Research Paper

Characterizing traditional rice varieties grown in temperate regions of Italy: free and bound phenolic and lipid compounds and in vitro antioxidant properties

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Abstract

Objectives: A comparative study regarding the fatty acid profiles and antioxidant properties of the two most common varieties of rice (Carnaroli and Karnak) cultivated in Sybaris (Southern Italy) was conducted. Carnaroli variety from Pavia district (Northern Italy) was also investigated.

Methods: Free and bound lipids were separately extracted. Fatty acid composition was determined by high resolution gas chromatography (HRGC). Free and bound phenolic acids were determined using high performance liquid chromatography (HPLC). The antioxidant properties were assessed using 2,2’-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay.

Results: The total lipid content was found to be higher in both Sybaris species with respect to the Pavia variety. The lipid distribution was also significantly different. In particular, the amount of bound lipids (14.2–16.3 mg g⁻¹) was higher than the free lipids (7.2–7.5 mg g⁻¹), and unsaturated fatty acids (USFA) were significantly higher in the free-FA fraction than in bound-FA in both Sybaris varieties. By contrast, Carnaroli variety from Northern Italy displayed a higher content of free (5.3 mg g⁻¹) than bound lipids (2.5 mg g⁻¹) and a higher content of USFA in the bound-FA. The antioxidant activity of Pavia rice extract was higher than that of both Sybaris varieties.

Conclusions: The two Sybaris varieties are richer in lipids than the Pavia rice, while exhibiting the same qualitative fatty acid profile with some differences in the content of individual fatty acids. The distribution of lipids (free or bound) was found to be significantly different in the Carnaroli varieties, highlighting the effects of different climatic and pedological areas.

Key words: free and bound lipids; radical scavenging; free and bound phenolics.

Introduction

Rice (Oryza sativa) is a major cereal crop and is consumed by 53 per cent of the world’s population as a staple grain, according to the FAO (FAO, 2016). It is the grain with the second-highest worldwide production, after maize (Mir et al., 2016). Since a large portion of maize crops are grown for purposes other than human consumption, rice represents the most important grain with regard to human nutrition and caloric intake, providing more than one fifth of the calories consumed worldwide (Oli et al., 2014; Hu et al., 2017). Rice is a gluten-free carbohydrate food and thus it is suitable for everyone...
including people with celiac disease. It is a good source of starch, and it also contains small amounts of proteins (6%–7%) and lipids (3 per cent). Brown rice contains small amounts of B vitamins and is a useful source of folate. Numerous studies have shown that the essential phytochemicals in rice are significantly associated with reduced risk of developing chronic diseases such as cardiovascular disease, type 2 diabetes, and some cancers (Yawadio et al., 2007; Kondo et al., 2017).

Rice production is geographically concentrated in Western and Eastern Asia. Asia is the biggest rice producer, accounting for 90 per cent of the world’s production and consumption of rice. China and India, which account for more than one-third of global population, supply over half of the world’s rice. Brazil is the most important non-Asian producer, followed by the USA. Italy is currently the largest rice producer in the European Union (EU): Italian production is around 1.5 million tons and represents 48 per cent of EU total production (Ferrazzi et al., 2017). Approximately 90 per cent of the rice cultivation in Italy is located in the northern regions (Po Valley), mainly in the regions of Piemonte and Lombardia, in the triangle Vercelli, Novara, Pavia. The most important rice varieties cultivated are Carnaroli, Arborio, Baldo, and Volano, all with typical characteristics that distinguish them from the other Italian varieties. Nevertheless, rice cultivation extends over some small areas cropped in the Sardinia Island and Southern Italy which represent only 1.4 per cent of the total arable area.

