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Characterization of color, phenolic profile, and antioxidant activity of Italian pigmented rice varieties after different technological treatments

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ABSTRACT

Pigmented rice varieties are rich in antioxidant and anti-inflammatory compounds (e.g. anthocyanins and proanthocyanidins). Therefore, their consumption could exert beneficial effects, particularly in people suffering from chronic diseases (e.g., celiac disease). Pigmented rice is commonly consumed as brown rice, but technological treatments could be applied to reduce its cooking time and improve its nutritional value (vitamins and minerals). In this study, two relatively new pigmented varieties (Violet and Orange) were characterized in terms of phenolic content and antioxidant capacity and the impact of two technological treatments (e.g., milling and parboiling) on their phytochemical composition was evaluated. Two pigmented and one non-pigmented Italian varieties were included for comparison. Both technological processes affected the concentration of phenolic compounds and their relative antioxidant property. Although milling mainly reduced the phenolic content and the antioxidant activity, anthocyanins seem to be more affected by parboiling (reduction of 91.5%). Despite the effects of technological treatments on active compounds, pigmented varieties still represent an interesting antioxidant source when compared to the non-pigmented ones.

1. Introduction

Rice is one of the most widely consumed cereals in the world. The European Food-Based Dietary Guidelines recommend the consumption of whole grains because the healthiest components (essential fatty acids, fibres, vitamins, and minerals) are concentrated in the aleurone layer and embryo (Mbanjo et al., 2020). Indeed, with respect to milled rice (obtained by removing bran layers and germ), whole brown rice (obtained by removing only the hull) is richer in antioxidant molecules, including lipophilic (e.g., vitamin E) and phenolic compounds (Bhattacharya, 2004).

In particular, the pigmented varieties of rice are rich in phenolic compounds and different studies have underlined their potential beneficial effects for the 1) anti-inflammatory activity (Limtrakul et al., 2016), 2) anticancer activity in terms of growth inhibition in cancer cell lines (Mbanjo et al., 2020; Upanan et al., 2019), 3) antioxidant and 4) anti-diabetic properties (Boue et al., 2016; Mbanjo et al., 2020). Reactive oxygen species (ROS) are by-products generated by the metabolism of biological systems and, in low concentration, are essential for several physiological processes. However, an increasing concentration of ROS causes oxidative stress, which seems to play a major role in the development and progression of numerous human diseases (such as cardiovascular diseases, hypertension, inflammation, and diabetes). Among chronic diseases involving the gastrointestinal tract, different studies have suggested that ROS play an important role in the etiology and progression of inflammatory bowel diseases (IBDs) and celiac disease (Patlevič et al., 2016). Indeed, among several other factors, the worsening of oxidative stress is considered a feature involving intestinal mucosa alteration in celiac disease (Ferretti et al., 2012). Dietary antioxidants could mitigate this oxidative stress. Pigmented rice varieties are rich in flavonoids, anthocyanins and proanthocyanidins, molecules with known antioxidant and anti-inflammatory effects. Their inclusion in the diet could therefore contribute to improving the health of the general population as well as those of people suffering from chronic diseases involving the gastrointestinal system, such as celiac and IBDs subjects.

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Different pigmented rice varieties are present on the market; they are commercialized as brown rice or they are subjected to technological treatments (e.g., parboiled and milling) aimed mainly at reducing their cooking time. Parboiling is a hydrothermal process consisting in soaking, steaming, and, finally, slowly drying paddy rice (Bhattacharya, 2004). Milling consists of the mechanical removal of the external layer of the kernel. Parboiling and/or milling are responsible for some advantages for both producers and consumers (higher milling yield, shorter cooking time, and longer shelf-life) (Min et al., 2014), and they could have positive effects on rice nutritional values in terms of starch availability, glycemic index and/or vitamins and mineral contents. Although it is known that parboiling increases the concentration of tocotrienols, tocopherols and γ-oryzanol (Min et al., 2014), it could influence the phenolic compounds present in rice.

Different studies conducted in Asia have evaluated the impact of milling on the phenolic compounds' content. Reddy et al. observed a reduction (up to 89.97%) in phytochemicals and antioxidant activity after the polishing of three pigmented rice varieties (Reddy et al., 2017). Paiva et al. studied the effect of milling and parboiling on the phenolic compounds' stability in two pigmented rice varieties (red and black) from Brazil, observing that polishing strongly reduced the free forms that were partially preserved after the parboiling procedure (Paiva et al., 2016). The studies conducted on European rice varieties were mainly focused on the phytochemical characterization of brown rice and the impact of different home cooking methods (boiling and "risotto" cooking). Only a few studies evaluated the impact of industrial technological treatments. Finocchiaro et al. evaluated the impact of milling on two Italian white and red rice varieties (Finocchiaro et al., 2007), while Petroni et al. studied the proximate composition, polyphenol content and anti-inflammatory properties of Italian rice varieties, including a type of white rice receiving different processes (milled, parboiled and integral) (Petroni et al., 2017). Considering that Italy, with a cultivated area of 218.000 ha, represents a European leader in rice production and few data are available on pigmented Italian rice and the impact of industrial technological treatments on it; the aim of the present study was the phytochemical characterization of different Italian rice varieties, in term of polyphenols content and antioxidant capacity, and the parallel evaluation of the effects of parboiling and milling on their phytochemical composition. For these purposes, five Italian rice varieties were collected (white, red, and black), and the impact of milling and parboiling on two pigmented samples was deeply explored.

