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Characterisation of different typical Italian breads by means of traditional, spectroscopic and image analyses

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Abstract

In this paper a complete characterisation of four typical durum wheat breads produced in Italy was performed, from the starting semolinas to the final product, also considering the intermediate dough. An evaluation of the quality of durum wheat re-milled semolinas was carried out by means of routine investigations, together with nuclear magnetic resonance (NMR) and isotope ratio mass spectrometry (IRMS) analyses, that were also applied to dough ready for baking and bread to monitor the variations that occur during processing. The experimental data obtained from routine and spectroscopic determinations were investigated using multivariate statistical analysis to evaluate the possibility of differentiating flours, doughs and breads according to their geographical origin. Computerised image analysis was applied to quantify the crumb grain features of different bread types, and to try to characterise each bread type through a set of crumb morphological and colour parameters.

Keywords: Bread; Quality; Geographic origin; Multivariate statistical analysis; Nuclear magnetic resonance; Isotope ratios; Image analysis

1. Introduction

Historically, the production of durum wheat has been an important industry for some region of Italy. Wheat production and sales are subject to strong competition in international markets. In addition, traditional wheat bread and pasta are gaining an increasing interest from consumers. It is, therefore, important to facilitate and to boost the initiative of access to PDO (protected designation of origin) for production of typical bread and pasta, integrated with the local production of durum wheat. Genzano and Altamura breads obtained, respectively, the protected geographical indication (PGI) and the PDO and Matera bread is going to attain the PGI from the European Community. The provenance of wheat used to produce the semolina must be indicated on these products in order to guarantee their geographical origin.

In this regard, high resolution NMR spectroscopy was successfully applied to liquid food products (Belton et al., 1997; Mannina, Patumi, Proietti, Bassi, & Segre, 2001; Sacco et al., 2000) whereas, in the case of solid matrices, the "proton high resolution magic angle spinning" (^{1}H HR-MAS) NMR has been used (Garrod et al., 1999; Shintu, Ziarelli, & Caldarelli, 2004). ^{1}H HR-MAS was employed in the investigation regarding the characterisation

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of durum wheat flours coming from different areas of Southern Italy (Sacco et al., 1998); in the analysis of wheat products to study the kinetic release of oligosaccharides due to hydrolases in the amylolytic starch degradation of flours (Amato et al., 2004) and to investigate the effect of hydration on the major constituents of gluten, ω-gliadins and glutenins (Belton, Gil, Grant, Alberti, & Tatham, 1998; Gil et al., 1997). A preliminary study conducted on two cultivars of durum wheat flours coming from different production areas situated in Italy showed that ^{1}H HR-MAS NMR spectroscopy provides a fast and reproducible method for the differentiation of the samples according to their geographical origin and cultivar (Brescia et al., 2002a).

Recently, it has been shown that IRMS may provide unique information about the geographical origin of food samples, because the isotopic compositions are governed by the geo-climatic conditions under which plants grow. This technique has been applied to differentiate food products like wines (Bréas, Reniero, & Serrini, 1994), juices (Gonzalez et al., 1998), and durum wheat flours (Brescia et al., 2002b).

Finally, computerised image analysis has been established as a very effective tool to quantify the appearance of food products (Brosnan & Sun, 2002; Cheng-Jin & Da-Wen, 2004), and there are several examples from the bread crumb grain area (Bertrand, Le Guerneve, Marion, Devaux, & Robert, 1992; Crowley, Grau, & Arendt, 2000; Peri, Amodio, Romaniello, & Colelli, 2003; Sapirstein, Roller, & Bushuk, 1994; Sapirstein, 1999; Zghal, Scanlon, & Sapirstein, 1999). The internal structure of yeast-leavened bread when sliced, commonly referred to as crumb grain, can be described as a complex of interconnected cells in a heat set glutinous-stark matrix (Kamman, 1970). Crumb grain (or crumb visual texture) is an important element of the bread quality, and reflects flour characteristics, dough formulation, and processing (Scanlon & Zghal, 2001).

The aim of this paper was to evaluate the possibility of differentiating some types of premium Italian durum wheat bread, including their intermediate and raw materials, according to their geographical origin using, besides the classical routine techniques, HR-MAS NMR, IRMS and computerised image analysis. The resulting data were treated by means of multivariate statistical analysis.

2. Materials and methods

2.1. Samples

Four towns, with an established bread-making tradition in Italy, were considered: Altamura (Apulia), Laterza (Apulia), Matera (Basilicata) and Bonorva (Sardinia). The bakers in two of these towns have already requested a protected designation of origin (PDO) or protected geographical indication (PGI) for their product (Italian Official Bulletin, 2004; Official Journal of the European Communities, 2003). A total number of 16 loaves of bread, four per town, were collected at local bread-makers, together with the durum wheat flours used in their production. The preparation of all the breads examined was completed with traditional baking methods, which involved a prolonged sponge-dough procedure and the use of sourdough starter for leavening.

