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Chemometrical Characterization of Four Italian Rice Varieties Based on Genetic and Chemical Analyses

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This paper describes a method for achieving qualitative identification of four rice varieties from two different Italian regions. To estimate the presence of genetic diversity among the four rice varieties, we used polymerase chain reaction−randomly amplified polymorphic DNA (PCR-RAPD) markers, and to elucidate whether a relationship exists between the ground and the specific characteristics of the product, we studied proximate composition, fatty acid composition, mineral content, and total antioxidant capacity. Using principal component analysis on genomic and compositional data, we were able to classify rice samples according to their variety and their district of production. This work also examined the discrimination ability of different parameters. It was found that genomic data give the best discrimination based on varieties, indicating that RAPD assays could be useful in discriminating among closely related species, while compositional analyses do not depend on the genetic characters only but are related to the production area.

KEYWORDS: Rice; variety characterization; RAPD; fatty acids; metals; antioxidant activity; PCA

INTRODUCTION

Rice (*Oryza sativa*), a major cereal crop, is an excellent source of calories, in the form of starch, and has the added benefit of providing protein with higher nutritional quality than other cereal grains. Improving and increasing the world's supply will depend on the development of improved varieties, improved production practices, and new products that capitalize on rice's unique components and functional properties (*1*).

A small amount of milled and brown rice is used as material for processing of foods, whereas a large amount of rice grain is used in cooked form (*2*). Rice bran oil is a coproduct of the rice milling industry (*3*). Over the past few years there has been an increased interest in rice bran oil because of its content of tocotrienols, sterols, and γ-oryzanol, which have been found to lower serum cholesterol (*4*, *5*), to have high antioxidant activity (*6*) and anti-inflammatory properties (*7*), and to inhibit tumor formation (*8*, *9*). However, these unsaturated lipids are liable to degrade during storage, which results in deterioration of flavor, palatability, and eating quality (*10*). The cultivated rice ecotypes in the world mainly belong to two types: (i) *Oryza glaberrima*, limited to West Africa, and (ii) *Oryza sativa*, the most common in Asia and Europe.

Oryza sativa can be classified into two groups: the ecotype called Indica or Patna with long grains, the ecotype Japonica characterized by short grains, the most diffused in Japan, Korea, northern China, Egypt, Turkey, Italy, and Spain. The worldwide production of rice in 2004 was 609 million tons, with the majority from Asia (550 million tons), followed by South America and Africa (23 and 19 million tons, respectively). The European and Australian production of rice represent together only 1% of the world amounts, with about 4 million tons. Italian production is around 1.5 million tons (major regions of production are Piemonte, Lombardia, Emilia-Romagna, and Veneto). Only a third of the Italian production is reserved for local consumption, while the remainder is exported in Europe and worldwide, where the Italian rice is more and more appreciated. Approximately 90% of the Italian production is concentrated in Piemonte and Lombardia regions, while the remaining 10% is in Emilia-Romagna and Veneto. The rice cultivated on the delta of the Po river (located between Emilia-Romagna and Veneto regions) covers approximately 9000 hectares of territory, and the most important rice varieties cultivated are Carnaroli, Arborio, Baldo, and Volano, all with typical characteristics that distinguish them from the other Italian varieties. The use of molecular markers in taxonomy and evolutionary studies enhances the ability to make inferences about phylogeny and provides vital information about genetic mechanisms for observed evolutionary patterns (*11*). Recently RAPD molecular markers have been used to study genetic

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diversity among Italian rice varieties (*12*), but this study focused on the identification of Japonica ecotype between tropical and temperate. To our knowledge there are no reports on the evaluation of genetic variability of the four cultivars considered in our study. The first part of the present study regarded the evaluation of genetic diversity among the four rice varieties cited above, analyzing the RAPDs patterns through principal component analysis (PCA). Principal component analysis is among the most versatile of all chemometric methods. It seeks to maximize the variance information present in a data set in as few new dimensions as is possible. The main element of this approach consists of the construction of a small set of new orthogonal (i.e., noncorrelated) variables derived from a linear combination of the original ones (*13*). Current PCA applications on food analysis are centered on some particular kind of food, such as wine (*14*−*16*), olive oil (*17*), or cheese (*18*). The second part of this study concerned the determination of the elemental and fatty acid composition of the four selected rice varieties and the use of these data to classify these samples according their geographical origin of production through PCA.