2. Materials and methods

2.1. Chemicals

Methanol, ethanol, water HPLC grade, acetonitrile, acetone, toluene, formic acid, and 1 M HCl were purchased from VWR International (Fontenay-sous-Bois, France). Folin-Ciocalteau reagent, 1,1-diphenyl-2-picryl-hydrazyl free radical (DPPH), salts and gallic acid standard were purchased from Sigma Aldrich (Steinheim, Germany). Anthocyanin standards were purchased from Extrasynthese (Genay, France).

2.2. Samples

In Italy, there is a tradition of growing and consuming non-pigmented rice varieties. However, pigmented rice has recently received increasing attention among Italian consumers and farmers. Some of the pigmented rice varieties currently available in Italy have been obtained from Asian rice and made suitable for the growing conditions of Italian rice fields (Melini et al., 2019).

Rice could be consumed as raw rice or could undergo different treatments to improve some technological and nutritional characteristics. In general, rice is "covered caryopsis", it is harvested as "paddy rice" or "rough rice", where the kernel is still within the hull; after the harvest, paddy rice is dehulled to obtained brown rice. Subsequently, the milling procedure could be applied as an additional step in which the aleurone is removed with the pericarp and the seed coat, following an abrasive process that allows the separation of milled rice and bran. Rice bran is generally considered a by-product widely used in animal feeding.

In this study, two new pigmented rice varieties grown in Piedmont (Italy) and registered at the Community Plant Variety Office (CPVO) were characterized and the impact of two technological treatments (parboiling and milling) on their phytochemical composition was evaluated. The samples considered were: Violet rice (medium-grain rice, black variety – patent n° 46269/2017) (CPVO, 2017a), and Orange rice (medium-grain rice, red variety - patent n° 46270/2017) (CPVO, 2017b). The brown Violet and Orange rice were polished to 7% and 10% degrees of milling, respectively. In parallel, bran was collected, sieved to remove the large particles (> than 500 μm) and analyzed. The parboiled Violet and Orange rice were obtained by applying a standardized process by placing brown rice in hot water (65–70 °C) for 3 h (ratio rice: water about 1:1.15 w/w). The rice was steamed at 110°, for 3 min, up to 2.5 bar, and dried until the moisture content was about 13%.

In addition, considering the wide biodiversity of Italian rice, other pigmented and non-pigmented varieties were included as a comparison: Venere rice (medium-grain rice, black variety), Ermes rice (long-grains rice type B, red variety), and Carnaroli rice (medium-grain rice, non-pigmented variety). The brown rice Carnaroli was polished to 10% degree of milling and the bran was collected as previously reported.

Venere and Ermes rice varieties came from the Italian market, while Violet, Orange, Carnaroli and the treated samples (parboiled and milled) were provided by "Azienda Agricola Bertolone Eleonora di Bertolone Giovanni", Collobiano (VC).

The identification codes for rice samples are reported in Fig. 1, while bran samples were identified as follows: Violet bran (VB), Orange bran (OB) and Carnaroli bran (CB).

Rice samples were grounded at room temperature (around 20 °C) by a laboratory dish-mill (Buhler MLI-204, Segrate, Italy) to obtain rice powders smaller than 500 μm, that were stored in plastic bags (Reber S.r.l., Ruzzara, RE, Italy), under vacuum, in the dark, at 4 °C till their characterization.

2.3. Color evaluation

According to Cappa et al. (Cappa et al., 2021), grounded rice samples (approximately 4 g) levelled in a round (25 mm diameter) cup were evaluated by using a Minolta Chroma Meter II (Minolta, Osaka, Japan) with standard illuminant C. Results were expressed in the CIE L*a*b* space as L* [lightness; from black (0) to white (100)], a* [from green (−) to red (+)], and b* [from blue (−) to yellow (+)] values. In order to quantify the effect of treatments, (for parboiled and milled samples with respect to brown samples) ΔE values were calculated by using the following equation:

$$\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2}$$

2.4. Characterization of anthocyanin profile

2.4.1. Samples extraction

Rice anthocyanins were extracted using acidified methanol (Colombo et al., 2021): 0.5 g flour or 0.25 g bran were suspended in 10 mL of methanol:1 M HCl 85:15 (v/v) and maintained under stirring for 30 min at room temperature (around 20 °C) in the dark. Samples were centrifuged at 8000×*g* for 20 min at 4 °C (Avanti J-25, Beckman Coulter, Brea, CA, USA) and filtered using 0.45 μm filters (VWR International, Fontenay-sous-Boys, France). Extracted solutions were stored at −20 °C until analysis.

