DOI 10.1002/mnfr.200700011

Research Article

Characterization of antioxidant compounds of red and white rice and changes in total antioxidant capacity during processing

Franca Finocchiaro[1], Barbara Ferrari[1], Alberto Gianinetti[1], Chiara Dall'Asta[2], Gianni Galaverna[2], Francesca Scazzina[3] and Nicoletta Pellegrini[3]

[1] C.R.A. – Experimental Institute for Cereal Research, Italy
[2] Department of Organic and Industrial Chemistry, University of Parma, Italy
[3] Department of Public Health, Human Nutrition Unit, University of Parma, Italy

Although most rice cultivars have whitish kernel, some varieties have a red testa. Aim of this work was to compare the total antioxidant capacity (TAC) and the antioxidant chemical composition (namely tocols, γ-oryzanols, and polyphenols) of red and white rices. In addition, the effect of milling and cooking on antioxidants was investigated in both rices. Dehulled red rice showed a TAC more than three times greater than dehulled white rice and its high TAC was essentially characterized by the presence of proanthocyanidins (PA) and associated phenolics. Milling caused a significant loss of TAC, even if red rice maintained a higher TAC. Cooking caused a further loss of antioxidants, but when there was a full uptake of cooking water by the grains ("risotto") this loss was limited. Thus, the consumption of whole or partially milled rice cooked as risotto would be preferred to preserve its nutritional properties.

Keywords: Oryzanols / Red rice / TEAC assay / Tocols / Total antioxidant capacity

Received: January 10, 2007; revised: April 13, 2007; accepted: April 15, 2007

1 Introduction

Rice is one of the most important cereal crops for human consumption in the world. Among rice varieties, the most commonly consumed rices have whitish kernels, though there are also several varieties with a colored testa (black, purple, or red), visible after removing the hull (floral bracts) that covers the caryopsis. Varieties with white kernels were selected during the past, presumably because of their preferred appearance, whereas red (reddish-brown) caryopses are mainly found in wild rice species. Farming and consumption of colored varieties is limited in Western countries, but in some growing areas of Asia, traditional varieties with pigmented caryopsis are particularly valued on local markets.

Experimental studies have reported that colored rice supplementation decreased oxidative stress *in vivo* and simultaneously increased antioxidant capacity *in vivo* and *in vitro* [1–3]. Such healthy properties have been related to three well-known classes of antioxidant compounds present in rice: γ-oryzanols, tocols, and polyphenols. γ-Oryzanols, a family of ferulic acid esters of unsaturated triterpenoid alcohols, have been characterized in rice bran [4]. Similarly, rice bran oil contains tocotrienols and tocopherols [5]. Rice polyphenols include phenolic acids, that are present in kernels of white rice [6], and flavonoids that characterize pigmented rices [7]. The color of purple and black kernels has been associated with the presence of anthocyanins [7], whereas the nature of red grain pigments is still unclear. Reddy *et al.* [7] concluded that the reddish colored testa is associated with the presence of a class of polymeric compounds, the proanthocyanidins (PA). However, native PAs are *per se* colorless [8], therefore other compounds should be responsible for the color of red rice. Thus, notwithstanding the clear role of PAs as radical-scavenging components in red rice [9], other compounds could also be involved in the high antioxidant capacity observed in red rice.

Although black rice contains anthocyanin pigments with notable antioxidant and anti-inflammatory properties [10],

---

Correspondence: Dr. Franca Finocchiaro, C.R.A. – Experimental Institute for Cereal Research, Via S. Protaso, 302, 29017 Fiorenzuola d'Arda (PC), Italy
E-mail: fin.fra@libero.it
Fax: +39-0523-983750

Abbreviations: ABTS, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt; **ANOVA,** analysis of variance; **PA,** proanthocyanidins; **TAC,** total antioxidant capacity; **TEAC,** Trolox equivalent antioxidant capacity red rice has been shown to have a higher antioxidant capacity [9]. Red rice is thus potentially more interesting than black rice, but its antioxidant composition is still not fully characterized. Oki *et al.* [9] reported that the high radical-scavenging property of red rice acetone extract was linked to oligomeric PAs. PAs (or condensed tannins) are complex flavonoid polymers naturally occurring in some cereals and legume seeds and are particularly abundant in some fruits and fruit juices [11]. The antioxidant effect of these compounds has been demonstrated [12, 13]. PAs can undergo oxidative processes of rearrangement and condensation, which produce complex compounds called phlobatannins (or phlobaphenes) that have a lower solubility than the PAs and an altered reactivity [14] as well as a red-brick color [15].

The aim of this work was to characterize both the antioxidant chemical composition (*i.e.*, tocols, γ-oryzanols, and polyphenols) and the total antioxidant capacity (TAC) of red-kernel rice, and compare these characteristics with those of white-kernel rice. Thereafter, because rice is commonly milled and cooked prior to consumption, we investigated the effect that these processes have on the level of antioxidants in both rice types.

2 Materials and methods

2.1 Chemicals

6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) and 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) were purchased from Sigma-Aldrich (St. Louis, MO). Cyanidin chloride was provided by Extrasynthese S. A. (Lyon, France). γ-Oryzanol courtesy of R. Cornelli (Prodotti Gianni, Milano, Italy). Tocopherol and tocotrienol kits (α, β, γ, δ single homologs) were obtained from VWR International (Milano, Italy). Caffeic, sinapic, *p*-coumaric, and *o*-coumaric acids; (+)-catechin hydrate and arabic gum were purchased from Fluka Chemica (Milano, Italy); ferulic acid was purchased from Ega Chemie (Steinhem, Germany). All solvents were of HPLC grade and purchased from VWR International and Carlo Erba (Milano, Italy).

2.2 Rice materials

All the rice materials (*i.e.*, red rice cultivar S. Eusebio and white cultivar Carnaroli) were harvested in 2003 in farm fields located near Novara (Italy). Rices with three milling degrees were compared: dehulled rice (*i.e.*, whole-kernel or "brown" rice) with only the hulls removed; undermilled rice, which had a low milling degree (3% of external tissues were removed from the dehulled caryopsis, including germ and part of the bran layer); milled rice, from which bran and germ were removed (about 6% with respect to dehulled rice). The bran resulting from this milling process was collected, stored at −20°C, and analyzed a short time after. Although weedy rice (unsuitable for agricultural purposes) is also defined as "red rice", in this paper a cultivable variety was indeed considered. Analogously, though "white rice" can also mean a rice that is fully processed (milled rice), throughout the paper we refer to genotypes with a whitish testa, as opposed to red rice.

All the samples were milled with a Cyclotec Sample Mill (Foss Italia S.p.A., Padova, Italy) equipped with a 0.5 mm screen. The moisture content was determined with a Precisa HA60 IR moisture analyzer (Precisa Instruments, Diekinton, Germany). Moisture content of dehulled rice samples ranged from 10.7 to 13.6% (mean value of 12.9%). Each sample was stored at −20°C before analysis. All the analyses as well as the cooking tests were carried out in duplicate.

2.3 Cooking tests

2.3.1 Preparation of water-cooked rice

Rice samples (40 g) were cooked in 800 mL of deionized water previously brought to the boil. After the first 10 min, and then after every 2 min, ten kernels were removed, pressed on a glass, and starch gelatinization was observed on a black surface. Rice was considered cooked (and the corresponding time was recorded) when nine out of ten kernels showed full gelatinization [16]. In the referred method [16], demineralized water was used, but also the possibility to use 1% salt in water (as can be reasonable) was considered. The salt was reported to produce a slight increase in the cooking time. Thus, in a preliminary experiment we tested the cooking time both without salt and with 0.5% salt (this % gives a better taste) and we did not observe any significant difference, neither for water-cooked rice nor for "risotto" (not shown). Analogously, no significant differences in the loss of dry matter were observed (not shown). The cooked rice samples were immediately freeze-dried overnight, then ground as reported above and stored at −20°C.

2.3.2 Preparation of risotto

Deionized water (180 mL) was brought to the boil, then 50 g of rice was added. Cooking times were established as reported for water-cooked rice. The initial volume of water was determined prior to the experiment so as to guarantee that at the end of cooking all the water was absorbed by the rice. Samples were stored and milled as described above.

The moisture content of the freeze-dried samples was determined with a Precisa HA60 IR moisture analyzer (Precisa Instruments). Moisture content of cooked rice samples ranged from 7.5 to 10.5% (mean value of 8.5%).

Cooking times of boiled rice and risotto are reported in Table 1. The time to obtain 90% gelatinization is a conventional time and is a very few minutes longer than the time commonly used at home by the average Italian consumer.