2.2. Routine classical analyses of durum wheat flours

Ash content was determined according to the AACC approved method 08-01 (AACC, 2000). Gluten content and gluten index were determined by means of Glutomatic (Perten Instruments, Hamburg, Germany) as in AACC method 38-11 (AACC, 2000). Alveograph analysis was adapted to durum wheat as reported by D'Egidio, Mariani, Nardi, Novaro, and Cubadda (1990) and performed by Chopin alveograph (Chopin, Villeneuve La Garenne, France). Yellow index was determined by the Chromameter CR300 (Minolta, Osaka, Japan) tristimulus colorimeter, considering the b^* value. The content of yellow pigments was determined as described in Fares, Platani, Tamma, and Leccese (1991). All the analyses were carried out in duplicate.

2.3. Spectroscopic analyses of durum wheat flours, dough and bread

Bread and dough samples were freeze-dried and pulverized for spectroscopic investigations, while semolina samples were directly analyzed. Isotopic contents were determined by using an isotopic mass spectrometer Finnigan MAT delta S mass (Thermo Finnigan, San Jose, USA). A Carlo Erba 1110 elemental analyzer (Carlo Erba, Milano Italy) was connected to the spectrometer to convert carbon and nitrogen to CO_2 and N_2, respectively. Isotopic ratios were expressed as isotopic deviations (δ^{13}C and δ^{15}N) defined as $\delta = (R_s - R_{ref})/R_{ref} \cdot 1000$, where R_s is the isotopic ratio measured for the sample and R_{ref} is the isotopic ratio of the reference. The latter is the PDB (Pee Dee Belemnite) for carbon and atmospheric N_2 for nitrogen.

Samples for ^{1}H HR-MAS measurements were prepared by mixing 40 mg of semolina, dough and bread with 33 mg of D_2O. Spectra were acquired at 300 K on an AVANCE 500 MHz (Bruker Analytik GmbH, Rheinstetten, Germany) spectrometer equipped with a high-resolution HR-MAS probehead suitable for 4 mm rotors. The following experimental conditions were applied: spectral width = 8400 Hz (∼14 ppm); time domain = 32 K points; number of transients = 128; mixing time = 80 ms. Spectra were acquired by using the mono-dimensional version of NOESY sequence, with water signal suppression by presaturation and were processed by applying a 0.3 Hz line broadening factor.

2.4. Chemometric methods

The statistical analysis of data was performed by using the Statistica package (Statsoft Inc., Tulsa, Oklahoma, USA). The results of determinations on flour, dough and bread samples obtained with routine, NMR and IRMS analyses were collected in separate data sets.

Multivariate statistical analysis was applied on all the data sets. In particular, two approaches were investigated: principal component analysis (PCA) to explore the distribution of the samples, and discriminant analysis (DA) to evaluate flour, dough and bread samples from various producing areas could be mathematically distinguished on the basis of their geographical origin.

2.5. Image acquisition, segmentation and measurement

For image analysis leavened breads produced in Altamura, Laterza, and Matera were selected. Each loaf of bread was sliced in regular slices of 25 mm thickness using a slice regulator and bread knife. The four inner slices of each loaf of bread were considered for crumb grain features measurements.

The images of the two sides of each slice were separately captured using a flatbed scanner (HP G95, Hewlett–Packard, USA) and its supporting software. The images were scanned in the RGB (24 bit) standard format. The spatial resolution was approximately (70 μm^2/pixel). A total of 96 images (three bread types × four loaves × four slices × two sides) were acquired. A single 70 × 70 mm square field of view (FOV) of the crumb area was considered for each image. This FOV was saved in the hard disk of a PC in tagged image file format (TIFF) without compression. Subsequently, the FOV of each image was processed and analysed with a code written in Matlab 6.5 (Math Work, USA). Fig. 1 shows typical fields of view for Altamura, Laterza and Matera bread types.

A dynamic thresholding technique was utilised for image segmentation and cells detection in each FOV (Gonzalez & Woods, 1993). The dynamic threshold was determined by the method described in a previous study (Peri et al., 2003).

