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Impact of traditional and innovative cooking techniques on Italian black rice (*Oryza sativa* L., Artemide cv) composition

Antonio Colasanto, Fabiano Travaglia, Matteo Bordiga, Jean Daniel Coïsson, Marco Arlorio, Monica Locatelli *

Dipartimento di Scienze del Farmaco, Università degli Studi del Piemonte Orientale, 28100 Novara, Italy

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ABSTRACT

Due to its high polyphenol content, black rice plays a significant role in good nutrition; however, these antioxidant compounds are affected by heat treatments required for the rice consumption. The aim of this work was to investigate how cooking affects the composition of Artemide black rice, comparing innovative methods, such as *sous vide*, with traditional domestic techniques (risotto and pilaf). Proteins and ashes were not affected by cooking, except for pilaf rice, where a 42 % ashes decrease was observed; fiber content increased after all cooking methods, reaching a 29 % increase in the risotto. Antioxidant activity, total polyphenols, anthocyanins and proanthocyanidins were reduced on average of 40 %, 34 %, 43 % and 39 %, respectively. Individual anthocyanins decreased, while phenolic acids and other flavonoids presented different behaviours, also depending if considered in their free or bound form. Cyanidin-3-O-glucoside was reduced up to 56 % in the *sous vide* cooked rice at 99 °C, and only by 45 % and 37 % in the risotto and *sous vide* cooked rice at 89 °C, respectively. Traditional risotto preparation and the innovative *sous vide* cooking at 89 °C also maintained the highest antioxidant polyphenols content, saving 63 % of the antioxidant activity in respect to the raw black rice. Concluding, these last techniques can be suggested for a better preservation of bioactive compounds.

1. Introduction

Due to its proteins, carbohydrates, vitamins and essential elements content, rice (*Oryza sativa* L.) is probably the most important food crop in the world. For more than half of the world population it is considered a staple food, particularly in Europe, America, and Asia (Kukusamude, Phitchan, Nunticha, & Supalak, 2021). The greatest production of rice in Europe comes from Italy, especially from two regions (Piedmont and Lombardy), in which more than 90 % of the Italian rice is produced (Bordiga et al., 2014). Brown rice is the whole-grain rice; the bran layers have not been removed by polishing, so brown rice is richer in fiber, lipids and bioactive phytochemicals, such as phytosterols and phenolic compounds, compared to dehulled one. White rice varieties are currently the most cultivated and consumed, contrary to pigmented ones, whole cultivation is limited to restricted areas of the globe (including Italy, France and north Africa). However, pigmented rice varieties in the last few years have received an increased attention due to their phenolic content, antioxidant activity and potential beneficial effects on human health (Huang et al., 2020).

Pigmented varieties from different cereals are considered as health-promoting foods for their high content of anthocyanins, the pigments mainly responsible of the blue/red/black rice colour. They are located primarily in the bran, reason why these varieties are consumed preferably as whole rice. Cyanidin-3-O-glucoside is the most abundant anthocyanin in black rice, followed by peonidin-3-O-glucoside (Hou, Qin, Zhang, Cui, & Ren, 2013). The Italian Artemide black rice, a natural hybrid obtained from Venere rice and a white *indica* rice variety, is particularly rich in these healthy compounds (Bordiga et al., 2014).

Notwithstanding anthocyanins have numerous beneficial effects on human health, they are highly unstable compounds, in fact they are

Abbreviations: AA, antioxidant activity; AC, anthocyanins content; Cat, catechin; CE, catechin equivalents; CnE, cyanidin-3-O-glucoside equivalents; Cn-3-gent, Cyanidin-3-O-gentiobioside; Cn-3-glc, Cyanidin-3-O-glucoside; Cn-3-rut, Cyanidin-3-O-rutinoside; Coum, coumaric acid; Fer, ferulic acid; Gal, gallic acid; MAC, monomeric anthocyanins; Myr, myricetin; PAC, proanthocyanidin content; PC, phenolic content; PIL, Pilaf; Pn-3-glc, Peonidin-3-O-glucoside; Pn-3-rut, Peonidin-3-O-rutinoside; Proto, protocatechuic acid; RIS, risotto; SV89, *Sous vide* 89 °C; SV99, *Sous vide* 99 °C; TE, Trolox equivalents; Van, vanillic acid.

* Corresponding author.
E-mail addresses: antonio.colasanto@uniupo.it (A. Colasanto), fabiano.travaglia@uniupo.it (F. Travaglia), matteo.bordiga@uniupo.it (M. Bordiga), jeandaniel.coisson@uniupo.it (J.D. Coïsson), marco.arlorio@uniupo.it (M. Arlorio), monica.locatelli@uniupo.it (M. Locatelli).

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influenced by pH, light, oxygen, enzymes and thermal treatments (Hou et al., 2013). Since cereals must be consumed after cooking, it is important to investigate how heat treatments could influence the phenolic profile of these foods. A lot of techniques can be used to cook black rice; the methods vary depending on the culture and habits of the various countries, but the most common are boiling, in which rice is boiled in an excess of water, and other methods, in which rice is cooked in an amount of water that should be completely adsorbed, such as "risotto" and "pilaf" modes. In addition, microwave, pressure cooker and steam cooking are largely used (the last one particularly in Indonesia) (Daomukda, Moongngarm, Payakapol, & Noisuwan, 2011; Juliano, 2016).

In recent years, innovative techniques for food cooking have become increasingly popular, including *sous vide* cooking, that consists in cooking raw foods under controlled time and temperature, under vacuum, in heat-resistant bags (Baldwin, 2012). This technique is principally applied to cook meat, fish, and vegetables. The low temperatures generally used in the *sous vide* cooking determine in meat and fish a minor shrinkage of the myofibrillar structure, comparing to traditional techniques, with a consequent reduction of water loss (Ayub & Ahmad, 2019). Some Authors have also observed that the impact of this technique on lipid oxidation and hydrolysis, as well as on the loss of mineral salts, is lower than other traditional techniques. Rasinska, Rutkowska, Czarniecka-Skubina, and Tambor (2019) demonstrated that the peroxide value in rabbit meat subjected to sous vide cooking is lower than that observed following boiling and roasting. Nieva-Echevarría, Manzanos, Goicoechea, and Guillén (2017) did not detect species deriving from either lipid oxidation or hydrolysis in *sous vide* cooked European sea bass. da Silva et al. (2017) observed that the loss of mineral salts in *sous vide* cooked bovine liver was almost nil, differently from boiling. Interesting results were obtained also on polyphenolic compounds. In their study on chicory subjected to various types of cooking (boiling, steam cooking, *sous vide* and microwave cooking), Renna, Gonnella, Giannino, and Santamaria (2014) observed that the concentration of total polyphenols after *sous vide* cooking did not vary compared to the raw matrix, while after boiling a 46 % decrease of these compounds was observed. Moreover, the antioxidant activity had a partial reduction after boiling and steaming, while it increased after *sous vide* cooking.

Many studies investigated the impact of cooking on bioactive compounds of black rice (Catena et al., 2019; Colasanto et al., 2021; Fracassetti, Pozzoli, Vitalini, Tirelli, & Iriti, 2020; Ryu & Koh, 2017). It was demonstrated that boiling is the worst cooking method to preserve polyphenols. However, none of these works has considered alternative cooking techniques to cook black rice. This is the reason why in this work we have investigated the impact of *sous vide* cooking, a method poorly applied to cereals, but highly used in other food matrices to minimize the polyphenolic degradation, on the chemical and nutritional composition of Artemide black rice, in comparison to other usual techniques (risotto and pilaf).

2. Materials and methods

2.1. Chemicals

Anthocyanins (cyanidin-3-O-glucoside, peonidin-3-O-glucoside, cyanidin-3-O-rutinoside and peonidin-3-O-rutinoside) were purchased from LGC Standard S.r.l. (Sesto San Giovanni, Milan, Italy). All the other polyphenol reference compounds (protocatechuic acid, ferulic acid, *p*-hydroxybenzoic acid, gallic acid, vanillic acid, coumaric acid, myricetin, catechin), chemicals, and reagents were of analytical grade from Merck KGaA (Darmstadt, Germany). Acetonitrile, methanol (all HPLC grade), and formic acid (50 %, LC-MS grade) were obtained from Carlo Erba Reagents S.r.l. (Cornaredo, Milan, Italy). Ultrapure water (18.2 MΩ cm at 25 °C) was produced by MAINA ULTRAPURE system (G. Maina, Pecetto Torinese, Turin, Italy).