Table 1. Cooking times of red and white rices. Values are presented as means ± standard error ($n = 2$). Degree of milling: D: dehulled, U: undermilled; M: milled. ANOVA showed a significant effect of variety, milling and cooking as well as of the interaction of variety × milling on the cooking time ($P \leq 0.05$)

Rice sample		Time (min)
Water-cooked	cv. S. Eusebio (D)	35.0 ± 1.0
	cv. S. Eusebio (U)	18.0 ± 0.0
	cv. S. Eusebio (M)	17.0 ± 0.0
Risotto	cv. S. Eusebio (D)	38.0 ± 0.0
	cv. S. Eusebio (U)	21.5 ± 0.5
	cv. S. Eusebio (M)	21.0 ± 0.0
Water-cooked	cv. Carnaroli (D)	36.0 ± 0.0
	cv. Carnaroli (U)	18.5 ± 0.5
	cv. Carnaroli (M)	17.0 ± 0.0
Risotto	cv. Carnaroli (D)	40.0 ± 0.0
	cv. Carnaroli (U)	21.5 ± 0.5
	cv. Carnaroli (M)	21.0 ± 0.0

2.4 Total antioxidant capacity (TAC)

To assay free soluble antioxidants (*i.e.*, antioxidant compounds that are not covalently linked to cellular components and do not require for the extraction a preliminary hydrolysis), rice samples (0.5 g) were extracted with 5 mL of methanol for 20 min on a horizontal shaker (220 oscillations/min) at room temperature. Supernatant was separated by centrifugation at 1000 × *g* for 10 min and the extraction was repeated twice. The three supernatants were combined. The residue was re-extracted by the addition of 5 mL of acetone/water (70:30 v/v), centrifuged at 1000 × *g* for 10 min and the supernatant recovered. This extraction was repeated twice and supernatants collected. The residue was further used to extract the bound phenolic compounds (*i.e.*, polyphenols that are covalently linked to cellular components [17]). For this, the residue was digested with 10 mL of 2 M NaOH at room temperature for 1 h with shaking under nitrogen gas. The pH was then corrected with 10 mL of 3 M acetic acid. The antioxidants released during the basic hydrolysis were extracted with 10 mL of ethyl acetate. After centrifugation at 1000 × *g* for 10 min, the supernatant was collected and the extraction repeated twice. Then, the solvent was evaporated under vacuum and the residue recovered by adding deionized water. All rice extracts were adequately diluted (depending on their activity) in the corresponding solvent and immediately analyzed for their antioxidant capacity. To analyze TAC released in water during boiling, 35 mL of acetone was added to 15 mL of cooking water. Supernatant was separated from precipitated starch by centrifugation at 1000 × *g* for 10 min, adequately diluted and analyzed for its TAC.

The TAC was evaluated according to the Trolox equivalent antioxidant capacity (TEAC) method based on ABTS radical cation decoloration in the presence of antioxidants in a flow-injection system [18]. The ABTS radical cation was prepared by reacting a 7 mM aqueous solution of ABTS with 2.45 mM potassium persulfate (final concentration), allowing the mixture to stand in the dark at room temperature for 12–16 h before use [19]. The solution was then diluted in ethanol to an absorbance of 0.70 (±0.20) AU at 734 nm, and used as the mobile phase in a flow-injection system, according to Pellegrini *et al.* [18]. Results were expressed in mmol of Trolox *per* kg of dry matter (solid sample) or L (liquid sample). The variation in TAC values for replicates was always between 3 and 10% RSD. When the RSD was higher than 10%, the analyses were repeated to confirm the value.

2.5 Determination of tocols and γ-oryzanols

The extraction was performed according to Peterson and Qureshi [20]. Briefly, 1 g of milled sample was extracted with 7 mL of methanol on a horizontal shaker for 20 min at 220 oscillations/min at room temperature. Supernatant was separated by centrifugation (15 min at 2000 × *g*) and the residue was re-extracted with 7 mL of methanol. Extracts were combined. Methanol was evaporated in a vacuum rotary evaporator (Heidolph Instrument, Kelheim, Germany) at 38°C and the flask was flushed with nitrogen. The dried residue was extracted with 4 mL of *n*-hexane by vortexing for 3 min. Tocols and γ-oryzanols were separated by HPLC; the analyses were carried out with a Beckman-System Gold chromatograph (Beckman Instrument, CA, USA). Each sample (20 μL) was injected in an autoinjector and eluted from a Lichrospher Si60 column (250 mm × 4 mm, 5 μm id). The mobile phase used was 0.5% isopropanol in *n*-hexane v/v with a flow rate of 1.5 mL/min. Tocol homolog peaks were detected according to the AOCS method [21] with a Perkin Elmer LS 30 fluorimeter (Perkin Elmer, Connecticut, USA) set at 290 nm (excitation wavelength) and at 330 nm (emission wavelength). Data were recorded and the peak area measured using a Varian 2200 integrator (Varian, CA, USA). Stock standard solutions for tocol homologs were prepared according to the AOCS method [21]. A mixed tocopherol and tocotrienol standard working solution was prepared daily mixing appropriate volumes of stock solutions of tocopherol and tocotrienol standards and diluting them with *n*-hexane to give a solution containing between 1 and 5 μg/mL of each homolog. Each homolog was determined separately and the sum of all homologs is reported as total tocol content and expressed as mg *per* kg of dry matter (either of the raw or of the cooked sample). Total γ-oryzanols were quantified [22], with the photo diode array (PDA; Beckman Instrument) at the maximum wavelength of 315 nm [22]. The γ-oryzanol standard was used to build up a calibration curve in *n*-hexane. Results are expressed as mg *per* kg of dry matter.

2.6 Determination of hydroxycinnamic acids

Hydroxycinnamic acids were determined after alkaline hydrolysis and extraction [23]. To 5 mL of clear extract, after addition of the internal standard *o*-coumaric acid (about 30 mg/kg), 10 mL of 1 N NaOH was added. Complete hydrolysis of the bound forms of hydroxycinnamic acids occurred in 4 h, at room temperature and in the dark. The solution was then acidified to pH 2 with 1 N HCl and free hydroxycinnamic acids were extracted with ethyl acetate (3 × 20 mL). After evaporation of the solvent under vacuum, the residue was dissolved in $THF/H_2O = 80:20$ v/v (2 mL) for HPLC analysis. HPLC analyses were performed with a Waters model 2690 separation module (Waters, Milford, MA, USA) equipped with a Waters model 2487 dual-band UV-Vis detector (set at 330 nm) with a C_{18} Spherisorb RP-column (250 mm × 4.6 mm, 5 μm id) (Waters), thermostatted at 30°C. The elution conditions were as follows: flow rate, 1 mL/min; temperature, 30°C. The solvent system used was a gradient of solvent A (water with 2% v/v formic acid) and solvent B (water/THF = 20:80 v/v). The following gradient was applied: 0–20 min isocratic 84% A and 16% B, 20–25 min linear to 40% B, 25–30 min isocratic 40% B, 30–31 min linear to 16% B, and 31–40 min 16% B for reconditioning the column. Quantification was performed using calibration curves of five standards (ferulic acid, sinapic acid, caffeic acid, *o*-coumaric acid, and *p*-coumaric acid) in the range of 0.1–20.0 μg/g. The calibration curves were linear in the range studied, showing correlation coefficients ≥ 0.99. The interday repeatability of the method was proved by performing five injections of standard solutions at three different concentration levels: the RSDs were 3.3% for 0.5 μg/g, 4.2% for 5 μg/g, and 5.1% for 15 μg/g.

The LOD (S/N = 3:1) and LOQ (S/N = 10:1) were 0.01 μg/g and 0.05 μg/g for all the cinnamic acids, respectively.

2.7 Determination of total polyphenol content

To measure total polyphenols, milled samples of dehulled rice (200 mg) were extracted for 2 h with 2 mL of 2 M NaOH vortexing every 10 min. Total polyphenols were quantified with the modified Prussian blue assay [24]. Briefly, 0.1 mL of acidified rice extracts and 3 mL of distilled water were dispensed into test tubes. Then, 1 mL of 0.016 M $K_3Fe(CN)_6$ and 1 mL of 0.02 M $FeCl_3$ were added in rapid succession. After 15 min, 5 mL of stabilizer (30 mL distilled water, 10 mL 85% H_3PO_4, 10 mL 1% arabic gum) were added to each sample. Absorbancies were measured at 700 nm by using a DU-64 spectrophotometer (Beckman Instruments). Increasing concentrations of catechin standard (between 0.3 and 1.0 mmol/L, initial concentration) were used to build up a calibration curve. As we verified the linearity of the curve in the range found in our samples, the assay was standardized against 0.001 M catechin and polyphenols were expressed as g *per* kg (of dry matter) of catechin equivalents.

2.8 Determination of PAs

PAs were quantified by butanol/HCl depolymerization on the TAC extracts (*i. e.*, methanol and acetone) according to Porter *et al.* [25]. Briefly, into a glass tube sealed with Teflon-lined screw cap, 6 mL of butanol/concentrate HCl (95:5 v/v) and 100 μL of 2% w/v solution of $NH_4Fe(SO_4)_2 \cdot 12H_2O$ in 2 M HCl were added to 1 mL of methanol or acetone extracts. The tube was thoroughly mixed and suspended in a constant-level water bath run at 95.0 ± 0.2°C and heated for 50 min. Absorbance of the reaction mixture was measured at 540 nm by using a DU-64 spectrophotometer (Beckman Instruments). The quantification was performed based on a calibration curve of cyanidin chloride. Results of duplicate analyses are given as mg *per* kg (of dry matter) of cyanidin equivalents. Total PA content was established as the sum of PA content in the methanol and acetone extracts.

