, respectively) and the pulp ($r \approx 0.870$ for both assays). This positive correlation was previously reported in a study of ancient apple cultivars from Northeast Italy [18] and by VIEIRA et al. [19] in research of 11 apple cultivars in Brazil, where *AOA* was determined using ferric-reducing antioxidant power (FRAP), DPPH, and ABTS assays. Additionally, it was highlighted that the correlation was slightly stronger in the pulp compared to the peel, which is unlike the previous study by LECCESE et al. [20].

All these findings, along with the high correlation between the two *AOA* assays (peels: $r = 0.954$; pulp: $r = 0.998$), indicated that the *AOA* assays are comparable and interchangeable for characterizing antioxidant activity. They also suggest that polyphenolic compounds, which are prevalent in the peels, are the primary contributors to the antioxidant properties of these apple cultivars.

A significant relationship was also found between *AOA* and the content of epicatechin in both peel and pulp tissues (Tab. 5). In contrast, no clear trend was obtained for catechin. However, it suggested that the structure of epicatechin plays a more significant role in contributing to *AOA*, even if catechin and epicatechin exhibit similar scavenging abilities [21]. Infact their structures are quite similar with different stereochemical configuration at the C2 position of the C-ring.

Principal component analysis

PCA is a statistical technique used to reduce the number of dimensions in large datasets to principal components that retain most of the original information. The main goal is to highlight differences between samples and identify which variables contribute most to those differences. PCA has been previously employed in studies for the comprehensive evaluation of apple fruits, wine, honeys and many more food matrices [18, 22–24].

In this study, PCA was used to establish the similarity/dissimilarity among the chemical compositions of apple samples based on their cultivars and on the part of the fruit.

In Fig. 1, the scores and loading resulting from PCA are displayed. Principal component 1 (PC1) explained up to 53.7 % of the total variance and principal component 2 (PC2) explained 13.5 %. Thus, the two-dimensional graph presented was able to explain 67.2 % of the variability in the experimental data.

The variables that contributed most to PC1 were chlorogenic acid, epicatechin, phlorizin and quercetin. PC2 was associated with catechin, phloretin, gallic acid and cyanidin. The samples are clearly grouped into three separate areas on the plot along PC1, two related to the peel and one to the pulp. The peel of apple cultivars such as Rosata, Annurca, Pinova, Limoncella and Kissabelrouge (M1, M2, M6, M3 and M9, respectively) was

Tab. 5. Pearson's correlation coefficients between antioxidant activity, total polyphenolic content and single phenolic compounds.

	Antioxidant activity				*p*
	DPPH assay		ABTS assay		
	Peel	Pulp	Peel	Pulp	
Total polyphenolic content	0.709	0.874	0.748	0.881	< 0.01
Antioxidant activity (ABTS assay)	0.954	0.998	1.000	1.000	< 0.01
Gallic acid	0.414	–0.183	0.190	–0.222	< 0.01
Catechin	0.125	0.234	–0.025	0.223	< 0.01
Chlorogenic acid	0.742	0.544	0.719	0.504	< 0.01
Epicatechin	0.756	0.723	0.721	0.792	< 0.01
Rutin	–0.020	–	0.174	–	< 0.01
Phloridzin	0.143	–	0.091	–	< 0.01
Quercetin	0.230	–	0.146	–	< 0.01
Cyanidin	0.497	0.064	0.368	0.074	< 0.01

DPPH – 2,2-diphenyl-1-picrylhydrazyl, ABTS – 2,2'-azinobis-(3-ethylbenzthiazolin-6-sulfonic) acid.

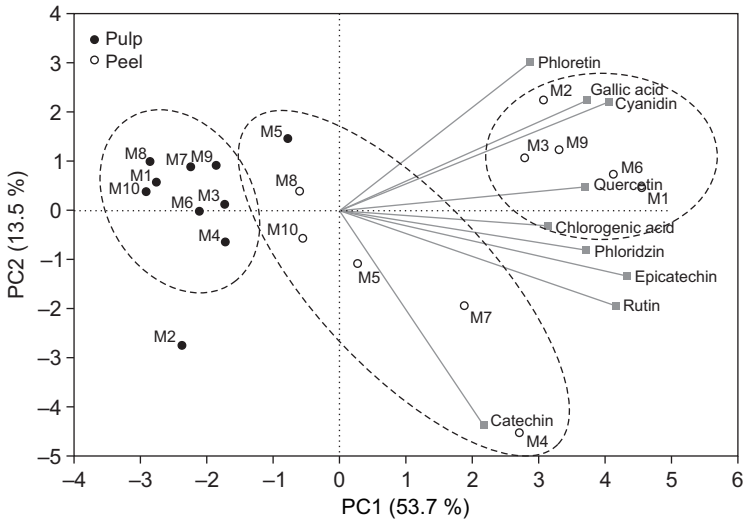

Fig. 1. Principal component analysis of apple cultivars.

Identification of apple varieties is given in Tab. 1.

therefore characterized by the presence of high levels of phloretin, cyanidin, quercetin and chlorogenic acid, phenolic compounds that resulted instead less present in the peel of the remaining apples Gala, Renetta, Golden delicious, Topaz and Golden Brown. The group with the data on apple pulp remains separated on the other side of the plot, characterized by lower levels of bioactive substances, with samples strictly close to each other, showing poor dissimilarity.

CONCLUSIONS

The data presented in this research indicate that the peel had a notably higher level of bioactive substances and a different polyphenolic profile from that of the pulp, with subdivisions based on polyphenol components and antioxidant activity, while the pulp profile showed lower diversity.

The findings of this study are in accordance with the previous literature that highlights the importance of the consumption of the whole apple in order to maximize the beneficial effects deriving from the antioxidant content of this fruit. Furthermore, this study on different apple cultivars shows how peel, often discarded as waste, can, on the contrary, be considered an important by-product to be exploited as a source of bioactive ingredients for functional foods or additives. In this contest, the potentiality of organically grown apples with high polyphenol content can be valorized in order to meet consumer preferences for healthier and environmentally friendly products.

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