MATERIALS AND METHODS

Plant Material. The four rice cultivars employed in this study, Arborio, Baldo, Carnaroli, and Volano, were obtained from the Pavia district (Lombardia region) and Ferrara district (Emilia-Romagna region): these two cities are 311 km apart and are completely independent. Plants were harvested at physiological maturity and grain samples were taken for chemical analyses. For genetic analyses, a cleanup has been used prior to grinding. The cleanup consisted of an immersion in NaOCl at 10% for 5 min, two washes of 5 min (each one with Milli-Q water), and a treatment with denatured alcohol. The samples were milled and then dried in an oven at 110 °C for 2 h.

Only for genetic analyses, about 100 mg of kernels for each variety was milled by use of liquid nitrogen as cooling method. The analyses were carried out in triplicate.

Genetic Analysis: (A) DNA Reagents and Instrumentation. Taq polymerase, $MgCl_2$ solution, and 10× buffer were from Genenco (Milan, Italy); dNTPs and primers were obtained from M-Medical (Milan, Italy). Gels were prepared with agarose LE from Euroclone (Milan, Italy). The remaining chemicals were from Fluka (Buchs, Switzerland). Amplification was carried out on a Perkin-Elmer 9700 machine (Applied Biosystems, Monza, Italy). The amplification products were separated on 2% agarose gels, by use of a Power Pack 300 power supply equipped with a subcell agarose gel electrophoresis system (Bio-Rad, Segrate, Milan, Italy).

(B) Genomic DNA Extraction. The extraction of the genomic DNA was carried out with Gene Elute (Sigma), specific for vegetable material. DNA concentration was quantified on a Smart-Spec 3000 spectrophotometer (Bio-Rad, Segrate Milan, Italy). Purity of the DNA was evaluated by means of 260/280 nm absorbance ratio and by running the DNA on agarose gel electrophoresis with qualitative standards.

(C) DNA Amplification and Detection. The optimized PCR amplification mixture (25 μL) contained 11.5 μL of ultrapure H_2O, 2.5 μL of 10× buffer [100 mM Tris-HCl, 500 mM KCl, 500 mM $(NH_4)_2SO_4$, and 15 mM $MgCl_2$, pH 8.7, at 20 °C], 2 μL of dNTPs (dATP, dCTP, dGTP, and dTTP, 2.5 mM each), 1 μL of 10 μM primer, 2.5 μL of 25 μM $MgCl_2$, 0.5 unit of Taq polymerase, and 5 μL of genomic DNA (0.1 ng/μL). The mixture was overlaid with a drop of mineral oil to prevent evaporation and cross contamination. PCR conditions were as follows: one cycle at 94 °C for 4 min (initial denaturation), followed by 45 cycles at 94 °C for 40 s (denaturation), 32 °C for 40 s (annealing), 72 °C for 90 s (extension), and ending with a final step at 72 °C for 7 min (extension). First more than 20 random decameric primers, synthesized by M-Medical (Milan, Italy), were screened to test their ability to generate reproducible amplification products: 12 of them were selected and used for the amplification (**Table 1**).

Reaction products (12 μL) were separated by electrophoresis in 2% agarose gels (100 min at 80 V) by use of Tris−borate−EDTA (TBE) buffer (8.8 mM Tris-HCl, 8.8 mM boric acid, and 0.2 mM EDTA). The samples were added to loading buffer (1×) and then stained with 0.5 μg/μL ethidium bromide. All RAPD profiles were photographed with the Fluor-S MultImager detector, equipped with Quantity One software from Bio-Rad (Segrate, Milan, Italy).

Table 1. Details of the Random Primers (10-mer) Used in This Study

primer	sequence 5′–3′	T_m, °C
A1	CAGGCCCTTC	34.7
A2	TGCCGAGCTG	38.6
A3	AGTCAGCCAC	22.9
A4	AATCGGGCTG	35.8
A5	AGGGGTCTTG	28.2
A9	GGGTAACGCC	35.3
C1	TTCGAGCCAG	32.2
C4	GATGACCGCC	35.1
D2	GGACCCAACC	33.1
D3	GTCGCCGTCA	33.1
D5	TGAGCGGACA	33
D8	GTGTGCCCCA	36.7

Proximate Composition: (A) Moisture. The samples were carefully cleaned, finely milled, and then dried in an oven at 110 °C to constant weight. Moisture was expressed as a percentage (w/w).