2.2. Rice samples and cooking procedures

The local company "Azienda Agricola Luigi e Carlo Guidobono Cavalchini, tenuta La Mondina", located in Casalbeltrame, Novara (Italy), kindly supplied the "Artemide" black rice (harvest year: 2018) in under-vacuum commercial packages kept at room temperature.

Rice was analyzed raw, previously ground in a laboratory blender (Sterilmixer 12, International PBI, Milan, Italy) and then reduced to a fine flour using a mixer mill (MM 400, Retsch GmbH, Haan, Germany), and after cooking.

Three cooking techniques were applied: risotto (Ris), pilaf (Pil) and *sous vide* (SV). The cooking parameters derived from previous tests, carried out to guarantee a similar texture of the rice, independently from the cooking method. 100 g of rice, corresponding to about 1 portion, was chosen as rice amount, and the ratios rice/water were optimized to allow a complete absorption of the water by the rice.

2.2.1. Risotto (RIS)

100 g of black rice was placed in a cooking pot and set on the largest plate of the electric hob "Electrolux PQX320C" (Stockholm, Sweden). The plat was set to the power level "4", corresponding to medium/high cooking, and the rice was toasted for 3 min, mixing continuously with a steel spoon. The start of the toasting phase coincides with the start of the cooking time. Then, 100 mL of distilled hot water (previously heated on a plate at 80 °C) was added and the pot was closed with a lid. After the almost complete adsorption of the water by the rice (5 min), eight aliquots of 50 mL of distilled hot water were added, at 3 min and 30 s from each other, for a total volume of 500 mL. The total cooking time was 35 min (during the last minute the lid was removed), then the rice, maintained in the pot covered by the lid, was left to cool at room temperature (20 °C). At the end of the cooking the water was completely absorbed by the rice or evaporated. The temperature of rice during cooking, measured with a kitchen thermometer (Habor HCP1, Dongguan, Guangdong, China) varied in the range 98–100 °C.

2.2.2. Pilaf (PIL)

100 g of black rice was placed in a thin layer in an aluminium pan and 150 mL of boiling distilled water was added. The pan was covered by an appropriate lid and then was put in an oven (Candy FCS201X, Brugherio, Monza e Brianza, Italy) previously preheated at 175 °C (static mode). The cooking was conducted for 30 min, after which the rice was left to cool at room temperature (20 °C), in the pan covered by the lid.

2.2.3. Sous vide (SV)

100 g of black rice was placed in a plastic bag suitable for *sous vide* cooking and 100 mL of distilled water was added. Vacuum packaging was carried out using the chamber vacuum packer "LAVEZZINI UNICA" (UNIVAC Group S.r.l., Fiorenzuola d'Arda, Monza e Brianza, Italy). The black rice contained in the hermetically sealed bags was cooked at 89 °C (SV89) or 99 °C (SV99) for 1 h in a cooking bath (SEVERIN SV 2447, SEVERIN Elektrogeräte GmbH, Sundern, Germany) able to maintain the set temperature in a range of ± 1 °C. The rice was left to cool at room temperature (20 °C), inside the bags and in the dark.

Cooking conditions are summarized in Table 1; each cooking mode was carried out in triplicate. Prior to the analyses, cooked rice samples were freeze-dried (Heto Drywinner 8, Copenhagen, Denmark) according to the following procedure: pre-freezing −25 °C for 1 h; primary drying −10 °C for 16 h and 0 °C for 16 h; secondary drying 10 °C for 30 h and 20 °C for 10 h. Finally, as raw grains, lyophilized cooked samples were ground (Sterilmixer 12, International PBI, Milan, Italy) and reduced to a fine flour (MM 400, Retsch GmbH, Haan, Germany).

2.3. Proximate composition

The determination of the proximate composition was performed as previously described in Colasanto et al. (2021). The thermo-balance

Table 1
Cooking conditions applied.

Cooking procedure	Ratio rice/water (g/mL)	Cooking time (min)	Temperature (°C)	Notes
Risotto (RIS)	100/500	35 (including 3 min toasting)	100	Temperature of the rice during cooking
Pilaf (PIL)	100/150	30	175	Temperature of the oven
Sous-vide 99 °C (SV99)	100/100	60	99	Temperature of the cooking bath
Sous-vide 89 °C (SV89)	100/100	60	89	Temperature of the cooking bath

Sartorius MA30 (Sartorius AG, Goettingen, Germany), the Kjeltec system I (Foss Tecator AB, Höganäs, Sweden) and the Megazyme total dietary fiber analysis kit were used to determine moisture, total protein (conversion factor: 5.95) and total dietary fiber contents, respectively. The ash content was determined according to the AOAC (1990) procedure.

2.4. Phenolic characterization

2.4.1. Extraction of phenolics

All the samples, both raw and freeze-dried cooked black rice, underwent an extraction procedure for the characterization of both free and bound phenolics, based on the protocol described by Papillo et al. (2018) and Giordano, Reyneri, Locatelli, Coïsson, and Blandino (2019), with some modifications. 100 mg of ground sample was extracted with 1.5 mL of a 50 % *v/v* aqueous ethanol in ultrasonic bath for 2 min at room temperature. Then, the test tube was centrifuged (Eppendorf Centrifuge 5417 R, Hamburg, Germany) at 15,000 *g* for 2 min and the upper phase was collected in a 15 mL test tube. The extraction was repeated two times on the solid residue, collecting every time the upper phase in the 15 mL test tube. This phase represents the free fraction of phenolics, while the solid lower phase was used for the extraction of bound phenolics. The total extract was divided in aliquots for the analyses and stored at −20 °C until use. For each rice sample the extraction was performed in triplicate.

The solid residue from the extraction of free phenolics was quantitatively transferred in a 50 mL Erlenmeyer flask using a total volume of 10 mL of NaOH 4 M. The flask was placed on a magnetic stirring plate and mixed for 3 h and 30 min, after which the suspension was acidified with HCl 6 M to a pH of 2.30. Then, the flask content was transferred into a separating funnel and three extractions with ethyl acetate, using 30, 20 and 10 mL, sequentially, were carried out. Following each extraction step, the organic phase was collected in a 100 mL flask and finally the solvent was removed by a rotary evaporator (Rotavapor® BUCHI R-210, Flawil, Switzerland). The dry extract obtained was dissolved in 2 mL of HPLC grade methanol, filtered on a 0.45 μm filter (Spartan^{TM} 30/0.45 RC) and transferred in a 2 mL test tube, representing the bound fraction of phenolics. The extract was stored at −20 °C until use. For each cooked rice sample the extraction was performed in triplicate.

2.4.2. Spectrophotometric analyses

Spectrophotometric analyses were performed on the hydroalcoholic extracts (free phenolic fraction).

2.4.2.1. Antioxidant activity. The antioxidant activity (AA) was determined as DPPH radical scavenging, following the method described in Locatelli et al. (2009). More specifically, samples were opportunely diluted in MeOH up to a final volume of 700 μL, then 700 μL of 100 μM DPPH$^{\bullet}$ methanolic solution was added; MeOH added to the same volume of DPPH$^{\bullet}$ solution was used for the control. The solutions were shaken and left to stand in the dark for 20 min, then the absorbance (A) was read at 515 nm (SHIMADZU UV-1900 spectrophotometer, Shimadzu, Tokyo, Japan). Inhibition percentage of the radical was calculated as follows:

$$\text{DPPH}^{\bullet}\text{ inhibition (\%)} = [(A_{control} - A_{sample})/(A_{control})] \times 100 \quad (1)$$

Final results were expressed as Trolox equivalents (TE) by means of a calibration curve, and referred to rice weight (d.w.).

