

Characterization of Italian Durum Wheat Semolina by Means of Chemical Analytical and Spectroscopic Determinations

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ABSTRACT

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Proton high-resolution magic angle spinning nuclear magnetic resonance (¹H HR-MAS NMR) has been applied for the analysis of two cultivars of durum wheat produced in different Italian geographical areas. Furthermore, on the same samples, isotopic ratios were measured by mass spectrometry (IRMS). The application of chemometrics to these results permitted the discrimination of semolina by cultivar and geographical origin. A similar approach has been applied to the results obtained from

chemical analyses. The comparison showed that NMR spectroscopy can provide a faster method for the detection of differences among the durum wheat semolina according to geographical and varietal origin. Furthermore, IRMS data are suitable to provide information about the geographical origin of samples. This present investigation is part of an extensive research project to find a scientific method capable of classifying wheat foods for the assignment of a “denomination of origin” trademark.

Consumers are showing an increasing interest in the geographical or varietal origin of foodstuffs they buy; this information is regarded as an additional warranty of their quality and authenticity. This requirement is expanding to various products, including pasta and durum wheat breads. In fact, in some Italian regions, the promotion of the Denomination of Protected Origin (DOP) certifying durum wheat bread authenticity is requested by producers. It could be important to facilitate DOP for production of typical bread, integrated to the local production of durum wheat and to the traditional working technique. For this purpose, it is necessary to set objective tests to verify the authenticity of geographical origin of the durum wheat utilized. Therefore, analytical monitoring is necessary on durum wheat samples produced in different geographical areas to find authenticity markers that could be employed to characterize typical products.

Chemometric methodologies applied to food composition sometimes successfully proved that the geographical and varietal origin of the samples can be related to chemical compounds. In recent years, several attempts have been made to differentiate the origin of wines coming from different regions by means of multivariate statistical analysis of various classes of compounds (Etiévant et al 1988; Arvanitoyannis et al 1999; Sivertsen et al 1999). Analytical determinations have been used to differentiate olive oils (Armanino et al 1989) and honeys (Latorre et al 1999) according to geographical origins. Armanino et al (1996) found differences in the origin and cultivar of wheat samples by chemical analysis. However, these methods have several limitations; they are time-consuming and need too many analytical methods to obtain information about the presence of different classes of compounds. On the other hand, nuclear magnetic resonance (NMR) spectroscopy provides the possibility of obtaining information on the presence of many compounds in a single spectrum. For this reason, the interest for the applications of NMR to food science is growing, as confirmed by the large number of publications in the last few years (Martin et al 1995; Belton et al 1996; Vlahov 1999) and by the International

Conference on Application of Magnetic Resonance in Food Science (Belton et al 1999). Durum wheat flours have been analyzed by means of proton high-resolution magic angle spinning nuclear magnetic resonance (¹H HR-MAS NMR), which revealed that spectral data contain useful information for sample discrimination (Sacco et al 1998). The remarkable advantage of this technique is the possibility of directly analyzing solid food products, avoiding the drawbacks connected with the pretreatment of the samples for analysis such as the possible modification of the sample composition due to extraction processes. Another source of information for the determination of geographical origin is the analysis of stable isotope ratios. Stable isotopes are now used more and more to verify the authenticity of foodstuffs as well as origin (Danho et al 1992; Martin et al 1995).

The goal of this work was to characterize the geographical and varietal origin of durum wheat samples coming from different Italian locations using analytical determinations, ¹H HR-MAS NMR, and IRMS. Furthermore, we compared results from all analytical determinations with those from spectroscopic analyses to evaluate the discriminating capability of the two methods.

MATERIALS AND METHODS

Field Materials

Two durum wheat cultivars, Simeto and Colosseo, were grown during 1998-99 in experimental plots with three replicates at 13 different locations in Italy. These have been distinguished according to geographical position in north, central, and south Italy. Samples (3 kg) were obtained from a blend of the three replicates from each location and from each cultivar for analyses.

