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Analytical Methods

Gas chromatographic–mass spectrometric characterisation of the Italian Protected Designation of Origin "Altamura" bread volatile profile

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ABSTRACT

Dynamic headspace extraction technique coupled to the gas chromatography–mass spectrometry analysis was applied to characterize volatile compounds of both crust and crumb of the Protected Designation of Origin Italian durum wheat sourdough "Altamura" bread. Volatile compounds of crust and crumb were characterized and statistically compared and their relative abundance was also calculated. A total of 89 compounds belonging to different chemical classes were identified in the crust. More abundant compounds detected in the crust were ethanol (20 ± 6%), 2-furfural (14 ± 7%) and 3-methyl-1-butanol (9 ± 5%). A lower number of volatile compounds (74) was identified in crumb, among which ethanol (32 ± 7%), 3-methyl-1-butanol (23 ± 6%) and 3-pentanol (7 ± 3) were the most abundant. The influence of different baking modes (wood- or gas-fired) on volatile compounds, macroscopic appearance and selected physico-chemical parameters (colour and texture) of bread samples were also evaluated.
Samples baked in wood-fired oven showed larger amount of volatile compounds such as furans and aldehydes that could positively influence the flavour of the product.
Crust of wood-fired breads showed higher amounts of compounds from Maillard reaction, resulting in harder and browner breads than gas-fired samples. Macroscopic appearance of crumb of wood-fired breads showed higher percentages of larger pores, being also less hard and cohesive than gas-fired samples.

1. Introduction

"Altamura" bread is a traditional durum wheat sourdough bread produced in Altamura (Bari, Apulia, Southern Italy) and it is the only bakery product that obtained the Protected Designation of Origin (PDO; European Union, 2003). This bread is produced in a restricted geographical area by a limited number of small artisanal bakeries and its production requires, among the others, the use of flour of durum wheat varieties (Appulo, Arcangelo, Duilio and Simeto) grown in the Altamura area (at least 80% of the total flour) and a prolonged sponge-dough procedure (refreshed at least three times). PDO "Altamura" bread is largely appreciated by the consumers, due to its characteristic taste and its prolonged shelf-life (Raffo et al., 2003). Its restricted production has largely encouraged manufacture of industrial "Altamura" like bread that was found to show poorer volatile compound profile and significantly different macroscopic appearance and physico-chemical properties (Chiavaro, Vittadini, Musci, Bianchi, & Curti, 2008). The objective evaluation of the authenticity of "Altamura" bread may guarantee the consumers and avoid fraudulent mislabelling, as preservation and assurance of the organoleptic and nutritional quality of traditional foods is an emerging topic in the European Community (Trichopoulou, Vasilopoulou, Georga, Soukara, & Dilis, 2006). In particular, the characterization of the flavour profile of food could act as a chemical "fingerprint" of the product, since the nature and the relative amount of the volatile compounds present are distinctive features of the product. Thus, the flavour profile could be used to guarantee authenticity of food products, as already reported (Careri et al., 1993; Radovic et al., 2001; Virgili et al., 1994).

The flavour profile of "Altamura" bread is expected to be highly distinctive, as the production process was based on the use of sourdough (a piece of dough saved from the previous baking and then mixed with flour, salt and water to produce bread). During storage, lactic acid fermentation occurs because of the metabolic activity of lactic acid bacteria (LAB), and yeasts selectively multiplied from the flour on sourdough. "Altamura" bread sourdoughs were found to be rich in facultative heterofermentative LAB (*Lactobacillus plantarum, L. paracasei* and *L. casei*; Ricciardi, Parente, Piratino, Paraggio, & Romano, 2005), whereas *Saccharomyces cerevisiae* was the only yeast isolated (Ricciardi et al., 2002).

Sourdough is, therefore, a complex biological system which confers specific flavour characteristic to breads (Damiani et al., 1996; Gobbetti, 1998; Hansen & Hansen, 1994a; Rehman, Paterson,

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& Piggott, 2006). Other factors were also reported to influence volatile compound formation in sourdough breads; in particular, the type of flour (Hansen & Hansen, 1994b) and long fermentation time (Hansen, Lund, & Lewis, 1989) increase the production of flavour precursor amino acids, which can act as substrate for Maillard reactions during baking (Gobbetti et al., 1995; Hansen & Schieberle, 2005).

The aim of this work was to characterize volatile compounds present both in the crust and in the crumb of PDO "Altamura" bread samples obtained from several local bakeries, by means of the dynamic headspace extraction (DHS) technique coupled to the gas chromatography–mass spectrometry (GC–MS) analysis and to evaluate the influence of different baking mode (wood- or gas-fired oven) on volatile compounds, macroscopic appearance and selected physical–chemical parameters of bread samples.

2. Materials and methods

2.1. Materials

PDO "Altamura" bread loaves were obtained from six artisanal bakeries (A, B, C, D, E, F) located in Altamura (Bari, Italy). All loaves were produced according to the procedure described in the EC regulation (European Union, 2003), consisting in a prolonged sponge-dough procedure where the sourdough was refreshed three times before final dough was made. All the loaves had the same size (1 kg) and shape which is locally known as 'U sckuanète' (folded loaf), formed by folding the dough from either side towards the centre and cooked without contact between loaves' edges.

All breads were subject to indirect heating, by means of a wood-fired oven (bakeries A, B and C) or a gas-fired oven (bakeries D, E and F). Only one bakery (B) produced breads with both type of ovens.