2.4.2.2. Phenolic content. The phenolic content (PC) was determined following the modified version of the Folin-Ciocâlteu method previously described (Locatelli, Travaglia, Coïsson, Bordiga, & Arlorio, 2016). Briefly, 50 μL of Folin-Ciocâlteu reagent and 175 μL of aqueous Na_2CO_3 (5 % *w/v*) were added to an appropriate volume of hydroalcoholic rice extract, then the final solution volume was brought to 1450 μL with distilled water. After 1 h of incubation the absorbance was read at 760 nm, using a SHIMADZU UV-1900 spectrophotometer (Shimadzu, Tokyo, Japan).

Final results were expressed as catechin equivalents (CE) through a calibration curve, and referred to rice weight (d.w.).

2.4.2.3. Anthocyanin content. The total content of anthocyanins (AC) and monomeric anthocyanins (MAC) was obtained through the pH differential method, as previously described in Colasanto et al. (2021).

Extracts were opportunely diluted with potassium chloride buffer (0.025 M), pH 1.0 and, in the same manner, with sodium acetate buffer (0.4 M), pH 4.5. Solutions at pH 1.0 were let to stand for 5 min and those at pH 4.5 for 15 min; then the absorbance was measured at both 520 and 700 nm (SHIMADZU UV-1900 spectrophotometer, Shimadzu, Tokyo, Japan). The AC and MAC in the extracts were expressed as cyanidin-3-O-glucoside (Cn-3-Glu) equivalents, accordingly to the equations reported by Lavelli, Harsha, and Spigno (2016):

$$\text{MAC (μg/mL)} = [(A_{520\ nm} - A_{700\ nm})_{pH\ 1} - (A_{520\ nm} - A_{700\ nm})_{pH\ 4.5}] \text{ x MW x d x } 1000/\varepsilon \quad (2)$$

$$\text{AC (μg/mL)} = [(A_{520\ nm} - A_{700\ nm})_{pH\ 1}] \times MW \times d \times 1000/\varepsilon \quad (3)$$

where

MW=molecular weight of cyanidin-3-glucoside (449.2 g/mol),
d = dilution factor,
ε = molar extinction coefficient of cyanidin-3-O-glucoside (26,900 M^{-1} cm^{-1}).
Results were finally expressed based on rice weight (d.w.).

2.4.2.4. Proanthocyanidin content. The quantification of proanthocyanidin content (PAC) was obtained following the protocol described by Prior et al. (2010), with some modifications. Phenolic extracts were opportunely diluted with a mixture of acetone, water and acetic acid (75:24.5:0.5, *v/v*). Then, 280 μL of diluted extract (or 280 μL of solvent for the control) were added to 840 μL of DMAC (4-(dimethylamino) cinnamaldehyde) ethanolic solution (0.01 % *w/v*). The solution was left to react until the maximum absorbance value was reached (20 min), at 20 °C and away from the light, then the absorbance was read at 640 nm (SHIMADZU UV-1900 spectrophotometer, Shimadzu, Tokyo, Japan). Results were expressed as catechin equivalents (CE) through a calibration curve.

2.4.3. Chromatographic analysis

Chromatographic analysis was performed on both the hydroalcoholic (free fraction) and hydrolysed (bound fraction) phenolic extracts.

RP-HPLC-DAD analysis was performed as described by Colasanto et al. (2021), with only minor adjustments. The program gradient was as follows: from 6 to 20 % B (20 min), from 20 to 40 % B (15 min), from 40

to 60 % B (5 min), from 60 to 90 % B (5 min), isocratic 90 % B (5 min), from 90 to 6 % B (1 min), isocratic 6 % B (29 min), with a total run time of 80 min. The flow rate and the injection volume were 400 μL/min and 7 μL, respectively. Polyphenolic rice extracts were centrifuged (15,000 *g* for 20 min, microcentrifuge 5417R, Eppendorf, Milan, Italy) prior to the injection in the chromatographic system. Cyanidin-3-O-glucoside, peonidin-3-O-glicoside, cyanidin-3-O-rutinoside and peonidin-3-O-rutinoside were tentatively identified by comparison with retention times of individual authentic standard molecules and their UV–Vis spectra; the quantification was performed on the basis of calibration curves obtained with the corresponding standards. Cyanidin-3-O-gentiobioside, for which the commercial standard compound was not available, was identified based on the characteristics reported in the literature (Bordiga et al., 2014). Its concentration was expressed as equivalent of cyanidin-3-O-glucoside.

2.5. Statistical analysis

All the statistical analyses were performed using the free statistical software R 4.1.1 version (R Core Team, 2020) and results were expressed as mean ± standard deviation (SD) of at least three independent experiments. Differences were estimated by analysis of variance (ANOVA) followed by Tukey's honest significant difference test, and were considered significant for $p < 0.05$.

3. Results and discussion

In this paper we focused our interest on Artemide rice, an Italian black cultivar characterized by higher polyphenol content and major antioxidant capacity than other Italian pigmented varieties (Bordiga et al., 2014). Based on its functional characteristics, Artemide rice has been studied for the production of microencapsulated phenolic extract to be used as anthocyanin-rich ingredients, useful also in the bakery sector (Papillo et al., 2018). However, beyond this innovative application, Artemide rice can primarily be considered as a "natural functional food" to be consumed in common human diet. Nevertheless, antioxidant compounds are influenced by thermal treatments required for rice consumption; for this reason, as mentioned in the Introduction section, several papers have already investigated the impact of cooking on bioactive compounds of black rice, but alternative cooking techniques, and/or optimized conditions to cook black rice, have not been yet deeply investigated.

Based on these observations, in addition to more conventional cooking techniques, in this work we have investigated the impact of *sous vide* cooking, a novelty for rice and cereals preparation, but highly used for cooking other food matrices to minimize the polyphenolic degradation. Artemide black rice was thus subjected to different cooking techniques: risotto, pilaf and *sous vide*. The "risotto", a traditional preparation of the northern Italian tradition, includes a toasting phase that causes a structural change of rice and leads to the formation of a harder external layer in the caryopsis, inducing a slower release of the starch during cooking. The "pilaf" is a typical Middle Eastern cooking technique, considered a hybrid between boiling and "risotto" cooking, in which rice is oven-cooked, allowing a more homogeneous diffusion of heat, and it is not mixed during the entire cooking time (Li & Gilbert, 2018). Finally, the *sous vide* technique requires that foods are first vacuum-packed in special heat-resistant bags, and then cooked at controlled temperatures (generally lower than usual) and times (generally longer), in order to improve texture and, in some cases, reduce the degradation of bioactive substances (Baldwin, 2012; Dos Reis et al., 2015; Petersen, 1993). For the rice *sous vide* cooking, two temperatures were applied (89 and 99 °C), in order to evaluate the impact of this parameter.

3.1. Proximate composition

The proximate composition of both raw and cooked rice was evaluated; the results are summarized in Table 2, and expressed on a dry weight (d.w.) basis, except for the moisture content. The moisture percentage of raw rice (11.52 %) is in line with the average moisture values of black rice varieties (10.35 %-13.00 %) (Ito & Lacerda, 2019) and with the value observed in a previous work on Artemide black rice (11.70 %) (Colasanto et al., 2021). After cooking, a moisture percentage increment was observed, due to the water absorption by the rice grains, thus swelling, softening and becoming edible. Cooked rice showed comparable moisture values, with the exception of PIL, which presented a significant lower humidity (45.30 %). This value could be due to the non-hermetic closure of the lid and/or high temperature applied during oven-cooking, thus determining a major drying of the grain during the pilaf cooking in respect to the other methods.

Concerning the ashes values, no statistically significant differences were found between raw rice and RIS, SV99 and SV89 samples, while a relevant decrease was observed after pilaf cooking (−42 %). Concerning PIL, it is possible that the inorganic substances dissolved in the cooking-water, following its evaporation, remained on the surfaces of the pan, thus obtaining a reduction of their concentration in the rice.

Regarding proteins, no significant differences ($p > 0.05$) were found between raw and cooked rice, with an average value of 8.93 % (d.w.), suggesting that cooking does not affect the total proteins content of Artemide black rice.