2.4.2. Determination of total anthocyanin content

The total anthocyanin content (TA) of rice samples was determined according to the AOAC method (AOAC International, 2006): samples,

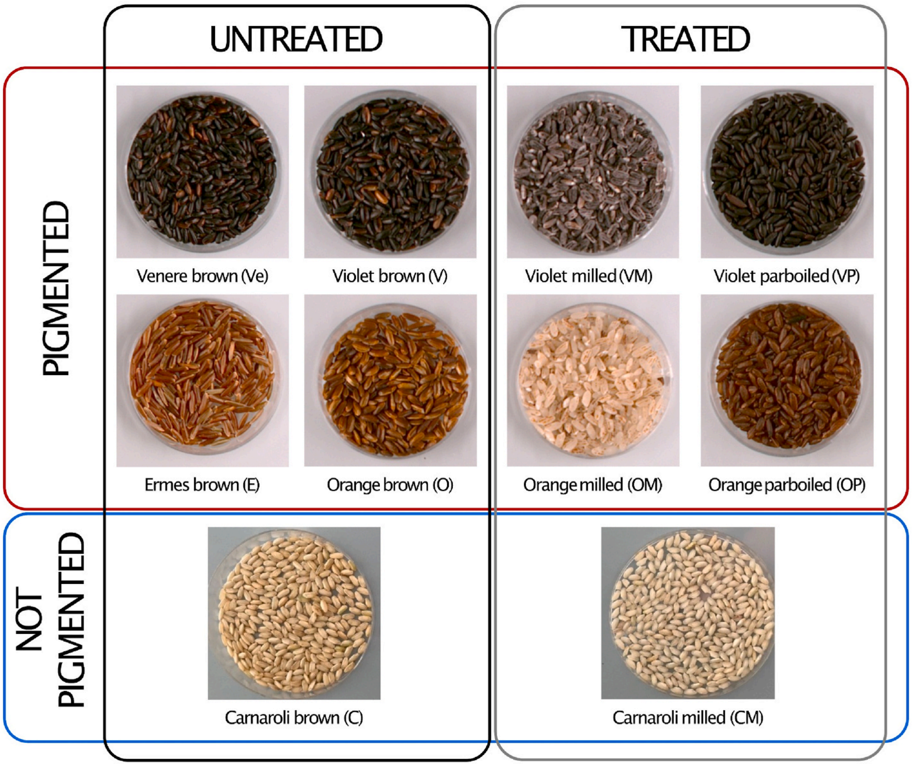

Fig. 1. –Pigmented and not pigmented rice samples included in this study.

extracted as described in section 2.4.1, were suitably diluted with 0.025 M KCl at pH 1 and 0.4 M CH_3COONa at pH 4.5. Solutions were analyzed by spectrophotometry at 520 nm and at 700 nm (to correct for haze).

The results were expressed as equivalents of cyanidin-3-*O*-glucoside (mg CY/g), according to the following equation:

$$\text{TA (mg CY/g)} = \Delta\text{A} \times \text{MW} \times \text{DF} \times 1000 \times \text{V/e} \times \text{l} \times \text{W}$$

where: ΔA = (A520 nm–A700 nm) pH 1.0 - (A520 nm–A700 nm) pH 4.5; MW: molecular weight (449.2 g/mol for CY); DF: dilution factor; 1000: conversion factor from g to mg; V: extraction volume (L); e: molar extinction coefficient (26,900 for CY); l: path length in cm (1 cm); W: sample weight (g).

2.4.3. Quantification of anthocyanins using HPLC-DAD

The identification and quantification of anthocyanins were obtained by applying the HPLC-DAD method reported by Colombo et al. (Colombo et al., 2021). Briefly, the analysis was performed using an HPLC equipment Jasco (Jasco, Tokyo, Japan) equipped with a pump (PU-980), an interface (LC-NETII/ADC), a diode array detector (MD-2010 Plus), a mixer (LG-150-0.4), a degasser (DG-2080-54), and an injection valve (Rheodyne, Cotati, CA, USA) with a 20 μL loop. The chromatographic column used was a Synergi 4u MAX-RP 80 A (250 × 4.60 mm 4 μm) (Phenomenex, Torrance, CA, USA) with a Security Guard C12 4 × 3.0 mm ID (Phenomenex).

The separation was performed at a flow rate of 0.8 mL min^{-1}. The gradient was set up as follows: 0–15 min: 94%–70%, A, 15–30 min: 70%–50% A, 30–35 min: 50%–10% A, 35–38 min: 10% An isocratic and 38–48 min: 10%–94% A; where (A) water:acetonitrile:formic acid 96:3:1 (v/v/v); and (B) acetonitrile:water:formic acid 50:49:1 (v/v/v). The anthocyanins were detected at 520 nm.

Stock solutions of cyanidin-3-*O*-glucoside (CY) and peonidin-3-*O*-glucoside (PE) were prepared in 0.1 M HCl at a final concentration of 200 μg mL^{-1}. The working solutions were suitably diluted in 0.1 N HCl at the concentration range of 0.5–20 μg mL^{-1} for CY and 0.5–10 μg mL^{-1} for PE. Samples, prepared as described in section 2.3.1, were analyzed in triplicate.

2.5. Determination of soluble phenolic content

2.5.1. Samples extraction

The soluble phenolic compounds were extracted using a hydro-alcoholic solution: 0.5 g flour or 0.25 g bran were suspended in 10 mL of ethanol:water 60:40 (v/v) (Catena et al., 2019) and maintained under stirring for 2 h at room temperature (around 20 °C) in the dark. Samples were centrifuged at 2000×*g* for 15 min at 4 °C (5810-R, Eppendorf, Hamburg, Germany) and filtered using 0.45 μm filters (VWR International, Fontenay-sous-Boys, France). Extracted solutions were stored at −20 °C until analysis. Each sample was extracted at least in triplicate.