In the Sybaris Valley, the largest plain of Calabria (Southern Italy), situated on the northern Ionian slope of the region between the Pollino and Sila massif, the tradition of rice cultivation is rather old, and, at present, the rice fields have reached the remarkable area of 562 hectares. The total production reaches about 15 000 quintals between rice and paddy rice, and it is now possible to find the rice produced in the Sybaris area in the supermarkets. Plant sowing takes place in May and harvest in mid-October: a production cycle of 140 days in total, 15 less than the cousins in the North. The intensity of the sun and the mild temperature allow the ears to reach a full maturation. The proximity to the sea allows, thanks to the salt, the creation of unique microclimate for the cultivation of rice. These factors combined with the purity of the water that floods the fertile soil and the wind that dominates the cultivation areas contribute to obtaining excellent rice quality. Among the most cultivated varieties in addition to Carnaroli (white or brown), it is possible to find other varieties, such as Karnak, round or balilla, Roma, Arborio, Thaibonnet, and Ganges, a kind of aromatic rice that resembles basmati. The characteristics of the soil and microclimate in Sybaris are favorable for the cultivation of Carnaroli rice. This is due to the clay soil that is rich in silt. Karnak variety is derived from a genetic mutation of Carnaroli rice and it is about 40 cm shorter than its progenitor, on average; it is a more productive and disease-resistant variety with respect to Carnaroli. Nevertheless, no information regarding the antioxidant components and the lipid and phenolic composition of rice extracts from commercially available varieties in Sybaris has been reported so far. The objective of the present research was to investigate the genetic diversity of the two most common Sybaris rice varieties (Carnaroli and Karnak) regarding lipid composition, phenolic acid profile, and antioxidant properties. Moreover, the Carnaroli variety from Pavia district was compared with the Sybaris varieties to highlight possible differences in composition due to differences in climatic and pedological conditions of their respective growing regions.

**Experimental**

**Rice samples**

The two rice varieties Carnaroli and Karnak were obtained from the Sybaris district (Calabria region). Plants were harvested at physiological maturity in the Favella Spa factory (Cantinella-di-Corigliano, Cosenza) and packed in the site of Contrada Santa Maria, Villapiana. The variety Carnaroli (Curritiro) from Pavia was purchased at a local supermarket. The rice was washed and dried for about 2 h at room temperature; it was immediately ground with an electric immersion grinder and dried in an oven at T = 110°C until constant weight, avoiding production of a yellow toast (about 3 h); time and temperature are important to inactivate endogenous lipase. Moisture was determined by drying at 110°C to constant mass. The samples were stored at −20°C prior to analysis. All analyses were performed using triplicate samples; the results are expressed on a dry matter basis.

**Chemicals and reagents**

Diethyl ether, ethanol, propan-1-ol, methanol, benzene, hexane, dimethyl sulfoxide (DMSO, ACS grade), acetonitrile (HPLC grade), sodium hydroxide, anhydrous sodium sulphate, and potassium carbonate were purchased from Carlo Erba Reagents (Milano, Italy). The methyl esters of fatty acids were obtained from Sigma-Aldrich Co. (Milan, Italy). Methyl tridecanoate (C13:0, Sigma-Aldrich Co., Italy) was used as an internal standard. Acetyl chloride, purchased from Sigma-Aldrich, was used after distillation. 2,2’-Diphenyl-1-picrylhydrazyl (DPPH), 2,6-di-ter-butyl-4-methylphenol (BHT), Folin-Ciocalteu’s reagent, authentic standards of gallic, ferulic, p-hydroxybenzoic, protocatechic, p-coumaric, and sinapic acids were also purchased from Sigma-Aldrich Fine Chemicals.

**Extraction of saponifiable lipids**

Rice lipids are usually divided into free lipids and bound lipids where free lipids are ether-extractable whilst bound lipids are typically extracted with hot aqueous alcohols.

**Free lipids**

The dried rice flour (50 g) was extracted with diethyl ether (350 ml, Bp 34°C) using a Soxhlet apparatus at 50°C for 24 h. The solvent was evaporated in a rotary evaporator at 35°C to dryness; the residual was dried under vacuum. The free lipid content was expressed as the percentage of the dry rice flour. Solvent-less lipids were transferred to brown airtight containers and stored at −25°C until analysis.