2.9 Thiolysis reaction

Thiolysis reaction was performed as reported by Guyot *et al.* [26]. Briefly, toluene-α-thiol (5% in methanol, 800 μL) was added to each sample (30 mg), followed by the addition of 400 μL of 0.4 N HCl in methanol. Each reaction tube was closed and incubated at 40°C for 60 min with mixing on a vortex mixer every 10 min, then the tubes were put on ice to stop the reaction. The mixture, 200 μL, was immediately filtered through a PTFE membrane (0.45 μm) and stored at 4°C for RP-HPLC analysis.

2.10 Characterization of polyphenolic compounds by HPLC-ESI-UV-MS

Polyphenol characterization was performed using rice bran and dehulled caryopses. Rice samples (5 g) were first defatted with *n*-hexane (3 × 50 mL) to remove lipophilic compounds. After centrifugation at 2000 × *g*, the remaining solids were extracted with a methanol/water (80:20 v/v, 3 × 50 mL) solution acidified with 1% formic acid to extract polyphenolic compounds. After centrifugation, the insoluble material was extracted with aqueous acetone (70:30 v/v, 3 × 50 mL) acidified with 1% formic acid. The combined methanol and acetone extracts were reduced to dryness by rotary evaporation under vacuum, redissolved, and further fractionated through a Sephadex LH-20 column (20 g stationary phase). Stepwise gradient elution was performed first with 200 mL methanol/water 80:20 and then with 200 mL acetone/water 70:30 (20 mL fractions). These subfractions were analyzed by RP-HPLC-UV(DAD)-ESI-MS to characterize the polyphenol composition.

HPLC-MS analyses were performed with a Waters model 2690 separation module equipped with a PDA detector (PDA model 996) and a Micromass ZMD mass spectrometer (Micromass, Manchester, UK), equipped with an electrospray source and a single quadrupole mass analyzer. A C18 column (5 μm, 300 Å, 250 mm × 4.6 mm id) (Jupiter Phenomenex, Torrance, CA, USA) was used. The elution conditions were as follows: flow rate, 1 mL/min; temperature, 30°C. The solvent system used was a gradient of solvent A (water with 0.2% v/v formic acid) and solvent B (water/ACN = 60:40 v/v with 0.2% formic acid). The following gradient was applied: 0–30 min linear from 100% A to 100% B, 30–32 min linear to 100% A, and 32–45 min isocratic 100% A for reconditioning the column. Data were acquired by the software Masslynx 3.4. PDA chromatogram (200–600 nm scan range) and mass chromatogram (positive ion mode, capillary voltage 3500 V, cone voltage 40 V, desolvation flow (N_2) = 4631 L/h, nebulizer flow (N_2) 981 L/h, scan range *m/z* = 100–2000 Da, scan time 4.1 s, source block temperature 80°C, desolvation temperature 150°C) were compared in order to identify the different peaks according to UV and mass spectra.

Thiolysis products were identified by LC-DAD-ESI-MS in the negative ion mode, using a gradient elution. The elution conditions were as follows: flow rate, 1 mL/min; temperature, 30°C. The solvent system used was a gradient of solvent A (water with 0.1 mM NaCl) and solvent B (water/ACN = 60:40 v/v with 0.1 mM NaCl). The following gradient was applied: 0–30 min linear from 100% A to 100% B, 30–32 min linear to 100% A, and 32–45 min isocratic 100% A for reconditioning the column. Data were acquired by the software Masslynx 3.4. PDA chromatogram (200–600 nm scan range) and mass chromatogram (negative ion mode, capillary voltage 3200 V, cone voltage 20 V, desolvation flow (N_2) = 4631 L/h, nebulizer flow (N_2) 981 L/h, scan range *m/z* = 100–2000 Da, scan time 4.1 s, source block temperature 80°C, desolvation temperature 150°C) were compared in order to identify the different peaks according to UV and mass spectra.

2.11 Statistical analysis

All the data were statistically analyzed according to a three-way analysis of variance (ANOVA) with interactions by using the Systat 9.0 software (SPSS, Chicago, IL, USA). Variety, milling, and cooking were considered as fixed factors.

3 Results

3.1 TAC

The TAC values of raw (dehulled, undermilled, and milled) rice samples are shown in Table 2. TAC value of dehulled red rice S. Eusebio was significantly higher than that of white cultivar Carnaroli, 35.9 mmol Trolox/kg and 10.2 mmol Trolox/kg, respectively. By comparing antioxidant capacities of dehulled rice extracts obtained by each solvent, it appeared that red rice antioxidant capacity was mainly due to free antioxidants; in fact, the TAC values of red rice methanol and acetone/water fractions were, respectively, about five and six times higher than those of dehulled Carnaroli. Conversely, the TAC of the dehulled white genotype was mainly characterized by bound antioxidant compounds (*i. e.*, polyphenols that are covalently linked to cellular components); the antioxidant capacity of the hydrolyzed extract was 5.5 mmol Trolox/kg and represented more than 50% of its total TAC. Interestingly, the TAC of this fraction was similar in both rice types, but slightly higher in red rice.

Table 2. TAC of methanol, acetone/water, and hydrolyzed extracts of red and white rices. Values are presented as means ± standard error (*n* = 2–4). Degree of milling: D: dehulled; U: undermilled; M: milled. ANOVA showed a significant effect of variety and milling as well as of their interaction on the TAC of either methanol, acetone/water, and hydrolyzed fractions ($P \leq 0.001$)

Rice sample	TAC (mmol Trolox/kg)		
	Methanol	Acetone/water (70:30 v/v)	Hydrolyzed
cv. S. Eusebio (D)	12.33 ± 0.46	17.19 ± 0.12	6.39 ± 0.20
cv. Carnaroli (D)	2.53 ± 0.07	2.18 ± 0.09	5.46 ± 0.11
cv. S. Eusebio (U)	2.34 ± 0.08	5.38 ± 0.08	2.92 ± 0.34
cv. Carnaroli (U)	1.01 ± 0.17	0.84 ± 0.05	3.08 ± 0.11
cv. S. Eusebio (M)	0.88 ± 0.04	1.72 ± 0.05	2.09 ± 0.08
cv. Carnaroli (M)	0.83 ± 0.10	0.67 ± 0.05	2.98 ± 0.24

As expected, rice milling caused a reduction in the TAC, particularly for red rice: undermilled and milled red rice lost 70 and 87% of the initial TAC found in the dehulled sample, respectively (Table 2), whereas the white rice had a loss of about 52 and 51% for the undermilled and milled samples, respectively. It can be noted that the major reduction of TAC values was obtained in the case of the acetone/water and methanol extracts, especially for undermilled red rice (Table 2).

Water cooking caused a loss of TAC values in all samples (Fig. 1), though this loss was more evident for red rice. In fact, dehulled white rice lost about 20% of the original TAC of the raw sample (Fig. 1), whereas the TAC of dehulled red rice dramatically decreased from 35.9 in the raw sample to 15.2 mmol Trolox/kg in the water-cooked sample, for a 60% loss (Fig. 1). Similarly, after water cooking, undermilled red rice lost 42% of its TAC, and milled red rice 40%, as compared to their respective raw samples (Fig. 1). The antioxidant power of the cooking water of dehulled red rice confirmed that almost all the TAC of red rice was lost during boiling (TAC of cooking water: 15.8 mmol Trolox/L). Due to the consistent loss observed during water cooking, a traditional Italian procedure (*i. e.*, risotto cooking) in

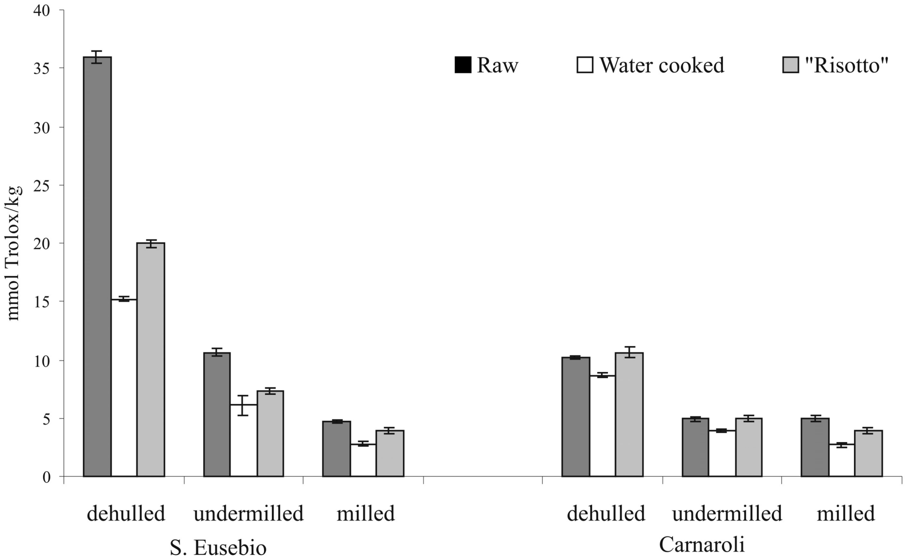

Figure 1. Effects of cooking procedures on TAC (mmol Trolox/kg of dry weight): (■) raw, (□) water cooked, and (▨) risotto rice samples. Values are presented as mean ± standard error ($n = 2$). ANOVA showed a significant effect of variety, milling, and cooking as well as of all their interactions on TAC ($P \leq 0.001$).

which all the cooking water is absorbed by the rice was decided to be performed. Although in risotto some TAC was still lost (Fig. 1), the loss was lower than that caused by water cooking. In fact, risotto of dehulled red rice lost only 44% of the TAC of the raw sample, while undermilled and milled red rice lost 31 and 17% of their respective raw samples. As observed during water cooking, most of the loss of antioxidant capacity occurred in methanol and acetone extracts, though, using the risotto cooking procedure, the TAC value of acetone extract was to some extent preserved (data not shown). In white rice, risotto cooking did not cause any significant loss of TAC (Fig. 1).