2.5.2. Folin-Ciocalteau's assay

The soluble phenolic compounds were quantified using the Folin-Ciocalteau method (Singleton & Rossi, 1965): 300 μL of samples suitably diluted or water for blank, were added with 1.5 mL of 0.2 N Folin–Ciocalteau's reagent and 1.2 mL of 7.5% sodium carbonate. Samples were maintained for 30 min in the dark and the absorbance was detected at 765 nm in a UV–visible spectrophotometer (Varian Cary 50 SCAN, Palo Alto, CA, USA). Results were expressed as mg/g equivalent of gallic acid (GAE) using a calibration curve of gallic acid in the range of 5–50 μg mL^{-1}.

2.6. Determination of antioxidant capacity

2.6.1. DPPH assay

The antioxidant capacity of samples was evaluated as a measure of radical scavenging activity using the DPPH spectrophotometric test (Brand-Williams, Cuvelier, & Berset, 1995). Samples (prepared as described in Section 2.4.1 and suitably dilute), or water used as a blank (0.5 mL), were added with 1 mL of 0.005% DPPH in methanol and maintained for 30 min in the dark. The absorbance was measured at 517 nm against methanol. The concentration of antioxidants was calculated using a calibration curve built plotting the concentration of gallic acid (from 1.0 to 5.0 μg mL^{-1}) versus the difference between the absorbance of the blank and the absorbance of standards. The results were expressed as mg/g equivalents of gallic acid (GAE).

2.6.2. HPTLC method

High-Performance Thin Layer Chromatography (HPTLC) is a chromatographic technique useful to obtain the fingerprint of samples, usually visually evaluated, allowing the identification of specific components, which can be tested in parallel for their antioxidant properties. Thanks to a standardized methodology, HPTLC fingerprints are reproducible and useful for a semi-quantitative evaluation. The fingerprints represent the identity of a sample. Molecules contained in samples are separated on an HPTLC plate during the chromatographic run and visualized at different wavelengths as bands characterized by a certain retention factor, color, and intensity. The comparison between bands in the samples and reference standards, loaded in parallel on the plate, allows the identification of compounds present in samples.

- Samples preparation

Each sample (0.5 g) was added with 10 mL of ethanol:water 60:40 (v/v) and extracted as described in Section 2.4.1. Extracted solutions were dried using rotavapor followed by a freeze-dryer (Edwards Modulyo, UK) and solubilized in 1 mL of methanol to concentrate compounds.

- Chromatographic conditions

The analyses were conducted as reported by Colombo et al. (Colombo et al., 2021). Briefly: 10 μL of rice samples were loaded onto HPTLC silica-gel plates 60 F254 (10 × 20 cm, Merck, Darmstadt, Germany), using a semi-automatic sample applicator (Linomat 4, CAMAG, Muttenz, Switzerland). After the chromatographic separation, using a solution of 10 mL of acetone:toluene:formic acid 4.5:4.5:1 (v/v/v), the plates were derivatized with 0.05% DPPH methanolic solution, maintained for 30 min in the dark and revealed at visible light (software VisionCats, CAMAG, Muttenz, Switzerland).

2.7. Statistical analysis

Results were expressed as mean ± standard deviation values. Data were subjected to a one-way analysis of variance (ANOVA), followed by the Duncan test to identify significant differences among the rice samples ($p < 0.05$). The correlation between variables was assessed by Pearson linear correlation. The statistical analyses were carried out with IBM SPSS Statistics, Version 27.0 (New York, USA).

3. Results and discussion

Numerous studies in the literature have focused on Asian pigmented rice varieties (Melini et al., 2019), while the nutritional and nutraceutical characteristics of Italian pigmented rice have been less investigated. In addition, the studies conducted on European rice varieties were mainly focused on the phytochemical characterization of brown rice. A few studies focused only on specific rice varieties (red and non-pigmented) have evaluated the impact of industrial technological treatments, observing a reduction in soluble phenolic content and antioxidant activity (Finocchiaro et al., 2007; Petroni et al., 2017). In this context, the aim of this study was the characterization of the phytochemical profile of different pigmented rice varieties, before and after technological treatments (parboiling and milling), focusing on the Violet and Orange rice that represents relatively new varieties.

According to technical data sheets, rice samples differed in their nutritional composition. In particular, pigmented samples had carbohydrate content (g/100 g) ranging between 62.8 (Venere brown) and 70.0 (Orange Parboiled); protein (g/100 g) from 7.4 (Ermes brown) and 9.7 (Violet Parboiled); fat (g/100 g) from 1.4 (Ermes brown) to 3.3 (Violet brown), and fibre content (g/100 g) of 3.8 (Orange brown and Orange Parboiled) to 4.9 (Violet Parboiled). On the opposite, the non-pigmented milled Carnaroli had a higher level of carbohydrates (76.5 g/100 g), fewer proteins (6.8 g/100 g), intermediate fats content (2.1 g/100 g) and less fibre (1.1 g/100 g). Although the non-pigmented rice varieties are more traditionally consumed, these data suggested that the consumption of pigmented rice may exert some nutritional benefits (e.g., lower glycemic response, gastrointestinal regulation, etc.).