Three loaves were used for characterization of each bakery (A, C, D, E, F) whereas B bakery provided six loaves, three cooked by means of wood-fired oven (B_w) and three by means of gas-fired oven (B_g) for a total of 21 loaves of "Altamura" bread.

2.2. Dynamic headspace extraction

Each loaf was analyzed by separating crust and crumb. Two sub-samples (crust and crumb) were obtained for each loaf and they were frozen under liquid nitrogen, ground in a domestic blender then stored in screw-cap glass vials at −20 °C until GC–MS analysis. Three independent DHS extractions were performed for each sub-sample in different days, in order to verify the absence of instrumental drift and to evaluate the repeatability of the whole procedure.

Frozen bread (1.5 g) sample (crust or crumb) were placed in a 200 ml Erlenmayer flask at 40 °C. Purified helium (40 ml min^{-1}) was passed through the system for 15 min and the entrained volatiles were adsorbed on a glass tubes (16 × 0.4 cm i.d.) trap filled with Tenax TA (90 mg, 20–35 mesh; Chrompack, Middelburg, The Netherlands). The volatile compounds were subsequently thermally desorbed and transferred to the GC system by using a TCT thermal desorption cold trap (TD800, Fisons Instruments, Milan, Italy). Desorption was performed at 280 °C for 10 min under a helium flow (10 ml min^{-1}); the volatiles were cryofocused in a glass lined tube at −120 °C with liquid nitrogen and injected into the GC capillary column by heating the cold trap to 230 °C.

In order to verify possible environmental contamination, blank analyses were carried out using an empty 200 ml Erlenmayer flask following the same procedure as for the sample. To assess the presence of carry-over effects, the adsorbent trap was also desorbed before and after each entire sampling procedure.

2.3. Gas chromatography–mass spectrometry

Gas chromatography–mass spectrometry (GC–MS) analysis was carried out using a system consisting of a TRACE GC 2000 gas chromatograph and of a TRACE MS quadrupole mass spectrometer (Thermo Electron Corporation, Milan, Italy). The interface and the source temperatures were kept at 230 and 200 °C, respectively. Electron impact mass spectra were recorded at 70 eV ionization energy (scan time, 0.5 s; electron multiplier voltage, 350 V) by scanning the mass spectrometer from m/z 35 to 350. The carrier gas was helium (pressure, 70 kPa). Chromatographic separation was performed on a fused-silica bonded-phase capillary column Supelcowax 10™ (30 m × 0.25 mm; d.f. = 0.25 μm; Supelco, Palo Alto, CA, USA).

The mass spectrometer was tuned before analyses by optimizing the intensity of the signals of reference gas (PTFBA, perfluorotributylamine) across the full mass range and calibrated, in order to obtain the best instrumental performance.

The temperature program was isothermal at 40 °C for 8 min, then raised to 160 °C at 6 °C min^{-1}, from 160 °C to 200 °C at 20 °C min^{-1}, holding this temperature for 1 min. The mass spectrometer data acquisition was performed using the release 1.2 Xcalibur™ software (Thermo Electron Corporation). The identification of the volatile compounds was achieved by comparing their mass spectra with those stored in the National Institute of Standards and Technology (NIST) US Government library. In addition, retention indices (RIs) were calculated for each peak with reference to the normal alkanes C_6–C_{16} series according to the following equation (van den Dool & Kratz, 1963):

$$RI_i = 100z + 100\frac{(RT_i - RT_z)}{(RT_{z+1} - RT_z)} \quad (1)$$

where RI_i is the retention index of the unknown peak, RT_i is the retention time for the unknown peak, RT_z and RT_{z+1} are the retention times for the *n*-alkanes that bracket the unknown peak, *z* is the number of carbon atoms in the *n*-alkane standard that elute just before the unknown peak.

Calculated RIs were then compared with those stored in a proprietary database obtained by injecting 250 volatile compounds usually found in a variety of food samples (Bianchi, Careri, Mangia, & Musci, 2007).

In order to evaluate relative quantitative differences in the aromatic profile of the samples investigated, GC peak areas were calculated as total ion current (TIC) for the identified compounds.

2.4. Colour analysis

Colour determination was carried out on crust and crumb using a Minolta Colourimeter (CM 2600d, Minolta Co., Osaka, Japan) equipped with a standard illuminant D65. L^* (lightness), a^* (redness) and b^* (yellowness) were quantified on each sample using a 10° position of the standard observer. The instrument was calibrated before each analysis with white and black standard tiles.

Crust colour was determined on nine pre-selected locations on the crust of each loaf. Crumb colour was determined on three points on the three central slices of the six loaves collected from the B bakery.

2.5. Textural measurement

Instrumental texture evaluation of crust and crumb was performed using a TA.XT2 Texture Analyzer equipped with a 25 kg load cell (Stable Micro Systems, Goldalming, UK) and Texture Expert for Windows software (version 1.22) for data analysis. The load cell calibration was performed daily according to the TA.XT2 manual (Stable Micro Systems). A puncture test was used to

measure crust hardness using a 3 mm diameter stainless steel probe which penetrated into the crust for 5 mm at a test speed of 60 mm min^{-1}. Maximum peak force (N) was measured from the penetration curve and taken as crust hardness. Six measurements were taken on the crust of each loaf at pre-selected locations. Texture profile analysis (TPA) was carried out to evaluate crumb texture using a cylindrical aluminium probe (35 mm diameter) and crosshead speed of 60 mm min^{-1} to compress a crumb sample to 50% of their original height. Measurements were carried out on crumb samples (20 × 20 × 20 mm) extracted from the centre of three slices (20 mm thickness) taken from the centre of the loaf.