Semolina Sample Preparation

After cleaning, each grain sample was conditioned with tap water overnight to 16.5% (dmb) of final humidity. The grain samples (3 kg) were milled on a Buhler MLU 202 durum wheat mill with six breaking and six sizing passages, equipped with a semolina purifier. The semolina extraction rate was ≈59–65%.

Chemical Characterization

The protein content (N × 5.7) was determined by Kjeldhal analysis in duplicate (Approved Method 46-13, AACC 2000). Moisture (%) was determined in oven drying for 1 hr at 130°C on 5 g of sample in duplicate (Approved Method 44-19). Gluten content (%) was determined on 10 g of semolina by manual washing with a 2% NaCl solution buffered at pH 6.8 (Approved Method 38-10). Ash content (%) was determined in duplicate on 5 g of sample by dry combustion for 16 hr at 580°C (Approved Method 08-01). Semolina yellow index was measured by chromameter (CR-200, Minolta,

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Osaka, Japan) equipped with a pulsed xenon lamp and measure surface 8 mm in diameter.

Fatty Acids Analyses

Fatty acids were determined by gas chromatography in duplicate. They were extracted from 1 g of semolina with 5 mL of diethyl-ether and stirred for 5 hr. Extract (300 μ L) were dried under N₂ stream, methylated with 20 μ L of methyl-acetate and 150 μ L of NaOH 2N in methanol, and stirred in vortex for 30 sec at room temperature. Afterward, 5 mL of hexane were added to extract methyl-ester of fatty acids that were separated by GC (model HRGG Mega Series 5300 Carlo Erba Instruments, Rodano, Mi Italy) equipped with a capillary column (Supelcowax, 30 m \times 0.25 mm ϵ 0.25 μ m), and a detector flame ionization detector (FID) (Supelco Park-Bellefonte, PA). Oven temperature was programmed from 100°C for 10 min to 240°C at a rate of 20°C/min.

α and β Carotene Determination

α and β Carotene were determined by HPLC in duplicate. Semolina (1 g) was blended with a 3:4, v/v, mixture of 5 ml of hexane and ethanol for 5 hr in the dark at room temperature. The semolina extract was dried with sodium sulphate anhydrous. The determination was made by HPLC (LKB, Bromma) equipped with a pump (model 2150), a detector UV/VIS (model 2141), and a recorder (model 2221). The separation was performed with a Supelcosil LC-SI (250 \times 4.6 min, 5 μ m) column. The mobile phase was a 85:15, v/v, blend of hexane and ethyl-alcohol, and the flow was 0.8 mL/min with λ 454 nm. The analysis was conducted at 25°C.

α and γ Tocopherol Determination

α and γ Tocopherols were determined by HPLC in duplicate. Semolina (1 g) was mixed with 5 mL of diethyl-ether and stirred for 5 hr in the dark at room temperature. The determination was made by HPLC (LKB, Bromma) equipped with a pump (model 2150), a detector UV/VIS (model 2141), and a recorder (model 2221). The separation was performed with a Supelcosil LC-SI (250 \times 4.6 min, 5 μ m) column. The mobile phase was a (99.2:0.8, v/v) blend of hexane and 2-propanol, and the flow was 0.8 mL/min with λ 290 nm. The analysis was conducted at 25°C.

TABLE I
Loadings of Original Set of Variables Associated with Principal Components from Chemical Characterization

Variables	PC1	PC2	PC3	PC4
Gluten	-0.136	0.855	-0.245	0.225
Yellow index	-0.734	-0.086	-0.481	0.035
Ash content	0.014	0.277	-0.411	-0.724
Protein content	-0.377	0.808	-0.316	0.041
α -carotene	-0.451	-0.263	-0.491	0.437
β -carotene	-0.309	-0.528	-0.275	0.533
α -tocopherol	0.667	-0.119	-0.371	-0.045
γ -tocopherol	-0.801	0.006	-0.070	-0.246
Palmitic acid	0.625	0.405	0.041	0.431
Stearic acid	0.857	0.199	-0.073	0.083
Oleic acid	0.795	0.213	-0.202	0.232
Linoleic acid	0.785	-0.304	-0.102	-0.174
Linolenic acid	0.540	-0.366	-0.626	-0.210