Subsequent to cells detection, crumb morphological and colour features analysis was performed. Crumb morphological features included: mean cell area, cell density (number of cells/cm^2), and cell shape distribution. Cell shape was quantified using a shape factor SF ($SF = 4 \times \pi \times$ cell area × cell perimeter^2). This measurement calculates circularity of an object. A perfect circle has a shape factor of 1, and a line has a shape factor approaching 0. Cell shape distribution analysis was performed by counting the percentages of cells that fall into four predefined shape factor classes: $0.0 < SF \leqslant 0.2$, $0.2 < SF \leqslant 0.5$, $0.5 < SF \leqslant 0.8$, and $0.8 < SF \leqslant 1.0$. Before crumb colour analysis, each FOV was converted from RGB space to $L^*a^*b^*$ space to obtain a better representation of colour (Pitas, 1993). The $L^*a^*b^*$ colour space is derived from the CIE (Commission Internationale de 1'Eclairage) XYZ tristimulus values, and it is a perceptually uniform colour space in which it is possible effectively quantify small colour differences as seen by the human eye (Cheng, Jiang, Sun, & Wang, 2001). Crumb colour analysis was performed by averaging brightness (L^*), red/green balance (a^*), and yellow/blue balance (b^*) for the cell wall material pixels of each FOV.

Crumb feature data were analysed using a one-way analysis of variance (ANOVA). ANOVA was performed by a statistical software (STATGRAPHICS PLUS 5, Manugistics, USA). Tukey's honestly difference test was used to detect significant differences at $P < 0.05$.

3. Results and discussion

3.1. Routine classical determinations

The results of the main routine classical analyses carried out on durum wheat flours, collected in four Italian towns well known for their established tradition of bread-making from durum wheat, are shown in Table 1. The flours examined could be classified into two different categories: durum wheat re-milled semolinas (those from Altamura, Laterza

Fig. 1. Typical FOV for Altamura (a), Laterza (b), and Matera (c) bread types.

Table 1
Technologic characteristics of the durum wheat flours examined

Sample	Dry gluten (% d.m.)	Gluten index	*P*/*L*	W (10^{-4} J)	Yellow pigments (ppm β-carotene)	Yellow index	Ashes (% d.m.)
Durum wheat re-milled "semolato"							
Bonorva 1	12.6	92	0.64	101	4.60	18.62	1.14
Bonorva 2	10.7	92	1.38	107	3.99	18.26	1.09
Bonorva 3	11.0	93	1.39	121	3.96	19.22	1.13
Bono rva 4	11.4	93	0.92	119	4.34	18.19	1.12
Mean	11.7	93	1.08	112	4.22	18.57	1.12
Standard deviation	0.7	1	0.37	10	0.31	0.47	0.02
Durum wheat re-milled semolina							
Matera 1	11.6	86	1.11	133	4.03	19.00	0.92
Matera 2	12.2	82	0.98	153	4.26	19.68	0.87
Matera 3	12.2	92	1.03	138	4.62	19.51	0.90
Matera 4	11.4	87	1.00	147	4.53	18.77	0.89
Mean	11.9	87	1.03	143	4.35	19.24	0.90
Standard deviation	0.4		0.06	9	0.27	0.43	0.02
Altamura 1	11.0	76	0.34	71	4.39	18.35	0.85
Altamura 2	10.2	80	0.50	89	4.46	19.90	0.87
Altamura 3	11.6	83	1.03	120	5.07	22.65	0.90
Altamura 4	11.3	80	1.05	135	4.62	19.52	0.86
Mean	11.0	80	0.73	104	4.63	0.11	0.87
Standard deviation	0.6	3	0.36	29	0.31	1.82	0.02
Laterza 1	11.4	86	1.35	104	4.53	19.28	0.90
Laterza 2	10.8	94	2.50	176	4.87	19.07	0.84
Laterza 3	10.9	83	0.83	101	4.48	18.54	0.92
Laterza 4	10.1	94	1.90	166	4.20	19.24	0.87
Mean	10.8	*89*	0.65	137	4.52	19.03	0.88
Standard deviation	*0.5*		0.72	40	0.27	0.34	0.03

and Matera) and durum wheat re-milled "semolato" (the samples from Bonorva), being the *semolato* "the product obtained from the milling and sieving of durum wheat after the extraction of semolina" (Italian Official Bulletin, 2001). This was confirmed by the ash content of each flour, which accomplished the current law requirements, with values ranging from 1.09% to 1.14% d.m. for the *semolato* samples and an overall mean content of 0.88% d.m. for the re-milled semolinas. Two samples showed a slightly higher ash content that the required: Matera 1 and Laterza 3, with a value of 0.92% d.m.

Regarding the bread-making characteristics of the flours under examination, the alveographic indices (W and P/L), and the gluten content and index were considered. On the whole, the alveograph data showed a great variability, with the samples from Laterza having higher P/L values (mean value 1.65) than the other examined samples, revealing a gluten characterised by tenacity more than by extensibility. Besides, the highest W values were found in re-milled semolinas from Laterza and from Matera (mean values 137×10^{-4} J and 143×10^{-4} J, respectively). Even if for bread-making purposes the P/L ratio should not exceed 2, with an optimum ranging from 0.4 to 0.8 (Boggini, Pagani, & Lucisano, 1997; Boyacioglu & D'Appolonia, 1994), durum wheat flours can usually show P/L values much higher than the optimum (Simeone, Blanco, Pasqualone, & Fares, 2001), leading to a kind of bread that is characteristically more compact than that from soft wheat. Indeed, the samples with lower P/L values were found to contain soft wheat flour (data not shown).