The textural parameters considered were hardness (peak force of the first compression cycle in N), cohesiveness (ratio of positive force area during the second compression to that during the first compression area, dimensionless) and springiness (ratio of the time duration of force input during the second compression to that during the first compression, dimensionless; Bourne, 1978).

Crust hardness was evaluated in all loaves. TPA was performed on the crumb of the six loaves collected from the B bakery.

2.6. Image acquisition and analysis

Crumb grain of the six loaves collected from the B bakery was evaluated by means of a digital image analysis system, as previously reported (Chiavaro et al., 2008). Images of both faces of the three central slices (20 mm thickness) from each loaf were captured with a flatbed scanner (Model Scanjet 8200, HP, Cupertino, USA), with a resolution of 600 dots per inch (dpi) and converted from true colour to 256 level grey scale.

The images were calibrated, standardized and optimized applying appropriate filters to measure pore size and their distribution using an Image-Pro Plus 4.5 (Media Cybernetics Inc., USA) software. The image of the entire crumb of each slice was evaluated considering the different slice profile of each bread type. Crumb grain was characterized by enumerating the pores present in five pre-selected dimensional classes based on their area (class 1 = 0.05–0.49 mm^2; class 2 = 0.50–0.99 mm^2; class 3 = 1.00–4.99 mm^2; class 4 = 5.00–49.99 mm^2; class 5 = >50 mm^2) and the number of pores and the area occupied by each class (expressed as percentage of the total number of pores and total pore-area, respectively) was evaluated, as previously reported (Chiavaro et al., 2008).

Crust thickness was also measured (size function of Image-Pro Plus software) on three pre-selected points of the crust of the six loaves collected from the B bakery.

2.7. Statistical analysis

Means and standard deviations were calculated with SPSS statistical software (Version 13.0, SPSS Inc., Chicago, IL, USA).

SPSS was used to perform a Student t-test ($p \leqslant 0.05$) in order to compare the volatile profile of crust and crumb. Texture and colour data of crust were subjected to one-way-analysis of variance (ANOVA) and Least Significant Difference test (LSD) at a 95% confidence level ($p \leqslant 0.05$). A Student t-test ($p \leqslant 0.05$) was also used to evidence differences for all variables considered related to the baking mode.

3. Results and discussion

3.1. Volatile compounds analysis

The volatile profile of different bread types (i.e., wheat, sourdough or yeast fermentation) has been widely investigated during the past years (Rehman et al., 2006; Schieberle & Grosch, 1987, 1994). These studies demonstrated that bread flavour is composed by different volatile compounds, belonging to several chemical classes, mainly heterocyclic compounds, alcohols, aldehydes, ketones, etc. Depending on the characteristic of each kind of bread, volatile compounds are present in well defined ratios (Grosch & Schieberle, 1991, 1997). In addition, it is well known that the characteristic flavour profile of bread is mainly generated in two different steps of the breadmaking process: fermentation and baking (Martinez-Anaya, 1996).

A comparison among volatile profiles of different bread types is difficult to be carried out, due to their different ingredients and preparation procedure. In addition, different experimental conditions and/or instrumentation used to perform the analysis may be considered.

The physico–chemical properties and the volatile profile of "Altamura" bread (both artisanally or industrially produced) were previously reported both in the fresh product and during storage (Chiavaro et al., 2008). To the authors' knowledge, the volatile fraction of "Altamura" bread was the object of only one study where volatile compounds of two differently shaped Altamura bread samples, obtained from a single bakery, were compared with an "Altamura like" product (Chiavaro et al., 2008).

In this study, the volatile compounds of crust and crumb were separately analyzed as the fermentation generally influence the crumb volatile profile while the baking process is mainly responsible for the production of the crust aroma (Hansen & Hansen, 1996). Volatile compounds were determined on all loaves (crust and crumb) from the different bakeries and reported in Table 1. Identification of the volatile compounds was performed by comparing their mass spectra with those reported in the NIST library and by using RIs, that allow to distinguish among compounds producing similar spectra on the basis of their different chromatographic behaviour. Compounds were considered positively identified when both mass spectra and retention indices led to the same identification, taking into account that a difference of 10 RI units can be considered acceptable.

TIC GC–MS chromatogram of the volatile compound profiles was reported in Fig. 1a for crust and in Fig. 1b for crumb of an "Altamura" bread sample, respectively. Eighty nine volatile compounds, belonging to different chemical classes, were detected in the crust of "Altamura" bread; 17 furans, 13 aldehydes, 11 aliphatic hydrocarbons, 9 alcohols, 9 aromatic hydrocarbons, 8 ketones, 9 pyrazines, 5 sulfuric compounds, 3 terpenes, 3 pyrroles and 2 esters.

Crumb of "Altamura" bread samples showed lower recognized volatile compounds (74) grouped as follows: 16 aldehydes, 13 furans, 12 alcohols, 10 aromatic hydrocarbons, 9 ketones, 3 aliphatic hydrocarbons, 3 pyrazines, 3 terpenes, 2 pyrroles, 2 sulfuric compounds and 1 ester.

Two sub-samples (crust and crumb) were analyzed for each loaf to determine peak areas and calculate relative abundance. Three independent analyses were performed on each sub-sample showing a very good repeatability (RSD $\leqslant$ 10%) for most of the compounds. Three loaves produced in different days for each bakery were analyzed obtaining RSD $\leqslant$ 25%.