(B) Total Nitrogen Compounds. Total nitrogen compounds were determined on 1 g of sample, according to the Kjeldahl method (International Dairy Federation, Determination of nitrogen content, Kjeldahl method, Norma FIL-IDF, no. 20B, Brussels, Belgium, 1993).

(C) Lipid Extraction. About 3 g of dried product was put in a thimble and introduced into the extraction unit (Velp Scientifica, Usmate, Milan, Italy). Extraction phase: 50 mL of diethyl ether (Carlo Erba, Rodano, Milan, Italy). Boiling stones: 30 min with thimble immersed in boiling solvent, 30 min of reflux washing. The extraction vessel was put in an oven at 100 °C for 30 min, then in a desiccator, and then it was weighed to obtain the total lipid content.

Chemical Analysis: (A) Atomic Absorption Spectrometry. Na, K, Mg, Zn, Ca, Fe, Cu, Ni, Mn, Cr, Pb, and Cd were determined according to described methods. The rice samples were thinly milled, accurately homogenized, and dried at 110 °C in a laboratory oven. Aliquots (2 g) of each sample were mineralized in CEM digestion vessels [poly(tetrafluoroethylene) (PTFE) model SV140, FKV] with HNO_3−H_2O_2 in a microwave digester (Millestone MLS 1200, FKV) coupled with a module for steam extraction (EM 5, FKV). Triplicate mineralizations were done on each sample. A Perkin-Elmer graphite furnace mounted on a Perkin-Elmer (model 1100B) atomic absorption spectrometer (AAS) was used with an autosampler. The spectrometer was equipped with deuterium background corrector and single-element Intensitron (Perkin-Elmer). Hollow cathode lamps were used for the measurements of copper at 324.8 nm. The accuracy of the measurement was evaluated by means of recovery tests and the precision, expressed as coefficient of variation (CV%), was in the range of 0.6−2.4. Standard solutions of each element were prepared by diluting reference standard solutions for AAS (BDH certified atomic absorption reference solutions). All reagents and chemicals were of "pro-analysis" grade and the water used was obtained by means of a Milli-Q system (Millipore, Bedford, MA). The samples were checked against reference standards and measured for their absorbance, after instrument calibration. The average of five readings of absorbance was taken in all samples.

(B) Fatty Acid Composition. The fatty acid composition was determined in duplicate by gas chromatography−mass spectrometry (GC-MS). After extraction, fats were dissolved in 2 mL of hexane and were saved in a glass tube in the freezer (−25 °C) until the day of analysis. Fatty acid methyl esters were prepared by transesterification with 1 mL of 5% of sodium hydroxide in metanol solution. The supernatant phase was transferred to a vial, capped, and then injected into the GC-MS immediately. The GC-MS equipment consisted of a Varian Saturn 2100 MS/MS ion trap mass spectrometer coupled to a Varian 3900 gas chromatograph equipped with a model 1177 split/splitless injector and a CP 8400 autosampler. The separation was achieved by a 30 m × 0.25 mm i.d., 25 μm film thickness, DB5 capillary column (Varian) supplied with helium carrier gas at 1 mL/

Table 2. Component Loading for Extraction of Three Principal Components from the Complete Data Set[a]

component	% variance	cumulative % variance
1	87.7	87.7
2	5.1	92.9
3	3.2	96.1

[a] There were 82 original variables.

min costant flow. The injector temperature was 280 °C and the oven temperature program was the following: start 100 °C for 2 min, ramp to 200 °C at 10 °C/min, and hold for 16 min. The MS acquisitions were performed by electron ionization (EI), full scan mode, scan time 1 s/scan; emission current 10 μA. Temperatures: trap, 180 °C; transfer line, 200 °C; manifold, 70 °C. Mass range, *m*/*z* 40−650.

(C) Antioxidant Activity. The antioxidant capacity was detected in triplicate by photochemiluminescence on the oil extracted as already described above. The oil (0.15 g) was dissoved in 1 mL of hexane/diethyl ether/methanol (1:1:1).