Table 1 reported sample identification, pigmentation classification and colorimetric indices. As attended, samples were significantly ($p < 0.05$) different in terms of colorimetric indices and the two technological treatments significantly ($p < 0.05$) affected them: parboiling caused a reduction of lightness (L*) and an increase of redness (a*) and yellowness (b*) due to the thermal treatment applied, while milling determined an increase of lightness (brighter samples) and a reduction of the other color coordinates due to the removal of the external rice layer. Furthermore, ΔE values were always higher than 3, suggesting the color difference with respect to the brown sample is visible by human eyes (de Souza & Fernández, 2011).

3.1. Anthocyanins biodiversity in pigmented rice and impact of technological treatments

Anthocyanins are responsible for the purple and blue colors of rice varieties (Mbanjo et al., 2020) and are known for their antioxidant properties. Anthocyanins in rice samples were quantified using the spectrophotometric pH differential method (AOAC International, 2006) and the HPLC-DAD method, previously developed by Colombo et al. (Colombo et al., 2021). The spectrophotometric pH differential method is a simple, low-cost and widespread assay for the total anthocyanin determination (Catena et al., 2019), useful for rapid screening and comparison among samples. However, this method generally overestimates the anthocyanin content, due to the interference from other colored compounds (Catena et al., 2019). Therefore, the HPLC-DAD analysis, allowing the identification and quantification of each specific anthocyanin compound, is an important tool to better characterize the samples and study the impact of technological treatment on this class of molecules.

According to the literature, anthocyanins were detected in black and purple varieties (Fig. S1), indeed red rice is generally rich in proanthocyanidins (Hosoda et al., 2018; Pereira-Caro et al., 2013).

Table 2 lists the total anthocyanin content (TA), quantified by the spectrophotometric method, and their identification and quantification by the HPLC-DAD method.

The HPLC-DAD method allowed the detection of cyanidin-3-*O*-glucoside and peonidin-3-*O*-glucoside, which are the most representative anthocyanins in rice (Bhuvaneswari et al., 2020; Min et al., 2014).

As expected, both the treatments applied drastically reduced the anthocyanin content of Violet samples. Anthocyanins accumulate in the outer layers of the kernels (Finocchiaro et al., 2010); this is the reason why Violet milled rice showed a reduction in the content of these compounds when compared to the corresponding brown rice (Violet brown) and high anthocyanins content was found in the bran obtained by the milling process (25.62 ± 3.45 mg CY/g and 18.09 ± 0.26 mg/g measured with spectrophotometric and chromatographic techniques, respectively). Furthermore, anthocyanins are heat-labile molecules (Min et al., 2014); in fact, a reduction of 97.5%, of their concentration was found in Violet parboiled rice; this was evidenced also by Min et al. who reported a reduction of anthocyanins after parboiling from 88% to 96% (Min et al., 2014).

In addition, these analyses underline differences between the considered black varieties: the Violet brown rice showed the significant ($p < 0.05$) highest anthocyanins content also compared to Venere brown rice (1.06 mg/g). However, these results suggested that the brown

Table 1
Sample identification, pigmentation and colorimetric indices.

Pigmentation	Untreated				Treated				
	Sample	L*	a*	b*	Treatment	L*	a*	b*	ΔE
Black	Violet (V)	$54.2 \pm 1.0^{b; B}$	$1.9 \pm 0.2^{d; A}$	$1.5 \pm 0.2^{b; B}$	Parboiled (VP)	$47.0 \pm 0.5^{a; A}$	$3.5 \pm 0.2^{f; C}$	$4.9 \pm 0.3^{d; C}$	8.2
					Milled (VM)	$62.1 \pm 0.5^{d; C}$	$2.7 \pm 0.3^{e; B}$	$0.4 \pm 0.1^{a; A}$	8.0
	Venere (Ve)	56.6 ± 0.7^{c}	1.6 ± 0.2^{c}	2.1 ± 0.4^{c}					
Red	Orange (O)	$66.6 \pm 0.4^{e; B}$	$1.9 \pm 0.3^{d; B}$	$10.7 \pm 0.^{h; B}$	Parboiled (OP)	$62.2 \pm 0.9^{d; A}$	$2.5 \pm 0.2^{e; C}$	$14.2 \pm 0.7^{j; C}$	5.6
					Milled (OM)	$82.7 \pm 1.5^{f; C}$	$-0.7 \pm 0.2^{b; A}$	$8.5 \pm 0.4^{f; A}$	16.5
	Ermes (E)	66.9 ± 0.6^{e}	4.3 ± 0.1^{g}	13.2 ± 0.3^{i}					
Not pigmented	Carnaroli(C)	$86.1 \pm 0.9^{g; A}$	$-1.7 \pm 0.1^{a; A}$	$10.0 \pm 0.7^{g; B}$	Milled (CM)	$90.2 \pm 0.3^{h; B}$	$-1.9 \pm 0.2^{a; B}$	$6.8 \pm 0.4^{e; A}$	5.2

For the same variable, samples having different lower-case letters are significantly different ($p < 0.05$).
For the same variable and variety, upper-case letters indicate statistically significant differences among the different technological treatments ($p < 0.05$).

Table 2
Total anthocyanin content (TA) measured by spectrophotometric method and expressed as mg equivalent of cyanidin-3-*O*-glucoside (CY)/g (n = 3), and their identification and quantification in mg/g by the HPLC-DAD method (n = 6).