Relative abundance was calculated in both crust and in crumb for all the detected volatile compounds. Crust samples showed ethanol (20 ± 6%), 2-furfural (14 ± 7%), 3-methyl-1-butanol (9 ± 5%), 3-pentanol (6 ± 2%), 3-methylbutanal (6 ± 3%), 2-methylbutanal (5 ± 4%), acetone (5 ± 3%), 2,3-butanedione (4 ± 1%) and 2,3-pentanedione (4 ± 2%) as more abundant compounds. Ethanol (32 ± 7%), 3-methyl-1-butanol (23 ± 6%), 3-pentanol (7 ± 3), 2,3-butanedione (5 ± 2%), acetone (5 ± 2%), 3-methylbutanal (4 ± 2%) and 2-methylbutanal (4 ± 2%) were the most abundant volatiles in crumb.

Table 1
Volatile compounds identified in "Altamura" bread samples

N[a]	Volatile compound	RI_{calc}	RI_{tab}[b]	ID[c]	Occurrence
	Furans				
3	Furan	792	802	MS, RI	Crust/crumb
7	Tetrahydrofuran	858		MS	Crust/crumb
8	2-Methylfuran	872	876	MS, RI	Crust/crumb
12	3-Methyl furan	907		MS	Crust
17	2-Ethyl furan	957	945	MS, RI	Crust/crumb
25	2,3,5-Trimethylfuran	1060		MS	Crust/crumb
50	2-Propenyl-2-furan	1218		MS	Crust
54	2-Pentylfuran	1242	1240	MS, RI	Crumb
65	2(5H)-Furanone 5-furfuryl-5-methyl	1300		MS	Crust/crumb
87	3-Furancarboxaldehyde	1448		MS	Crust/crumb
90	2-Furfural	1483	1474	MS, RI	Crust/crumb
91	2-Acetyl-5-methylfuran	1488		MS	Crust
94	Furfuryl formate	1516		MS	Crumb
95	2-Furfurylmethyl ketone	1521		MS	Crust/crumb
99	Furfuryl alcohol	1554		MS	Crust/crumb
100	5-Methyl-2-furfural	1590	1589	MS, RI	Crust/crumb
102	2-Methyl benzofuran	1614		MS	Crust
103	2,2′-Bifuran	1629		MS	Crust/crumb
104	2-Furanmethanol	1678		MS	Crust/crumb
	Aldehydes				
2	Propanal	784	801	MS, RI	Crust/crumb
6	2-Propenal	843		MS, RI	Crust/crumb
9	Butanal	878	878	MS, RI	Crust/crumb
10	2-Methyl-2-propenal	884		MS	Crumb
14	2-Methylbutanal	917	914	MS, RI	Crust/crumb
15	3-Methylbutanal	921	917	MS, RI	Crust/crumb
19	Pentanal	985	977	MS, RI	Crust/crumb
29	Hexanal	1089	1080	MS, RI	Crust/crumb
31	2-Methyl-2-butenal	1102		MS	Crust
48	Heptanal	1194	1186	MS, RI	Crumb
64	Octanal	1296	1286	MS, RI	Crust/crumb
69	*trans*-2-Heptenal	1333	1320	MS, RI	Crust/crumb
80	2-Nonanal	1402		MS, RI	Crust/crumb
86	2-Octenal	1440	1446	MS, RI	Crust/crumb
93	Decanal	1510	1502	MS, RI	Crumb
97	Benzaldeyde	1539	1528	MS, RI	Crust/crumb
98	2-Nonenal	1550	1546	MS, RI	Crumb
	Alcohols				
16	Ethanol	942	932	MS, RI	Crust/crumb
23	1-Propanol	1045	1052	MS, RI	Crust/crumb
32	3-Pentanol	1117	1112	MS, RI	Crust/crumb
41	1-Butanol	1170	1152	MS, RI	Crust/crumb
42	2-Penten-1-ol	1179		MS	Crumb
51	3-Methyl-1-butanol	1226	1215	MS, RI	Crust/crumb
59	1-Pentanol	1267	1256	MS, RI	Crust/crumb
74	1-Hexanol	1366	1354	MS, RI	Crust/crumb
82	2-Hexenol	1414	1492	MS, RI	Crumb
89	1-Octen-3-ol	1461	1456	MS, RI	Crust/crumb
92	2-Ethyl-1-hexanol	1499	1492	MS, RI	Crust/crumb
105	3-Nonen-1-ol	1697		MS	Crumb
	Aliphatic Hydrocarbons				
5	2-Octene	834	814	MS, RI	Crust/crumb
36	Hydrocarbon	1139		MS	Crust
43	Hydrocarbon	1182		MS	Crust
44	Hydrocarbon	1186		MS	Crust
53	Hydrocarbon	1237		MS	Crust
55	Branched hydrocarbon	1245		MS	Crust
56	Hydrocarbon	1247		MS	Crust
57	Hydrocarbon	1259		MS	Crust
62	Hydrocarbon	1292		MS	Crust
66	Hydrocarbon	1311		MS	Crust
76	Cyclic hydrocarbon	1378		MS	Crust/crumb
78	Hydrocarbon	1399		MS	Crumb
	Aromatic hydrocarbons				
22	Toluene	1040	1040	MS, RI	Crust/crumb
28	Aromatic hydrocarbon	1084		MS	Crust
34	Ethylbenzene	1129	1125	MS, RI	Crust/crumb
35	*p*-Xylene	1136	1127	MS, RI	Crust/crumb
37	*m*-Xylene	1142	1132	MS, RI	Crust/crumb
45	*o*-Xylene	1189	1182	MS, RI	Crumb
52	1-Ethyl-2-methyl benzene	1228		MS	Crumb
61	Aromatic hydrocarbon	1285		MS	Crust/crumb
67	Butyl benzene	1319		MS	Crumb
75	Aromatic hydrocarbon	1374		MS	Crust/crumb
85	Aromatic hydrocarbon	1428		MS	Crust/crumb
86	Aromatic hydrocarbon	1433		MS	Crust
	Ketones				
4	Acetone	814	814	MS, RI	Crust/crumb
13	2-Butanone	908	901	MS, RI	Crust/crumb
20	2,3-Butanedione	992	986	MS, RI	Crust/crumb
26	2,3-Pentanedione	1071	1071	MS, RI	Crust/crumb
38	2,3-Hexanedione	1147	1143	MS, RI	Crust
46	2-Heptanone	1191	1185	MS, RI	Crust/crumb
63	2-Octanone	1294	1280	MS, RI	Crumb
72	6-Methyl-5-hepten-2-one	1349	1340	MS, RI	Crust/crumb
84	3-Octen-2-one	1419		MS	Crumb
101	2-Cyclopenten-1,4-dione	1606		MS	Crust/crumb
	Pyrazines				
60	2-Methylpyrazine	1278	1274	MS, RI	Crust/crumb
68	2,5-Dimethylpyrazine	1331	1328	MS, RI	Crust
70	2,6-Dimethylpyrazine	1337	1335	MS, RI	Crust
71	2-Ethylpyrazine	1344	1344	MS, RI	Crust/crumb
73	2,3-Dimethylpyrazine	1355	1355	MS, RI	Crust
77	2-Ethyl-3-methylpyrazine	1393	1402	MS, RI	Crust/crumb
79	2-Ethyl 5-methylpyrazine	1399	1406	MS, RI	Crust
81	2-Ethyl-6-methylpyrazine	1413	1411	MS, RI	Crust
88	2-Ethyl-3,5-dimethylpyrazine	1454	1443	MS, RI	Crust
	Sulfuric compounds				
1	Carbon disulfide	700		MS	Crust
24	*S*-Methyl acetic acid	1052		MS	Crust
27	Dimethyl disulfide	1077	1075	MS, RI	Crust/crumb
30	2-Methyl thiophene	1096	1090	MS, RI	Crust
58	Thiazole	1262		MS	Crust/crumb
	Terpenes				
21	Terpene	1035		MS	Crust
33	Terpene	1128		MS	Crumb
40	α-Phellandrene	1164	1160	MS, RI	Crust/crumb
47	Limonene	1192	1194	MS, RI	Crust/crumb
	Pyrroles				
39	1-Methyl pyrrole	1150		MS, RI	Crust/crumb
49	2-Acetyl-1-pyrroline	1204		MS, RI	Crust
96	Pyrrole	1534		MS, RI	Crust/crumb
	Esters				
11	Ethyl acetate	896	893	MS, RI	Crust/crumb
18	Ethyl propanoate	963	957	MS, RI	Crust