The gluten content of the re-milled semolinas from Matera showed the highest values, with a mean of 11.9% d.m. The lowest values were found in semolinas from Laterza (10.8% d.m.). Gluten content was found to be negatively correlated to P/L values ($r = -0.50$, $P < 0.05$), as already observed by other authors (Peña, 2000). The gluten index showed values ranging from 76 to 94, with the highest values, found in *semolato* from Bonorva (mean gluten index 93), that were very high for bread-making purposes.

Finally, the colour characteristics of the durum wheat flours were considered. The yellow colour is typical of both durum wheat flour and durum wheat bread crumb, and it influences the acceptance of the end-product by the consumer, so that the official production process of Altamura bread foresees a yellow index of the starting re-milled semolina higher than 20 (Official Journal of the European Communities, 2003) and that of Matera bread foresees a minimum value of 21 (Italian Official Bulletin, 2004). The yellow index of the flours ranged from 18.19 to 22.65, with a yellow pigment content between 3.96 and 5.20 ppm of β-carotene. These two parameters showed a significant correlation ($r = 0.55$, $P < 0.05$).

3.2. Spectroscopic determinations

^{1}H HR-MAS NMR spectra of the analyzed samples reveal principally signals due to lipids and polysaccharides as described in Brescia, Sgaramella, Ghelli, and Sacco (2003), where flour and bread spectra were compared to investigate the compositional modifications of flours due to the bread-making process.

In this work, the study was extended to the dough before baking in order to monitor the compositional modifications occurring in the different processing stages.

In Fig. 2, the spectrum of a durum wheat semolina and of the dough and bread obtained from it are shown. In the dough spectrum it is possible to observe the appearance of two signals resonating at 1.91 and 2.43 ppm and due to acetic and succinic acid, respectively. The same signals are also present in the bread spectrum, but with lower intensity.

δ^{13}C and δ^{15}N ratios were determined on the flours, dough and bread samples by means of the IRMS technique and are reported in Table 2. The average values of δ^{15}N significantly discriminated flour, dough and bread samples according to geographical origin while δ^{13}C values were not significantly different for the samples and this result could be expected because they are mainly affected by the pathway of CO_2 fixation used by the plant species, that is common for all the studied samples.

Flour samples from Bonorva had the highest average ^{15}N content. This result can be ascribed to the geographical origin of the samples and to the characteristics of the soil or to the different agronomic practices. It has been demonstrated that synthetic and organic nitrogenous fertilizers show different δ^{15}N value (Bateman, Kelly, & Jickells, 2005). It should be noted that flour samples Altamura 3

Table 2
Isotopic parameters of the analyzed samples

Sample	Flours		Dough		Bread	
	δ^{15}N	δ^{13}C	δ^{15}N	δ^{13}C	δ^{15}N	δ^{13}C
Matera 1	2.59	−24.5	2.47	−24.7	2.83	−24.7
Matera 2	2.68	−24.6	2.82	−24.7	3.07	−25.0
Matera 3	2.67	−24.7	2.42	−24.8	2.97	−24.7
Matera 4	2.27	−25.5	2.18	−25.0	3.13	−25.5
Mean	2.55	−24.9	2.47	−24.8	3.00	−25.0
Standard deviation	0.93	0.5	0.26	0.1	0.13	0.4
Altamura 1	2.63	−25.3	2.88	−24.7	3.68	−25.1
Altamura 2	2.44	−24.3	2.46	−24.7	3.45	−25.0
Altamura 3	3.77	−25.0	2.63	−24.9	3.37	−25.5
Altamura 4	3.33	−24.9	2.61	−24.8	3.03	−25.5
Mean	2.68	−25.1	2.64	−24.8	3.38	−25.2
Standard deviation	0.28	0.1	0.17	0.1	0.27	0.2
Laterza 1	2.47	−25.2	2.71	−25.2	3.08	−25.4
Laterza 2	2.41	−25.2	2.87	−25.3	2.96	−25.6
Laterza 3	2.95	−25.1	2.82	−25.2	3.02	−25.3
Laterza 4	2.89	−25.0	2.76	−25.2	3.25	−25.2
Mean	2.68	−25.1	2.79	−25.2	3.08	−25.4
Standard deviation	0.28	0.1	0.07	0.1	0.15	0.2
Bonorva 1	3.19	−25.1	3.61	−25.0	2.81	−25.6
Bonorva 2	3.56	−24.7	3.35	−24.5	2.43	−25.2
Bonorva 3	3.01	−25.1	3.20	−25.0	2.75	−24.9
Bonorva 4	3.87	−25.0	3.95	−25.2	2.93	−25.5
Mean	3.41	−25.0	3.53	−24.9	2.73	−25.3
Standard deviation	0.38	0.2	0.33	0.3	0.21	0.3