Table 3. Total content of tocols (tocopherols and tocotrienols; mg/kg of dry weight) in dehulled (D), undermilled (U), and milled (M) rice samples cooked under different conditions. Values are presented as mean ± standard error ($n = 2$). ANOVA showed a significant effect of milling and cooking, but not variety as well as of all the interactions of the three factors on tocol content ($P \leq 0.001$)

Rice samples	Total tocols (mg/kg)		
	Raw	Water-cooked rice	Risotto cooked rice
cv. S. Eusebio (D)	50.4 ± 0.9	66.5 ± 1.9	40.3 ± 0.9
cv. S. Eusebio (U)	30.7 ± 1.6	22.5 ± 1.3	9.0 ± 0.4
cv. S. Eusebio (M)	14.7 ± 0.1	10.9 ± 0.7	6.3 ± 0.1
cv. Carnaroli (D)	43.4 ± 0.2	59.4 ± 0.9	50.0 ± 1.5
cv. Carnaroli (U)	10.1 ± 1.6	10.1 ± 0.0	16.1 ± 1.6
cv. Carnaroli (M)	10.2 ± 0.4	8.6 ± 0.3	8.2 ± 0.0

3.2 Tocols and oryzanols

The concentration of tocols is reported in Table 3. There were only slight differences of total tocols between red and white rice. Since tocols are mainly concentrated in the outer layers of the grain, during red rice processing the level of tocols decreased by 39% in the first milling step (from dehulled to undermilled rice) and by 71% after the second milling step (from dehulled to milled rice). In the case of Carnaroli rice, the major reduction of tocols was observed during the first milling step. Interestingly, water cooking increased the tocol concentration of all the dehulled samples. Indeed, a 23% loss of soluble dry matter occurred during water cooking, so that a correspondingly higher concentration of lipophilic compounds *per* kg of dry matter would be expected in the cooked sample. In addition, release of bound tocols (*i. e.*, tocol molecules that are strongly linked to cellular components) during heat treatment was observed by Qureshi *et al.* [27], at least when dry heating the rice bran. No increase of tocol concentration was observed in undermilled and milled samples, presumably because damaging the tissutal integrity made these antioxidants more susceptible to oxidative removal during cooking. Contrasting effects of oxidative removal of tocols at high-temperature and purported release of bound tocols were apparent in the "risotto cooking" where no loss of dry matter occurs.

The total γ-oryzanol content of dehulled rices was 470 mg/kg for cv. S. Eusebio and 744 mg/kg for cv. Carnaroli (Table 4). As was seen for tocols, rice milling caused a consistent decrease of γ-oryzanol concentration (Table 4), and cooking of dehulled rices provoked an increase of γ-oryanol concentration in S. Eusebio but not in Carnaroli dehulled rice, possibly due to the contrasting effects of

Table 4. Total content of γ-oryzanol (mg/kg of dry weight) in dehulled (D), undermilled (U), and milled (M) rice samples cooked under different conditions. Values are presented as mean ± standard error ($n = 2$). ANOVA showed a significant effect of milling and cooking, but not variety, as well as of variety × milling and variety × milling × cooking interactions on γ-oryzanol content ($P \leq 0.05$)

Rice samples	Total γ-oryzanol (mg/kg)		
	Raw	Water-cooked rice	Risotto cooked rice
cv. S. Eusebio (D)	470 ± 43	568 ± 25	715 ± 20
cv. S. Eusebio (U)	255 ± 70	90 ± 15	103 ± 3
cv. S. Eusebio (M)	90 ± 5	69 ± 2	45 ± 2
cv. Carnaroli (D)	744 ± 10	564 ± 25	686 ± 3
cv. Carnaroli (U)	191 ± 35	65 ± 0	110 ± 14
cv. Carnaroli (M)	58 ± 5	n.d.[a)]	25 ± 6

a) Not detectable.

releasing some bound γ-oryzanol molecules and oxidative removal.

3.3 Hydroxycinnamic acids

Hydroxycinnamic acids were quantified as reported in Table 5. The hydroxycinnamic profile of the two varieties was slightly different, in particular for the sinapic acid content (160.7 mg/kg in S. Eusebio and 14.2 mg/kg in Carnaroli rice). HPLC analysis showed the presence of ferulic acid, sinapic acid, and *p*-coumaric acid in all the analyzed samples, whereas caffeic acid was detected in a low amount in red rice only.

The milling degree had a great influence on the hydroxycinnamic acids content, causing a consistent decrease, especially in ferulic and sinapic acid content (Table 5). Likewise, after both water cooking and risotto cooking, the hydroxycinnamic acids content dropped, with a consistent fall of ferulic and sinapic acids for both white and red genotypes (Table 5).

3.4 PAs and total polyphenols

PAs were absent in white rice (Table 6), whereas they were detectable in the S. Eusebio samples. However, with milling and cooking, red rice PA levels decreased dramatically, and in cooked milled red rice PA were no longer detectable (Table 6).

Total polyphenol concentration (Table 6) of dehulled red rice was about two-fold higher than that of dehulled white rice Carnaroli.

3.5 Identification of polyphenolic compounds by HPLC-ESI-UV-MS

After extraction and Sephadex fractionation, the main polyphenolic compounds present in the bran and in the whole rice of the red variety were identified *via* HPLC-ESI-UV-MS analysis by comparing the mass spectra, the UV spectra and, when possible, by spiking with original standards.

Table 5. Content of hydroxycinnamic acids (mg/kg of dry weight) in dehulled (D), undermilled (U), and milled (M) rice samples cooked under different conditions. Values are presented as mean ± standard error ($n = 2$). ANOVA showed a significant effect of variety, milling, and cooking on the content of each hydroxycinnamic acid ($P \leq 0.05$)

		Sinapic acid (mg/kg d. w.)	Caffeic acid (mg/kg d. w.)	Ferulic acid (mg/kg d. w.)	*p*-Coumaric acid (mg/kg d. w.)
cv. S. Eusebio	Raw (D)	160.7 ± 4.8	2.4 ± 0.1	120.8 ± 3.6	22.5 ± 0.7
	Water cooked (D)	2.1 ± 0.1	n.d.[a)]	12.1 ± 0.4	2.6 ± 0.1
	Risotto cooked (D)	0.7 ± 1.6	n.d.[a)]	1.1 ± 0.1	1.3 ± 0.1
	Raw (U)	52.3 ± 1.6	1.4 ± 0.1	52.9 ± 1.6	11.4 ± 0.1
	Water cooked (U)	0.5 ± 0.1	n.d.[a)]	2.1 ± 0.1	1.0 ± 0.1
	Risotto cooked (U)	1.9 ± 0.1	n.d.[a)]	2.1 ± 0.1	1.2 ± 0.1
	Raw (M)	19.2 ± 0.6	0.7 ± 0.1	25.6 ± 0.8	9.6 ± 0.3
	Water cooked (M)	0.6 ± 0.1	n.d.[a)]	2.7 ± 0.1	1.7 ± 0.1
	Risotto cooked (M)	1.2 ± 0.1	n.d.[a)]	7.3 ± 0.1	3.6 ± 0.1
cv. Carnaroli	Raw (D)	14.2 ± 0.5	n.d.[a)]	66.2 ± 1.5	5.5 ± 0.1
	Water cooked (D)	5.8 ± 0.4	n.d.[a)]	20.3 ± 1.9	3.1 ± 0.2
	Risotto cooked (D)	1.3 ± 0.1	n.d.[a)]	7.5 ± 0.7	8.9 ± 0.3
	Raw (U)	6.6 ± 0.1	0.3 ± 0.0	11.1 ± 0.3	2.5 ± 0.3
	Water cooked (U)	1.3 ± 0.2	n.d.[a)]	n.d.[a)]	9.6 ± 0.3
	Risotto cooked (U)	2.2 ± 0.1	n.d.[a)]	15.4 ± 0.5	5.1 ± 0.1
	Raw (M)	0.6 ± 0.1	n.d.[a)]	5.9 ± 0.2	1.7 ± 0.1
	Water cooked (M)	1.2 ± 0.1	n.d.[a)]	2.2 ± 0.1	7.2 ± 0.2
	Risotto cooked (M)	4.3 ± 0.1	n.d.[a)]	7.4 ± 0.2	2.2 ± 0.1

a) Not detectable.