[a] Peak number according to retention time.
[b] Tabulated RI (proprietary database).
[c] Identification method: MS, identification by comparison with mass spectra stored in NIST library; RI, identification by comparison with tabulated RI.

In particular, 3-methyl-1-butanol, 2-furfural, 2,3-butanedione and 3-methylbutanal were previously found to be positively correlated with bread flavour by sensory evaluation on wheat and sourdough bread (Hansen & Hansen 1996; Hansen et al., 1989).

Differences among volatile profiles on both crust and crumb were evaluated in terms of nature and relative amount of the volatiles for all the compounds and peak areas were statistically compared by means of a Student *t*-test.

It is well known that the volatile fraction of the crust is strongly influenced by thermal reactions occurring during baking as caramelization of sugars, Stecker degradation of carbonyl compounds leading to aldehydes and overall non-enzymatic browning which formed furans, pyrazines, pyrroles and sulfuric compounds (Hansen & Schieberle, 2005; Martinez-Anaya, 1996). On the contrary, as previously stated, crumb flavour was more markedly influenced by volatile compounds produced by enzymatic reactions during dough fermentation, such as alcohols, aldehydes and ketones (Hansen & Hansen, 1996).

Among furans, 3-methylfuran, 2-propenyl-2-furan, 2-ethylbenzofuran and 2(5H)-furanone-5-methyl were found only in crust, whereas 2-pentylfuran and furfuryl formate were just