**Bound lipids**

Dried rice flour (50 g) was mixed with propanol-water (320 ml, 3/1 v/v) and 2,6-di-ter-butyl-4-methylphenol (BHT; 50 mg) (Davies, 1985) to inhibit the oxidative degradation of lipids during analysis. The suspension was heated under reflux using a Soxhlet apparatus at 110°C for 15 h, changing the solvent every 3 h (five extractions) and the combined propanol-water extracts were evaporated to dryness in a rotary evaporator. The dried extract was dissolved in chloroform and filtered using a glass fiber funnel. The filtrate and washings were evaporated to dryness in a rotary evaporator. The dried rice flour (50 g) was extracted with diethyl ether (350 ml, Bp 34°C) using a Soxhlet apparatus at 50°C for 24 h. The solvent was evaporated in a rotary evaporator at 35°C to dryness; the residual was dried under vacuum. The free lipid content was expressed as the percentage of the dry rice flour. Solvent-less lipids were transferred to brown airtight containers and stored at −25°C until analysis.

**Fatty acid composition**

The fatty acid composition was determined in triplicate by high-resolution gas chromatographic analysis (HRGC) of the oils after transesterification.

**Direct transesterification**

One hundred milligrams of dried oil were precisely weighed in borosilicate glass tubes. Tridecanoic acid (50 μg) dissolved in 2 ml of methanol-benzene 4:1 (v/v) was added to the sample, as internal
standard. Acetyl chloride (200 μl) was slowly added, with stirring, over a period of 1 min to the mixture. The tube was tightly closed with teflon-lined caps. After stirring at 100°C for 1 h (Fazio et al., 2013), the mixture was cooled to room temperature, and 5 ml of a 6 per cent K₂CO₃ solution was slowly added with stirring to stop the reaction and neutralize the mixture. The esters were extracted with hexane (4 × 2 ml). The supernatant containing the fatty acid methyl ester was combined, dried over Na₂SO₄, filtered, and evaporated to dryness. The resulting oil was then transferred to a vial under nitrogen gas. The dried extracts were analysed immediately.

HRGC-FID analysis

HRGC analyses were carried out on a Shimadzu GC-2010 system equipped with an AOC-20i autosampler, a split/split-less injector, and a flame ionization detector (FID, Shimadzu, Milan, Italy) and were performed at the following experimental conditions: the column used was a fused-silica capillary column (Supelcowax™ 10) (30 m × 0.22 mm id × 0.25 μm); the oven temperature was programmed from an initial temperature of 80°C (5 min hold), raising to 230°C at a rate of 3.0°C/min; the injection volume was 1.0 μl in the split mode (17:1). Helium was used as carrier gas. The detector temperature was set at 250°C. The hydrogen flow rate was 47.0 ml min⁻¹; the air flow rate was 400 ml min⁻¹; the make-up flow rate (N₂/Air) was 30 ml min⁻¹. All samples were dissolved in n-hexane for injection (1/10 v/v). Fatty acids (FA) were identified by comparison of the retention times observed with those of standard fatty acid methyl esters (FAMES). The quantitative determination was obtained by the external standard method and the results are reported as mg per 100 g of extracted oil. Data are reported as a mean value ± SD of three samples of each variety, analysed individually in triplicate.

Extraction of phenolic compounds

The rice powder (10.0 g) was extracted with methanol (150 ml) for 24 h in an electrical shaker at room temperature; the extract was filtered through Whatman No.1 filter paper and the solvent was removed. The residual rice powder was further extracted twice with methanol, filtered and the extracts were combined before evaporating under vacuum. The residual crude methanolic rice extract was weighed and stored at −20°C under a nitrogen gas stream. Extractions were performed in triplicate.

Determination of total phenolic content

The total phenolic content (TPC) of each fraction was determined using the Folin–Ciocalteu method with some modification (Plastina et al., 2012). Briefly, 200 μl of extract solution (10 mg in 1 ml of DMSO) were shaken for 1 min with 1 ml of diluted (1:10) Folin–Ciocalteu reagent. Then 800 μl of 10 per cent Na₂CO₃ were added and the final volume was made up to 5.0 ml with distilled water. After the mixture was left to stand for 2 h at room temperature, the absorbance at 760 nm was measured by using a UV-vis spectrophotometer (model V-550, Jasco Europe). The results of TPC were estimated using a standard curve prepared using gallic acid and expressed as mg of gallic acid equivalents per gram of extract.