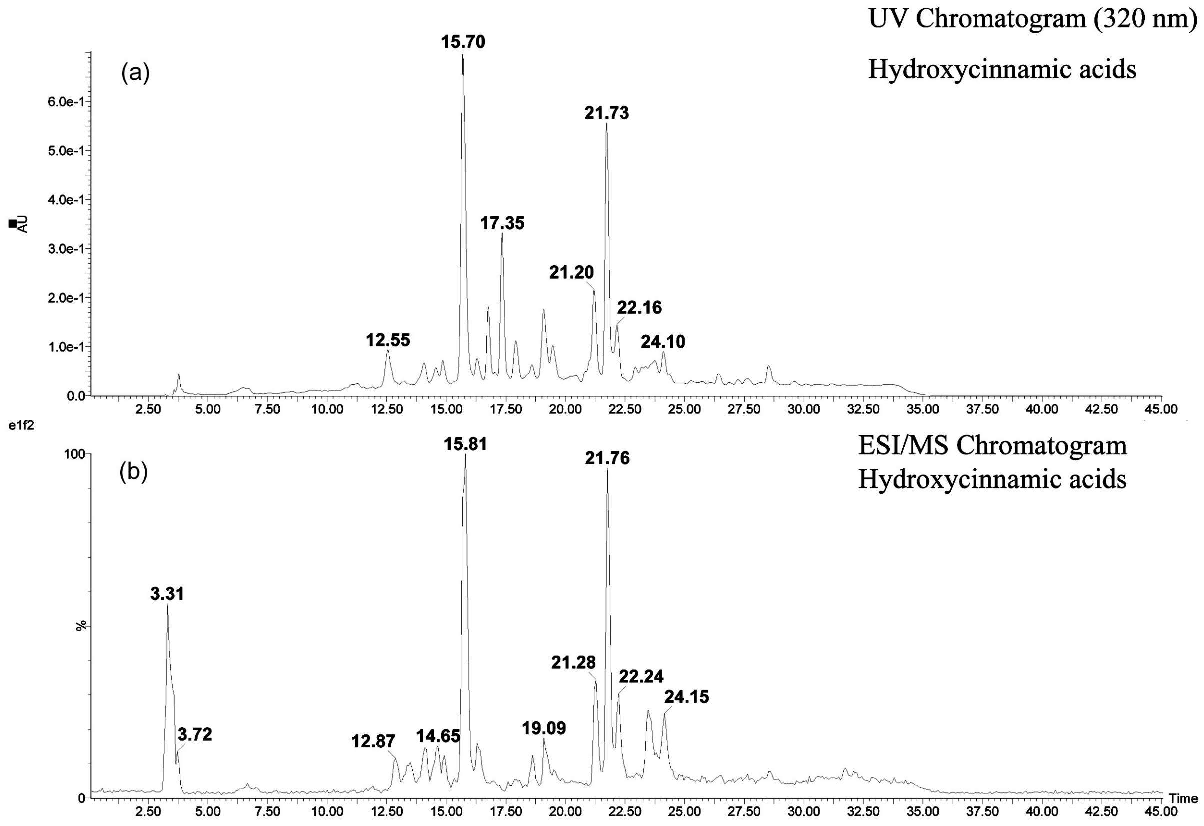

Figure 2. HPLC-DAD-ESI-MS chromatogram of the methanol/water fraction of red rice: (a) UV profile at 320 nm; (b) ESI/MS chromatogram.

Table 6. Content of PAs (mg/kg of cyanidin equivalents of dry weight) in dehulled (D), undermilled (U), and milled (M) rice samples cooked under different conditions and total polyphenol content (g/kg of cyanidin equivalents of dry weight) in dehulled rice sample. Values are presented as mean ± standard error ($n = 2$). ANOVA showed a significant effect of variety and milling as well as of their interaction on the PA content ($P < 0.001$)

Rice sample	PA content (mg/kg cyanidin)			Total polyphenols (g/kg catechin)
	Raw	Water-cooked	Risotto cooked	
cv. S. Eusebio (D)	76.00 ± 1.2	2.64 ± 0.74	23.53 ± 2.80	2.57 ± 0.01
cv. S. Eusebio (U)	17.95 ± 0.34	0.73 ± 0.00	4.79 ± 0.13	
cv. S. Eusebio (M)	2.47 ± 0.23	n.d.[a)]	n.d.[a)]	
cv. Carnaroli (D)	n.d.[a)]	n.d.[a)]	n.d.[a)]	1.21 ± 0.02
cv. Carnaroli (U)	n.d.[a)]	n.d.[a)]	n.d.[a)]	
cv. Carnaroli (M)	n.d.[a)]	n.d.[a)]	n.d.[a)]	

a) Not detectable.

In the first fractions eluted from Sephadex with methanol/water eluent, several polyphenolic compounds were identified. In particular, there were hydroxycinnamic acids, mainly present as esters with glucose or other carbohydrates and organic acids, such as tartaric and quinic acid, as reported in Fig. 2. Feruloyl glucoside and synapoyl glucoside were identified on the basis of mass spectra: diagnostic is the presence of $[M + H]^+$ ($m/z = 357$ for ferulic acid and $m/z = 387$ for sinapic acid, respectively) and of $[A - OH]^+$ (acylium ion: $m/z = 177$ for ferulic acid and $m/z = 207$ for sinapic acid). Feruloyl quinic acid was identified by the $[M + H]^+$ ($m/z = 369$) and by the acylium ion $[A - OH]^+$ at $m/z = 177$. Sinapoyl rutinoside and sinapoyl tartrate were identified by the $[M + H]^+$ ($m/z = 533$ for rutinoside and $m/z = 357$ for tartrate derivative) and of $[A - OH]^+$ (acylium ion: m/z 207 for sinapic acid). Coumaroyl derivatives were present only in traces as well as caffeoyl derivatives as shown in Fig. 3. Analyses for the presence of derivatives of

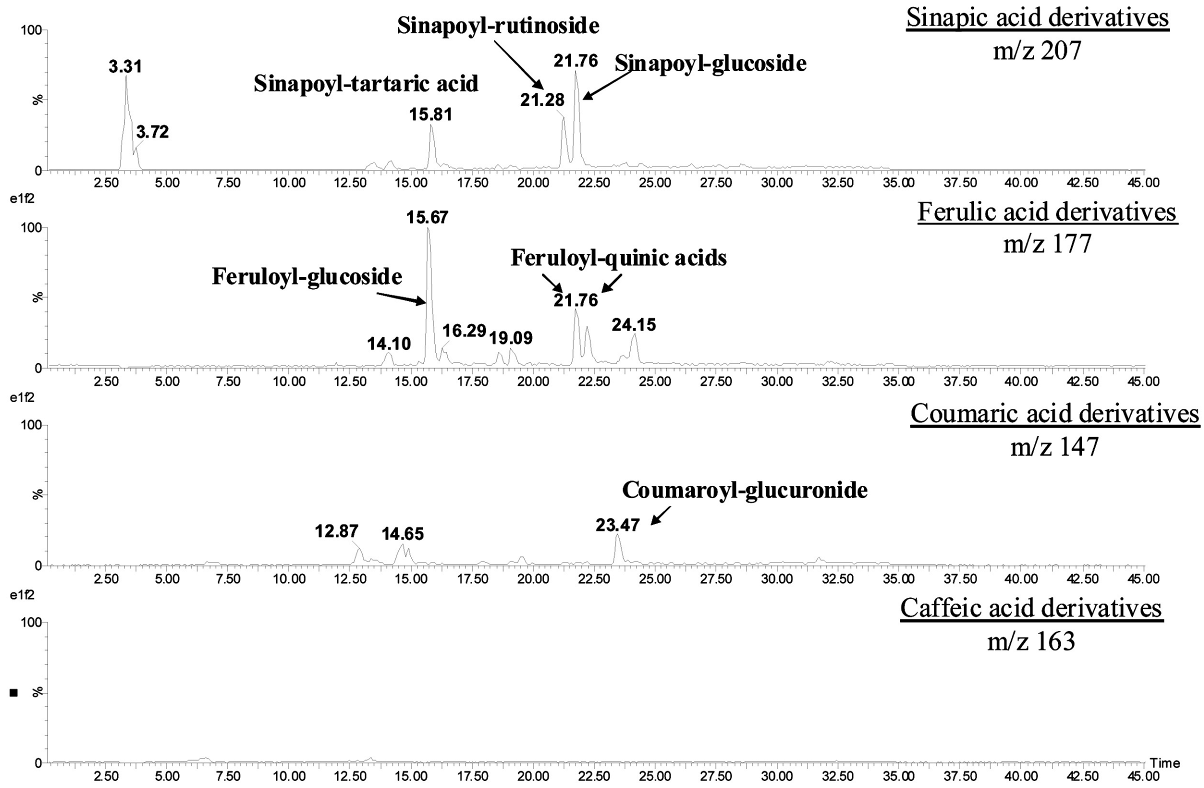

Figure 3. Extract ion chromatogram (XIC) for the characteristic cinnamate fragments (*m/z* 207 for sinapoyl derivatives, *m/z* 177 for feruloyl derivatives, *m/z* 163 for caffeoyl derivatives, and *m/z* 147 for coumaroyl derivatives).

these compounds was then performed after hydrolysis of the extract to release the bound form of the hydroxycinnamic acids: sinapic, ferulic, and *p*-coumaric acid were confirmed to be present.