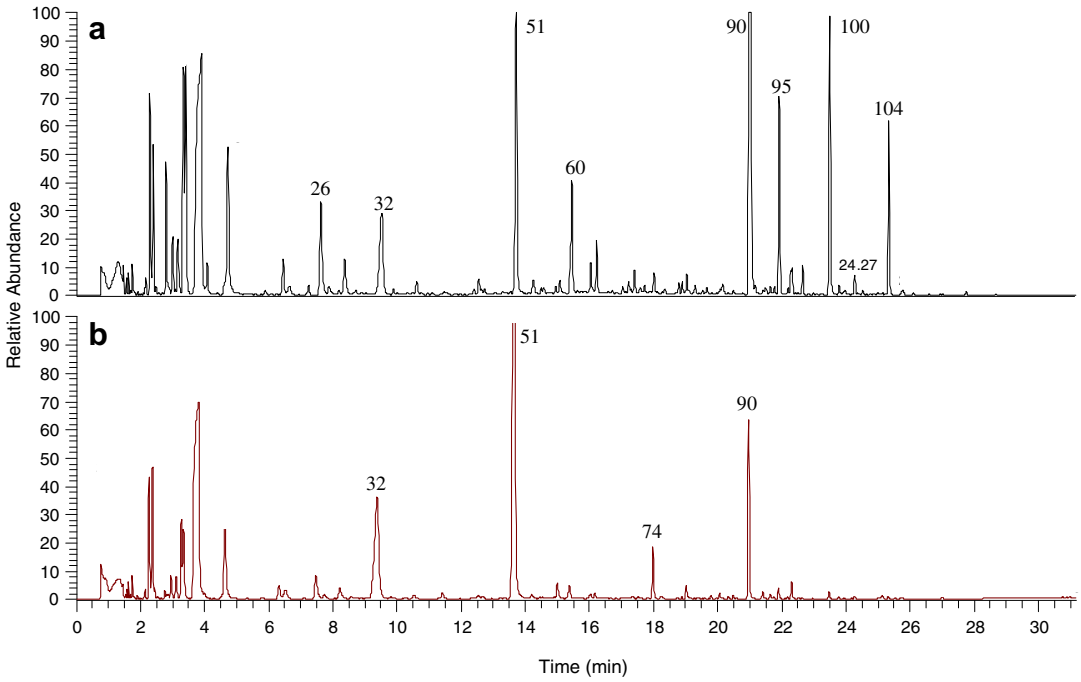

Fig. 1. Representative TIC GC–MS chromatograms of the volatile fraction obtained from (a) crust and (b) crumb of an "Altamura" bread sample.

detected in crumb. Significant differences were found for all the compounds, with the exception of tetrahydrofuran, 2-ethylfuran, 3-furancarboxaldehyde and 2,2′-bifuran. In particular, a 3–40 fold higher content of furan derivatives was found in crust samples in comparison with crumb, as these compounds were principally formed by thermal degradation of sugars (Martinez-Anaya, 1996).

Among aldehydes, 2-methyl-2-propenal, decanal and 2-nonenal were found only in crumb, whereas 2-methyl-2-butenal only in crust. 2-Methylbutanal, 3-methylbutanal and benzaldheyde were significantly higher (threefold) in crust than in crumb. In particular, 3-methylbutanal, responsible for malty and tallowy notes in crust (Schieberle & Grosch, 1987), was reported to have a greater influence on flavour of crust than crumb in sourdough rye bread, as consequence of its higher concentration (Schieberle & Grosch, 1994). Crumbs of all samples also showed an high content of acetone and 2,3-butanedione (diacetyl), which was reported to impart positive characteristics to bread flavour giving buttery note (Martinez-Anaya, 1996).

Alcohols found in crust and crumb did not significantly differ, except for 2-penten-1-ol, 2-hexenol and 3-nonen-1-ol which were found only in crumb. Crumbs also showed high amount of 3-methyl-1-butanol, besides ethanol, which was related to the fermentation activity of yeast (Hansen & Hansen, 1996). Among ketones, 2,3-hexandione was found only in crust, whereas 2-octanone and 3-octen-2-one in crumb. Significantly higher amounts of 2,3-pentanedione, 2-heptanone and 2-cyclopenten-1,4-dione (3–10 folds) were found in crust in comparison with crumb, probably due to Maillard reactions (Martinez-Anaya, 1996) .

More pyrazine derivatives were found in crust (9) than in crumb (3), showing a 5–12 fold higher amount in crust, thus confirming their origin from baking process. In particular, 2-ethyl-3-methylpyrazine, which was reported as the most important roasted odour compounds in sourdough rye bread crust (Schieberle & Grosch, 1987), was significantly higher in crust than crumb, as already observed for sourdough bread (Schieberle & Grosch, 1994). As expected, 2-acetyl-1-pyrroline, the characteristic impact volatile compound responsible for the roasty, popcorn-like aroma of the bread crust, was found only in crust. Pyrroles, also formed during non-enzymatic browning reaction, were found both in crust and crumb, resulting in a content sixfold higher in crust than in crumb.

Among esters, only ethyl acetate, which was referred as dominant heterofermentative LAB product (Spicher, Rabe, Sommer, & Stephan, 1982) was detected in large amount both in crusts and in crumbs, as esters are largely lost during baking due to their high volatility (Hansen et al., 1989).

Volatile profile of PDO "Altamura" bread was found similar to those previously reported (Chiavaro et al., 2008) showing that the volatile compound profile may be an useful tool for the assessment of the authenticity of PDO "Altamura" bread in comparison with Altamura like product.

3.2. Effect of different baking mode on volatile compounds and selected physical–chemical properties

The effect of different baking mode (wood- or gas-fired oven) was evaluated on crust and crumb samples taking into account not only volatile profiles but also colour, texture and macroscopic appearance of the breads.

Generally, volatile compounds of differently baked samples were found to not significantly differ both for crust and crumb. Lack of significant differences among wood- and gas-fired samples could be probably ascribed to a high intrinsic variability of the samples, as they were obtained from different producers. The use of different durum wheat varieties (i.e. mixed in different ratios) and/or different fermentation processes (i.e. microbiological composition of sourdough due to different environment), may have influenced volatile profiles of bread more than baking mode, reducing the differences among compounds for samples originating from different bakeries.