Determination of DPPH radical–scavenging activity

The free-radical scavenging activity of the extracts was evaluated using the DPPH method. DPPH is a stable free radical, and when it reacts with a radical scavenger, its maximum absorbance at 517 nm fades rapidly. The antioxidant effect is proportional to the disappearance of DPPH in test samples. The free radical scavenging activity against DPPH was determined at three different concentrations according to a known protocol (Gabriele et al., 2012) with some modifications. Thus, DMSO solutions containing known amounts of the extract and of DPPH were prepared, and the colorimetric decrease in absorbance of DPPH was measured at 517 nm after a period of 30 min since preparation. More specifically, standard DMSO solutions of the extract (10 mg in 1 ml of DMSO) and DPPH (3.9 mg in 10 ml of MeOH, corresponding to 1 mM) were prepared. Different volumes of the extract solution (100, 250, and 500 μl) were mixed with 100 μl of the DPPH solution, and the final volume adjusted to 3 ml by the addition of the necessary amount of MeOH. In this way, three different solutions with extract concentration of 0.33, 0.83, and 1.67 mg ml⁻¹ were obtained for each extract. The mixtures were shaken vigorously and left standing at room temperature in the dark for 30 min The absorbance of each sample was then measured against blank (the DPPH solution obtained by diluting 100 μl of the DPPH standard solution with MeOH to give a final volume of 3 ml) at 517 nm using a UV-vis spectrophotometer (model V-550, Jasco Europe). Experiments were carried out in triplicate, and BHT was used as the positive reference antioxidant. The percentage of scavenging activity on DPPH was calculated according to the following formula:

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\text{Scavenging Ability (\%)} = \left( \frac{\text{Absorbance}_{17\text{min of control}} - \text{Absorbance}_{17\text{min of sample}}}{\text{Absorbance}_{17\text{min of control}}} \right) \times 100.
\]

Determination of EC₅₀ values

The EC₅₀ values (50 per cent free scavenging concentration values) of the extracts were determined using the correlation and regression programmer of the Graph Pad Prism program, and the results are given as mean values ± SD (n = 3).

Determination of free and bound phenolic acids

Free and bound phenolic acids were determined according to a previously reported method (Sumczynski et al., 2016), with some modifications. For phenolic acid determination, the rice bran samples were defatted by extraction with hexane. The defatted rice (10 g) was extracted with 70 per cent (v/v) aqueous ErOH to recover the free phenolic acids. The residue was hydrolysed with 1 M NaOH (2 x 200 ml) and then centrifuged at 10000 rpm for 20 min (T = 5°C). The supernatants were collected and acidified with 4 N HCl to pH 1 and then were extracted (4 times) with ethyl acetate (200 ml each). The solvent was evaporated to dryness, and the residue was dissolved with DMSO (1 mg ml⁻¹) and analysed. The extracts were separated by using a Shimadzu HPLC system, equipped with two SCL-10-AVP pumps, an SLC-10-AVP controller, and an SPD-20A UV/Vis detector: the column used was a discovery HS C18 (Supelco) (250 mm × 4.6 mm id, 5 μm particle size). The mobile phase was a mixture of purified water with 0.1 per cent TFA (solvent A) and acetonitrile (solvent B) at a flow rate of 1.5 ml min⁻¹. Gradient elution was performed as follows: 0–15 min, linear gradient from 5% to 9% solvent B; from 15 to 30 min 9% solvent B; from 30–37 min, linear gradient from 9% to 13% solvent B; from 37 to 55 min, linear gradient from 13% to 18% solvent B; from 55 to 60, linear gradient from 18% to 20% solvent B, linear gradient from 20% to 90% solvent B, linear gradient from 90% to 5% solvent B. The detector was set at 280 nm to detect hydroxybenzoic acids and at 325 nm to detect hydroxycinnamic acids. The separated phenolic acids were identified comparing their retention times with authentic compounds. Quantitative determination was carried out by using the external standard method. Calibration curves were constructed with the external standards.
Results and Discussion