Additional polyphenolic compounds identified in the first fraction, eluting from the Sephadex in very small amounts were: cyanidin, identified on the basis of the molecular ion at $m/z = 287$ $[A + H^+]$, peonidin-rutinoside on the basis of $m/z = 609$ $[M]^+$, 463 $[M - \text{rhamnose}]^+$, 301 $[A]^+$, and malvidin–rutinoside on the basis of $m/z = 639$ $[M]^+$, 493 $[M - \text{rhamnose}]^+$, 331 $[A]^+$.

The fractions eluting from the column with acetone/water (70:30 v/v) were characterized, in addition to the well-separated low molecular weight constituents, by the presence of a very large unresolved hump, attributable to unresolved highly polymerized phenolic compounds (Fig. 4). However, mass spectrum of these fractions showed the presence of different molecular weights of dimers, trimers, and oligomers of PAs. PAs (condensed tannins) are polymers of flavan-3-ol units linked by carbon–carbon bonds. Oligomeric units consist mainly of (+)-catechin, (–)-epicatechin, and their 3-*O*-gallates, linked by either 4–8 or 4–6 bonds (B-type). Also identified in many plants are the oligomers with a second interflavanoid bond formed between 2 and 7-*O* (A-type). On the basis of the observed molecular weight and of the data reported in the literature, the main PA components are probably oligomers of epicatechin singly linked (B-type) with the interflavan link between the 4 and the 8 positions. The identified components are reported in Table 7. The presence of other oligomers with a higher degree of polymerization is highly probable, but not easily detectable with electrospray MS due to their low volatility. Depolymerization in the presence of benzyl thiol showed the monomeric unit of epicatechin together with other still unidentified fragments (Figs 5 and

Table 7. PA components identified in red rice by HPLC-ESI-UV-MS

Oligomer	Ions *m/z*
Monomer	291
Dimer	579
Doubly linked dimer (type A)	577
Digallated dimer	883
Trimer	867
Tetramer	1155
Pentamer	1443
Hexamer	1754
Heptamer	2042
Octamer	2330

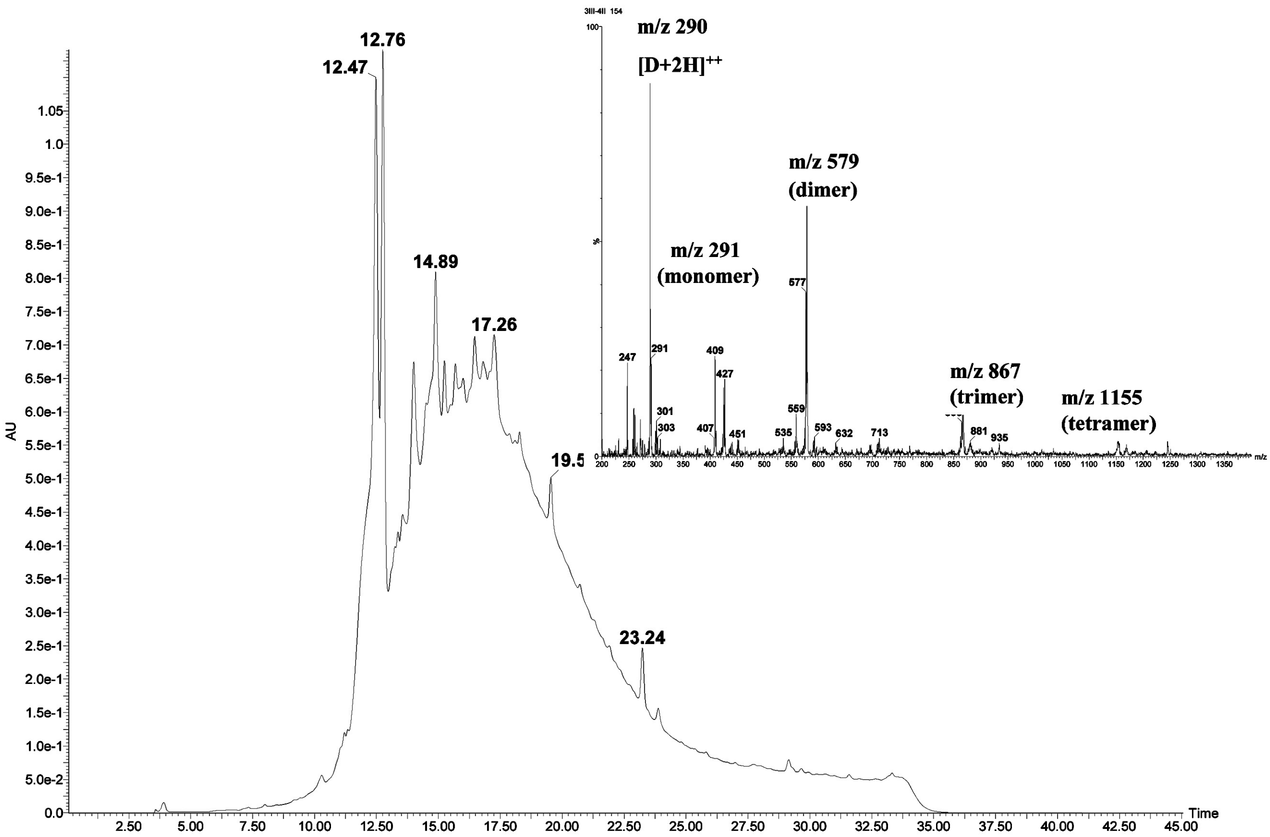

Figure 4. UV chromatogram (280 nm) of acetone/water fraction of red rice: the broad hump is due to unresolved complex phenolic compounds. The mass spectrum revealed the presence of PA.

6). The PAs are particularly concentrated in the bran fractions and are thus progressively lost during processing.

As for the white variety, a quite similar hydroxycinnamic acids profile was detected, but no anthocyanins and PAs were found.

4 Discussion

The present study demonstrates that the red rice variety analyzed has an antioxidant capacity more than three times greater than that of white rice. This difference was not due to the antioxidant capacity associated to lipophilic compounds, since both varieties of rice had similar contents of total tocols and γ-oryzanols (Tables 3 and 4). The tocol concentrations were comparable to those reported for other cereals [20, 28], and the levels of γ-oryzanols were high and within the range reported in the literature [29]. However, dehulled caryopses of red rice had double the concentration of polyphenols as found in dehulled white rice (Table 6). Consequently, based on their greater content in red rice, polyphenols were hypothesized to be the antioxidant fraction most probably causing the huge difference of TAC observed between the two varieties of rice. These results confirm previous data obtained by Oki *et al.* [9] and Goffman and Bergman [30], who showed that the antioxidant capacity of colored rice bran extracts was highly correlated with total phenolic content, suggesting that phenolics are the main compounds responsible for the free radical-scavenging activity in rice bran extracts.

It has been recently reported that the antioxidants capacity in grains was commonly underestimated [17, 31] because bound phytochemicals were not included. In common cereals, most grain polyphenols are in insoluble forms, bound to cell walls, and these insoluble forms are the major contributors to the TAC: 90% in wheat, 87% in corn, 71% in rice, and 58% in oats [31]. Interestingly, the antioxidant capacity of the fraction of bound polyphenols analyzed after alkaline hydrolysis was similar in the two types of rice (Table 2). However, due to the higher TAC of red rice, the percentage contribution to TAC of this hydrolyzed fraction was considerably different: high in dehulled white rice (about 55%), and low in the dehulled sample of the red variety (about 18%). Thus, the higher antioxidant capacity observed in red rice was associated with an higher level of free phenolic compounds extractable in methanol and acetone/water solvents, again in agreement with Oki *et al.* [9]. In particular, as red rice was shown to be characterized by