On the contrary, the influence of the baking mode was clearly evident on colour and hardness of the crusts, as shown in Table 2. Crusts of wood-fired samples showed significantly higher a^* (redness) values than gas-fired ones whereas L^* (lightness) and b^* (yellowness) did not show differences among the samples dif-

Table 2
Colour and textural parameters of crust for "Altamura" bread samples ($n = 3$)

Samples	L*	a*	b*	Hardness (N)
Wood-fired samples				
A	52.6 a,b (2.4)	9.1 a,b (1.0)	14.9 a,b (1.6)	16.5 a (1.9)
B_w	47.9 c,d (1.8)	10.3 a (1.2)	11.1 c,d (1.5)	18.9 a (1.2)
C	50.9 a,b,c (2.6)	9.2 a,b (0.8)	14.0 b,c (1.6)	17.9 a (1.5)
Gas-fired samples				
B_g	46.4 d (1.9)	8.0 c (0.3)	10.1 d (1.5)	7.0 c (1.7)
D	54.6 a (1.4)	8.3 b,c (0.7)	17.1 a (1.1)	10.7 b (1.8)
E	49.5 b,c,d (3.3)	8.4 b,c (0.7)	12.3 c,d (1.2)	10.5 b (1.6)
F	51.3 a,b (1.8)	8.2 c (0.2)	13.0 b,c (1.3)	4.6 d (0.8)

Same letters within each column do not significantly differ ($p \leqslant 0.05$), standard deviation given in parenthesis, RSD of colour indices < 15%; sample size = 9, RSD of texture parameter < 25%; sample size = 9.

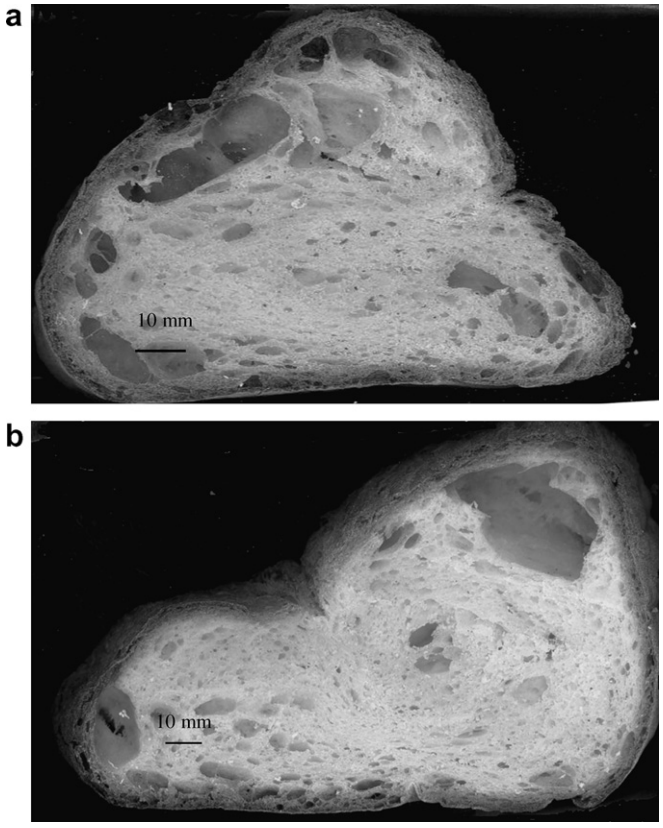

Fig. 2. Characteristic images of the central slice of B_w (a) and B_g (b) Altamura bread samples.

ferently cooked. Crusts of A, B_w and C breads were found significantly harder than gas-fired samples. The superficial layer of dough may be probably dehydrated quickly during baking in a wood-fired oven leading to the production of a crust richer of coloured compounds active in the browning process (non-enzymatic browning).

A more detailed investigation on the effect of baking mode on breads may be carried out comparing samples from bakery B that were prepared with ingredients of the same origin (i.e., flour, water and sourdough).

Significant differences were found among furans and aldehydes on the crusts of the differently cooked loaves, as expected by a more pronounced Maillard reactions in wood-fired samples. In particular, significantly larger amounts of furan, tetrahydrofuran, 2-methylfuran and 2-propenyl-2-furan were found in for wood-fired samples. In addition, 2-furanecarbonenitrile and 3-methylfuran were detected only in wood- and gas-fired breads, respectively.

Butanal, pentanal and 2-methyl-2-butenal were found 4–5 fold higher in B_w crust than in B_g. Differences were less marked for all the other compounds with the exception of 1-butanol, ethylpropanoate and limonene which were found in significantly higher amounts on the crust of wood-fired bread.

Significant differences were found among some furans and aldehydes for crumb, likewise crusts. In particular, furan, tetrahydrofuran, 3-methylfuran were found only in B_w samples while furfural peak area was significantly higher in B_g sample. Among aldehydes, 2-propenal and pentanal were found only in B_w crumbs whereas 2-octenal was fourfold higher in B_w than in B_g samples.