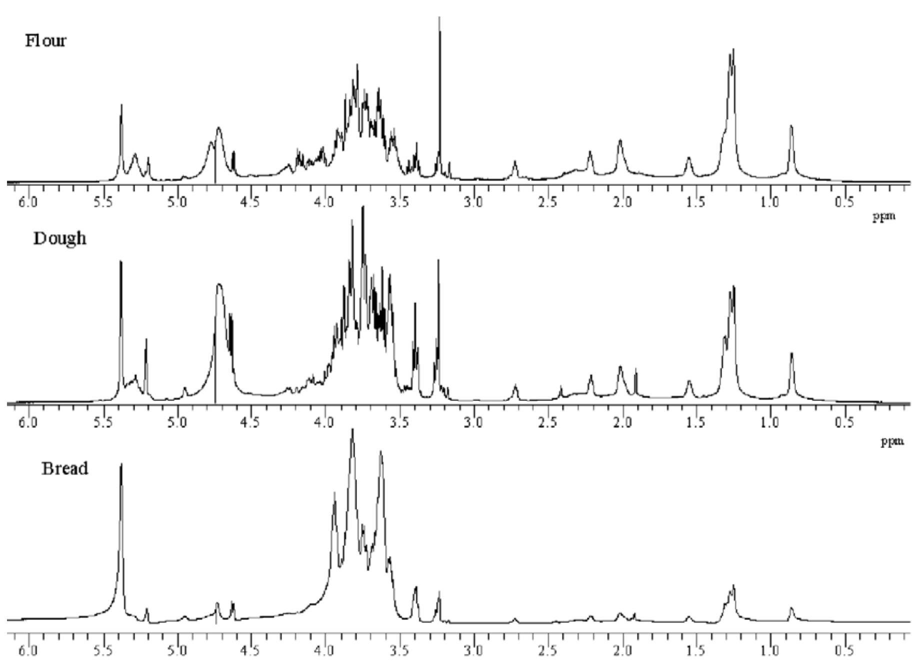

Fig. 2. ^{1}H HR-MAS NMR 400 MHz spectra of a durum wheat flour, and of the related dough and bread.

and 4 possess $\delta^{15}N$ values that fall in the range of the Bonorva samples. This could be attributable to different batches of durum wheat that have been grown with different types of nitrogen fertiliser.

This differentiation between Bonorva and samples of other regions was maintained in the dough samples but for the bread samples an unexpected change was verified since samples from Bonorva had the lowest ^{15}N content. This result reflects an opposite trend of $\delta^{15}N$ parameter compared with the general observed behavior. Indeed, for all the other samples an increase in the ^{15}N content with the baking process was noticed. This can be due to the degradation of the amino acids, deriving from proteolytic events during dough fermentation (Thiele, Grassi, & Ganzle, 2004), due to processes (e.g. the Maillard reaction) that occur during baking. This process could be favored for molecules containing the lighter isotopes. As a consequence, the ^{15}N content of the sample increases. In the samples from Bonorva the opposite behavior was observed. This behavior is still under study and must be confirmed analyzing a higher number of samples. It could be explained considering the particular baking process of