(D) Photochemiluminescence. The luminol PCL assay was carried out with the procedure described by Popov and Lewin (*19*). The rice oil was measured in the Photochem with the ACL kit (Analytikjena, Jena, Germany). Reagent 1 (solvent and dilution reagent) (2.3 mL), 200 μL of reagent 2 (buffer solution), 25 μL of reagent 3 (photosensitizer), and 10 μL of standard or sample (rice oil dissolved in hexane/diethyl ether/methanol (1:1:1) solution were mixed and measured. A light emission curve was stopped at 180 s, using inhibition as the parameter to evaluate antioxidant effect. The antioxidant capacity was then determined by use of the integral under the curve and was expressed as micromoles per gram of Trolox used as standard to obtain a calibration curve.

Statistical Analysis. In this work, principal component analysis (PCA) was performed by using Mathematica software (*20*). We constructed a set of *n* independent, orthogonal variables (i.e., linear combinations of the original variables), such that each of these new variables accounts for as much of the variance in the entire data set as possible. This was done algebraically by finding the eigenvectors and eigenvalues of the covariance matrix. For the chemical data, we performed a standardization of the variables to an equal variance of one by dividing them by their respective standard deviations (*21*). A brief description of the methods applied follows. For the first PCA, all 82 RAPD bands obtained from 12 primers were used as the data set; in the second PCA, the original data set of 82 RAPD bands was reduced. **Table 2** give a measure of how much of the variance of the data is accounted for by each of the three principal components. Only the first three principal components are listed (since the others contribute less than 4% to the total variation). In the first column of **Table 2** are listed the components extracted, and in the second column the variances of the new PCs, while the third column contains the variance values expressed as a percent of the cumulative sum of the variances extracted. PC 1 accounts for 87.7% of the variance, PC 2 for 5.1%, and PC 3 for only 3.2% of the variance. The analysis of variance (ANOVA) was carried out on all data by StatMost program.

RESULTS AND DISCUSSION

Genomic Profile. The first aim of this work was to develop a tool for identification of rice samples from the same variety grown in two different districts (Pavia and Ferrara). We chose to use RAPD markers for this purpose because in recent years they have been widely used in genetic analysis of crops, since they are technically simple to use, good at detecting polymorphisms, time−saving, and require small quantities of DNA. Reecntly, the RAPD technique has been successfully used in crop genetic analysis, to evaluate the phylogenetic diversity among 40 rice accessions from Africa (*22*) and to estimate the genetic diversity in a set of land rices in comparison to a representative sample of improved rice varieties (*23*).

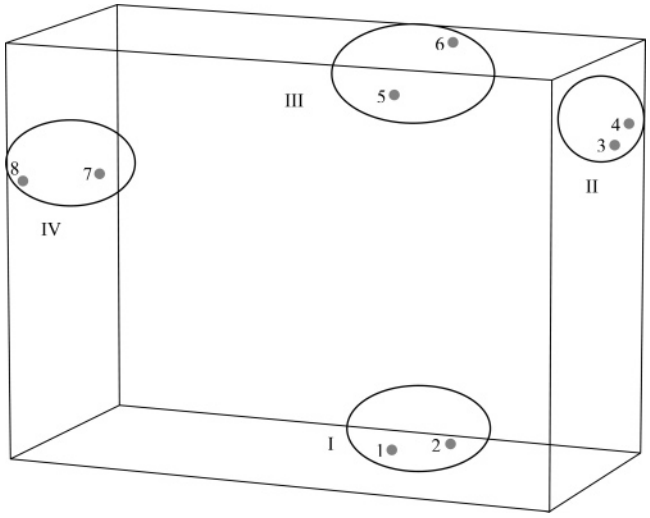

Figure 1. Principal component analysis of the 82 RAPD bands (12 random primers) on eight rice samples, where the three first principal components explain 96.1% of the total variation. Four clusters corresponding to the four rice varieties are seen (I, Arborio; II, Baldo; III, Carnaroli; IV, Volano).