TABLE II
¹H Chemical Shift and Signal Assignment from HR-MAS Spectrum for Statistical Analysis

ppm	Group	Multiplicity	Compound
6.46	Not determined	Broad Singlet	Unknown compound
5.35	Anomeric proton (H1)	Doublet	Polysaccharides
5.26	Anomeric proton (H1)	Doublet	Polysaccharides
4.59	Anomeric proton (H1)	Doublet	Polysaccharides
3.41	H2	Triplet	Polysaccharides
3.13	Not determined	Broad Singlet	Unknown compound
1.98	-CH ₂ -CH-CH-	Broad Singlet	Triacylglycerols

NMR Determinations

NMR measurements on semisolid samples were performed on an Bruker Avance 600 MHz spectrometer using an HR-MAS probe-head suitable for 4-mm rotors. Samples for ¹H HR-MAS measurements were prepared as follows: 33 mg of D₂O were mixed in the rotors using a syringe needle with 40 mg of semolina to obtain homogeneous samples with similar degrees of swelling. The NMR spectra for each sample were obtained using a presaturation sequence for water suppression. The experimental conditions were 32,768 data points, 512 scans, spectral width of 7,184 Hz (\approx 12 ppm), and 2.28 sec of acquisition time.

Isotopic Ratio Determinations

Isotopic contents (¹³C/¹²C, ¹⁸O/¹⁶O, ¹⁵N/¹⁴N) were expressed as isotopic deviations δ defined as: $\delta (^{i}/_{j}) = (R_s - R_{ref})/R_{ref} \times 1,000$ where R_s is the isotopic ratio measured for the sample and R_{ref} is the isotopic ratio of the reference. The results obtained by IRMS were expressed in $\delta (^{i}/_{j})$ versus the PDB (Pee Dee Belemnite), V-SMOW (Vienna Standard Mean Ocean Water) international standards for carbon and oxygen and versus atmospheric N₂ for nitrogen.

The mass spectrometric determinations of the C, O, and N content were made by online analysis using an elemental analyzer (NA 1108 Fisons Instruments, Rodano, Italy) connected to an isotopic mass spectrometer (Delta S Finnigan MAT, Bremen, Germany).

Samples placed in tin containers were submitted to a flash combustion in a stream of helium enriched with pure oxygen. A conventional procedure was used for carbon and nitrogen determinations (Bréas et al 1994).

Measurements of ¹⁸O/¹⁶O ratio were obtained by modifying an elemental analyzer device for pyrolysis of the sample. Afterward, ¹⁸O/¹⁶O was determined with the isotope ratio mass spectrometer (Bréas et al 1998) in the CO resulting from the pyrolysis.

Multivariate Statistical Analyses

Chemometrics were performed using Statistica 5.0 (StatSoft, Tulsa, OK). Results of analytical and spectroscopic determinations were collected in two data sets. Applied chemometric methods were principal component analysis (PCA) and discriminant analysis (DA). PCA describes individuals initially present in an m -dimensional space of the variables, in a space of two or three dimensions. These new axes (principal components [PC]) are linear combinations of the original variables calculated to maximize the dispersion of individuals. Coefficients by which the original variables must be multiplied to obtain the PC are called loadings. The numerical value of a loading of a given variable on a PC indicates how much the variable has in common with that component (Massart et al 1988).

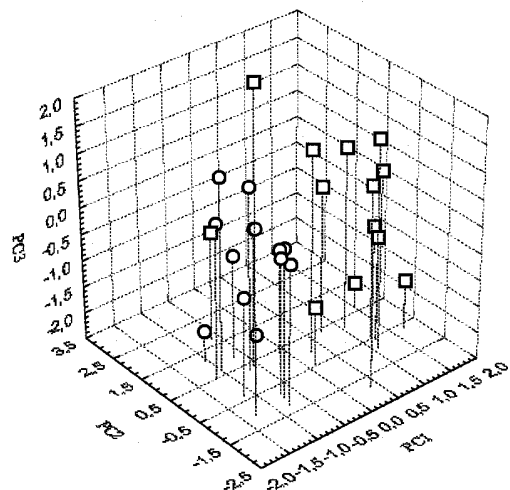


Fig. 1. Scatter plot of sample scores from first two principal components (PC1 and PC2) using analytical data. Simeto (○); Colosseo (□).