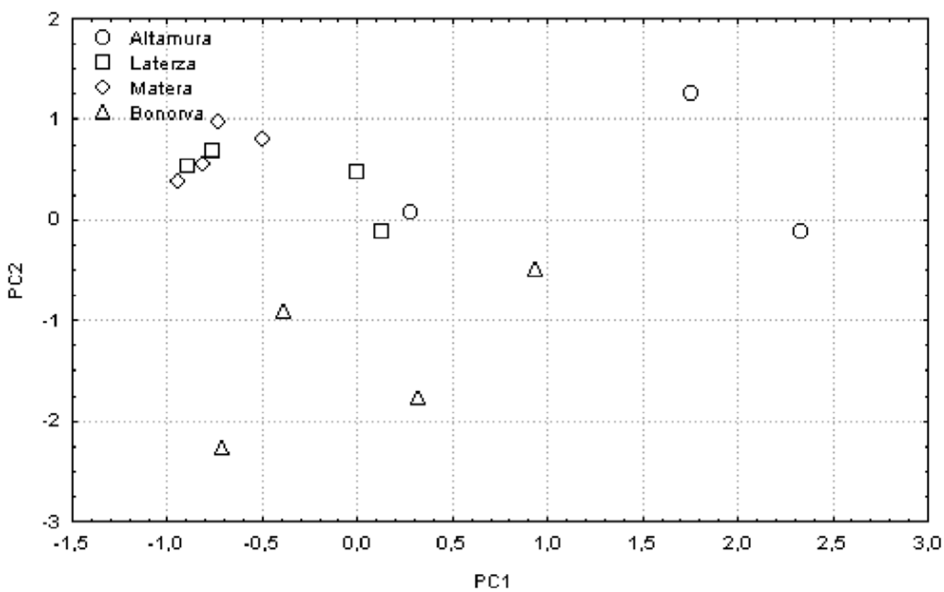

Fig. 3. Scatter plot of the scores from the first two principal components PC1 and PC2 obtained using NMR and IRMS data of flour samples.

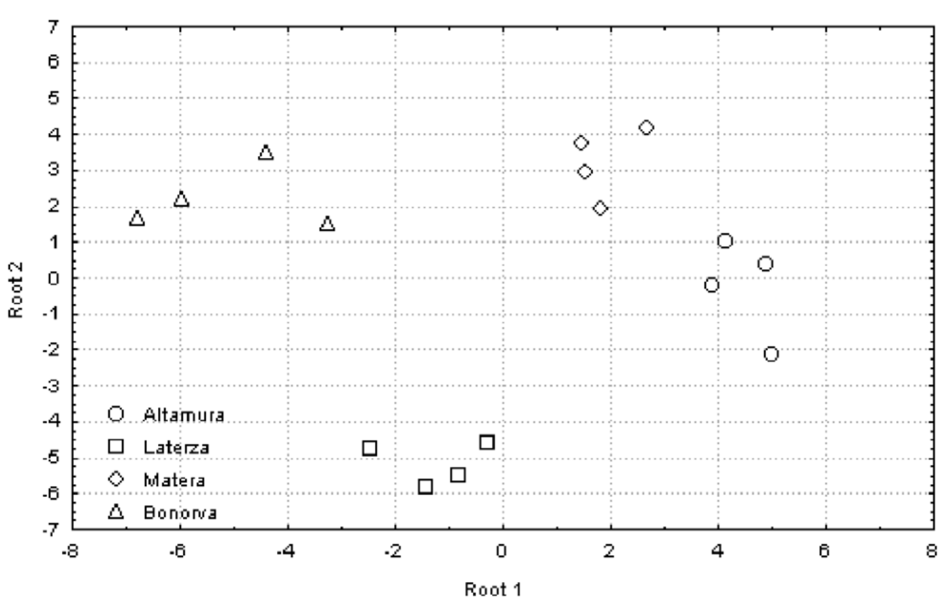

Fig. 4. Plot of the four geographical origins for dough samples on the first two discriminant functions for NMR and IRMS data.

samples from Bonorva, consisting in a two-step cooking of a very thin dough layer so as to obtain a crispy, almost dry, thin bread with pale golden appearance and very scarce incidence of the Maillard reaction, without neither the soft and slightly humid crumb nor the brown crust of the other three types of bread examined. This effect was confirmed by carrying out the same baking process of typical Bonorva bread with another semolina sample.

3.3. Statistical analysis

The intensities of the signals present in the ^{1}H HR-MAS NMR of all the samples, proportional to the concentration of the individuated compounds, were calculated and normalized to the intensity of the highest signal of the spectrum, due to the methylenic protons of the fatty acid chains. The data obtained and the isotopic parameters were gathered in three data sets for flours, dough and breads.