The average content of total dietary fiber in raw rice was 6.10 %, a lower value that than obtained in a previous work (10.8 %) (Colasanto et al., 2021); this discordant result could be given by factors related to cultivation such as the climate conditions, the use of fertilizers and the degree of ripeness of the grain at the time of harvest (Mae et al., 2006) and/or by major impact of the rice dehusking. Following cooking there was an increase in the values, in particular for RIS, SV99 and SV89, which increased their fiber content by 28.52 %, 15.76 % and 23.28 %, respectively. This result could be linked to a degradation of the grain structure, which facilitated the fiber extraction.

3.2. Phenolic composition

3.2.1. Phenolic, anthocyanins, proanthocyanidins content and antioxidant activity

Spectrophotometric determinations were performed as a preliminary step on free soluble fraction of polyphenols (aqueous ethanolic extracts) to obtain a preliminary wide-ranging characterization and comparison of raw (uncooked) and differently cooked black rice samples. The phenolic content determined through the Folin-Ciocâlteu method and expressed as milligrams of catechin equivalents (CE) per gram of rice (dry weight, d.w.), is represented in Fig. 1 (panel A). A significant

Table 2
Proximate composition of raw and cooked Artemide rice.

	Moisture (%)	Ashes (% d.w.)	Proteins (% d.w.)	Total dietary fiber (% d.w.)
RAW	11.6 ± 0.3^{c}	1.49 ± 0.08^{a}	8.96 ± 0.01^{a}	6.10 ± 0.13^{d}
RIS	61.5 ± 1.1^{a} (+434 %)	1.49 ± 0.07^{a}	9.02 ± 0.14^{a}	7.84 ± 0.46^{a} (+29 %)
PIL	45.3 ± 0.8^{b} (+293 %)	0.87 ± 0.02^{b} (− 42 %)	8.90 ± 0.16^{a}	6.61 ± 0.50^{cd} (+8%)
SV99	62.2 ± 0.4^{a} (+441 %)	1.41 ± 0.07^{a}	8.83 ± 0.16^{a}	7.06 ± 0.45^{bc} (+16 %)
SV89	62.5 ± 1.4^{a} (+442 %)	1.42 ± 0.10^{a}	8.95 ± 0.09^{a}	7.52 ± 0.19^{ab} (+23 %)

Results are expressed as mean ± standard deviation. Values with different letters in the same column are significantly different ($p < 0.05$). Values between brackets indicate significant percentage variations in respect to raw rice.

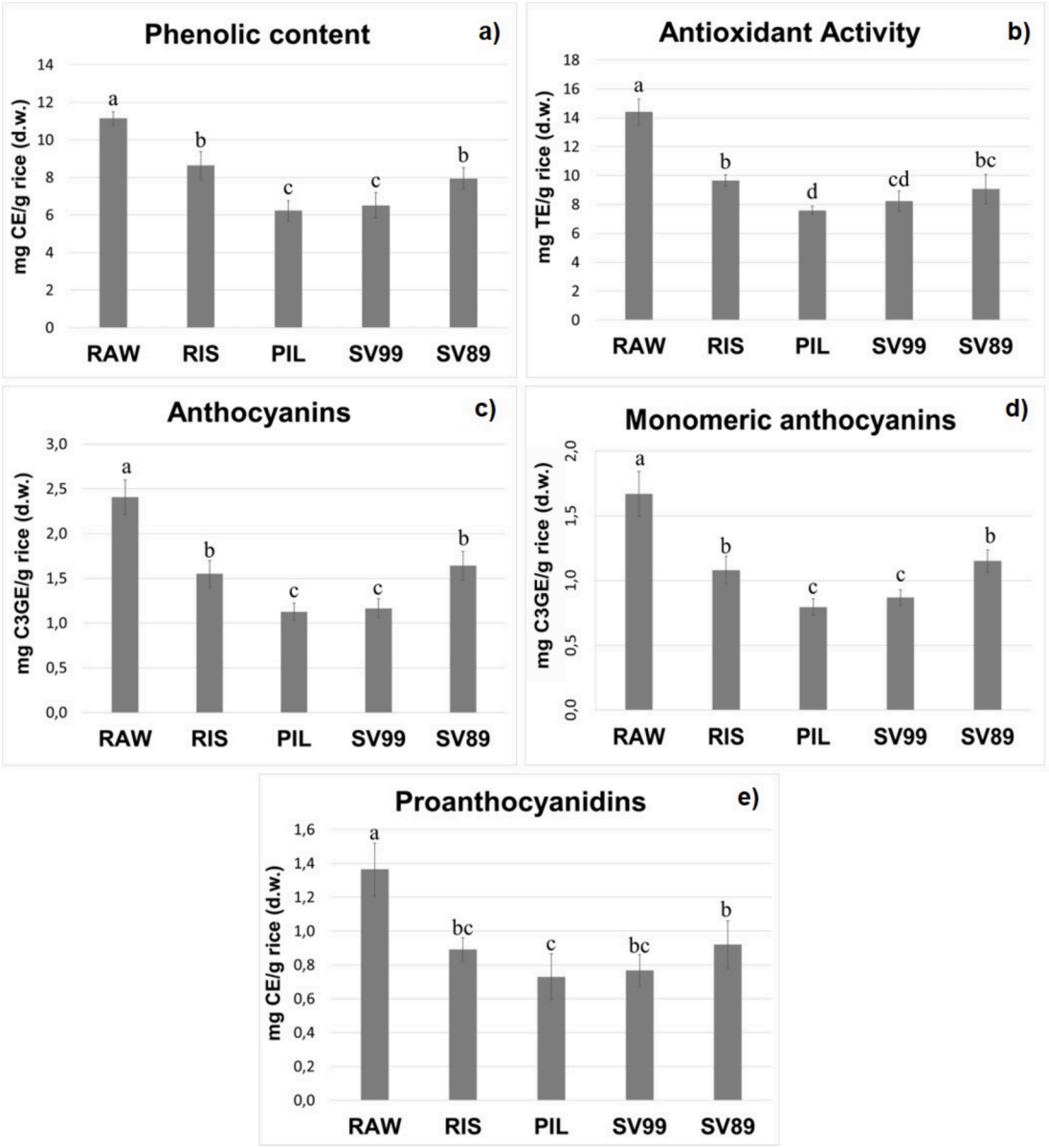

Fig. 1. Free phenolic content (mg CE/ g d.w.) **(a)**, antioxidant activity (mg TE/ g d.w.) **(b)**, anthocyanins (mg CnE/ g d.w.) **(c)**, monomeric anthocyanins (mg CnE/ g d.w.) **(d)**, and proanthocyanidins (mg CE/ g d.w.) **(e)** quantified in raw and cooked black rice, expressed as mean ± standard deviation. For each parameter, values with different letters are significantly different ($p < 0.05$). CE: catechin equivalents; CnE: cyanidin-3-O-glucoside equivalents; TE: Trolox equivalents.

decrease of PC in all cooked sampled was observed respect to the raw rice. The average decrease of PC was 34 %, that is a lower reduction compared to that observed in our previous work (average decrease: −83 %) (Colasanto et al., 2021). The greatest loss of polyphenols occurred in PIL rice (−44 %) and SV99 rice (−42 %), while RIS and SV89 cooking allowed to preserve a greater quantity of polyphenols (−29 % and –22 %, respectively). The PIL cooking led to a great loss of polyphenols, probably caused by the high temperatures applied (175 °C). In addition, as observed for ashes, water-soluble polyphenols could have been deposited on the pan after water evaporation; in fact, a coloured layer on the edges of the pan appeared at the end of the cooking, thus suggesting a further loss of phenolic compounds. The lower impact of cooking in the SV89 sample seems to be linked to the reduced temperature used; in fact, by increasing the temperature to 99 °C, a relative greater loss of polyphenols was observed (−42 %). These results, together with other previous literature evidences (Colasanto et al., 2021; Fracassetti et al., 2020; Catena et al., 2019), suggest a significant impact of temperature values on the polyphenolic component of rice. However, even if temperatures around 100 °C were reached, in the RIS sample the highest PC values, similar to that obtained for the SV88, were observed. In this case, it could be hypothesized a role of the toasting process (not foreseen for the other cooking methods); in this cooking phase, following structural changes of the grain, a protective barrier could be formed, permitting to retain polyphenolic compounds inside the kernel. Furthermore, the optimization of cooking conditions for the risotto preparation (toasting phase: 3 min; total cooking time: 35 min; ratio rice/water (g/mL): 100/500) allowed to limit the loss of PC (−29 %) respect to our previous work (−79 %), where the toasting phase lasted 5 min, the total cooking time was 40 min and the ratio rice/water (g/mL) was 200/500 (Colasanto et al., 2021).