Extractable lipid content of rice

Rice lipids, which are predominantly in the form of triacylglycerides, are usually divided into free lipids and bound lipids, where free lipids are ether-extractable whilst bound lipids are typically extracted with hot aqueous alcohols (Morrison, 1998). The free lipids are adsorbed on the surface of starch granules, whereas bound lipids (also referred to as starch lipids) are located inside starch granules (Zhou et al., 1999) where they are complexed with amylose (Ho and Izzo, 1992). The majority of the bound lipids are either free fatty acids or monoacylglycerides. The terms like free lipids and bound (or starch) lipids thus refer to a functional rather than chemical classification.

As shown in Table 1, the content of bound lipids (14.2–16.3 mg g⁻¹) was higher than that of free lipids (7.2–7.5 mg g⁻¹) in both Sybaris varieties as well as in the Carnaroli variety from Pavia. The percentage of total lipids (referred to the starting dry rice flour, and expressed in % w/w) was slightly higher in the Carnaroli variety (2.3 per cent) than that in Karnak variety (2.1 per cent), whereas the percentage of free as well as bound lipids in the Carnaroli variety from Pavia was lower than those of both Sybaris varieties (1.6 per cent).

Fatty acid composition

Table 2 reports the fatty acid composition (expressed in terms of the esters by weight) of the analysed varieties (Carnaroli and Karnak from Sybaris and Carnaroli from Pavia). The fatty acid profiles of both fractions (free and bound lipids) were determined by gas chromatography using methyl tridecanoate as an internal standard.

Table 1. Quantitative recovery of the extracts (mg).

| Extracts      | Free lipids | Bound lipids | Methanol extracts | Total phenolic acids |
|---------------|-------------|--------------|-------------------|----------------------|
| Sybaris varieties | Carnaroli    | 375          | 813               | 965                  | 405                   |
|                | Karnak      | 382          | 710               | 720                  | 322                   |
| Pavia variety  | Carnaroli    | 98           | 695               | 503                  | 245                   |

Extract contents are referred to the starting dry rice flour (50 g).

Table 2. Fatty acid composition of Sybaris and Pavia rice varieties. SFA, saturated fatty acids; USFA, unsaturated fatty acids; ∑FA, total content of fatty acids (free or bound); ∑ FA + ∑ bound-FA, total content of diethyl ether extractable and propanol-water extractable fatty acids.

| Fatty acids | Sybaris varieties | Carnaroli | Karnak | Pavia variety | Carnaroli |
|-------------|-------------------|-----------|--------|---------------|-----------|
|              | Free lipids       | Bound lipids | Methanol extracts | Total phenolic acids |
| C12:0       | 0.25 ± 0.02       | 0.39 ± 0.03 | 0.11 ± 0.01      | 0.63 ± 0.03      | 0.06 ± 0.01       | 0.26 ± 0.03       |
| C14:0       | 33.87 ± 1.33      | 87.78 ± 1.33 | 24.72 ± 0.35     | 96.82 ± 4.12     | 33.61 ± 0.21      | 129.24 ± 1.32     |
| C16:0       | 129.54 ± 7.98     | 79.85 ± 1.33 | 95.35 ± 1.28     | 82.91 ± 1.79     | 0.72 ± 0.01       | 114.79 ± 1.45     |
| C16:1       | 1.11 ± 0.03       | 0.37 ± 0.01 | 0.91 ± 0.02      | 0.97 ± 0.03      | 0.71 ± 0.01       | 0.52 ± 0.03       |
| C18:0       | 15.29 ± 0.67      | 3.68 ± 0.07 | 12.25 ± 0.03     | 4.43 ± 0.02      | 10.57 ± 0.01      | 5.64 ± 0.02       |
| C18:1       | 278.29 ± 15.04    | 1.78 ± 0.04 | 209.58 ± 3.72    | 1.61 ± 0.01      | 178.13 ± 1.02     | 68.38 ± 0.08      |
| C18:2       | 290.62 ± 12.79    | 4.92 ± 0.18 | 223.30 ± 2.51    | 2.06 ± 0.01      | 4.13 ± 0.08       | 205.64 ± 2.38     |
| C18:3       | 10.65 ± 0.47      | 6.29 ± 0.09 | 7.63 ± 0.02      | 4.24 ± 0.02      | 8.49 ± 0.16       | 8.85 ± 0.11       |
| C20:0       | 5.09 ± 0.27       | 0.71 ± 0.02 | 3.19 ± 0.08      | 0.82 ± 0.01      | 15.92 ± 0.31      | 0.88 ± 0.01       |
| SFA         | 184.04            | 172.71     | 135.61           | 185.61           | 60.88             | 250.81            |
| USFA        | 580.67            | 13.36      | 441.44           | 8.88             | 192.17            | 283.39            |
| ∑FA         | 764.71            | 186.07     | 577.05           | 194.49           | 253.05            | 534.2             |
| ∑ free-FA + | 950.78            |            | 771.54           |                 | 787.25            |                   |
| ∑ bound-FA  |                   |            |                  |                 |                   |                   |