All 12 primers selected for this study led to polymorphic products. The number of RAPD bands per primer varied from 3 to 9, and these primers resulted in polymorphic patterns both between and within rice varieties. A total of 82 amplification products were scored and 48 (58.5%) were polymorphic. The selection of bands for inclusion in the statistical data set was based on band reliability, clarity, signal strength, and resolution. All individuals were scored for the presence or absence of RAPD fragments, and the data were entered into a binary data matrix as discrete variables (1 for presence and 0 for absence). Principal component analysis has been successfully applied with molecular technologies (RAPD markers) to perform data reduction and classification (*24*−*26*). In this study, several PCA analyses were done in order to perform a variable reduction and to identify the most useful variables to discriminate the four rice varieties.

The plot of the first three principal components (**Figure 1**) showed the four varieties clearly separated (cluster I, Arborio rice from Ferrara and Pavia; cluster II, Baldo rice from Ferrara and Pavia; cluster III, Carnaroli rice from Ferrara and Pavia; and cluster IV, Volano rice from Ferrara and Pavia). Conversely, in this plot it is not possible to observe a discrimination of the geographic area of growth. In order to improve and simplify the genetic clustering of the rice samples, we performed a selection of the random primers used. The 12 primers were studied and the PCA results of each possible combination were considered. This approach led us to find a minimum number of eight RAPD primers, which provided a highly similar clustering of the rice varieties (data not shown). The selected RAPD primers were A1, A2, A3, A4, A5, A9, C1, C4, and F04, generating a total of 62 bands. It is interesting to note that by selecting the most useful primers, we obtained almost the same clustering effect, while the amount of variance slightly improved (cumulative variance 96.7%).

Proximate Composition. Chemical composition of plant foods can be influenced by genetic features as well as by cultivated area and fertilization methods. To characterize rice cultivars located in two different districts, we first studied proximate composition (moisture, lipids, ashes, total nitrogen compounds). Proximate compositions of Ferrara and Pavia rice are shown in **Table 3**.

Table 3. Proximate Composition[a]

rice samples	dry matter	lipids	ashes	total nitrogen compounds
Arborio Pavia	86.44	2.21	6.24	8.28
Arborio Ferrara	86.27	2.24	8.15	7.96
Baldo Pavia	86.34	1.81	6.01	9.21
Baldo Ferrara	86.51	2.06	4.32	9.36
Carnaroli Pavia	86.20	1.98	4.65	8.33
Carnaroli Ferrara	87.42	2.04	3.76	10.43
Volano Pavia	85.97	2.08	5.75	7.49
Volano Ferrara	87.71	1.96	5.32	9.08

[a] Data are expressed as percent dry matter. The average of three analyses is reported (CV% $\leq$ 2). Total nitrogen compounds = $N \times 6.25$ (conversion factor).

Table 4. Fatty Acid Composition of the Four Rice Varieties Studied[a]

rice samples	myristic, %	palmitic, %	linoleic, %	oleic, %	stearic, %	various, %
Arborio Pavia	0.31	18.04	24.52	41.28	3.53	12.31
Arborio Ferrara	0.30	18.39	23.01	39.79	4.37	14.15
Baldo Pavia	0.31	17.54	28.02	38.55	2.10	13.49
Baldo Ferrara	0.29	16.83	30.29	39.24	1.99	11.36
Carnaroli Pavia	0.38	19.70	21.53	44.61	2.44	11.34
Carnaroli Ferrara	0.27	17.34	27.36	45.02	1.45	8.56
Volano Pavia	0.28	16.48	27.59	40.92	2.85	11.87
Volano Ferrara	0.29	17.29	27.06	40.64	2.80	11.93

[a] Data are shown as mean values of triplicate data.

PCA analysis of proximate composition did not show any clustering of the rice samples according to their variety or area grown. There were no considerable differences in moisture in the four rice varieties and between the two geographical growing districts.

Lipid content was generally higher in Ferrara samples, ranging from 1.81% to 2.24%. The variety that exhibited the highest lipid and ashes content in both districts was Arborio. The variety with the lowest ashes amount was Carnaroli from Ferrara. The rice analyses proceeded with total nitrogen content determination (Kjeldahl method). Apart from Carnaroli from Ferrara, which exhibited the highest value, the comparison of the two geographic districts showed any noteworthy differences in nitrogen value.