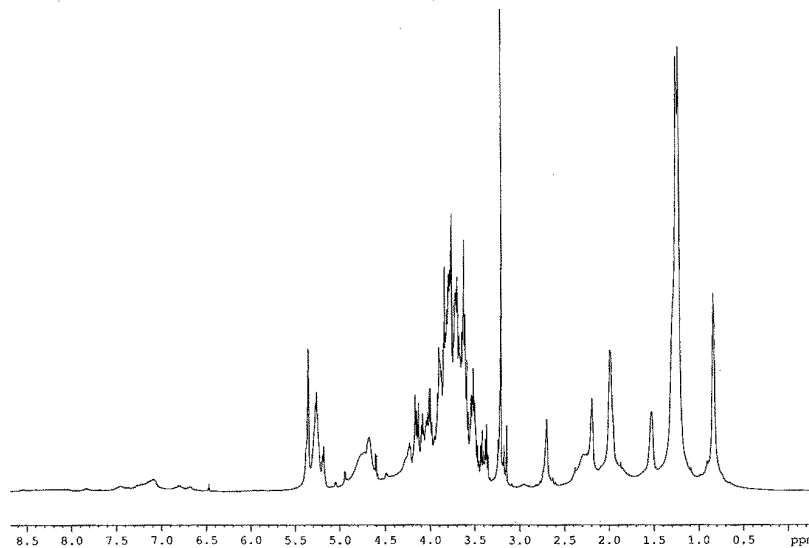


Fig. 2. ^1H HR-MAS NMR 600 MHz spectrum of durum wheat flour.

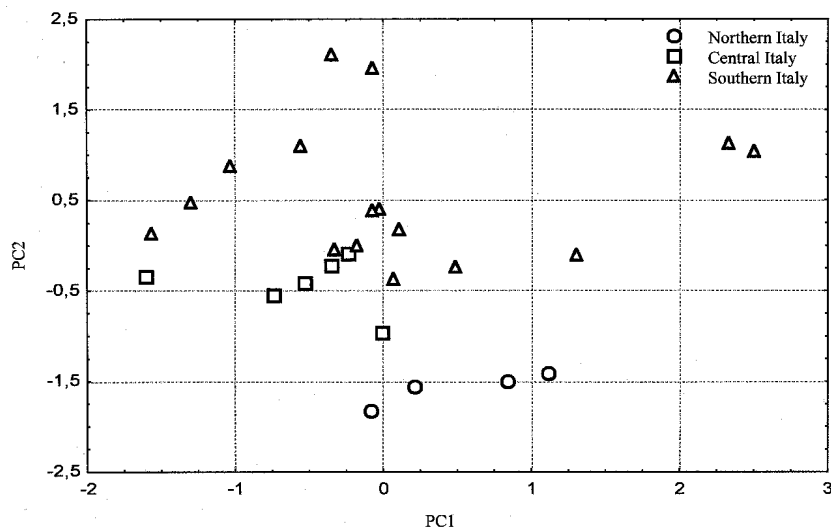


Fig. 3. Scatter plot of sample scores from PC1 and PC3 using spectroscopic data.

The aim of DA is to obtain discriminant axes which are linear combinations of the original variables, calculated to maximize distances between predefined groups. Its purpose is to calculate class models and boundaries, giving a rule of classification based on a set of known objects (training set). This rule can be applied to define the classification of unknown objects (test set).

RESULTS AND DISCUSSION

Analytical Determinations

Chemical analytical parameters shown in Table I were used to discriminate between the durum wheat samples. The statistical analysis was applied to the data matrix of 26 rows (samples) and 13 columns (analytical variables).