Values are mean ± SD of three samples of each variety, analysed individually in triplicate. Each value is expressed as g/100g of extracted oil.
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Table 3. Total phenolic content and radical scavenging ability of the extracts.

| Methanolic extracts | Total phenolic content (mg GAE/g extract) | Radical scavenging activity (%) |
|---------------------|------------------------------------------|--------------------------------|
| Sybaris Carnaroli   | 9.11 ± 0.18                              | 0.33 mg/ml: 27.4 ± 1.6; 0.83 mg/ml: 63.1 ± 1.9; 1.67 mg/ml: 83.2 ± 0.9 |
| Karnak              | 8.93 ± 0.16                              |                                |
| Pavia Carnaroli     | 9.06 ± 0.18                              |                                |

Figure 1. EC<sub>50</sub> values of the DPPH radical-scavenging activities of methanolic extracts of Sybaris and Pavia varieties (a shorter bar indicates a higher radical scavenging activity).

Antioxidant activity of the methanolic extracts

The methanolic extracts of Sybaris and Pavia rice varieties were examined and compared for their free-radical scavenging activities against the DPPH radical. Table 1 shows the recovery of the MoOH extracts, whereas the radical scavenging results are shown in Table 3. The scavenging effects on DPPH radicals increased with the concentration: it varied from 27.4 ± 0.33 mg ml<sup>-1</sup> to 83.2 ± 0.69 mg ml<sup>-1</sup> in the case of Carnaroli variety and from 36.9 ± 0.56 mg ml<sup>-1</sup> to 69.2 ± 1.2 mg ml<sup>-1</sup> in the case of Karnak variety at the same concentrations. The Curtiriso rice extract showed a higher antioxidant activity with respect to both Sybaris extracts (Table 3). Based on these results, the efficient concentration value (EC<sub>50</sub>) was determined (Figure 1). This represents the amount of the extract that causes a decrease in an initial DPPH concentration by 50 per cent. A lower EC<sub>50</sub> value therefore refers to a higher antioxidant activity. From Figure 1, it can be easily seen that the Karnak variety showed a higher radical-scavenging activity (EC<sub>50</sub> = 0.41 ± 0.02 mg ml<sup>-1</sup>) compared with the Carnaroli variety (EC<sub>50</sub> = 0.56 ± 0.03 mg ml<sup>-1</sup>), whereas the activity of Curtiriso rice extract was higher than that of Sybaris varieties (EC<sub>50</sub> = 0.35 ± 0.01 mg ml<sup>-1</sup>). In any case, the DPPH free radical-scavenging activity of the extracts was lower than that of BHT (EC<sub>50</sub> = 0.11 ± 0.02 mg ml<sup>-1</sup>) used as positive control.