Fatty Acid Composition. Rice lipids are commonly divided into free and bound lipids, where free lipids are solvent-extractable (ether) while bound lipids are typically extracted with hot aqueous alcohols. Free lipids, extracted with ethyl ether, represented the lipid fraction studied. Almost all the fatty acids detected were long-chain, although there were some differences in the content of individual fatty acid among varieties (see **Table 4**). The most represented fatty acids in all the samples analyzed were oleic (18:1) and linoleic (18:2) acids, followed by palmitic acid (16:0). These three fatty acids accounted for more than 80% of the total free lipids fraction in all varieties. Minor fatty acids included myristic and stearic acids. These results are consistent with those reported in literature (*27*, *28*).

Some studies reported the existence of a relationship between fatty acid composition and the mean temperature at ripening stages, which is almost completely determined by latitude (*29*, *10*). In these studies, myristic, stearic, and oleic acids were correlated negatively with latitude, while linoleic and linolenic acids were correlated positively with latitude. Since the two selected districts (Ferrara and Pavia) are almost located at the same latitude (thus they have the same temperature), we could suppose no temperature influence among the fatty acid values of the samples.

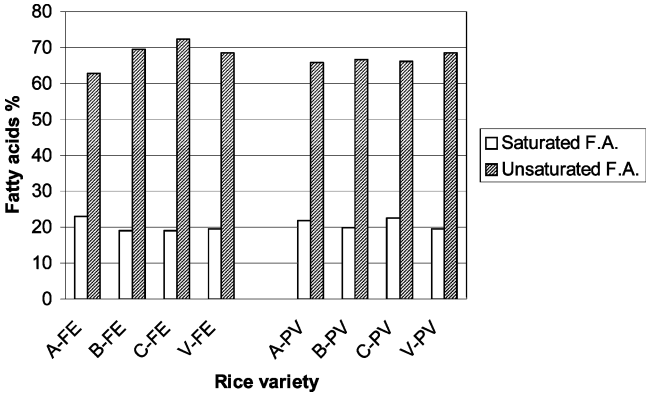

Figure 2. Total saturated and total unsaturated fatty acids in different rice varieties (A, Arborio; B, Baldo; C, Carnaroli; V, Volano; FE, Ferrara; PV, Pavia).

Distribution of fatty acids did not show any important difference between the two cultivated districts. **Table 4** shows there are not varietal differences in palmitic acid content between Ferrara and Pavia districts. The comparison of the four varieties exhibited similar differences in linoleic and oleic acid content. Linoleic acid percentages were included between 21% and 30%; Baldo variety presented the highest ratio of linoleic acid in both Ferrara and Pavia samples. It seemed interesting to focus attention on oleic acid content and compare the data of the different varieties and districts. The oleic acid amount showed a similar trend between the two cultivation districts, and in both cases, Carnaroli variety held the greater oleic acid content, while the Baldo variety showed the smallest content. These differences in individual fatty acids are reflected in the total saturated fatty acids, total unsaturated fatty acids (**Figure 2**), and total fatty acid contents.

The total unsaturated fatty acid content in rice cultivated in the Ferrara district was slightly higher than that from the Pavia district. Carnaroli variety grown in the Ferrara district had marginally higher total unsaturated acid content and lower total saturated fatty acid ratio. Principal component analysis was adopted for fatty acid data analysis. **Table 5** give a measure of how much of the variance of the data is accounted for by each of the three principal components.

Although the results of this analysis indicated a lower amount of variance, nevertheless the PCA plot obtained (see **Figure 3**) was similar to the previous one reported on genetic data, revealing four distinct clusters corresponding to the four rice varieties studied.

The graphical representation indicated that fatty acid composition was greatly affected by cultivar. In fact, fatty acid profile seemed to be regulated by genetic factors much more than by cultivation district. However, in the Arborio variety, the fatty acid composition was affected by cultivar as well as by cultivated district, even though the contribution ratio of cultivar was higher than the area of growth.