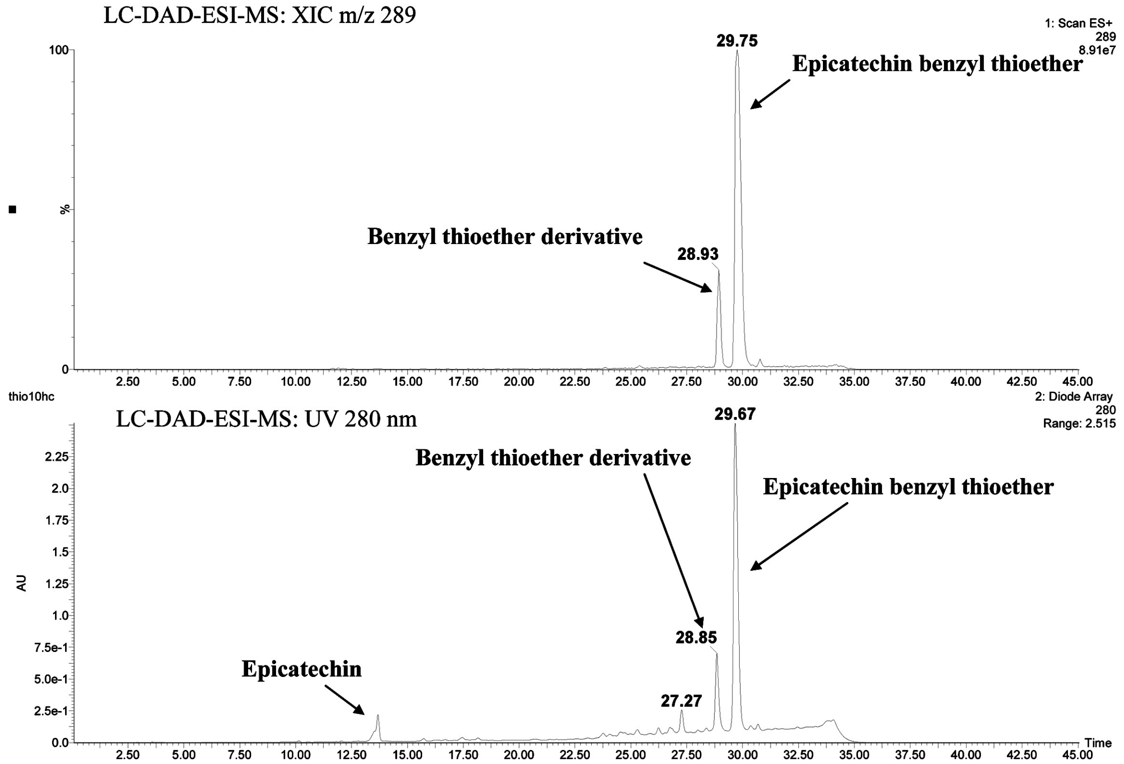

Figure 5. UV (280 nm) and XIC (*m/z* 289) in the negative ion mode of epicatechin and its benzylthioether derivatives.

the presence of PAs [7], these phenolic compounds were quantified as cyanidin equivalents [25] and confirmed to be exclusively present in red rice (Table 6). Moreover, the chemical structure of polyphenol compounds in red and white rice was investigated by HPLC-ESI-UV-MS analysis. Indeed, the mass spectrum of the red rice acetone/water fraction confirmed dimers, trimers, and oligomers of PAs to be present (Fig. 4, Table 7); they were absent in white rice.

It is however worth noting that the amount of PAs was not sufficient to explain the higher phenolic content of this rice (Table 6). In addition, as the antioxidant capacity of polymeric PAs is less than that of an equivalent weight of catechin (TEAC = 2.6 mmol Trolox/L [32, 33]), it can be calculated that, notwithstanding the food matrix results are always higher than standards compounds alone, the amount of PA detected in red rice is largely insufficient to explain the higher TAC of this rice. Analogously, the higher level of hydroxycinnamic acids found in red rice is by far too small to explain both the higher total phenolic content and the larger TAC of this rice (the TEAC of hydroxycinnamic acids is around 2 mmol Trolox/L [34], so they would explain only about 10% of the TAC of dehulled red rice). Hence, the higher antioxidant capacity of red rice with respect to white rice has to be explained with the larger amount of total polyphenols (Table 6) that in red rice is associated with the presence of PA. Although the fold difference in polyphenols between red and white rice (Table 6) is less than proportional to the TAC difference (Fig. 1), it is noteworthy to underlie that the TAC is an index that takes into account the antioxidant capacity of single compounds present in food as well as their potential synergistic and redox interactions. Thus, such interactions could make the polyphenols additionally present in red rice (including PA) more effective and could explain the more than proportional difference between the TAC and the antioxidant components quantified. In addition, the broad unresolved peak in the HPLC-ESI-UV-MS analysis of red rice is probably due to complex phenolics produced by PA oxidation and forming a red-brick precipitate in colorless PA solutions [15]. Such compounds, originally called phlobaphenes [15], are known to be responsible for the reddish-brown color of sorghum [15], wheat [35], and maize [36], and are a complex class of molecules, not yet well resolved [8, 14]. Accumulation of this kind of polyphenols, derived from PA, might explain both the red color and the high TAC of red rice.

The strong effect of milling on both varieties of rice, especially on red rice, clearly demonstrates that the antioxidant compounds that contribute to TAC are mainly localized in the outer layer of the kernel. In fact, milling deeply

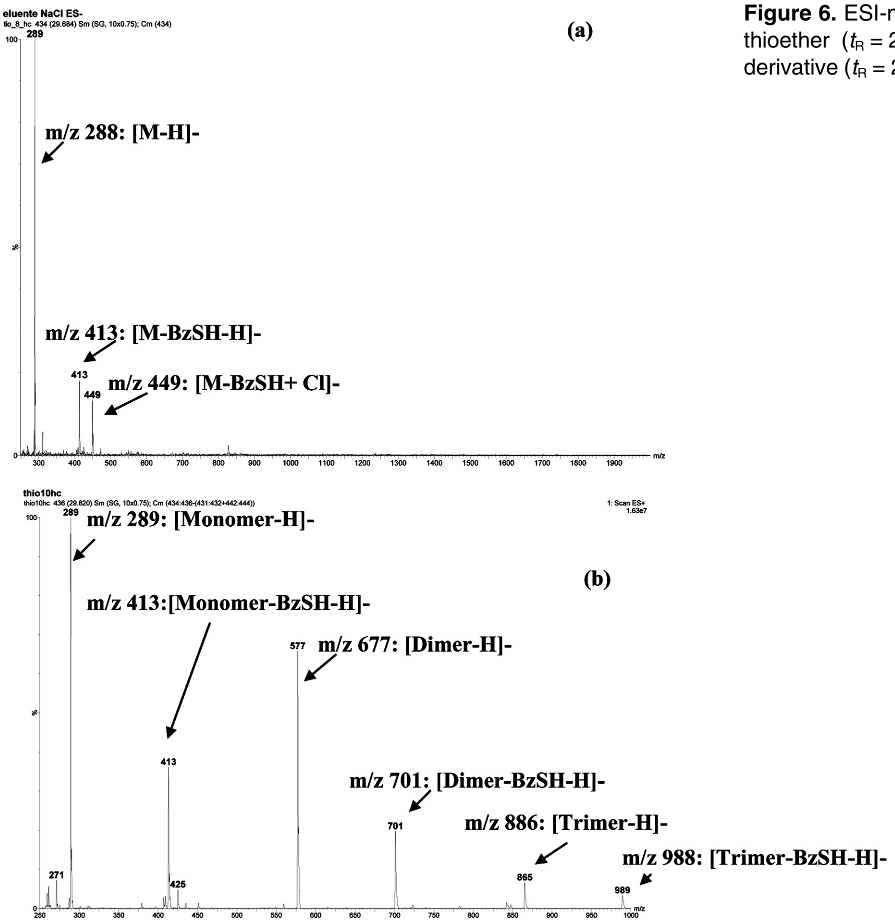

Figure 6. ESI-mass spectra of: (a) epicatechin benzyl thioether (t_R = 29.7 min) and (b) its benzyl thioether derivative (t_R = 28.8 min).

reduces the content of antioxidant compounds and in turn the TAC values. As a consequence, milled red rice exhibited a similar TAC value to that of milled white rice.

Recent data demonstrated that cereals and cereal products possess significant free radical-scavenging properties and may serve as a potential source of natural antioxidants in the diet [37–40]. In fact, grains contain unique phytochemicals that complement those in fruit and vegetables when consumed together. However, to our knowledge there is no information about the cooking effect on cereal TAC. As the rice is consumed cooked, the effect on the TAC of two different cooking processes commonly utilized for rice preparation was also evaluated. As expected, the water-soluble antioxidants present in red rice were almost all lost during water cooking, regardless of the milling degree, determining a strong TAC loss. However, also after water cooking the TAC of dehulled rice remained higher in the red than in the white variety. In addition, when the rice was cooked as risotto, wherein the cooking water is completely absorbed by the rice grains, a reduced loss of antioxidant compounds was observed. Thus, notwithstanding thermal and oxidative processes that occur during cooking, risotto of whole-kernel red rice retained 56% of the TAC of raw rice. On the other hand, risotto of dehulled and undermilled white rice maintained a TAC similar to that of the corresponding raw samples, despite a drop in the level of hydroxycinnamic acids. This was probably due to the release of bound molecules of lipophilic antioxidants. Thus, risotto of whole kernels is a good cooking method for red rice, partially preserving its antioxidant properties.

In conclusion, we demonstrated that whole-kernel red rice is a good source of antioxidants, but milling and cooking greatly reduce its TAC, due to removal of the outer layers of the caryopsis during milling and loss of free and soluble antioxidants during cooking. Therefore, to retain the antioxidant properties of red rice, more suitable processing methods should be developed. Because of the increasing importance acknowledged to the assumption of polyphenols, the consumption of whole, or poorly milled, cereals, represents a useful suggestion for the consumer health. Consumption of red rice, which has been shown to be a rich source of these compounds, could then be suggested in habitual diet to increase the daily intake of polyphenols.

We wish to thank Matteo Valeri and Nicola Ferrari for their excellent technical assistance. This work has been supported by the project "Qua.Si.Cer." of the Ministry of University and Research, by the project "Cerealab" of the Region Emilia-Romagna and by the project "Valoryza" of the Ministry of Agriculture and Forestry.