TPC of crude methanol extracts

The TPC was determined following the Folin–Ciocalteau method, and the results are expressed as mg gallic acid equivalents (GAE) per gram extract (Table 3). There were no significant differences detected amongst Sybaris varieties: the TPCs of Carnaroli and Karnak varieties were 9.11 mg GAE/g<sub>extract</sub> and 8.93 mg GAE/g<sub>extract</sub>, respectively. This might be due to similarities in growth conditions. Moreover, the phenolic content of rice from Pavia was also determined and found to be similar to that reported for Carnaroli variety from Sybaris (9.06 mg GAE/g<sub>extract</sub>). Considering that the antioxidant activity of the Pavia rice extract was higher with respect to those of the Sybaris extracts, it can be deduced that no clear correlation exists between the observed antioxidant activities and the TPCs. This result suggests that other substances contribute to the overall antioxidant activity.

Phenolic acid content

Phenolic acids constitute a group of antioxidants found in plants, including cereal grains. In rice, phenolic acids can be classified as bound and free phenolic acids. According to the literature, bound phenolic compounds are probably linked to other components through ester, ether, or acetol bonds (Robbins, 2003; Melini and Acquistucci, 2017; Panga et al., 2018). Table 1 shows the total phenolic acid content for the three varieties studied, whereas Table 4 shows the phenolic acid profiles. The profiles did not differ significantly among the three varieties (Carnaroli and Karnak from Sybaris and Carnaroli from Pavia). Previous studies have shown that two groups of phenolic acids are present in rice grain (Irakli et al., 2012): hydroxybenzoic acids (gallic acid, protocatechuic acid, p-hydroxybenzoic acid) and hydroxycinnamic acids (ferulic acid and sinapic acid). These six phenolic acids were identified in all rice extracts by comparison with the retention time and UV absorption of external standards and further confirmed using mass spectral data, as described previously (Ryan et al., 1999); gallic, protocatechuic, p-hydroxybenzoic, p-coumaric, ferulic, and sinapic acids (Table 4).

The phenolic acids listed in Table 4 were the only phenolic acids detected in the samples at significant concentrations (>1 µg/g). As highlighted by previous studies, the major portion of phenolic acids existed in bound (insoluble) form (Adom and Liu, 2002; Liyanapathirana and Shahidi, 2006; Shao et al., 2014; Zhang et al., 2015); thus, the bound phenolic acids, as a proportion of total phenolic acids, were about 90 per cent in Carnaroli variety, 94.4 per cent in Karnak variety, and 95 per cent in Pavia variety. Free phenolic acids were a minor fraction of phenolic acids, constituting only from 9.4 per cent (Carnaroli variety) to 5.0 per cent (Pavia variety) of the total phenolic acids present in these extracts. Gallic, protocatechuic, and p-hydroxybenzoic acids were found to be the major phenolic acids. The levels of individual phenolic acids, however, did not exceed the taste thresholds reported in the literature (Maga and Lorenz, 1973; Huang and Zavas, 1991). The ratio of bound phenolic acids to free phenolic acids was about 10 in the Carnaroli extracts and 17 in the Karnak extracts, whereas in the extract of Pavia rice, this ratio was about 20 even though the levels of total phenolic acids were lower than those of Sybaris rice extracts. The profiles of the bound phenolic acids fraction of both Sybaris varieties were dominated by lipids, whereas in the extract of Pavia rice, this ratio was about 1 in the bound lipids. This difference is probably due to the fact that amylose-lipid complexation shows a preference for SFA (Yamada et al., 1998; Soong et al., 2013). It is worth noting the difference in the oleic acid to linoleic acid ratios in the free-FA and bound-FA fractions. In the free-FA fraction, this ratio was close to unity in both Sybaris varieties but it was approximately 3 in the bound-FA fraction of Pavia variety and slightly higher than unity in Karnak variety.