Characteristic images of the central slice of the two breads were reported in Fig. 2a for B_w and b for B_g, respectively. The crust appeared to be detached from the crumb in both breads but more markedly in B_w samples with the presence of a hollow layer acting as an air cushion between crust and crumb (Fig. 2a and b). The average crust thickness of the breads was found to be 6.8 ± 0.7 mm, 5.2 ± 0.6 mm B_w and (b) for B_g., respectively, by objective measurement with the Image ProPlus Software. Very large pores were always observed in the B_w bread loafs. On the contrary, the presence of a finer and more homogenous pore distri-

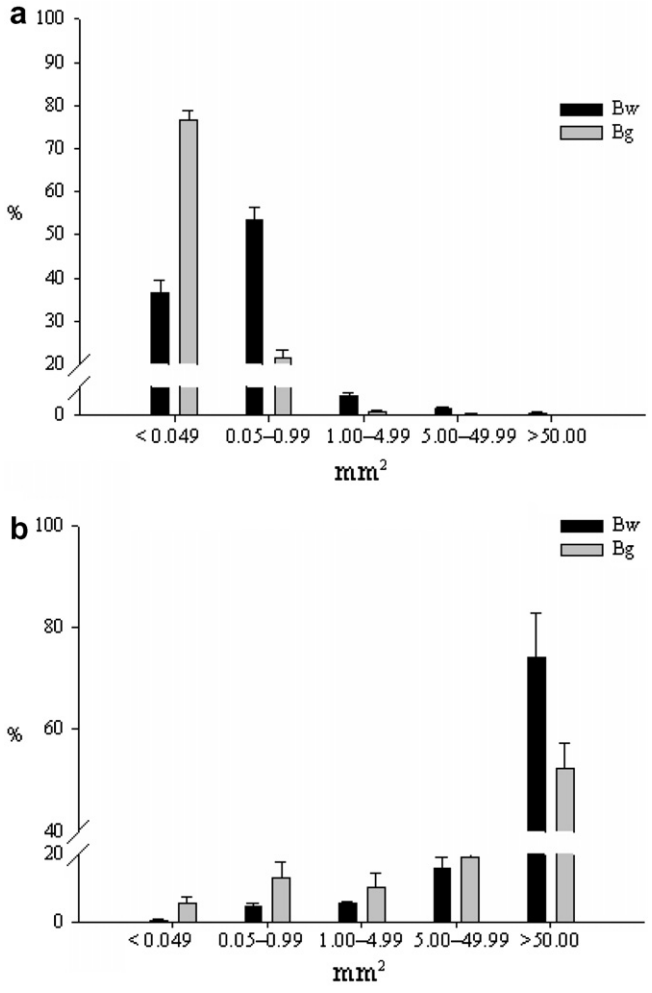

Fig. 3. Number of pores as percentage of the total number of pores (a) and area as percentage of total pore-area (b) for the selected five dimensional classes for B_w and B_g Altamura bread samples ($n = 3$, sample size = 6). Error bars represent +/−1 standard deviation, RSD < 15%.

bution characteristic was observed for the crumb of B_g samples, although large pores were present, especially under the crust.

The objective evaluation of number of pores and the total percent area occupied by the pores of each dimensional class for crumbs of B_w and B_g are reported in Fig. 3a and b, respectively. A significantly larger number of small pores were present in B_g samples (<0.049 mm^2) which accounted for more than 5% of the total pore-area. An opposite trend was observed also for pores in all the other dimensional classes. Larger pores (>50 mm^2) were found in both samples but, while they represented less than 55% in B_g crumb, they accounted for more than 70% of the total pore-area in the B_w bread, suggesting a different fermentation pattern during the first step of baking in wood-fired oven related to a different temperature gradient inside the dough.

Crumb colour of B_w and B_g samples was also measured. The colour of the crumb for wood-fired breads was found to have lightness of 70.0 ± 3.0 (L^*), redness of 0.6 ± 0.0 (a^*), yellowness of 16.3 ± 0.5 (b^*) and to not significantly differ from crumb of gas-fired samples. A TPA test was also performed on crumb of B_w and B_g samples. B_g crumbs showed significantly higher hardness (25.3 ± 2.4 N), cohesiveness (0.45 ± 0.02) and chewiness (9.7 ± 2.4 N), than B_w (hardness 15.6 ± 2.0 N, cohesiveness 0.40 ± 0.01, chewiness 6.9 ± 0.8 N, respectively). Springiness did not significantly differ between samples. The lower hardness of B_w crumb may be possibly ascribed to different water dynamics during baking where a more pronounced role could be played by the "air cushion" between crust and crumb that could have maintained higher crumb moisture content minimizing the sucking action of the drier crust, as previously observed in same shape "Altamura" samples (Chiavaro et al., 2008).

4. Conclusions

Volatiles of PDO "Altamura" durum wheat sourdough bread were for the first time fully characterized in this study, by means of DHE GC–MS technique, resulting in the identification of a large number of compounds, more numerous in crust than in crumb.

Effect of baking mode on volatile profile and selected physico–chemical properties was also evaluated. Bread samples cooked by means of wood- or gas-fired oven showed some differences in volatile profiles only for samples originating by the same bakery. In this case, differences in the volatile profile could be completely ascribed to the different baking mode, as ingredients and environmental conditions were the same. Samples baked in wood-fired oven showed larger amount of volatile compounds such as furans and aldehydes that could positively influence the flavour of the product.

Macroscopic appearance and crumb texture were also influenced by baking mode. In particular, wood-fired oven baking have led to product with a harder and more browned crust and a softer crumb which could be preferred by the consumers. These findings showed that the characterization of the volatile profile may be a useful tool to assess the PDO "Altamura" bread authenticity and to avoid economical frauds to guarantee both the consumers and the producers.

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