The calculations extracted four PC (eigenvalues > 1) explaining 77% of the sample variability. The loading values of the variables associated with each PC are reported in Table I. The examination of the loadings associated with each variable for the first four PC singles out the variables most influencing these PC. In the present case, the PC1 loadings are high and positive for yellow index and γ -tocopherol, and high and negative for stearic, oleic, and linoleic acids. Gluten and protein content have high loadings on PC2; linolenic acid, α -carotene, and yellow index have high weight on PC3; and

ash content contributes most to PC4. A four-dimensional display presents practical difficulties, so the first three PC were used for the score plot in Fig. 1. The clustering of the samples according to cultivar on the PC1 shows the discriminating capability of the analytical parameters in this context. This means that variables with higher loadings on PC1 hold information useful to discriminate between cultivars.

A further approach was used for each of the two cultivars to obtain information on the origin of the samples. No correlation was found from PCA between analytical data and geographical origin of the samples. The DA approach, as expected, gave a better discrimination of the samples but was marked by a 54% prediction ability.

Spectroscopic Data

A typical ^1H HR-MAS 600 MHz spectrum of wheat is reported in Fig. 2. More than 80 peaks can be distinguished resulting mainly from lipids and polysaccharides. Assignment was done by Sacco et al (1998). ^1H HR-MAS spectra were obtained for 26 samples. Heights of signals showing neither overlapping nor correlation with other signals were evaluated and normalized to one signal present in all the spectra. The chemical shifts of the selected resonances for the statistical analysis are reported in Table II. Isotopic parameters

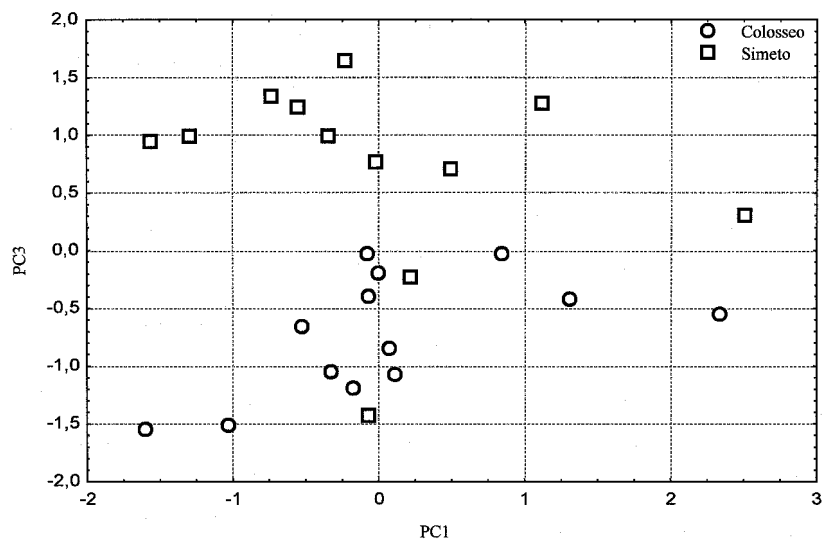


Fig. 4. Scatter plot of samples scores from PC1 and PC3 using spectroscopic data.