The antioxidant activity was determined by DPPH$^{\bullet}$ assay and expressed as milligrams of Trolox equivalents (mg TE/g d.w.) (Fig. 1, panel B). The AA of raw rice (14.41 mg TE/g d.w.) is in line with that reported by Bordiga et al. (2014) (13.79 mg TE /g d.w.), but lower than that found in our previous studies (21.4 mg TE /g d.w.) (Colasanto et al., 2021). This difference could be related to the different polyphenols'

concentration, which in turn varies depending on pedo-climatic conditions, agronomic treatments and/or attack by parasites, but also on the degree of dehusking process. After cooking a decrement of the antioxidant activity was observed. The most significant loss was observed in PIL sample (−47 %), while RIS and SV89 allowed to preserve greater antioxidant activity with a loss of 33 % and 37 %, respectively.

The anthocyanins content, expressed as cyanidin-3-O-glucoside equivalents (mg CnE/g d.w.), was determined as both anthocyanins (AC) and monomeric anthocyanins (MAC) content (Fig. 1 panel C and D). The AC value in Artemide raw rice (2.41 mg CnE/g d.w.) is slightly lower than that reported by Fracassetti et al. (2020) (3.71 mg CnE/g d.w.). Concerning the monomeric anthocyanins (1.67 mg CnE/g d.w.), they accounted for about 70 % of the total value. After cooking a significant decrease of AC and MAC was observed, evidencing in both cases a similar behaviour. PIL and SV99 samples were the mostly affected by the cooking, which determined a reduction of 53 % and 52 % for the AC, and 52 % and 48 % for the MAC, respectively. RIS and SV89 samples allowed to maintain a good concentration of anthocyanins, with a more contained loss of 35 % and 31 % for MAC and 36 % and 32 % for AC, respectively. These reductions in the anthocyanin content after cooking were observed also by other authors. The anthocyanin content determined by Melini, Panfili, Fratianni, and Acquistucci (2014) in the raw Artemide black rice (1.99 mg/g d.w.) decreased of about 50 % after "risotto" cooking (1.00 mg/g d.w.). Similarly, Fracassetti et al. (2020) observed a decrease in Artemide black rice anthocyanin content of about 48 % after cooking in a rice cooker with different additions of water (from 3.41 mg/g d.w. of raw rice to 1.76 mg/g d.w. of cooked one).

The proanthocyanidins content (PAC), expressed as milligrams of catechin equivalents (CE) per gram of rice (dry weight, d.w.), was determined through the BL-DMAC assay (Fig. 1, panel E). All the cooking methods examined led to a reduction in the concentration of proanthocyanidins. The greatest loss was found in PIL sample (−46 %), while SV89 sample gave the best results with a loss of 32 %. The RIS and SV99 cooking, on the other hand, gave an intermediate percentage of loss compared to the previous cooking (respectively 35 % and 43 %). Several factors could have led to the loss of proanthocyanidins, such as the degradation in the presence of oxygen (Joergensen, Marin, & Kennedy, 2004). In addition, proanthocyanidins are able to bind proteins in a non-specific way; during cooking, the formation of protein-proanthocyanidins complexes could made them insoluble in the extraction solvent, avoiding their detection and quantification. Furthermore, oxidative condensation reactions involving other tannins could occur, giving rise to high molecular weight insoluble proanthocyanidins; other chemical reactions could finally cause their cleavage in different molecules not detectable by this assay (Rauf et al., 2019).

3.2.2. *Characterization of individual phenolics through RP-HPLC-DAD analysis*

3.2.2.1. Anthocyanins. Individual monomeric anthocyanins were mainly detected in hydroalcoholic extracts (free form), with the only exception of cyanidin-3-O-rutinoside and peonidin-3-O-rutinoside, which were identified in the bound phenolic fraction, but at concentration under the limit of detection. The anthocyanin content in raw and cooked rice, expressed as μg/g (d.w.), is reported in Table 3.

Cyanidin-3-O-glucoside was the most abundant anthocyanin in Artemide black rice, representing about 86 % of total anthocyanins content in raw rice. This data agrees with the results obtained by Ito and Lacerda (2019), which reported for other black rice varieties a contribution of this anthocyanins to the total anthocyanin corresponding to about 88 %. Furthermore, the cyanidin-3-O-glucoside content quantified in Artemide black rice in this work (1258 μg/g) is greater than that determined by Bordiga et al. (2014) (1004 μg/g), while cianidin-3-O-gentiobioside and peonidin-3-O-rutinoside content (10.9 and 6.7 μg/g, respectively) were found in lower concentrations (42.0 and 36.9 μg/g).

Table 3
Anthocyanins (μg/g d.w.) identified in both uncooked and cooked black rice samples.

	Cn-3-glc	Pn-3-glc	Cn-3-rut	Pn-3-rut	Cn-3-gent
RAW	1258 ± 89^{a}	120 ± 88^{a}	68.6 ± 4.1^{a}	6.06 ± 0.42^{a}	10.9 ± 0.8^{a}
RIS	6921 ± 61^{c} (− 45 %)	62.5 ± 5.2^{c} (− 48 %)	37.4 ± 4.7^{c} (− 46 %)	3.14 ± 0.37^{c} (− 48 %)	7.29 ± 1.10^{b} (− 33 %)
PIL	564 ± 22^{d} (− 55 %)	51.2 ± 2.4^{d} (− 57 %)	29.8 ± 1.5^{d} (− 56 %)	3.28 ± 0.38^{c} (− 46 %)	5.31 ± 0.72^{c} (− 51 %)
SV99	554 ± 34^{d} (− 56 %)	50.5 ± 3.3^{d} (− 58 %)	27.4 ± 2.0^{d} (− 60 %)	3.71 ± 0.43^{bc} (− 39 %)	6.93 ± 0.58^{b} (− 36 %)
SV89	797 ± 11^{b} (− 37 %)	72.0 ± 0.9^{b} (− 40 %)	44.8 ± 1.6^{b} (− 35 %)	4.10 ± 0.66^{b} (− 33 %)	10.1 ± 0.7^{a}

Results are expressed as mean ± standard deviation. Values with different letters in the same column are significantly different ($p < 0.05$). Values between brackets indicate significant percentage variations in respect to raw rice. Cn-3-glc: Cyanidin-3-O-glucoside; Pn-3-glc: Peonidin-3-O-glucoside; Cn-3-rut: Cyanidin-3-O-rutinoside; Pn-3-rut: Peonidin-3-O-rutinoside; Cn-3-gent: Cyanidin-3-O-gentiobioside.

Differences in anthocyanins composition regarding different production batches of Artemide rice probably depend on year of cultivation, environmental climatic conditions and processing conditions (especially the husk removal).

For all the anthocyanins identified, a reduction in their content after cooking was recorded if compared to the raw sample. Regarding cyanidin-3-O-glucoside, the most abundant compound, SV89 cooking was found to be the best cooking method, which has resulted in a reduction of its content of 37 %, followed by RIS (−45 %). Significantly lower values were recorded for SV99 (554 μg/g) and for PIL (564 μg/g), with a loss compared to raw rice of 56 and 55 %, respectively. A similar trend was observed also for the peonidin-3-O-glucoside, for which SV89 determined a reduction of 40 % respect to the raw rice, against the 48, 57 and 58 % of RIS, PIL and SV99 cooking, respectively. In a similar way, SV89 was the cooking method that best preserved cyanidin-3-O-rutinoside and peonidin-3-O-rutinoside, with a loss of only 35 and 33 %, respectively, compared to the raw rice.

Finally, cyanidin-3-O-gentiobioside, even though present at low concentrations, is particularly interesting because seems more stable to the thermal treatment than to the other identified anthocyanins. Particularly, in the SV89 sample the reduction of this anthocyanin compared to raw rice is not statistically significant. A possible explanation for this result could lie in the chemical structure of this anthocyanin, as the presence of the disaccharide could have conferred greater stability to the molecule (Francavilla & Joye, 2020).