| Methanolic extracts | Total phenolic content (mg GAE/g<sub>extract</sub>) | Radical scavenging activity (%) |
|---------------------|------------------------------------------|--------------------------------|
| Sybaris Carnaroli   | 9.11 ± 0.18                              | 0.33 mg/ml: 27.4 ± 1.6; 0.83 mg/ml: 63.1 ± 1.9; 1.67 mg/ml: 83.2 ± 0.9 |
| Karnak              | 8.93 ± 0.16                              |                                |
| Pavia Carnaroli     | 9.06 ± 0.18                              |                                |
ferulic, sinapic, and p-coumaric acids (Figure 2). Ferulic acid and p-coumaric acid were bound to phytosterols which have the structure of oryzanols (Miller and Engel, 2006). Ferulic acid was the major phenolic compound in all varieties: the percentage of bound ferulic acid was 70 per cent in both Sybaris extracts and 75 per cent in the extract of Pavia rice. With the exception of protocatechuic and p-hydroxybenzoic acids, the concentrations of the other phenolic acids were higher in Karnak variety than in Carnaroli variety.

Conclusions

In conclusion, in this study, we have determined the lipid and phenolic content and in vitro antioxidant activity of two rice varieties, Karnak and Carnaroli, grown in the temperate region of Italy (Sybaris, Southern Italy). These results were compared with those obtained for Carnaroli rice grown in regions with continental climate (Po Valley, Northern Italy). We have found that the two Sybaris varieties are richer in lipids than the Pavia rice, while exhibiting the same qualitative fatty acid profile with some differences in the content of individual fatty acids. The distribution of lipids (free or bound) was found to be significantly different in the Carnaroli varieties deriving from different climatic and pedological areas. In fact, the variety from Sybaris exhibited a predominance of free lipids over bound lipids, in contrast with the Pavia rice. Moreover, both Sybaris rice varieties were richer in oleic acid than the Pavia variety.

This study has also shown that the Karnak variety from Sybaris possesses a higher antioxidant activity than the Sybaris Carnaroli variety, whereas the highest antioxidant activity was observed for the Pavia Carnaroli variety. However, no clear relationship could be drawn between the observed antioxidant activities and the TPCs, which were similar in all varieties studied. This lack of correlation suggests that other substances contribute to the overall antioxidant activity. Analysis of the phenolic acid contents showed that the major part of phenolic acids existed in bound form, with ferulic acid being the dominant phenolic acid compound in all rice varieties. In conclusion, our results highlight differences in the composition of rice of the same variety grown in Italian regions characterized by different pedoclimatic conditions.

Table 4. The content of free and bound phenolic acids.

| Phenolic acids          | Sybaris varieties | Pavia variety |
|-------------------------|-------------------|--------------|
|                         | Carnaroli         | Karnak       | Carnaroli |
|                         | Free | Bound | Free | Bound | Free | Bound |
| Gallic acid             | 77.7 ± 1.1        | 13.9 ± 0.2    | 81.5 ± 0.6 | 29.1 ± 1.1 | 23.8 ± 1.5 | 11.1 ± 1 |
| Protocatechuic acid     | 121.3 ± 2.1       | 84.9 ± 1.3    | 71.3 ± 0.6 | 78.8 ± 0.7 | 37.5 ± 0.5 | 49.6 ± 1.5 |
| p-Hydroxybenzoic acid   | 65.8 ± 0.9        | 92.3 ± 0.3    | 26.3 ± 1.1 | 103.4 ± 1.4 | 35.2 ± 1.1 | 51.3 ± 2.7 |
| p-Coumaric acid         | 16.5 ± 0.3        | 215.1 ± 1.5   | 33.9 ± 1.1 | 415.7 ± 0.8 | 19.2 ± 1.0 | 168.7 ± 0.3 |
| Ferulic acid            | 30.1 ± 0.8        | 2143.3 ± 8.8  | 47.7 ± 1.1 | 3243.8 ± 10.5 | 23.7 ± 0.6 | 2079.1 ± 10.3 |
| Sinapic acid            | 5.5 ± 0.1         | 502.1 ± 0.8   | 11.4 ± 0.5 | 764.6 ± 3.3 | 4.4 ± 0.1 | 422.7 ± 2.0 |
| Total                   | 316.9            | 3051.6        | 272.3      | 4635.4      | 143.8      | 2782.7     |
| % bound                 | 90.6             | 94.4          | 95         |            |            |            |

Values are mean ± SD of three samples of each species, analysed individually in triplicate. Each value was expressed as µg/g.

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