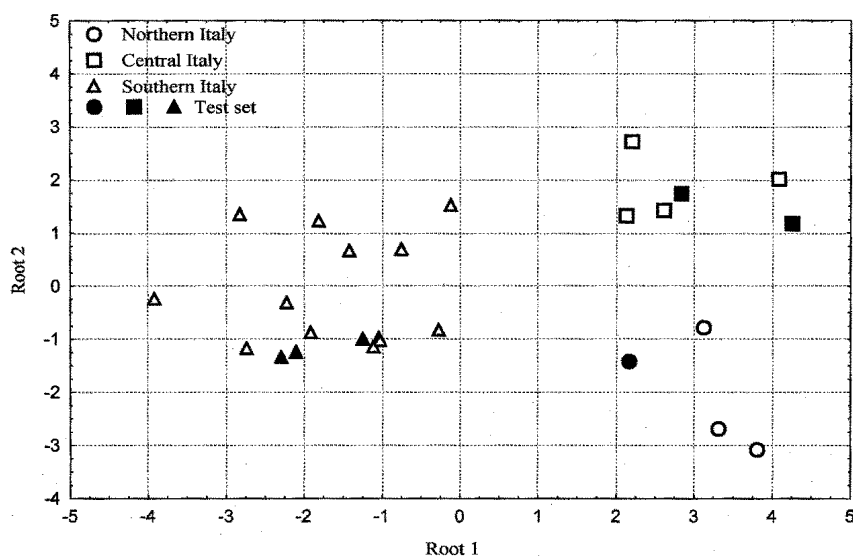


Fig. 5. Plot of three geographical origins for wheat flour samples on first two discriminant functions for spectroscopic data.

obtained from IRMS analysis have been included in this data set. IRMS results on the same samples analyzed by NMR will be reported separately.

Three factors were extracted from PCA explaining the 78% of the total variance. Figures 3 and 4, respectively, show the score plot of the samples on the first two PC and the score plot on PC1 and PC3. Loadings are reported in Table III. At first glance, samples seem to cluster in three groups on PC2, corresponding to different geographical origins (Fig. 3).

The sample cultivars were discriminated on PC3 (Fig. 4). It must be stressed that PCA is an unsupervised method (it does not know the origin or the cultivar of the samples). Therefore, it is noteworthy that a separation of the samples was achieved in the score plots, even if it is not satisfactory. Loading analysis showed that HR-MAS variables were correlated with PC1 and PC3, while PC2 was highly correlated to isotopic parameters. These results indicate the relevant influence of the sample cultivars on the concentration of the compounds revealed by HR-MAS and of the geographical origin on the isotopic parameters.

After the PCA treatment, DA was applied to the same parameters to classify the durum wheat samples into separate groups according to geographical origin. Samples were divided into a training set (three from the north, four from central, 13 from the south) to

develop a discriminant model and a validation set (one from the north, two from central, and three from the south) on which the model could be tested. The prediction ability of the model for the training set was 100%. The same model applied to the validation set gave 100% success (Fig. 5). A correct classification was also obtained applying DA for the discrimination of the samples according to cultivar.

CONCLUSIONS

The application of multivariate statistical methods to analytical and spectroscopic determinations on Italian durum wheat samples has permitted a discrimination of varietal and geographical origin. In particular, the chemical variables are useful in discriminating among cultivars, while NMR and IRMS give important information about the geographical and varietal origin of the samples. Isotopic data contributed most in the discrimination of the samples according to the geographical origin. Therefore, it seems reasonable to stress the importance of this versatile technique in the analysis of food products. In conclusion, the results obtained from the set of chemical variables give less information compared with the set of spectroscopic determinations. This is an important result because spectroscopic techniques seem the most advantageous. Samples do not

TABLE III
Loadings of Original Set of Variables Associated
with First Three Principal Components Obtained
from Spectroscopic Data (¹H HR-MAS, IRMS)

Variables	PC1	PC2	PC3
1.98 ppm	-0.606	0.049	-0.653
3.13 ppm	-0.650	0.187	0.670
3.41 ppm	-0.807	0.077	-0.262
4.59 ppm	-0.758	-0.089	-0.219
5.26 ppm	-0.819	0.315	0.265
5.35 ppm	-0.922	0.011	-0.267
6.46 ppm	-0.622	0.033	0.345
δ ¹³ C	0.363	0.800	-0.228
δ ¹⁸ O	-0.355	0.782	0.065
δ ¹⁵ N	0.606	0.695	-0.075

require any pretreatment and measurement time is usually very short. A development of this study could consist in the comparison of the characteristics of samples coming from Italy with those of samples coming from other countries (Canada, Turkey, Russia) and in the extension of the study to processed products like bread and pasta.

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