Untreated					Treated				
Sample	TA (mg CY/g) (mean ± SD)	HPLC-DAD Analysis (mg/g) (mean ± SD)			Treatment	TA (mg CY/g) (mean ± SD)	HPLC-DAD Analysis (mg/g) (mean ± SD)		
		CY	PE	Total (CY + PE)			CY	PE	Total (CY + PE)
Violet (V)	$3.982 \pm 0.383^{d; C}$	$2.261 \pm 0.181^{d; C}$	$0.136 \pm 0.010^{d; C}$	$2.398 \pm 0.191^{d; C}$	Parboiled (VP)	$0.340 \pm 0.018^{a; A}$	$0.055 \pm 0.003^{a; A}$	$0.005 \pm 0.0002^{a; A}$	$0.060 \pm 0.004^{a; A}$
					Milled (VM)	$1.401 \pm 0.175^{b; B}$	$0.661 \pm 0.010^{b; B}$	$0.038 \pm 0.002^{b; B}$	$0.699 \pm 0.011^{b; B}$
Venere (Ve)	2.137 ± 0.031^{c}	1.014 ± 0.063^{c}	0.050 ± 0.001^{c}	1.064 ± 0.062^{c}					

CY: cyanidin-3-*O*-glucoside; PE: peonidin-3-*O*-glucoside.
For the same variable, samples having different lower-case letters are significantly different ($p < 0.05$).
For the same variable and variety, upper-case letters indicate statistically significant differences among the different technological treatments ($p < 0.05$).

samples are a better source of anthocyanins compared to the treated samples. Therefore, they could represent an interesting source of active molecules, particularly for people suffering from chronic diseases involving the gastrointestinal system (such as celiac disease), generally affected by an inflammatory and oxidative status at the intestinal level (Ferretti et al., 2012).

These results agree with the findings reported by other authors. In fact, studying thirty pigmented varieties of rice from India using the LC-MS technique, Bhuvaneswari et al. reported an anthocyanin content ranging between 0.30 and 2.76 mg/g (Bhuvaneswari et al., 2020). From the analysis of four black Italian varieties, Melini et al. observed an anthocyanin content ranging between 0.80 and 2.09 mg/g (Melini et al., 2019). These data agree with our chromatographic results, as illustrated in Fig. 2.

Min et al. found a total anthocyanin content (TA) of 4.13 mg CY/g flour (d.w.) and 1.17 mg CY/g flour in two purple varieties from USA (Min et al., 2014); while Fracassetti et al. reported a total anthocyanin content between 1.83 and 3.41 mg CY/g in different batches of two black Italian varieties (Venere and Artemide) (Fracassetti et al., 2020). These data are comparable to our spectrophotometric results.

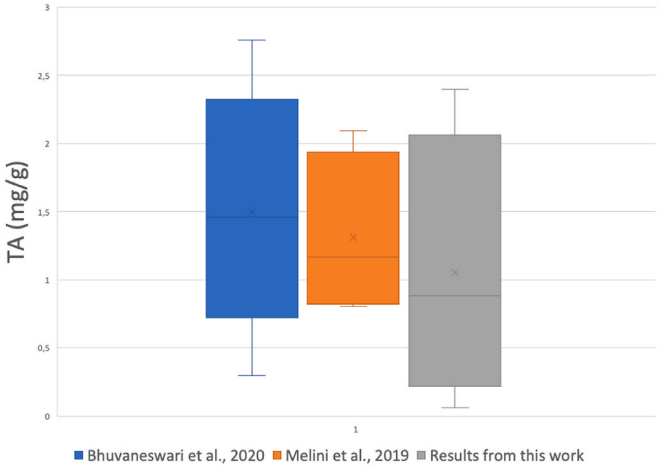

Fig. 2. Comparison between literature data (Bhuvaneswari et al., 2020; Melini et al., 2019) and results from the present study on the anthocyanin content.

3.2. Phenolic content biodiversity in pigmented and non-pigmented rice and the impact of technological treatments on phenolic compounds and antioxidant activity

Cereals are rich in soluble phenolic compounds (Paiva et al., 2016), which include anthocyanins but also other molecules such as phenolic acids and proanthocyanidins. The soluble phenolic compounds are rapidly absorbed in the small intestine (Wang et al., 2014), and this site is usually involved in chronic diseases, such as celiac disease. Considering the interest in this group of molecules, the soluble polyphenol content (SPC) and the associated antioxidant capacity (AOA) were evaluated (Table 3) in this study.

The content of soluble phenolic compounds ranged between 1.48 and 5.69 mg GAE/g for black varieties, from 0.31 to 1.33 mg GAE/g for red varieties and from 0.28 to 0.52 mg GAE/g for Carnaroli rice.

These results are comparable to data on rice from the USA published by Min et al. who found a SPC from 0.30 to 7.03 mg GAE/g flour (d.m.) in six rice varieties with different bran color, from white to purple (Min et al., 2014). Melini et al. found slightly higher data in seven pigmented brown rice varieties from Italy and France (5.44–15.08 mg/g d.m.) (Melini et al., 2019). Bordiga et al. observed a phenolic content between 1.4 and 11.9 mg GAE/g in six rice varieties with different pigmentation

Table 3
Soluble polyphenol content (SPC) and antioxidant capacity (AOA) of samples. Results are expressed as mg equivalents of gallic acid (GAE)/g (n = 6 for Orange samples; n = 9 for Ermes rice; n = 3 for other samples).