Total Antioxidant Activity. To estimate the quality of vegetal oils is more important to study the antioxidant capacity than the fatty acid composition. Antioxidants are substances that, when present or added to food products (especially lipids and lipid-containing foods), can increase the shelf life by retarding lipid peroxidation, which is one of the major causes of food deterioration during processing and storage (*30*). The PCL method, based on the photoinduced autoxidation inhibition of luminol by antioxidants mediated from the radical superoxide, is easy and rapid to perform. Luminol works as both photosensitizer and oxygen radical detection reagent and is suitable

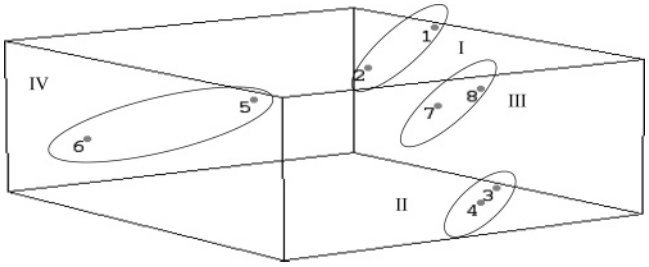

Figure 3. Principal component analysis of fatty acids measured on the rice samples studied, where the three first principal components explain 89.4% of the total variation. Four clusters corresponding to the four rice varieties are seen (I, Arborio; II, Baldo; III, Carnaroli; IV, Volano).

Table 5. Component Loading for Extraction of Three Principal Components from the Fatty Acid Data Set

component	% variance	cumulative % variance
1	49.9	49.9
2	24.3	74.2
3	15.2	89.4

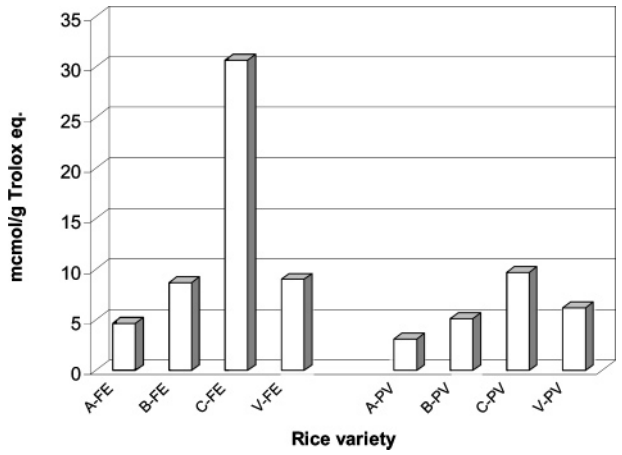

Figure 4. Antioxidant activity (photochemiluminescence, PCL, indicated as micromoles/gram of Trolox equivalents) of the eight rice samples from the four varieties (A, Arborio; B, Baldo; C, Carnaroli; V, Volano; FE, Ferrara; PV, Pavia).

to measure, in the nanomolar range, the radical scavenging properties of single antioxidants as well as more complex systems (*31*). The PCL assay, conducted following the ACL protocol, is particularly suitable for the determination of the radical-scavenging activity of rice-lipid-soluble antioxidants. **Figure 4** shows the antioxidant activity of the four different rice varieties grown in two separated regions.

All the rice samples tested in this study showed important antioxidant activity. It has to be noted that rice varieties grown in the Ferrara district presented a total antioxidant activity higher than those grown in the Pavia district. In particular, the results indicated that the highest antioxidant concentration was in Carnaroli rice from the Ferrara district. These results suggest that the rice antioxidant composition not only depends on the nutrient availability of the soil but also is probably related to genetic character.

Mineral Content. Fingerprinting techniques based on mineral composition and multivariate statistical analysis can be used for identification and classification of a specific agricultural product type according to its geographical origin (*32*). This is based on the assumption that the elemental composition of an agricultural product will reflect the composition of the provenance soil. Studies on wine (*33*−*35*) have shown the ability of this method to predict its geographic origin, but information about other products is still lacking. Since aqueous media used to irrigate rice fields can affect the distribution of heavy metals and other positive ions in soils, the determination of heavy metals (Cd, Cr, Pb, and Ni) and fundamental minerals such as K, Cu, Fe, Mn, Ca, Mg, Zn, and Na was carried out with a Perkin-Elmer atomic absorption spectrometer (AAS) as described in the Materials and Methods section. The metals Cd, Cr, Pb, and Ni, which are environmental pollutants and are known to interfere with nutrient uptake and with nutrient distribution into the plant (*36*), were close to the limit of detection (0.01 ppm for all metals) and therefore were excluded from the data set. The concentrations of metals in parts per million (ppm) are reported in **Table 6**.