From these results it is evident that the temperature has a strong impact on the total anthocyanin content. It's evident that the use of a lower temperature in *sous vide* cooking (SV89) better preserved anthocyanin content, compared to the same cooking method carried out at a higher temperature (SV99). Also, RIS cooking has allowed to obtain good results in terms of preservation of the anthocyanin content, even if a maximum temperature of 100 °C was reached. This could be explained by the toasting phase typical of this cooking mode, which could have created a sort of "barrier" effect on the surface of the rice, able to protect the anthocyanins from their possible degradation, as demonstrated in Colasanto et al. (2021). This effect could justify the limited loss of anthocyanins, even if higher temperatures are reached in RIS compared to SV89. Considering PIL cooking, in which higher temperature are reached (175 °C), it resulted in a type of cooking which, like SV99, in almost all cases (except for peonidin-3-O-rutinoside) determines a considerable reduction of the total content of anthocyanins. Our results agree with Catena et al. (2019), in which it was seen that PIL cooking

does not allow preserving good quantities of anthocyanins in the sample after cooking.

3.2.2.2. Free phenolic acids and flavonoids. In the free polyphenolic fraction, phenolic acids belonging to two different classes were found: 1) benzoic acid derivatives (gallic acid, protocatechuic acid and vanillic acid) and 2) cinnamic acid derivatives (ferulic acid and coumaric acid). In addition, a flavan-3-ol (catechin) and a flavonol (myricetin) were also found. The results of quantification obtained from the analysis of these compounds are shown in Table 4; all the results are expressed in μg/g of rice (dry weight) and presented as mean ± standard deviation.

Protocatechuic acid is the most abundant phenolic acid in the free phenolic fraction, both before and after cooking, with values of 86 μg/g in RAW and 208 μg/g, 185 μg/g, 228 μg/g and 197 μg/g in RIS, PIL, SV99 and SV89, respectively. All the cooking methods determined an increase of this phenolic acid compared to the levels detected in raw rice. In particular, SV99 is the cooking method that caused the greatest increase in the levels of protocatechuic acid (+165 %), while PIL caused the smallest one (+115 %).

The increase in the quantities of protocatechuic acid in black rice samples after cooking agrees with what observed by Hiemori, Koh, and Mitchell (2009), who demonstrated that the increase in protocatechuic acid derives from the degradation of cyanidin-3-O-glucoside.

High concentrations in free form were obtained also for vanillic acid, with a value of 54.5 μg/g in uncooked rice. RIS and PIL cooking did not cause significant variations in its content, while SV cooking determined an increase in its concentrations compared to the RAW sample, evidencing a positive relation with the temperature employed: the concentration of vanillic acid in SV89 (67.2 μg/g) was lower than that registered in SV99 (84.6 μg/g).

Regarding gallic acid in free form, its contribution is not very relevant (5.31 μg/g in uncooked rice); the concentration values were rather low both before and after cooking, also for the methods that caused a statistically significant increase (RIS, SV89 and SV99).

Considering the hydroxycinnamic acids in the free form, coumaric acid showed the highest average content (16.7 μg/g in RAW sample), while ferulic acid showed slightly lower values (9.34 μg/g in RAW sample). However, their trend after cooking is different: for coumaric acid a general decrease is observed after cooking, while for ferulic acid an increase is noted.

Finally, catechin and smaller quantities of myricetin were also found. About catechin all cooking types determined an increase in its concentration, compared to the raw rice (from 17 μg/g in RAW sample to 78.5 μg/g in SV99).

Summarizing, a general increase of free phenolic acids concentrations after cooking was observed, in contrast with our previous work in which all phenolic acids, except protocatechuic acid, decreased after cooking. These differences could be related to the different cooking conditions adopted for the risotto preparation and to the different cooking methods used in this work (Colasanto et al., 2021). However, in agreement with what observed in this work, an increase in free phenolic acids concentrations after cooking was observed also by Ryu & Koh (2017).

3.2.2.3. Bound phenolic acids and flavonoids. Phenolic acids present in this fraction are those bound to fiber components such as cellulose, hemicellulose, lignin, pectin and structural proteins. Five benzoic acid derivates (gallic acid, protocatechuic acid, vanillic acid, *p*-hydroxybenzoic acid and syringic acid), two cinnamic acid derivates (ferulic and coumaric acids) and three other flavonoids (catechin, myricetin and epicatechin) were found in this form. Their content in the samples is reported in Table 5.

Looking at the table in detail, it is clear that the cinnamic acid derivatives are more present than benzoic acid derivatives. Among the compounds that are present in both free and bound form there are gallic acid, protocatechuic acid, vanillic acid, ferulic acid, coumaric acid, catechin and myricetin, while *p*-hydroxybenzoic acid, syringic acid and epicatechin were identified in the only bound fraction.

Ferulic acid and coumaric acid are the most abundant compounds. In particular, ferulic acid (308 μg/g in the RAW sample) is present for 97 % in the bound form and for only 3 % in the free form. It is interesting to note that no type of cooking significantly affected the ferulic acid content of Artemide rice in bound form. A possible explanation of this phenomenon lies in the fact that in the structure of ferulic acid there is a methyl group linked to oxygen in position R1, which confers stability to the molecule (Chiremba, Rooney, & Beta, 2012).

The coumaric acid is present for 90 % in the bound form and only for 10 % in the free form (the values refer to the RAW sample). Following cooking, no significant changes were found in the content of bound coumaric acid compared to the values recorded in the RAW rice.

Vanillic acid is most abundant in the bound form (in the RAW sample it is present for the 62 % in this form) and after cooking its concentrations increased (+41 % in RIS and PIL, +38 % in SV99 and + 35 % in SV89). The other phenolic acids belonging to the same chemical group have been registered in bound form in very low quantities; in fact, the percentage of gallic acid and protocatechuic acid are only 19 % and 4 %, respectively.

The quantities of protocatechuic acid, *p*-hydroxybenzoic acid and syringic acid did not change after cooking, except for protocatechuic acid, that increased in SV99 sample in a significant manner (more than twice), and syringic acid, that slightly decreased after RIS cooking.

In general, the relative ratios between the free fraction and the bound fraction of phenolic acids after cooking do not change and remain almost similar to those recorded for the RAW sample. The only exception is the gallic acid: although its content in the bound form tends to increase after cooking, the free form increases more than the corresponding bound form, therefore the relative percentage of the bound form tends to decrease.

In addition to the various phenolic acids, some flavonoids in bound form (catechin, myricetin and epicatechin) were also quantified in the Artemide black rice samples. Catechin and myricetin were found in both free and bound forms, while epicatechin was found only in the bound

Table 4
Free phenolic acids (μg/g d.w.) in both uncooked and cooked black rice samples.

	Gal	Proto	Van	Fer	Coum	Cat	Myr
RAW	5.31 ± 0.48^{c}	86.0 ± 3.6^{e}	54.5 ± 1.7^{c}	9.34 ± 0.26^{c}	16.7 ± 0.7^{a}	17.0 ± 2.9^{d}	2.98 ± 0.29^{c}
RIS	6.06 ± 0.32^{b} (+14 %)	208 ± 10^{b} (+142 %)	51.2 ± 3.1^{c}	11.4 ± 1.3^{ab} (+22 %)	13.1 ± 0.8^{c} (− 22 %)	61.0 ± 4.2^{b} (+259 %)	5.81 ± 0.68^{a} (+95 %)
PIL	5.04 ± 0.28^{c}	185 ± 5^{d} (+115 %)	51.8 ± 10.6^{c}	10.3 ± 0.6^{bc}	13.4 ± 1.3^{c} (− 20 %)	50.0 ± 3.8^{c} (+194 %)	4.58 ± 0.19^{b} (+54 %)
SV99	7.32 ± 0.18^{a} (+38 %)	228 ± 5^{a} (+165 %)	84.6 ± 8.2^{a} (+55 %)	12.5 ± 0.3^{a} (+34 %)	15.2 ± 1.2^{ab}	78.5 ± 2.8^{a} (+362 %)	4.68 ± 0.48^{b} (+57 %)
SV89	6.09 ± 0.31^{b} (+15 %)	197 ± 3^{c} (+129 %)	67.2 ± 3.5^{b} (+23 %)	11.7 ± 0.6^{a} (+25 %)	13.6 ± 0.7^{bc} (− 19 %)	51.8 ± 1.1^{c} (+205 %)	4.32 ± 0.71^{b} (+45 %)

Results are expressed as mean ± standard deviation. Values with different letters in the same column are significantly different ($p < 0.05$). Values between brackets indicate significant percentage variations in respect to raw rice. Gal: gallic acid; Proto: protocatechuic acid; Van: vanillic acid; Fer: ferulic acid; Coum: coumaric acid; Cat: catechin; Myr: myricetin.