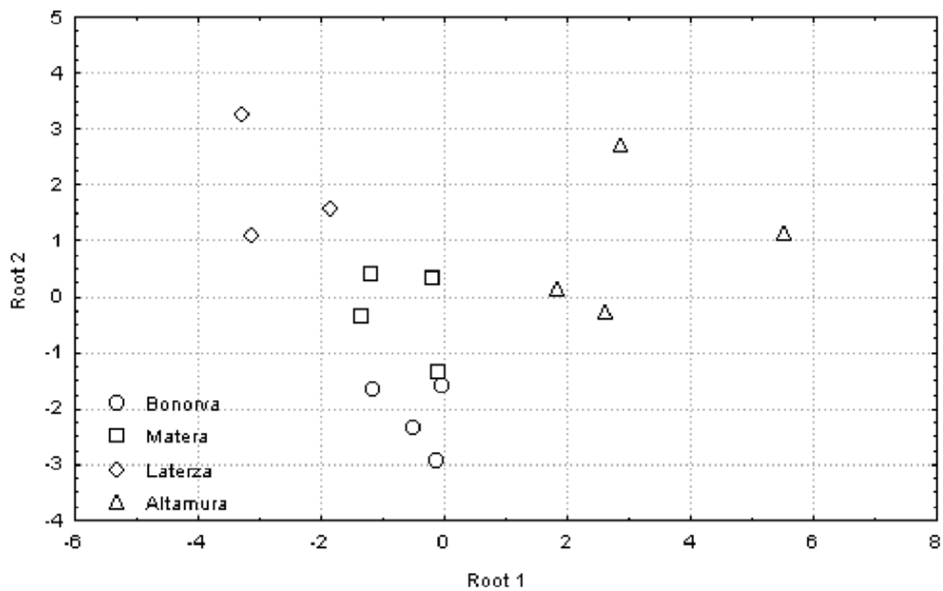

Fig. 5. Plot of the four geographical origins for bread samples on the first two discriminant functions for NMR and IRMS data.

Table 3
Crumb morphological features for bread of different types

Bakery	Mean cell area (mm^2)	Cell density (cell/cm^2)	Cell shape factor (% of total cells)			
			0.0 < SF < 0.2	0.2 < SF < 0.5	0.5 < SF < 0.8	0.8 < SF < 1.0
Matera 1	0.67a	30.2ab	10.14a	40.74a	32.70a	16.43a
Matera 2	0.61a	34.1b	11.86a	39.56a	33.40a	15.18a
Matera 3	0.67a	26.6a	11.27a	39.14a	34.72a	14.88a
Matera 4	0.71a	29.2a	25.67b	54.95b	17.31b	2.07b
Mean	0.66	30.0	14.73	43.60	29.53	12.14
Standard deviation	0.06	3.4	6.80	6.96	7.46	6.15
Altamura 1	0.86a	28.8a	10.71a	28.24a	35.31a	25.74a
Altamura 2	0.97a	29.4a	8.57a	27.56a	35.94a	27.92a
Altamura 3	1.05a	27.2a	10.66a	26.89a	35.98a	26.47a
Altamura 4	0.83a	30.5a	10.49a	28.27a	35.20a	26.03a
Mean	0.93	29.0	10.11	27.74	35.61	26.54
Standard deviation	0.13	2.1	1.27	1.18	1.19	1.34
Laterza 1	1.46a	16.9a	6.57a	35.48a	42.79a	15.16a
Laterza 2	1.56a	13.2a	5.92a	36.59a	43.25a	14.24a
Laterza 3	1.33a	16.5a	6.97a	34.07a	43.19a	15.85a
Laterza 4	1.56a	17.4a	6.52a	35.79a	43.11a	14.58a
Mean	1.48	16.0	6.50	35.48	43.06	14.96
Standard deviation	0.16	2.8	0.84	2.07	1.56	1.64

Values followed by the same letter in the same column are not significantly different ($P < 0.05$).

Multivariate statistical analysis was performed to evaluate if the data contained useful information for the discrimination of the samples according to their geographical origin.

As far as flours are concerned, a significant differentiation was found between samples from Bonorva (Sardinia) and the others (Fig. 3). The parameter that predominantly influenced the discrimination was the $\delta^{15}N$ ratio. Samples from Altamura, Laterza and Matera were not significantly differentiated. The data obtained by means of the routine analyses on the flour samples were also submitted to multivariate analysis showing that, basing on these data, Altamura samples were differentiated from the others (data not shown).

In addition, it was possible to observe a separation between dough samples from Altamura and Laterza and samples from Matera. The variables mostly responsible for this discrimination were the NMR data and $\delta^{13}C$. The discrimination of the samples was improved with respect to flours using the DA approach, with 87% of classification ability and 76% of predictive capacity (Fig. 4).

The differences between the samples were reduced when bread samples were considered (Fig. 5). In fact, the predictive ability of DA decreased to 60%. This behavior is certainly due to the chemical modifications that occur during the processing, and in particular after cooking. This result was expected from the observed features of the bread spectra. Nevertheless, due to the higher $\delta^{15}N$ values of Bonorva samples, these bread samples were very well differentiated from the others.

3.4. Image analysis

Crumb morphological and colour features for bread of different types are compared in Tables 3 and 4, respectively. ANOVA indicates that no significant differences were detected in crumb morphological features for loaves of different bakeries for Altamura bread. From the mean percentages of cells that fall into the four shape factor classes it resulted that more round cells then elongated cells are in cross-sectional area of Altamura bread. ANOVA indicates that no significant differences were detected for crumb colour features in loaves of Altamura different bakeries.

As far as crumb morphological features for Laterza bread are concerned, ANOVA indicates that no significant differences were detected in loaves of different bakeries. As in Altamura samples, more round cells then elongated cells are also in cross-sectional area of Laterza bread. No significant differences were detected for crumb colour features in loaves of different bakeries.