5 References

[1] Ling, W. H., Cheng, Q. X., Ma, J., Wang, T., Red and black rice decrease atherosclerotic plaque formation and increase antioxidant status in rabbits, *J. Nutr.* 2001, *131*, 1421–1426.

[2] Toyokuni, S., Itani, T., Morimitsu, Y., Okada, K., *et al.*, Protective effect of coloured rice over white rice on Fenton reaction-based renal lipid peroxidation in rats, *Free Radic. Res.* 2002, *35*, 583–592.

[3] Xia, M., Ling, W. H., Ma, J., Kitts, D. D., Zawistowski, J., Supplementation of diets with the black rice pigment fraction attenuates atherosclerotic plaque formation in apolipoprotein E deficient mice, *J. Nutr.* 2003, *133*, 744–751.

[4] Xu, Z., Godber, J. S., Purification and identification of components of γ-oryzanol in rice bran oil, *J. Agric. Food Chem.* 1999, *47*, 2724–2728.

[5] Dlack, M., Saska, M., Separation of vitamin E and γ-oryzanol from rice bran by normal-phase chromatography, *JAOCS* 1994, *71*, 1211–1217.

[6] Tian, S., Nakamura, K., Kayara, H., Analysis of phenolic compounds in white rice, brown rice and germinated brown rice, *J. Agric. Food Chem.* 2004, *52*, 4808–4813.

[7] Reddy, V. S., Dash, S., Reddy, A. R., Anthocyanin pathway in rice (*Oryza sativa* L.): Identification of a mutant showing dominant inhibition of anthocyanins in leaf and accumulation of proanthocyanidins in pericarp, *Theor. Appl. Genet.* 1995, *91*, 301–312.

[8] Haslam, E., in: Harborne, J. B., Mabry, T. J. (Eds.), *The Flavonoid: Advances in Research*, Chapman and Hall, London 1982, pp. 417–446.

[9] Oki, T., Masuda, M., Kobayashi, M., Nishiba, Y., *et al.*, Polymeric procyanidins as radical-scavenging components in red-hulled rice, *J. Agric. Food Chem.* 2002, *50*, 7524–7529.

[10] Hu, C., Zawistowski, J., Ling, W., Kitts, D., Black rice (*Oryza sativa* L. *indica*) pigmented fraction suppresses both reactive oxygen species and nitric oxide in chemical and biological model systems, *J. Agric. Food Chem.* 2003, *51*, 5271–5277.

[11] Santos-Buelga, C., Scalbert, A., Proanthocyanidins and tannin-like compounds- nature, occurrence, dietary intake and effects on nutrition and health, *J. Sci. Food Agric.* 2000, *80*, 1097–1117.

[12] Koga, T., Moro, K., Nakamori, K., Yamakoshi, J., *et al.*, Increase of antioxidant potential of rat plasma by oral administration of proanthocyanidin-rich extract from grape, *J. Agric. Food Chem.* 1999, *47*, 1892–1897.

[13] Stevens, J. F., Miranda, C. L., Wolthers, K. R., Schimerlik, M., *et al.*, Identification and in vitro biological activities of hop proanthocyanidins: Inhibition of nNOS activity and scavenging of reactive nitrogen species, *J. Agric. Food Chem.* 2002, *50*, 3435–3443.

[14] Porter, L. J., in: Harborne, J. B. (Ed.), *The Flavonoids: Advances in Research Since 1986*, Chapman and Hall, London 1993, pp. 23–55.

[15] Bate-Smith, E. C., Luteoforol (3′,4,4′,5,7-pentahydroxyflavan) in *Sorghum vulgare* L., *Phytochem.* 1969, *8*, 1803–1810.

[16] Ranghino, F., Valuation of rice resistance to cooking, *Il Riso* 1966, *15*, 117–127.

[17] Perez-Jimenez, J., Saura Calixto, F., Literature may underestimate the actual antioxidant capacity of cereals, *J. Agric. Food Chem.* 2005, *53*, 5036–5040.

[18] Pellegrini, N., Del Rio, D., Colombi, B., Bianchi, M., Brighenti, F., Application of the 2,2′-azynobis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation assay to a flow injection system for the evaluation of antioxidant activity of some pure compounds and beverages, *J. Agric. Food Chem.* 2003, *51*, 260–264.

[19] Pellegrini, N., Re, R., Yang, M., Rice-Evans, C. A., Screening of dietary carotenoids and carotenoid-rich fruit extracts for antioxidant activities applying the 2,2′-azynobis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation decoloration assay, *Methods Enzymol.* 1999, *299*, 379–389.

[20] Peterson, D. M., Qureshi, A. A., Genotype and environment effects on tocols of barley and oats, *Cereal Chem.* 1993, *70*, 157–162.

[21] American Oil Chemists' Society, Official Method Ce 8–89: Determination of tocopherols and tocotrienols in vegetable oils and fats by HPLC, in: *Official Methods and Recommended Practices*, *4th Ed.*, American Oil Chemists' Society, Champaign, IL 1989, pp. 1–5.

[22] Seetharamaiah, G. S., Prabhakar, J. V., Oryzanol content of Indian rice bran oil and its extraction from soap stock, *J. Food Sci. Technol.* 1986, *23*, 270–273.

[23] Rapisarda, P., Carollo, G., Fallico, B., Tomaselli, F., Maccarone, E., Hydroxycinnamic acids as markers of Italian blood orange juices, *J. Agric. Food Chem.* 1998, *46*, 464–470.

[24] Graham, H. D., Stabilization of the Prussian blue color in the determination of polyphenols, *J. Agric. Food Chem.* 1992, *40*, 801–805.

[25] Porter, L. J., Hrstich, L. N., Chan, B. G., The conversion of procyanidins and prodelphinidins to cyanidin and delphinidin, *Phytochem.* 1986, *25*, 223–230.

[26] Guyot, S., Marnet, N., Laraba, D., Sanoner, P., Drilleau, J.-F., Thiolysis-HPLC characterization of apple procyanidins covering a large range of polymerization state, *J. Agric. Food. Chem.* 2001, *49*, 14–20.

[27] Qureshi, A. A., Huanbiao, M., Packer, L., Peterson, D., Isolation and identification of novel tocotrienols from rice bran with hypocolesterolemic, antioxidant, and antitumor properties, *J. Agric. Food Chem.* 2000, *48*, 3130–3140.

[28] Cavallero, A., Gianinetti, A., Finocchiaro, F., Delogu, G., Stanca, A. M., Tocols in hull-less and hulled barley genotypes grown in contrasting environments, *J. Cereal Sci.* 2004, *39*, 175–180.

[29] Miller, A., Frenzel, T., Schmarr, H.-G., Engel, K.-H., Coupled liquid chromatography-gas chromatography for the rapid analysis of γ-oryzanol in rice lipids, *J. Chromatogr.* 2003, *985*, 403–410.

[30] Goffman, F. D., Bergman, C. J., Rice kernel phenolic content and its relationship with antiradical efficiency, *J. Sci. Food Agric.* 2004, *84*, 1235–1240.

[31] Adom, K. K., Liu, R. H., Antioxidant activity of grains, *J. Agric. Food Chem.* 2002, *50*, 6182–6187.

[32] Riedl, K. M., Hagerman, A. E., Tannin-protein complexes as radical scavengers and radical sinks, *J. Agric. Food Chem.* 2001, *49*, 4917–4923.

[33] Hagerman, A. E., Riedl, K. M., Jones, A., Sovik, K. N., *et al.*, High molecular weight plant polyphenolics (tannins) as biological antioxidants, *J. Agric. Food Chem.* 1998, *46*, 1887–1892.

[34] Pellegrini, N., Visioli, F., Buratti, S., Brighenti, F., Total antioxidant activity of olive oil and studies on the influence of heating, *J. Agric. Food Chem.* 2001, *49*, 2532–2538.

[35] Miyamoto, T., Everson, E. H., Biochemical and physiological studies on wheat seeds pigmentation, *Agron. J.* 1958, *50*, 733–734.

[36] Styles, E. D., Ceska, O., Pericarp flavonoid in genetic strains of *Zea mays*, *Maydica* 1989, *34*, 227–237.

[37] Miller, H. E., Rigelhof, F., Marquart, L., Prakash, A., Kanter, M., Antioxidant content of whole grain breakfast cereals, fruits and vegetables, *J. Am. Coll. Nutr.* 2000, *19*, 312s–319s.

[38] Halvorsen, B. L., Holte, K., Myhrstad, M. C. W., Barikmo, I. *et al.*, A systematic screening of total antioxidants in dietary plants, *J. Nutr.* 2002, *132*, 461–471.

[39] Wu, X., Gu, L., Holden, J., Haytowitz, D. B., *et al.*, Development of a database for total antioxidant capacity in foods: A preliminary study, *J. Food Compos. Anal.* 2004, *17*, 407–422.

[40] Martinez-Tomé, M., Murcia, M. A., Frega, N., Ruggeri, S., *et al.*, Evaluation of total antioxidant capacity of cereal brans, *J. Agric. Food Chem.* 2004, *52*, 4690–4699.