Table 5
Bound phenolic acids (μg/g d.w.) in both uncooked and cooked black rice samples.

	Gal	Proto	Van	*p*-OH	Syr	Fer	Coum	Cat	Myr	Epi
RAW	1.24 ± 0.30^{c}	3.93 ± 0.19^{b}	90.3 ± 4.7^{b}	3.46 ± 0.08^{ab}	1.72 ± 0.20^{a}	308 ± 10^{a}	147 ± 6^{a}	6.66 ± 0.76^{c}	88.3 ± 3.4^{a}	11.7 ± 1.3^{a}
RIS	4.39 ± 1.06^{ab} (+254 %)	5.00 ± 1.15^{b}	127 ± 20^{a} (+41 %)	4.47 ± 1.39^{a}	1.07 ± 0.42^{b} (−38 %)	282 ± 39^{a}	143 ± 10^{a}	16.8 ± 2.1^{a} (+152 %)	81.0 ± 12.8^{a}	18.4 ± 5.7^{a}
PIL	3.60 ± 1.06^{ab} (+190 %)	6.11 ± 1.31^{ab}	127 ± 9^{a} (+41 %)	3.53 ± 0.74^{ab}	1.52 ± 0.36^{ab}	288 ± 28^{a}	143 ± 14^{a}	13.1 ± 2.0^{b} (+97 %)	77.7 ± 13.5^{a}	18.8 ± 4.7^{a}
SV99	5.23 ± 0.68^{a} (+322 %)	8.24 ± 2.64^{a} (+110 %)	125 ± 23^{a} (+38 %)	3.78 ± 1.64^{ab}	1.24 ± 0.32^{ab}	301 ± 18^{a}	142 ± 10^{a}	12.1 ± 1.5^{b} (+82 %)	69.2 ± 14.8^{a}	19.9 ± 9.9^{a}
SV89	2.76 ± 0.67^{bc}	6.64 ± 0.64^{ab}	122 ± 14^{a} (+35 %)	2.47 ± 0.45^{b}	1.58 ± 0.16^{ab}	311 ± 18^{a}	143 ± 8^{a}	8.40 ± 1.02^{c}	76.0 ± 6.6^{a}	20.0 ± 2.2^{a}

Results are expressed as mean ± standard deviation. Values with different letters in the same column are significantly different ($p < 0.05$). Values between brackets indicate significant percentage variations in respect to raw rice. Gal: gallic acid; Proto: protocatechuic acid; Van: vanillic acid; p-OH: p-hydroxybenzoic acid; Syr: syringic acid; Fer: ferulic acid; Coum: coumaric acid; Cat: catechin; Myr: myricetin; Epi: epicatechin.

form. No variation in the content of myricetin and epicatechin were observed after cooking. Catechin is the only one of the three where significant increases in concentrations are recorded after cooking, compared to the RAW sample. In addition, although the catechin content in the bound form tends to increase after cooking, the free catechin increases more than in the corresponding bound form, therefore the relative percentage of the bound form tends to decrease. The most significant increase of catechin in the free fraction was recorded for SV99 (from 72 % in RAW to 87 % in SV99).

4. Conclusions

Our results demonstrated that conditions and methods of cooking significantly influence the final composition of black rice and, particularly, the polyphenolic fraction: the traditional risotto preparation and the innovative *sous vide* cooking at 89 °C maintained the best characteristics, saving up to 63 % of the initial antioxidant capacity of raw black rice, up to 69 % of total anthocyanins, and up to 78 % of polyphenols content.

Concerning individual compounds determined through HPLC, anthocyanins showed a little higher decrease in respect to spectrophotometric method; cyanidin-3-O-glucoside, the main anthocyanin in black rice, was reduced up to 56 % in the *sous vide* cooked rice at 99 °C, but only by 45 % and 37 % in the risotto and *sous vide* cooked rice at 89 °C, respectively. On the contrary, free phenolic acids increased after cooking, in a different extent depending on compound and cooking method; this increase could be related to a better extraction due to modifications of food structure and/or could derive from the degradation of anthocyanins or more complex phenolic compounds. Finally, the bound phenolic fraction remained substantially unchanged after cooking, with only few exceptions concerning gallic acid, vanillic acid and catechin, which increased after cooking.

Concluding, the study of the impact of different cooking methods is fundamental to understand changes in food composition, but it is it is also useful in view to educate the society in healthy nutrition and food preparation, at both domestic and commercial (canteens and restaurants) level. This concept is particularly relevant for black rice, a natural antioxidant polyphenol-rich food, which can be strongly affected in its composition during cooking.

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CRediT authorship contribution statement

Antonio Colasanto: Writing – original draft, Validation, Formal analysis. **Fabiano Travaglia:** Methodology, Investigation, Formal analysis. **Matteo Bordiga:** Writing – review & editing, Investigation. **Jean Daniel Coïsson:** Writing – review & editing, Supervision, Resources. **Marco Arlorio:** Writing – review & editing, Funding acquisition. **Monica Locatelli:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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References

AOAC (1990) Official Method of Analysis of the Association of Official Analytical Chemists. No. 934.06, AOAC, Arlington.

Ayub, H., & Ahmad, A. (2019). Physiochemical changes in sous-vide and conventionally cooked meat. *International Journal of Gastronomy and Food Science, 17*, Article 100145. https://doi.org/10.1016/j.ijgfs.2019.100145

Baldwin, D. E. (2012). Sous vide cooking: A review. *International Journal of Gastronomy and Food Science, 1*(1), 15–30. https://doi.org/10.1016/j.ijgfs.2011.11.002

Bordiga, M., Gomez-Alonso, S., Locatelli, M., Travaglia, F., Coïsson, J. D., Hermosin-Gutierrez, I., & Arlorio, M. (2014). Phenolics characterization and antioxidant activity of six different pigmented *Oryza sativa* L. cultivars grown in Piedmont (Italy). *Food Research International, 65*, 282–290. https://doi.org/10.1016/j.foodres.2014.03.007

Catena, S., Turrini, F., Boggia, R., Borriello, M., Gardella, M., & Zunin, P. (2019). Effects of different cooking conditions on the anthocyanin content of a black rice (*Oryza sativa* L. 'Violet Nori'). *European Food Research and Technology, 245*(10), 2303–2310. https://doi.org/10.1007/s00217-019-03337-6

Chiremba, C., Rooney, L. W., & Beta, T. (2012). Microwave-assisted extraction of bound phenolic acids in bran and flour fractions from sorghum and maize cultivars varying in hardness. *Journal of Agricultural and Food Chemistry, 60*(18), 4735–4742. https://doi.org/10.1021/jf300279t

Colasanto, A., Travaglia, F., Bordiga, M., Monteduro, S., Arlorio, M., Coïsson, J. D., & Locatelli, M. (2021). Cooking of Artemide Black Rice: Impact on Proximate Composition and Phenolic Compounds. *Foods, 10*(4), 824. https://doi.org/10.3390/foods10040824

Daomukda, N., Moongngarm, A., Payakapol, L., & Noisuwan, A. (2011). Effect of cooking methods on physicochemical properties of brown rice. *In 2nd International Conference on Environmental Science and Technology IPCBEE* (Vol. 6).

da Silva, F. L., de Lima, J. P., Melo, L. S., da Silva, Y. S., Gouveia, S. T., Lopes, G. S., & Matos, W. O. (2017). Comparison between boiling and vacuum cooking (sous-vide) in the bioaccessibility of minerals in bovine liver samples. *Food Research International, 100*, 566–571. https://doi.org/10.1016/j.foodres.2017.07.054