Table 6. Metal Content of the Four Milled Rice Varieties Studied[a]

rice samples	K, ppm	Cu, ppm	Fe, ppm	Mn, ppm	Ca, ppm	Mg, ppm	Zn, ppm	Na, ppm
Arborio Pavia	4208.9	5.78	31.69	105.9	418.6	920.2	35.97	25
Arborio Ferrara	3480.5	3.37	61.69	72.68	317.5	834.3	31.17	59
Baldo Pavia	3112.1	3.03	32.03	86.03	272.5	794.9	24.11	71
Baldo Ferrara	3040.2	3.15	37.26	45.03	294.6	868.6	28.61	84
Carnaroli Pavia	3671.4	2.49	34.55	149.9	280.3	803.7	27.84	28
Carnaroli Ferrara	3257.1	1.86	36.02	41.54	343	868.2	28.72	109
Volano Pavia	3494.6	3.22	27.27	55.25	311.6	891.3	25.23	29
Volano Ferrara	3214.9	3.71	31.16	54.61	298.9	868.0	29.78	91

[a] Data are shown as mean values of triplicate data.

The data reported in **Table 6** are consistent with literature values for rice (*27*). It was interesting to compare the Na concentration in the two cultivated districts. The data from the analysis revealed that the concentration of Na in Ferrara rice was significantly higher than the same varieties cultivated in the Pavia district ($p < 0.02$). This result was not surprising, considering the location near the sea of the Ferrara area, where rice was cultivated on an alluvial soil. Only the Baldo variety grown in the Pavia district presented a sodium ion amount comparable to Ferrara ones. Moreover, it has to be said that the production disciplinary recommends crop rotation. Crop rotation is the practice of growing a series of dissimilar type of crops in the same space in sequential seasons, which as a result improve soil structure and fertility. To perform a rational and statistical analysis of all elements, principal component analysis was used. **Table 7** gives a measure of how much of the variance of the metal data set is accounted for by each of the three principal components.

The analysis of all samples led to the identification of two clusters, located in different areas of the space, corresponding to the two cultivated districts. The plot obtained with this PCA analysis was highly satisfactory (**Figure 5**) since we obtained discrimination of the rice samples based on the geographical origin.

We investigated the PCA loading to see which metal had the greatest influence on each component. The first principal component accounted for the highest proportion of total variation and was mostly loaded on K. The second principal component was heavily loaded on Mn, while Fe dominated the third principal component.

In conclusion, the excellent PCA classification of the rice samples according to their varieties, obtained in this work with RAPD data, gave proof of the presence of a genetic distance among the Italian rice varieties studied. Moreover, the fatty acid profile and the total antioxidant capacity appeared to be regulated much more by genetic factors than by the different

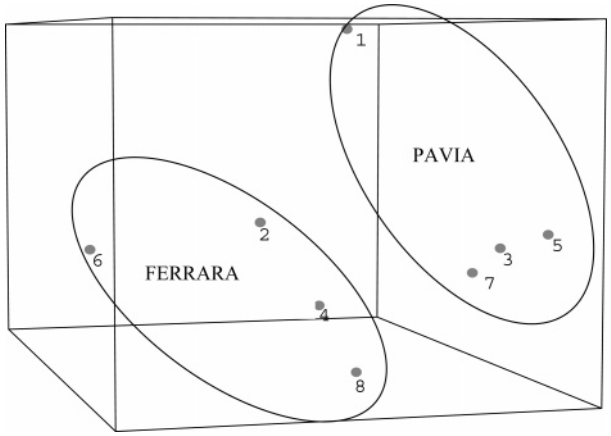

Figure 5. Principal component analysis of metal data measured on eight rice samples, where the three first principal components explain 83.2% of the total variation.

Table 7. Component Loading for Extraction of Three Principal Components from the Metal Data Set

component	% variance	cumulative % variance
1	38.6	38.6
2	26.4	65.0
3	18.2	83.2

growing area, showing the importance of the genetic characters on compositional parameters. Instead, the PCA analysis of mineral composition recognized the cultivated area of rice samples, suggesting a relationship between mineral composition of rice and cultivated area and revealing the great influence of the soil on crop features.

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