Pigmentation	Untreated			Treated		
	Sample	SPC(mg GAE/g) (mean ± SD)	AOA (mg GAE/g) (mean ± SD)	Treatment	SPC(mg GAE/g) (mean ± SD)	AOA (mg GAE/g) (mean ± SD)
Black	Violet (V)	$5.69 \pm 0.62^{g; B}$	$1.65 \pm 0.11^{g; B}$	Parboiled (VP)	$1.48 \pm 0.13^{de; A}$	$0.53 \pm 0.07^{d; A}$
				Milled (VM)	$1.71 \pm 0.05^{e; A}$	$0.67 \pm 0.04^{e; A}$
	Venere (Ve)	3.42 ± 0.40^{f}	1.07 ± 0.11^{f}			
Red	Orange (O)	$1.33 \pm 0.07^{cde; C}$	$0.25 \pm 0.01^{bc; C}$	Parboiled (OP)	$0.91 \pm 0.14^{bc; B}$	$0.17 \pm 0.01^{b; B}$
				Milled (OM)	$0.31 \pm 0.03^{a; A}$	$0.06 \pm 0.004^{a; A}$
	Ermes (E)	1.17 ± 0.46^{cd}	0.33 ± 0.11^{c}			
Not pigmented	Carnaroli (C)	$0.52 \pm 0.003^{ab; B}$	$0.14 \pm 0.02^{ab; B}$	Milled (CM)	$0.28 \pm 0.02^{a; A}$	$0.06 \pm 0.003^{a; A}$

For the same variable, samples having different lower-case letters are significantly different ($p < 0.05$).
For the same variable and variety, upper-case letters indicate statistically significant differences among the different technological treatments ($p < 0.05$).

(from white to black) (Bordiga et al., 2014). Fig. 3 illustrates the range of SPC detected in pigmented and non-pigmented rice originating from different parts of the world.

As expected, the brown black varieties showed the highest SPC, which was higher for Violet brown if compared to Venere brown; these results are in good agreement with the literature data (Rocchetti et al., 2019). The Orange and Ermes brown rice showed no significant differences ($p > 0.05$) in SPC values, although a great variability was observed in Ermes sample (1.17 ± 0.46), despite the extraction procedure being repeated nine times.

For all varieties considered, parboiling and/or milling determined a significant reduction ($p < 0.05$) in terms of polyphenol content. In particular, the hydrothermal process of parboiling induced a SPC reduction of 32% and 74% for Orange and Violet, respectively,suggesting that the parboiling process mainly affected the SPC in the Violet variety compared to the Orange ones.

The reduction of SPC concurs with Min et al. where hydrothermal processes (water cooking and parboiling) significantly decreased phenolic content and flavonoid content by 16%–91% in USA rice varieties with different pigmentation (Min et al., 2014).

It is interesting to highlight that the Violet variety seems to be equally affected by parboiling and milling; in fact, no statistically significant differences were observed between the SPC of Violet milled and Violet parboiled samples ($p > 0.05$).

Milling determined a SPC reduction of 46%, 70% and 77% for Carnaroli, Violet and Orange, respectively. This is due to the fact that these compounds accumulate mainly in the outer layers of rice and embryo, which are removed by the milling process (Mbanjo et al., 2020). Consequently, the bran obtained by the milling is particularly rich in phenolic compounds. In our study, SPC was 40.17 ± 0.65 mg GAE/g in Violet bran, 9.65 ± 0.10 mg GAE/g in Orange bran and 5.15 ± 0.68 mg GAE/g in Carnaroli bran. These results indicate that SPC in bran is from 7 to 9 times higher than the corresponding brown rice.

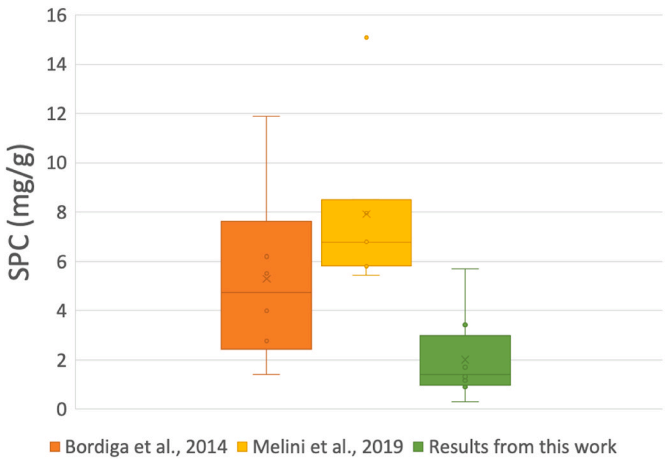

Fig. 3. Comparison between literature data (Bordiga et al., 2014; Melini et al., 2019) and results from the present study on the SPC.

The black varieties Violet and Venere showed the highest antioxidant capacity (AOA) (0.53–1.65 mg GAE/g) followed by the red varieties Orange and Ermes (0.33–0.06 mg GAE/g), and the non-pigmented rice Carnaroli (0.06–0.14 mg GAE/g). The technological treatments significantly ($p < 0.05$) affected the AOA, determining a reduction of values between 32% and 76%. In addition, lightness values, that increased after the milling process (Table 1), were linearly and negatively correlated with AOA ($r = 0.60$; $p < 0.05$), confirming that AOA was negatively affected by the milling process.