Dos Reis, L. C. R., de Oliveira, V. R., Hagen, M. E. K., Jablonski, A., Flôres, S. H., & de Oliveira, R. A. (2015). Effect of cooking on the concentration of bioactive compounds in broccoli (*Brassica oleracea* var. *Avenger*) and cauliflower (*Brassica oleracea* var. Alphina F1) grown in an organic system. *Food Chemistry, 172*, 770–777. https://doi.org/10.1016/j.foodchem.2014.09.124

Fracassetti, D., Pozzoli, C., Vitalini, S., Tirelli, A., & Iriti, M. (2020). Impact of cooking on bioactive compounds and antioxidant activity of pigmented rice cultivars. *Foods, 9* (8), 967. https://doi.org/10.3390/foods9080967

Francavilla, A., & Joye, I. J. (2020). Anthocyanins in whole grain cereals and their potential effect on health. *Nutrients, 12*(10), 2922. https://doi.org/10.3390/nu12102922

Giordano, D., Reyneri, A., Locatelli, M., Coïsson, J. D., & Blandino, M. (2019). Distribution of bioactive compounds in pearled fractions of tritordeum. *Food chemistry, 301*, Article 125228. https://doi.org/10.1016/j.foodchem.2019.125228

Hiemori, M., Koh, E., & Mitchell, A. E. (2009). Influence of cooking on anthocyanins in black rice (*Oryza sativa* L. *japonica* var. SBR). *Journal of Agricultural and Food Chemistry, 57*(5), 1908–1914. https://doi.org/10.1021/jf803153z

Hou, Z., Qin, P., Zhang, Y., Cui, S., & Ren, G. (2013). Identification of anthocyanins isolated from black rice (*Oryza sativa* L.) and their degradation kinetics. *Food Research International, 50*, 691–697. https://doi.org/10.1016/j.foodres.2011.07.037

Huang, H., Belwal, T., Li, L., Wang, Y., Aalim, H., & Luo, Z. (2020). Effect of modified atmosphere packaging of different oxygen levels on cooking qualities and phytochemicals of brown rice during accelerated aging storage at 37 °C. *Food Packaging and Shelf Life, 25*, Article 100529. https://doi.org/10.1016/j.fpsl.2020.100529

Ito, V. C., & Lacerda, L. G. (2019). Black rice (*Oryza sativa* L.): A review of its historical aspects, chemical composition, nutritional and functional properties, and applications and processing technologies. *Food chemistry, 301*, Article 125304. https://doi.org/10.1016/j.foodchem.2019.125304

Joergensen, E. M., Marin, A. B., & Kennedy, J. A. (2004). Analysis of the Oxidative Degradation of Proanthocyanidins under Basic Conditions. *Journal of Agricultural and Food Chemistry, 52*, 2292–2296. https://doi.org/10.1021/jf035311i

Juliano, B. O. (2016). Rice: Overview. In C. Wrigley, H. Corke, K. Seetharaman, & J. Faubion (Eds.), *Encyclopedia of Food Grains* (vol. 1, pp. 125–129). Oxford: Academic Press.

Kukusamude, C., Phitchan, S., Nunticha, L., & Supalak, K. (2021). Heavy metals and probabilistic risk assessment via rice consumption in Thailand. *Food Chemistry, 334*, Article 127402. https://doi.org/10.1016/j.foodchem.2020.127402

Lavelli, V., Harsha, P. S., & Spigno, G. (2016). Modelling the stability of maltodextrin-encapsulated grape skin phenolics used as a new ingredient in apple puree. *Food Chem, 209*, 323–331. https://doi.org/10.1016/j.foodchem.2016.04.055

Li, H., & Gilbert, R. G. (2018). Starch molecular structure: The basis for an improved understanding of cooked rice texture. *Carbohydrate Polymers, 195*, 9–17. https://doi.org/10.1016/j.carbpol.2018.04.065

Locatelli, M., Gindro, R., Travaglia, F., Coïsson, J. D., Rinaldi, M., & Arlorio, M. (2009). Study of the DPPH$^{\bullet}$-scavenging activity: Development of a free software for the correct interpretation of data. *Food chemistry, 114*(3), 889–897. https://doi.org/10.1016/j.foodchem.2008.10.035

Locatelli, M., Travaglia, F., Coïsson, J. D., Bordiga, M., & Arlorio, M. (2016). Phenolic composition of Nebbiolo grape (*Vitis vinifera* L.) from Piedmont: Characterization during ripening of grapes selected in different geographic areas and comparison with Uva Rara and Vespolina cv. *European Food Research and Technology, 242*(7), 1057–1068. https://doi.org/10.1007/s00217-015-2610-z

Mae, T., Inaba, A., Kaneta, Y., Masaki, S., Sasaki, M., Aizawa, M., … Makino, A. (2006). A large-grain rice cultivar, Akita 63, exhibits high yields with high physiological N-use efficiency. *Field Crops Research, 97*(2–3), 227–237. https://doi.org/10.1016/j.fcr.2005.10.003

Melini, V., Panfili, G., Fratianni, A., & Acquistucci, R. (2014). Bioactive compounds in rice on Italian market: Pigmented varieties as a source of carotenoids, total phenolic compounds and anthocyanins, before and after cooking. *Food Chem., 277*, 119–127. https://doi.org/10.1016/j.foodchem.2018.10.053

Nieva-Echevarría, B., Manzanos, M. J., Goicoechea, E., & Guillén, M. D. (2017). Changes provoked by boiling, steaming and sous-vide cooking in the lipid and volatile profile of European sea bass. *Food Research International, 99*, 630–640. https://doi.org/10.1016/j.foodres.2017.06.043

Papillo, V. A., Locatelli, M., Travaglia, F., Bordiga, M., Garino, C., Arlorio, M., & Coïsson, J. D. (2018). Spray-dried polyphenolic extract from Italian black rice (*Oryza sativa* L., var. Artemide) as new ingredient for bakery products. *Food Chemistry, 269*, 603–609. https://doi.org/10.1016/j.foodchem.2018.07.059

Petersen, M. A. (1993). Influence of sous vide processing, steaming and boiling on vitamin retention and sensory quality in broccoli florets. *Zeitschrift für Lebensmittel-Untersuchung und Forschung, 197*(4), 375–380. https://doi.org/10.1007/BF01242064

Prior, R. L., Fan, E., Ji, H., Howell, A., Nio, C., Payne, M. J., & Reed, J. (2010). Multi-laboratory validation of a standard method for quantifying proanthocyanidins in cranberry powders. *Journal of the Science of Food and Agriculture, 90*(9), 1473–1478. https://doi.org/10.1002/jsfa.3966

Rasinska, E., Rutkowska, J., Czarniecka-Skubina, E., & Tambor, K. (2019). Effects of cooking methods on changes in fatty acids contents, lipid oxidation and volatile compounds of rabbit meat. *LWT, 110*, 64–70. https://doi.org/10.1016/j.lwt.2019.04.067

Rauf, A., Imran, M., Abu-Izneid, T., Patel, S., Pan, X., Zaz, S., Silva, A. S., Saeed, F., & Suleria, H. A. R. (2019). Proanthocyanidins: A comprehensive review. *Biomedicine & Pharmacotherapy, 116*, Article 108999. https://doi.org/10.1016/j.biopha.2019.108999

R Core Team. R: A Language and Environment for Statistical Computing; R Foundation for Statistical Computing: Vienna, Austria, (2020); Available online: https://www.R-project.org/.

Renna, M., Gonnella, M., Giannino, D., & Santamaria, P. (2014). Quality evaluation of cook-chilled chicory stems (*Cichorium intybus* L., Catalogna group) by conventional and sous vide cooking methods. *Journal of the Science of Food and Agriculture, 94*(4), 656–665. https://doi.org/10.1002/jsfa.6302

Ryu, D., & Koh, E. (2017). Influence of cooking methods on free and bound phenolic acids in Korean black rice. *Journal of Food Processing and Preservation, 41*(2), Article e12873. https://doi.org/10.1111/jfpp.12873