ANOVA indicates that, with the exceptions of cell density for the loaf obtained from the bakery 2 and the percentages of cells that fall into the four shape factor classes for the loaf obtained from the bakery 4, no significant differences were detected in loaves of different bakeries for Matera bread. More elongated cells then round cells are in cross-sectional area of Matera bread. ANOVA indicates that, with the exception of the chromatic coordinate b* for the loaf obtained

Table 4
Crumb colour features for bread of different types

Bread type	L^*	Chromatic coordinates	
		a^*	b^*
Matera 1	80.97a	−1.14a	24.45a
Matera 2	81.53a	−1.45a	26.23a
Matera 3	79.08a	−1.42a	24.73a
Matera 4	82.61a	−1.04a	22.05b
Mean	80.93	−1.26	24.37
Standard deviation	2.03	0.41	1.76
Altamura 1	76.33a	−0.83a	26.88a
Altamura 2	79.43a	−0.67a	26.53a
Altamura 3	77.73a	−1.07a	26.17a
Altamura 4	78.34a	−1.29a	25.85a
Mean	77.96	−0.97	26.36
Standard deviation	1.76	0.37	0.93
Laterza 1	72.64a	−1.11a	24.37a
Laterza 2	72.36a	−0.94a	24.80a
Laterza 3	74.14a	−1.45a	26.16a
Laterza 4	73.53a	−1.46a	24.63a
Mean	73. 17	−1.24	24.99
Standard deviation	1.88	0.34	1.29

Values followed by the same letter in the same column are not significantly different ($P < 0.05$).

Table 6
Crumb colour features for bread of different types

Bread type	L^*	Chromatic coordinates	
		a^*	b^*
Altamura	77.96a	−0.97a	26.36a
Laterza	73.17b	−1.24a	24.99b
Matera	80.93c	−1.26a	24.37b

Table 5
Crumb morphological features for bread of different types

Bread type	Mean cell area (mm^2)	Cell density ($cell/cm^2$)	Cell shape factor (% of total cells)			
			$0.0 < SF \leqslant 0.2$	$0.2 < SF \leqslant 0.5$	$0.5 < SF \leqslant 0.8$	$0.8 < SF \leqslant 1.0$
Altamura	0.93a	29.0a	11.11a	27.74a	35.61a	26.54a
Laterza	1.48b	16.0b	6.50b	35.48b	43.06b	14.96b
Matera	0.66c	30.0a	14.73c	43.60c	29.53c	12.14b

from the bakery 4, no significant differences were detected for crumb colour features in loaves of different bakeries.

Finally, substantial differences were detected for many crumb features extracted from FOV of different bread types. ANOVA indicates that significant differences were detected for mean cell area, cell shape (Table 5), and cell wall material brightness (Table 6). These differences compare well with visual inspection of bread slices.

4. Conclusions

In general, the classical routine analyses of durum wheat flours were variable from one case to another and different bread-making characteristics were observed. Multivariate analysis indicated that, basing on the classical data, the Altamura re-milled semolinas could be differentiated to those from the other sampling areas considered. In any case, the majority of the flours examined produced a typically compact bread with good colour features. A poor correlation among the flour data set and the crumb grain morphological and colour data was observed. It could be attributed to the incidence of other variables linked to the leavening and baking process, that were not considered in the present study.

As far as the use of quantitative NMR data in the discrimination of typical breads was concerned, a significant contribution of this information was found out, though it has to be confirmed on a higher number of samples. Isotopic content, being influenced by geo-climatic factors, showed to be useful in the discrimination of the samples according to geographical origin. Moreover, it seems to be influenced by the bread production process, in particular by the baking stage. This interesting result will be deeply explored in a following study.

The results of this research confirm that computerised image analysis for quantitative assessment of bread crumb attributes is effective. This approach gives objective and consistent measurements for qualitatively relevant bread crumb features, and the measured features compare well with bread slice visual inspection.

From measured crumb grain morphological data (mean cell area, cell density, and cell shape distribution), and cell wall material colour data (average brightness, red/green balance, and yellow/blue balance) no significant differences were detected within each bread type according to the production bakery (with some exception for the Matera bread type), while several crumb features resulted to be significantly different according to the bread type. In the case of the Matera bread, significant differences were detected in morphological crumb grain features and cell wall yellow/blue balances according to the production bakery. This is an unexpected result that needs further investigation during the next planed research.

On the base of the data obtained from the present study, crumb grain of the Altamura and Laterza bread types can be objectively characterised through mean cell area, cell density, cell shape distribution, average brightness, and average yellow/blue balance of the cell wall material. Also, all the artisan bread types selected in the present study can be discriminated using a set of qualitatively relevant crumb features, and quantitative data that are easily interpretable.

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