According to the soluble phenolic content, the AOA measured in bran by-products was 16.14 ± 0.12 mg GAE/g in Violet bran, 2.26 ± 0.14 mg GAE/g in Orange bran and 1.21 ± 0.11 mg GAE/g in Carnaroli bran.

Generally speaking, few studies evaluated the impact of parboiling or milling on pigmented Italian rice varieties. The study of Petroni et al. considered the impact of these treatments only on one non-pigmented variety, underlying the reduction of SPC after the processes (Petroni et al., 2017). Finocchiaro et al. observed a reduction in total antioxidant activity in two Italian varieties (white and red) after a different degree of milling (3% and 6%), particularly in red rice, where a reduction of 70% and 80% was observed proportionally to the milling degree (Finocchiaro et al., 2007). The data collected in this study provided interesting information about the impact of technological treatments on different pigmented varieties considered: for example, the milling process mainly affected the phenolic content and the relative antioxidant activity in the pigmented rice, particularly the orange rice, compared to the parboiling treatment.

Natural antioxidants in foods are different, they include phenolic compounds, vitamins, enzymes, and carotenoids. The contribution of phenolic compounds to the total antioxidant activity of the samples was explored, evaluating the correlation between these two variables. The bran showed TA, SPC and AOA values considerably different from rice samples and for this reason, they were excluded from this evaluation. A positive linear correlation ($r = 0.950$, $p < 0.001$) was found between polyphenol content and antioxidant activity and between total anthocyanin content and antioxidant activity ($r = 0.953$, $p < 0.001$). Therefore, both phenolic content and anthocyanin content could be used as indicators of the antioxidant properties of the rice samples.

To sum up, pigmented varieties, particularly the brown samples, are characterized by an interesting phenolic profile that was directly correlated with their antioxidant capacity. These data confirmed that the pigmented rice could represent a promising "functional" ingredient for the general population, but particularly for people suffering from chronic gastrointestinal diseases.

The AOA was also evaluated by applying the High-Performance Thin Layer Chromatography (HPTLC) technique (Fig. S2). HPTLC is a rapid, suitable semi-quantitative method for screening different compounds classes, allowing the fingerprint characterization of complex products. In addition, the HPTLC technique could be used to evaluate some biological properties, such as antioxidant activity, which can be directly associated with each compound present in the samples.

Although this method does not fully consider the contribution of anthocyanins to the AOA, due to the characteristic color of these molecules, the HPTLC analysis is useful to evaluate the contribution of other classes of phenolic compounds (such as flavonols, flavan-3-ols and phenolic acids).

The results agree with those obtained by the spectrophotometric method: bran samples showed the highest AOA, followed by Violet brown (V), Venere brown (Ve) and Orange brown (O) samples. It is interesting to note that, excluding anthocyanins, the Violet varieties still showed the highest content of other phenolic compounds and the strongest associated antioxidant property. The technological treatments affected the AOA of samples, particularly in the milled rice (VM and OM), as previously observed. Indeed, the Violet parboiled (VP) showed an interesting phenolic content, compared to Violet milled (VM), therefore the parboiling process in the Violet variety seems to affect anthocyanins mainly, as observed in spectrophotometric and HPLC-DAD analysis.

4. Conclusions

This study focused on Italian rice varieties, with particular attention on two relatively new pigmented rice (Violet and Orange) and the impact of industrial technological treatments on the phytochemical composition of samples. Some of the rice collected were available on the Italian market, allowing us to evaluate real samples already available for consumers.

Our results confirmed that the phytochemical characteristics (e.g., antioxidant capacity, soluble polyphenols, and total anthocyanin content) of pigmented varieties are higher than the non-pigmented Carnaroli variety. Furthermore, a correlation was found between polyphenols content and antioxidant activity, and between total anthocyanin content and antioxidant activity, suggesting that both the phenolic content and anthocyanins content could be used as indicators of rice flour's antioxidant properties. The two investigated technological processes - that have well-known advantages in terms of cooking time, starch availability, glycemic index and/or vitamins and mineral contents-negatively affected the concentration of phenolic compounds and the corresponding antioxidant capacity of samples; however, some active compounds (e.g., anthocyanins, phenolic acids) withstand to the hydrothermal or the mechanical treatment with a different extent according to the rice variety and the process considered. This study, characterizing different Italian rice varieties, contributes to improving the knowledge about the biodiversity present in the country. These results could be therefore helpful in the selection of the most suitable raw material, in terms of phenolic compounds, to be used in the food industry, including those producing gluten-free products. Further studies need to be conducted to define the technological treatment and cooking conditions that allow to preserve the phytochemical content of the pigmented rice varieties as much as possible, as well as guaranteeing a reduction of the actual cooking time of the pigmented rice varieties that penalize their consumption.

CRediT authorship contribution statement

Francesca Colombo: Conceptualization, Data curation, Formal analysis, Writing – original draft. **Carola Cappa:** Conceptualization, Formal analysis, Supervision, Writing – review & editing. **Corinne Bani:** Formal analysis. **Marco Magni:** Formal analysis. **Simone Biella:** Formal analysis. **Patrizia Restani:** Conceptualization, Data curation, Supervision, Writing – review & editing. **Chiara Di Lorenzo:** Conceptualization, Data curation, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare no conflict of interest.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fbio.2023.102674.

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